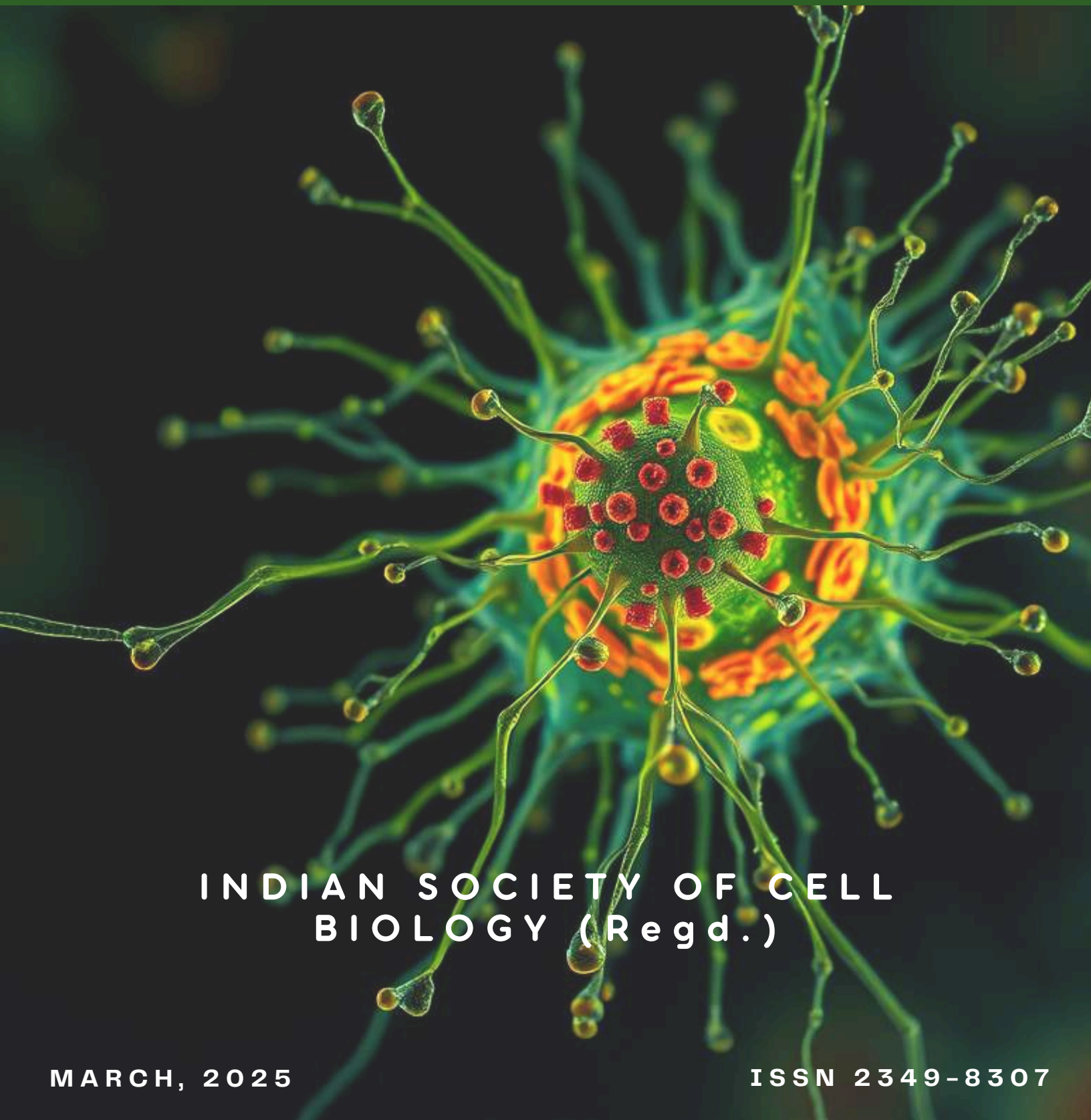
VOLUME 48, ISSUE 1

ISCB NEWSLETTER



INDIAN SOCIETY OF CELL BIOLOGY (Regd.)

MARCH, 2025

ISSN 2349-8307

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Coverpage: An AI-generated abstract depiction of a neuron, featuring a central cell body with extended processes resembling dendrites or an axon. The composition highlights the complexity of neural cells and their role in cellular communication within the nervous system.

Message from the President, ISCB

It is my pleasure to write the foreword for the last newsletter of Indian Society of Cell Biology (ISCB) being brought out by the current team. Prof. Sathees Raghavan and his entire team have done a marvellous job in bringing out interesting, informative, colorful and timely newsletters of the Society over the past two years. The contents included well written scientific articles from members from all over the country. The team's efforts led to several members voluntarily submitting articles, describing either their own work or recent findings in cell biology. In addition, the newsletters played a huge role in informing members of the activities of the society. These included announcements, detailed instructions and summary of the proceedings of two of the most vibrant annual meetings of ISCB, the first at Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Navi Mumbai in January 2024 and the second at National Institute of Science Education and Research, Bhubaneswar in December 2024. We now eagerly look forward to the next meeting to be held at Indian Institute of Technology, Kanpur in December 2025. On behalf of ISCB, I invite all the members as well as others to participate and contribute their research in the annual meeting and in other activities of the Indian Society of Cell Biology.

This has been a tremendous effort by the team and I congratulate and thank them on behalf of ISCB.

-Prof. Surendra Ghaskadbi, Ph.D., FNASc
President
Indian Society of Cell Biology

Charting New Frontiers in Cell Biology

Dear Members,

It is with great pleasure that I welcome you to this new issue of the Indian Society of Cell Biology (ISCB) Newsletter. Each issue of our newsletter not only reflects the dynamic rhythm of the scientific community but also bears witness to how far we have come as a nation in supporting cell biology research—from its nascent beginnings to the rapidly evolving frontiers of modern biomedical science.

Two major highlights of the year were the 46th All India Cell Biology Conference, held at ACTREC, Navi Mumbai in January 2024, and the 47th All India Cell Biology Conference, hosted by the School of Biological Sciences, NISER, in December 2024. Both events were resounding successes, bringing together researchers across disciplines and career stages to share cutting-edge science and foster meaningful collaborations.

This edition of the newsletter also offers an in-depth reflection on the Evolving Landscape of Cancer Research in India—a journey from early cytological studies to modern advances in genomics, precision oncology, and artificial intelligence. Our Research News section further highlights the richness of ongoing work—from groundbreaking studies on podocyte dysfunction and DNA damage repair to the role of securin in carcinogenesis and the evolutionary conservation of caspase-driven macrophage differentiation. These articles demonstrate the depth and diversity of cell biology research being undertaken across the country.

It is also a moment of joy and pride as we congratulate Professor Arun Kumar Shukla and Professor Sandeep M. Eswarappa, recipients of the 20th Prof. S. P. Ray-Chaudhuri 75th Birthday Endowment Lecture Award and the 5th Prof. Rita Mulherkar Award Lecture, respectively. Many congratulations to all student awardees for their commendable poster and oral presentations.

Finally, I extend my heartfelt thanks to all contributing authors for their timely, insightful, and valuable contributions to this edition. Many thanks to the President and Prof. J. K. Roy for inputs with the newsletter. I would also like to take this opportunity to formally acknowledge and thank the Journal of Cell Science and Cactus Global for their support to ISCB. Additionally, I would like to extend my thanks to Nikon, Juniper, Eppendorf, Samitek, JJ Biotech, and ATNT Laboratories for supporting the newsletter and ISCB activities.

In the spirit of our original vision, let us continue exploring, asking, and innovating—because at the heart of every discovery lies a cell, and in each cell, the key to opening life itself.

Warm regards,
Prof. Sathees C Raghavan
Secretary, ISCB

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NEWSLetter
Indian Society of Cell Biology
Banaras Hindu University, Varanasi, 221005

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ISCB Newsletter
ISSN 2349-8307
Volume 48, Issue 1, 2025

Exclusive Interview: Prof. Surendra Ghaskadbi on Science and Innovation

Curious about a scientist who has pushed the boundaries of cell and developmental biology? Dive into this fascinating conversation with Prof. Surendra Ghaskadbi as he shares insights on Hydra research, regeneration, and the power of scientific outreach!

Surendra Ghaskadbi is a cell and developmental biologist who held the positions of Senior and Emeritus Scientist at the MACS-Agharkar Research Institute in India. His lab has been particularly interested in cell signaling and pattern formation during early development, focusing on using Hydra as a research organism. More recently, Surendra has played a role in teaching and outreach in India, and he is also the current President of the Indian Society of Cell Biology. Dr Seema Grewal, Executive Editor at the Journal of Cell Science (JCS) chatted with him to find out more about his career, his advice for junior researchers and his role as the society President. This interview was originally published in JCS and can be accessed in full at: https://doi.org/10.1242/jcs.262156. We are pleased to include the interview in the ISCB newsletter with the approval of the interviewee.

EARLY INTEREST IN SCIENCE

Let's start right at the beginning: how did you first become interested in science?

There was no one pursuing science per se in my family - my father was a professor of English, my mother a Marathi graduate and both of my sisters became doctors - but somehow I was always interested in animals and wanted to learn more about them. I had no inkling that I was going to become interested in science, or even that it could become such an enjoyable and useful activity.

JOURNEY INTO CELL AND DEVELOPMENTAL BIOLOGY

How did you then become interested in cell and developmental biology?

I was lucky to go to one of the better colleges in the area that had a very strong science focus. They had good departments in zoology, botany and biochemistry. Initially, I opted for microbiology, along with zoology and chemistry, because microbiology was very popular at that time - it was fashionable. But I soon realized that I liked studying animals more than microbes, so I chose to focus on zoology. I then studied entomology for my MSc. The other influential thing that I realize in retrospect was that we had a very distinguished biochemistry Professor at my college - Professor John Barnabas. He was interested in the evolution of hemoglobin and, to study this, he used more than 50 species of vertebrates belonging to different classes. He and his group built phylogenetic trees based on amino acid sequence changes in hemoglobin molecules. His research, and particularly the animals he used, excited me and inspired me to pursue a career in science. After that, I moved to Pune University to do my MPhil, where I worked on the chromosomes of shrews and squirrels.

Soon after this, while I was doing a teaching job in a local college, I came across Professor Leela Mulherkar. She was an embryologist who had returned to India in the late 1950s after studying in the Institute of Genetics in Edinburgh with Conrad Waddington. She had worked on the chick embryo, doing lots of transplantation experiments, and she started teaching experimental embryology for the first time in India, using locally available materials. I had the chance to work with her for my PhD and that's when I became interested in embryology. We looked at lots of different model systems, like chick, frog and snail embryos as well as Hydra and sponges - whatever was available really.

PHD AND POSTDOCTORAL RESEARCH

What did you study during your PhD and postdoc?

I was looking at the cellular and teratological effects of a secondary mold metabolite called cytochalasin H, which was somewhat similar to cytochalasin B but had different effects on cells. Professor Mulherkar had initially got interested in teratology after the thalidomide tragedy had emerged as a big problem in Europe. She studied the teratological effects of several chemicals on development. Working with her was interesting and illuminating. In addition, I learned a lot of laboratory techniques. After a short post-doc with Professor Sohan Modak I became more interested in the basics

of development. Of course, interfering with normal development by various means, at organismal, biochemical, cellular and molecular levels, remains to be one of the most popular and rewarding approaches to study normal development. In fact, we know a lot about normal biological phenomena by studying the abnormal.

One of the things that I would like to mention is that, for a multiple reasons, I have never done a formal postdoc abroad. On the one hand, this meant that I was not able to learn many of the modern techniques that were being used at that time. But it did mean that I could get a faculty position quite early on in my career. I was also very comfortable with tackling problems locally, both at the laboratory as well as at the administrative levels, because I was used to the environment in India. Many people who did postdocs abroad, and got used to the facilities, support and granting systems in other countries, found it difficult to find their feet when they returned to India. Obviously, things are quite different now, but in the 1980s it wasn't easy to do science in India, so I think staying here proved to be an advantage for me.

BUILDING A LAB AND SELECTING A MODEL SYSTEM

How did you decide what direction you wanted to go in when making the transition to setting up your own research group?

While working with Professor Mulherkar, I got interested in development, especially the phenomenon of neural induction. I was trying to understand the kinds of proteins and genes that participate in neural induction in chick and frog embryos, and I decided that I wanted to continue looking at patterning and morphogenesis during early development. We contributed to the area over the first few years but the problem with the frog embryo was that you could only work with it seasonally in India. So, if a reviewer asked you to do an experiment, you had to wait until the next monsoon to get embryos. Chick embryo studies were a little easier but there was a period after the avian flu that happened in India when the quality of eggs - at least during early development - deteriorated. I have still not been able to understand why and how this happened as the poultry industry, overall, has been doing consistently well since after the outbreaks were contained. In addition, we couldn't do much in the way of genetics with these models. So that's when I started thinking about Hydra, which I had used as a part of my doctoral studies. Hydra has an organized nervous system so I thought it might be a good idea to look at how one of the first nervous systems arose. But I soon realized that that was a very ambitious question! However, I did find Hydra to be a very attractive model: it's easy to handle in the lab, it has an amazing regenerative ability, it seems to defy aging and it also has stem cells. There were many features that any developmental biologist would like, so I switched to working with Hydra. One practical consideration was that, since hardly any labs in India worked with vertebrate embryos and no labs were working with Hydra, I had relatively less competition within the country. Most laboratories in India that time worked with Drosophila. Even now, many labs continue to use Drosophila as a model, although other research organisms such as the worm and zebrafish are also used widely.

In the year 2000, working with Hydra in India was not terribly easy as, after the late 1970s, no lab had used Hydra for either teaching or research purposes. We started slowly and in the first 10 years, we were only able to publish a few papers. These studies, however, provided a solid foundation for what we did over the next 10 years. While reintroducing Hydra as a research and teaching organism in India, I continued my work with frog and chick embryos so that the graduate students and the lab could continue 'performing' in ways acceptable to peers and administrators of science. This allowed the students and me to move on in our careers. We avoided putting all our eggs in one basket, pun intended.

My parent institute not only allowed but encouraged me to start a developmental biology group, so we were

able to hire people working with other research organisms like zebrafish and Drosophila. I felt that putting too much emphasis on just one or two models was detrimental to teaching and research, so it was good to have this diversity. Ultimately, a model system is just that – it's one model. But organisms are all so different from each other so if one wants to learn and teach biology one needs to study a variety of systems. I worry that sometimes people forget this and design their research questions based on their model system when in fact they should think about the larger question first and should not be averse to using more than one model system if needed. I often see colleagues trying hard to 'sell' their model organism, which may be justified while defending a grant proposal but is unfair to students; they need to learn about organisms belonging to as many taxa as possible.

RESEARCH FOCUS AND MAJOR DISCOVERIES

What was the main question that your research tried to address?

We were really interested in cell-cell signaling molecules in Hydra since these can not only provide information about regeneration and pattern formation in Hydra but can also provide insights into the evolution of developmental mechanisms themselves. To begin with, we started looking at Noggin, which is a known BMP inhibitor that's important for neural induction in vertebrates. At that time, there was no report of Noggin from Hydra. We identified the Hydra Noggin and found it to be structurally conserved and, when we ectopically expressed it in Xenopus embryos, we got a phenotype we were very excited about. But we have still not been able to pinpoint its precise function in Hydra, though we now believe that it plays a role in the patterning of tentacles. We also discovered Hydra Gremlin - another BMP inhibitor - and have been looking at molecules like FGF and VEGF. Finding FGF in Hydra was not surprising but finding VEGF was a big surprise because Hydra is diploblastic and doesn't have mesoderm (and blood), which is the tissue that VEGF is usually associated with. But it's very much there and we believe that it has some role in the patterning of tentacles. We also discovered many receptor tyrosine kinases in Hydra.

In collaboration with my wife Saroj, who worked at Savitribai Phule Pune University, we have also been looking at DNA repair molecules in Hydra; this was the other major project in the lab. We found that nucleotide excision repair genes are all present in Hydra. Moreover, when we expressed Hydra homologs of Xeroderma pigmentosum genes in human cells deficient for these genes, they could rescue the phenotype to an extent. This is particularly interesting given Hydra's tremendous regenerative capacity and lack of organismal senescence. Our work so far has thrown out more questions that it has answered. However, this makes me happy rather than unhappy.

EXCITING QUESTIONS IN THE FIELD

And what, in your opinion, are the most exciting questions in the field?

I think the topics of regeneration and aging - and their interrelationship - are both exciting and important. We now know that as more complex organisms evolved, their regenerative capacity has decreased and there are many theories behind this. I don't think we have the real answer to why this happens but understanding this could be useful, for example in the context of in vitro tissue and organ regeneration. Most of the molecules that Hydra and other organisms use for regeneration are very much present in humans but there's still lots of work to be done to understand why humans have limited regenerative abilities.

The other area I find very intriguing is biodiversity and personalized medicine. On the one hand, we have lots of conservation, with almost all developmental and signaling pathways acting in concert. But on the other hand, we know we have so much biodiversity. How can the same set of genes make such different structures with almost unlimited outcomes? This question is being actively addressed but we are far from finding an

answer. And, in the case of humans, how does variation dictate how we might respond so differently to different medicines? Also, how do factors like the microbiome, which continuously changes in quality and quantity, influence physiology? It would be wonderful to find out what the connections are!

ADVICE FOR ASPIRING RESEARCHERS AND PIS

You've had your own group for some time now so what would be your advice to someone who is aspiring to set up their own group and become a PI?

I don't have much experience outside of India, so my advice is restricted to what I've learned here. One of the things that I have seen is that, very often, postdocs start their labs and try to continue the work that they have done with their mentor, but it takes a very long time for them to get established and get independent recognition in the field. My feeling is that if people try to do something that's unique and original to them, the work will be that much more rewarding. I know that's more risky but one can have a couple of parallel projects - one that is less risky, so that you'll be able to publish well and your graduate students can move on in their careers, but then a second project that is a bit more ambitious and high risk, which will allow you tackle something more interesting and satisfying in the long run.

The other piece of advice or suggestion I have relates to effective communication. Although people doing science come from all around the world, the common medium for communication is still English, so I think that young PIs really need to put effort into improving their oral as well as written communication in English. Because whatever good work you do, there is no point in doing it unless it's well presented and shared effectively with others. I often see good, established scientists with exciting data struggling to communicate their findings. They need to adjust their communication style to their audiences. I think there's still lots of work to be done here.

PASSION FOR TEACHING AND OUTREACH

I gather that you're winding down your lab and doing lots more teaching and outreach – can you tell us more about this?

Yes, I had a very active lab up until 2021 but I'm no longer accepting any graduate students. I'm still associated with Agharkar Research Institute, but I spend most of my time teaching, doing outreach and mentoring all over the country. I'm also involved in writing books for school children with the intention of getting them interested in science from a young age. We also need to make sure that everyone, even if they don't go on to study biology or even natural sciences, has some basic understanding of science. During the pandemic, one came across many so-called 'educated' people wearing their face masks on their chins. If only they knew and remembered the basics of infection and the advantages of using a face mask properly, things could have been a lot better.

Teaching is also one of my passions. I love to talk about biology and interact with people of all ages. I go all around the country, wherever I'm invited, to teach cell and developmental biology. I usually teach at the master's level and quite often at places where there are no developmental biology teachers. Students all over the country are equally curious and smart, and they come out with the most interesting and difficult questions. I also do some teaching and outreach with schools and colleges. I enjoy taking Hydra with me. When we look at them using microscopes, the school children can see them moving around and feeding – they get very excited! And the great thing is that I can just cut the Hydra using a sharp blade and the children can watch them regenerate over the next few days; they can easily see a biological phenomenon unfolding in front of their eyes. In fact, we ended up designing a Hydra kit that we provide to colleges and universities so that they can establish cultures of their own and use them for carrying out simple but interesting experiments.

LEADERSHIP, CHALLENGES, AND PERSONAL INSIGHTS

You are also President of the Indian Society of Cell Biology - how, when and why did you first become involved with the society?

Soon after starting my PhD, back in 1980, I went to an Indian Society of Cell Biology (ISCB) conference in Delhi. I really enjoyed the conference and found it to be very useful: there were lots of good talks, the general atmosphere was very good, and there was a lot of time allotted to posters. After that, I started going to the ISCB meetings almost every year. I always stay for the whole duration of the meeting; I'm not a great fan of the fly-in, fly-out culture. I became a lifetime member of the society in 1988 and developed an interest in society matters. With time, I slowly became associated with the running of the society and started to contribute to it. For example, I organized the annual meeting back in 2008. Not everybody likes this kind of society work but I really enjoy it. So, for me, becoming the president was no big deal - it was just part of the job.

What are the main aims and mission of the society?

Firstly, we hold an annual conference where we all meet and discuss science. We make it a point to invite some international speakers, which gives the meeting a different flavor. It's a great opportunity for students and postdocs to present their work. We made a rule to ensure that we don't repeat invited speakers for three years and that's been fantastic, as it's allowed us to get lots of young PIs speaking at the meetings. We also have some hands-on workshops that are organized by the society, for example on embryology or microscopy. These help teachers design laboratory exercises in their colleges. These take place across the country so that we can try to provide access for people across India. The society is also very interested in improving and supporting the teaching of cell biology, so we have generated some protocols for quality teaching.

And what are the main challenges that the society faces?

One of the problems is that there are very few people working for the society. Lots of people use it as a platform, for example to meet people and present their work, but getting people on board to run the affairs of the society is always a challenge. For a scientific society to function, you also need funds, but to get those funds, you need dedicated people who work continuously behind the scenes to find those funds and apply for them. It's largely a thankless job and does not get translated in recognitions and awards, but it can be very rewarding and satisfying if it goes well.

If you could change one thing in academia, what would it be?

I really feel quite strongly that the charges associated with publishing are getting out of control. It is becoming a big business without checks and balances. Many labs in India find it very difficult to raise that kind of money to publish their papers. I also find it perplexing that some of these publishers get so much work out of all of us - by asking us to review papers for them for free - and they never put back anything. JCS (and the other Company of Biologists journals) is obviously an exception, and I know there are also some very good society-run journals that support their communities. But most of the other journals don't do this, and this really creates a problem. There are some institutions in India that can afford to pay to publish in these expensive journals but there are people doing equally good work who end up publishing in journals that are not really read by many people, just because they do not have any other option. It creates a huge divide.

One of the other areas I would like to see a change in is the postdoc culture in India. It really hasn't developed, despite some attempts by individuals and funding agencies. People tend to leave the country for postdoc jobs or move on to do something else. There are also very few clinicians doing basic biology research in India, probably because they must spend most of their time dealing with patient care, but this means there are

limited opportunities to foster collaborations between basic scientists and clinicians. I think this is also something that needs to be addressed.

Finally, is there anything our readers would be surprised to find out about you?

I like to whistle Bollywood songs. It's a habit of mine that has become a hobby. During the pandemic, I started a small YouTube channel of myself whistling. I got a lot of support from students and friends, out of sympathy I suspect, but I now have quite a few followers. I even whistle at cell biology conferences and, so far, it has been tolerated well.

The other thing I would like to mention is that I'm a kidney donor - I donated a kidney to my wife 10 years ago and both of us are in good health. So, we help to counsel people who are potential donors and recipients; we tell them - very frankly - what we have gone through emotionally, financially and physically, to help them feel more comfortable with the whole process. We also participate in discussions on live organ donation. The number of men who go for live organ donation is extremely small, for multiple reasons and especially in India, so we try to pass on the message that it's possible and that it's good.

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Evolving Landscape of Cancer Research in Indian Science

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The field of life sciences has experienced a profound evolution, advancing from the fundamental study of cells to the forefront of molecular biology and omics technologies. This journey has led to significant breakthroughs in our understanding of life across multiple scales, with each discovery paving the way for the next, contributing to a more integrated and precise comprehension of living systems. Additionally, we are now closer than ever to understanding the underlying causes of various diseases, from chronic conditions to complex cancers and genetic disorders.

As technological advancements continued to unfold, cancer research in India has undergone a remarkable transformation. This evolution has been characterized by progress in both fundamental and applied research, an increase in funding, and the adoption of advanced technologies. The journey of Indian researchers through these changes—navigating emerging trends and aligning their work with global developments—has been shaped by significant government support, both through funding initiatives and the formulation of policies and guidelines. This dynamic progression has been an exciting chapter in the story of Indian science, particularly in the field of cancer research.

An overview has been described below:

1. Early Stages (1970s-1990s): Foundations in Cell Biology and Cytology

In the 1970s and 1980s, cancer research in India was largely centered around the study of the morphological characteristics of cancer cells through cytology and histopathology. Research in cell biology aimed at understanding the cellular changes that lead to cancer, primarily focusing on cancer's impact on tissue structure and function. Key research areas included basic cell biology research - understanding cellular transformations, the concept of oncogenes, and

tumor suppressor genes, etc; cancer etiology - research into the role of tobacco, diet, and environmental factors; cancer screening and diagnosis- pioneering work were done for developing cytological techniques for detecting cancer, such as the Pap smear (for cervical cancer) and was widely adopted in India.

Institutions like the Indian Institute of Science (IISc), Tata Memorial Centre (TMC) in Mumbai, and the All India Institute of Medical Sciences (AIIMS) in New Delhi, started laying the groundwork for cancer research. During this time, the Indian Council of Medical Research (ICMR) had set up the National Cancer Registry Programme (NCRP) in 1982, which continues to be one of the largest and most comprehensive cancer registries in the world. The NCRP began with three population based (existing Bombay registry and new registries at Bangalore and Madras) and today has expanded to 38 Population Based Cancer Registries (PBCRs), of which 35 PBCRs' are located in 20 states, and 3 PBCRs are in three Union Territories. In addition, there are around 215 Hospital Based Registries (HBCRs) across the country which contributes to the PBCRs in the given area. Together they provide information on burden and trends of cancer in the population over time and future estimates of incident number of cases.

In 1966 the Indian Cancer Research Center and the Tata Memorial Hospital were combined to form the first comprehensive center dedicated to cancer care and research in India – the Tata Memorial Center (TMC). Thereafter, many new additions to the institute were gradually made in terms of new approaches to research in cancer biology that included separate laboratories/departments for studies on genotoxicity, radiobiology, cellular chemotherapy and comparative oncology.

In 1975, India's active National Cancer Control Program was launched with focus on primary prevention and early detection of cancer, through establishment of Regional Cancer Centers –one in every state and developing oncology units in existing medical colleges across the country. Presently there are 62 such centres spanning all the states and union territories of India

In 1986, India took a revolutionary step by establishing one of the world's first dedicated Departments of Biotechnology (DBT), under the Ministry of Science and Technology. Since its inception, the Department of Biotechnology has played a crucial role in advancing research and innovation, spanning from fundamental science to translational research. It has fostered an ecosystem that nurtures innovation from initial ideas through to commercialization. Cancer research has been a key area of focus, with early recognition of the urgent need to address and overcome the challenges in this domain and thus a dedicated Task Force on Cancer Disease Biology was constituted ito support focused research in the area.

2. Progress in the 1990s to Early 2000s: Molecular Biology and Early Molecular Mechanisms

During the 1990s and early 2000s, research shifted towards molecular biology, where the focus expanded to understanding DNA

mutations, oncogenes, and tumor suppressor genes. Key developments during this period included identification of cancer-associated genes: studies on the p53 gene (a well-known tumor suppressor) and its mutations in cancers like breast, oral, and lung cancer in Indian populations, exploring cancer cell signaling pathways: research into the mechanisms by which cancer cells evade normal regulatory pathways, including studies on growth factors and the EGFR (epidermal growth factor receptor). Also interest grew in identification of biomarkers for early detection for cancers prevalent in India like oral cancer, cervical cancer, and breast cancer.

Tata Memorial Center (TMC), Mumbai & All India Institute of Medical Sciences (AIIMS), New Delhi continued to play a major role, alongside many other institutes that started working in this area such as the National Institute of Immunohaematology (NIIH), Mumbai, National Centre for Cell Science (NCCS), Pune, National Institute of Cancer Prevention and Research (NICPR) Noida, National Institute of Immunology, New Delhi etc

Indian Institute of Technology (IITs) and National Institutes of Technology (NITs) began to collaborate with clinical institutions for translational cancer research, especially in the development of new diagnostic tools and therapeutic interventions.

3. 2000s to 2020s: Emergence of Omics, Sequencing, and Big Data

The 2000s marked the introduction of omics technologies, which transformed cancer research globally, including in India. Advancements in genomics, proteomics, and transcriptomics allowed for high-throughput screening of molecular features of cancer. Notable achievements include initiatives like the Indian Genome Variation Consortium and Genome India project, the first large-scale comprehensive studies in the country to study and catalogue the variations in nearly thousand candidate genes for predictive marker discovery, founder identification and also to address questions related to ethnic diversity, migrations, extent and relatedness with other world population started mapping genetic variations in the Indian population. Another ambitious biomedical research effort was participation of India in the International Cancer Genome Consortium (ICGC) project to generate high resolution catalogues of genomic alterations of oral cancer in Indian population. Similar studies were undertaken by Tata Memorial Hospital at Mumbai on lung cancer, head and neck cancer, and breast cancer that revealed unique mutations common in the Indian population. Research into molecular targeted therapies became a focal point, with the development of Tyrosine Kinase Inhibitors (TKIs) for cancers such as chronic myeloid leukaemia (CML) and HER2-positive breast cancer.

The National Cancer Grid, an initiative of the Government of India through the Department of Atomic Energy and its grant-in-aid institution, the Tata Memorial Centre, was established in 2012 to create a network of cancer centres, research institutes, patient groups and charitable institutions across India with the objective of developing uniform standards of patient care for prevention, diagnosis, and

treatment of cancer. Today it has a network of more than 300 clinical centers across the country.

The Advanced Centre for Treatment Research and Education in Cancer (ACTREC) - came into existence which today serves as state-of-art R&D satellite of the Tata Memorial Centre (TMC). ACTREC comprises two arms—a basic research arm i.e Cancer Research Institute (CRI) and a Clinical Research Centre (CRC). National Cancer Institute (NCI) in Jhajjar (Haryana) approved by Union Cabinet in 2013 became fully functional by 2020 and is now a hub for clinical research and cancer treatment.

During this period, the Department of Biotechnology under the visionary leadership of Prof. M.K. Bhan had laid the foundation of some of the most advance institutions in the areas of genomics, precision medicine and translational research. In 2006, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram was accorded the status of an autonomous National Institute of the Department with focus on disease biology research and translation of biotechnology to knowledge economy. National Institute of Biomedical Genomics (NIBMG), Kolkata was established in February 2009explicitly devoted to research, training, translation, service, and capacity-building in biomedical genomics. Translational Health Sciences & Technology Institute (THSTI), Faridabad was established in 2009 with capabilities in indigenous vaccines, monoclonal antibodies, in vitro diagnostics, biotherapeutics, drug discovery and provides a scientific atmosphere for clinical research propelling healthcare advancements forward. The Institute for Stem Cell Science and Regenerative Medicine (inStem) at Bengaluru was established and was one of the first institutions in the country, dedicated to the study of stem cells and regenerative biology.

At the same time, IISERs established by Ministry of Education were declared as Institutes of National Importance by the Parliament of India in 2012. Many more IITs were launched across the country and an era of interdisciplinary and collaborative research was started.

Thus, as the field of cancer research evolved, the institutional landscape in the country also got transformed, expanding in both size and capability, becoming better equipped to tackle challenging research and explore new frontiers. The number of cancer-related research publications from India has shown a significant upward trend from 2010. In 2010, there were 1,147 publications, which steadily increased each year, reaching 8,080 in 2024 (as per pubmed search data). Overall, India's cancer research output has expanded nearly sevenfold in 14 years, indicating growing scientific focus, better funding opportunities, and enhanced research capabilities in the field of oncology

4. Present Day (2020s): Precision Medicine, AI, and Comprehensive Cancer Research

In the present decade, the cancer research landscape in India is increasingly shaped by the intersection of precision medicine, artificial intelligence (AI), and advanced bioinformatics tools. More and more Indian researchers in cancer are building large-scale cancer data repositories, focusing on specific cancer types

that are more prevalent in the country, like oral cancers, cervical cancers, and esophageal cancers. This is largely driven through multi-omic approaches, integrating genomics, epigenomics, transcriptomics, proteomics, and metabolomics to understand the complex biology of cancer. AI and Machine Learning in Cancer Diagnosis includes developing machine learning algorithms to analyze medical imaging (e.g., histopathology slides, CT scans) for early detection and diagnosis, which could reduce the burden on healthcare professionals and lead to quicker, more accurate diagnoses.

5. Key Focus Areas for Future Cancer Research in India

Despite the concerted efforts of all stakeholders across various fronts, the harsh reality remains the rising cancer burden, with projected increases in both incidence and mortality rates driven by a wide range of factors fuelling this unprecedented growth. There are critical gaps and key areas in cancer research that need urgent attention:

- Cancer Prevention and Early Detection: With the high burden of preventable cancers in India (e.g., oral, cervical, and breast cancer), research in early diagnostic tools, including affordable and accessible methods like mobile health technologies, is crucial. Currently, there is a lack of predictive biomarkers and comprehensive companion datasets, limiting precision in cancer treatment. Emerging technologies, such as artificial intelligence, offer the potential to discover new predictive biomarkers for early diagnosis as well as drug responses.
- Genomic and Epigenomic Research: Genomic data offers information that can inform treatment paths for patients. But large-scale genomic studies often lack sufficient representation of South Asians, which can lead to gaps in understanding cancer dynamics in this population. More research is needed on the unique genetic and environmental risk factors associated with cancer in the Indian population. This can help in the development of personalized treatments.
- Affordable and Accessible Cancer Therapies: While global research is advancing in many advanced therapeutics, most of these are still emerging fields in India, requiring more research and clinical trials. India has recently gathered pace in advanced cell & gene therapies with cost reduction in therapies such as CAR-T cell therapy, through collaborations like the US-India partnership. Other cutting-edge technologies, including immune checkpoint inhibitors, bispecific antibodies, antibody-drug conjugates, mRNA therapies, and personalized vaccines, are also set to have a significant impact and hence need for more such collaborative efforts.
- With advancement in genomics, data analytics, and digital health, it is important that the clinical trials are also re-designed integrating biomarker-driven approaches that allows more personalized treatment options. This necessitates better mechanism of data sharing and improved industry-academic collaboration. There is a need for more streamlined and faster drug and institutional study approvals, which can be achieved through enhanced collaboration

and multi-disciplinary expertise within the regulatory ecosystem.

- Multi-modality treatment, where drugs are combined to target cancer through multiple mechanisms simultaneously, is a growing trend in cancer care. Developing these combinatorial therapies, including drug repurposing, can make treatments more effective and affordable.

- To overcome cost barriers and enhance the efficiency of cancer care delivery it is essential to introduce evidence based uniform palliative care services. Palliative care should also extend beyond patients to include their families/support networks. Further, palliative care can be evolved to consider the emotional, psychological, and social impact of cancer.

- The important and evolving role of Complementary & alternative Medicine (CAM) systems such as Ayurveda, Yoga and Naturopathy, Unani, Siddha, Sowa Rigpa and Homoeopathy (AYUSH) offers an opportunity to bridge gaps and supplement treatment plans for better patient care.

- There is a considerable potential in exploring "holistic survivorship" and addressing socio-cultural stigma is essential for inclusive, supportive care and improved survivor well-being.

6. Are We Ready?

India is on the right path, but challenges remain. India faces limited access to both anti-cancer treatments and essential cancer medication & resources. Additionally, there is a significant shortage of critical facilities, such as radiotherapy, which approximately half of cancer patients will require at some point during their treatment. According to WHO guidelines, India needs 1,350 linear accelerator machines to meet patient demands, but as of 2022, there are only 365 available. Furthermore, India has a shortage of medical professionals to address its cancer burden. In 2018, there was only one medical oncologist for every 1 million people, compared to 161 medical oncologists per 1 million in the USA. These inadequacies results into delays of several months between a primary care consultation and arrival at a cancer center, leading to disease progression and raises concerns about completion of treatment regimens. The recent budget announcement to set up Cancer Day Care Centers in every district hospital is a welcome step.

In order to keep pace with the technological advancement, it is essential that both scientific & clinical research in the country is strengthened. Training plays a vital role in helping oncologists and scientists to understand the full care process and stay updated on new cancer therapies. However, most oncology training programs in India lack structured education in clinical trial methodologies, good clinical practice, and drug development. It is essential that the investment in research infrastructure is enhanced, clinical workforce is strengthened, training programs are encouraged and health care delivery is made accessible for all. Collaboration between public and private sectors to fast-track research findings into affordable clinical applications and policy-level support for integrating cancer research into public health systems is essential. Simplifying various regulatory standards and embracing

global best practices are vital to accelerate the translation of research discoveries into effective patient care.

Although India has made significant strides in cancer research over the last five decades, from the early work in cell biology to the advent of omics-based studies and precision medicine, continued investment, collaboration, and focus on specific high-burden cancers will be key to transforming the research and treatment paradigms for cancer in India.

The Department of Biotechnology (DBT) has been at the cornerstone of cancer research and innovation in India. By providing critical funding, fostering interdisciplinary collaboration, supporting the development of cutting-edge technologies, and promoting a culture of entrepreneurship, the DBT has contributed significantly to making India a leader in cancer research and biotechnology. The Department's efforts in developing targeted therapies, cancer diagnostics, and prevention strategies have not only advanced scientific knowledge but also made cancer care more accessible and affordable to the Indian population. The Department is also leveraging best of the international partnership to strengthened our skills and advance our technological capabilities. The first India-U. S Cancer Moonshot dialogue held in August 2024 led jointly by Department of Biotechnology from Government of India and the U.S. Office of Science & Technology Policy (OSTP) and the National Cancer Institute (NCI), U.S. committed to utilize the combined strengths of both the countries in our fight against cancer. As India moves toward the future of precision medicine, AI-driven healthcare, and biological therapies, the DBT's role in nurturing and sustaining cancer research will remain essential.

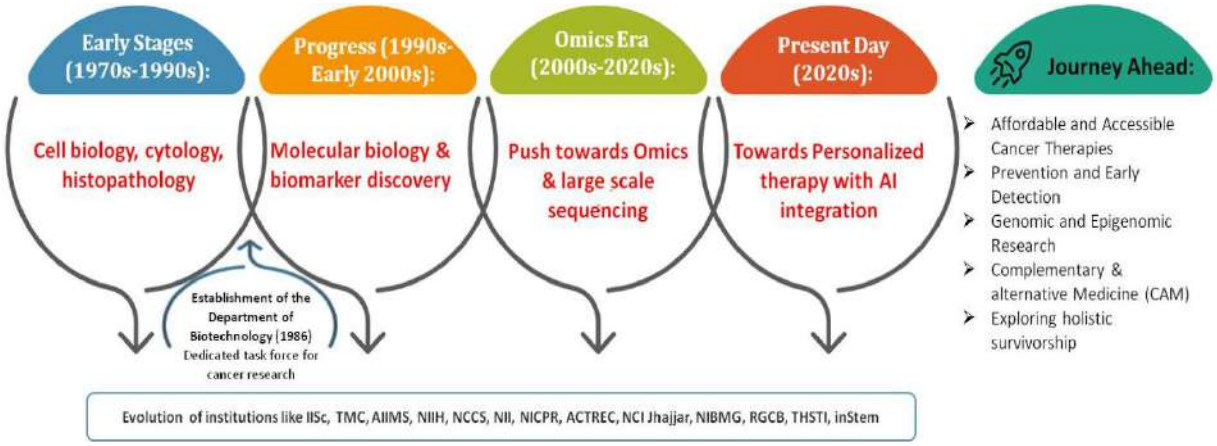

Figure 1: Evolving Landscape of Cancer Research in Indian Science

Acknowledgement:

The author expresses heartfelt gratitude to the Department of Biotechnology, Government of India.

Disclaimer: The views expressed are of the author and do not necessarily represent the opinions of the Department of Biotechnology, Government of India.

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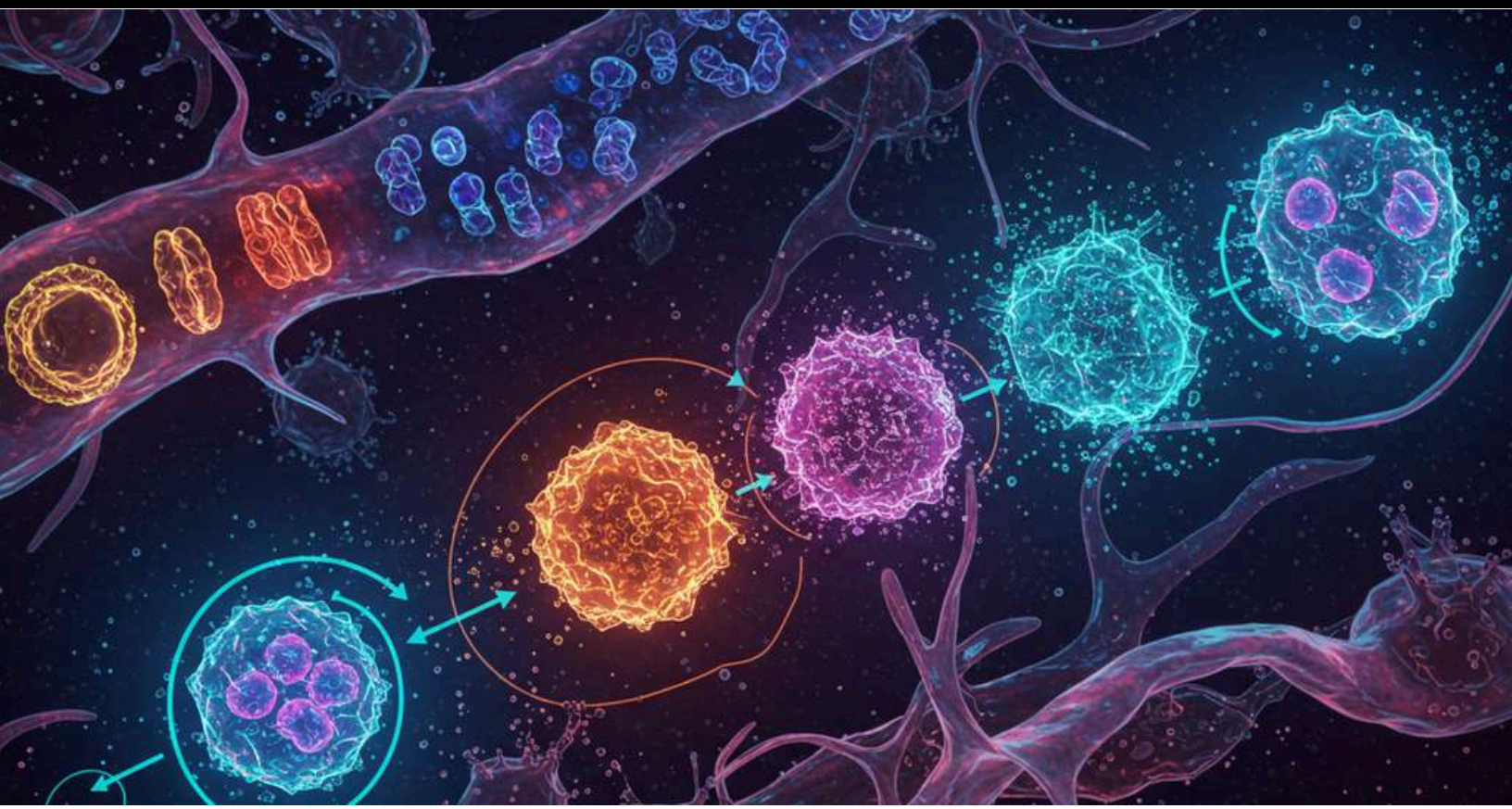
Research Article - Section 1

CELL CYCLE AND CELL DEATH MECHANISMS



The orchestration of cell cycle progression and programmed cell death is fundamental to maintaining tissue homeostasis and responding to stress or injury.This section highlights diverse roles of cell cycle regulation and programmed cell death in health and disease. Anil Kumar Pasupulati explores how growth hormone triggers mitotic catastrophe in podocytes, contributing to diabetic nephropathy. A. Chatterjee's study reveals securin overexpression and precocious anaphase as early markers of areca nut-induced carcinogenesis. Meanwhile, Bama Charan Mondal and Deepak Maurya uncover a novel role for caspase-activated DNase in driving macrophage differentiation via controlled DNA breaks. Collectively, these contributions offer fresh perspectives on how cell cycle dysregulation and controlled cell death can either undermine physiological stability or drive functional differentiation.

Mitotic Catastrophe of Podocytes: A Curious Case of Growth hormone in the Pathology of Diabetic Nephropathy

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Terminally differentiated (TD) cells have unique features: TD cells possess specialized properties, which are the result of tissue-specific gene expression, associated with an irreversible growth arrest. The TD cells acquire unique characteristics and represent highly specialized functions of the tissue. Some prominent examples of TD cells are myocytes (heart and muscle), neurons (brain), keratinocytes (skin), and glomerular podocytes (kidney). A key feature of TD cells is the irreversible exit from the cell cycle (G0 stage/Quiescent) to help maintain the structural and functional integrity of the tissue (Figure 1). By their nature, TD cells must live as long as the organism and it requires extraordinary measures to ensure their survival. Despite their physiological importance to the organism, the inability to proliferate represents the Achilles' heel of the TD cells and poses strategic problems to the organism. The organs whose function entirely depends on TD cells make them vulnerable to irreplaceable cell loss caused by various noxious stimuli and manifest in an array of diseases. For instance, damage of neurons leads to neurodegenerative disease; injury to cardiomyocytes results in cardiac damage and heart failure; similarly, damage or injury to glomerular podocytes results in impaired nephron function and culminates in proteinuric kidney disease.

Podocytes are crucial for permselective filtration of the kidney: Podocytes are terminally differentiated visceral epithelial cells of the glomerulus. The podocyte cell body gives rise to primary processes that branch into secondary processes, which arise the protrusions known as foot processes (FP). Podocyte FP eventually extends and enwraps the entire surface of the glomerular capillaries and provides epithelial coverage. The podocyte actin cytoskeleton is an

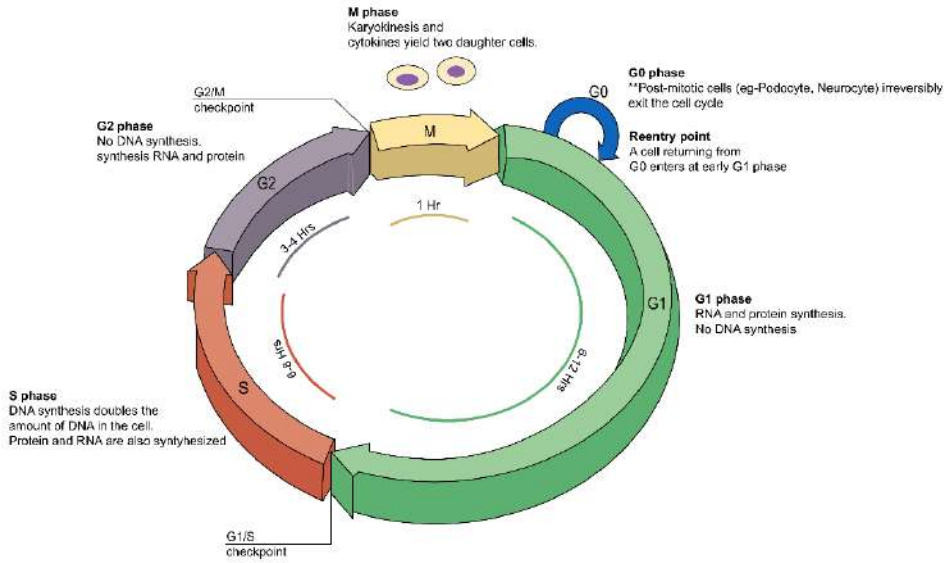

Figure 1: Representation of cell cycle events. Terminally differentiated cells accumulate in post-mitotic and quiescent (G0 stage). Normal cells either halt reversible at G0 stage and re-enter the cell cycle upon appropriate mitogenic stimuli. Each phase of cell cycle is tightly controlled by stage specific activators and check points.

important mediator of cell shape and traction force generation, which also offers resistance to capillary hydrostatic pressure. FP of adjacent podocytes interdigitates, leaving between them long filtration slits that are bridged by a modified adherens junction known as slit-diaphragm (SD). An intact SD serves as a permselective (size, charge, and shape) barrier to ensure ultra-filtered almost protein-free primary urine composed of small molecules such as urea, creatinine, and glucose (1,2). important mediator of cell shape and traction force generation, which also offers resistance to capillary hydrostatic pressure. FP of adjacent podocytes interdigitates, leaving between them long filtration slits that are bridged by a modified adherens junction known as slit-diaphragm (SD). An intact SD serves as a permselective (size, charge, and shape) barrier to ensure ultra-filtered almost protein-free primary urine composed of small molecules such as urea, creatinine, and glucose (1,2).

Podocyte injury results in proteinuria and implicates in diabetic nephropathy: Since podocytes are the crux of the glomerular filtration barrier, injury to the podocyte cytoskeleton, organelles, or its FP compromises podocyte structure and function that eventually resulting in proteinuria. Since podocytes are constantly exposed to blood and are vulnerable to several insults that are prevalent in metabolic and infectious diseases such as diabetes, obesity, COVID, and HIV. Podocyte damage is considered one of the earliest manifestations of renal injury and a significant contributor to proteinuric kidney disease. Since podocytes counteract the intraglomerular pressure, the loss of podocytes results in an abnormal glomerular

filtration rate (GFR). Both proteinuria and decline in GFR are hallmarks of nephropathy, which is a major contributor to end-stage kidney disease (ESKD). As said above, podocytes are TD cells, newer cells cannot replenish injured and detached podocytes. Therefore, podocyte loss possibly results in permanent alterations in the glomerular filtration unit. Podocyte injury is implicated in several subtypes of glomerular diseases such as glomerulosclerosis, minimal change disease, and nephrotic syndrome, and is a predominant pathological event implicated in loss of renal function.

A large body of evidence suggests that podocyte dysfunction is one of the earliest glomerular manifestations in progressive diabetic nephropathy (DN). Podocyte abnormalities such as hypertrophy, epithelial-mesenchymal transition, cytoskeletal rearrangement, programmed death, and reduced podocyte density compromise the fullest potential of its instrumental role in glomerular filtration. Patients with diabetes are presented with a reduction in podocyte count per glomerulus. The renal manifestations in diabetic subjects were similar to animal models of DN. In the conditions of a diabetic milieu, several factors such as endocrine disruptions, hyperglycemia, oxidative stress, lipotoxicity, hemodynamic abnormalities, dysfunction of cell organelles, and impaired autophagy could contribute to podocyte injury. Owing to their importance in glomerular biology and renal function, podocytes become a choice for intense investigations to understand the pathobiology of DN and ESKD. In the setting of diabetes, reduced insulin levels and signaling disrupts the normal feedback loop that regulates pituitary growth hormone (GH) secretion. Elevated serum GH levels in diabetes or acromegaly are associated with kidney injury and proteinuria (3,4). Since podocytes offer primary defense against proteinuria we longed to investigate the direct role of GH on podocytes to detail the pathology of DN and to design therapeutic options.

GH-induces cell-cycle re-entry of terminally differentiated podocytes: An unbiased microarray analysis revealed the up-regulation of Notch signaling components (Notch1 and Hes1) in GH-treated human podocytes. At the same time, we also observed the upregulation of Notch signaling genes in glomerulus from diabetic mice (5). Notch signaling play a crucial role in the proliferation of embryonic stem cells and particularly during nephrogenesis a high notch activity was observed. Matured human podocytes are quiescent (G0 Phase) and express high levels of cyclin-dependent kinase (CDK) inhibitors, suggesting they cannot proliferate. However, GH-treated podocytes showed upregulation of cyclin B1, which signifies the transition of a cell from a resting state (G0) into the G1 phase of the cell cycle, initiating the process of cell division (6). GH-treated podocytes showed upregulation of Ki-67, a nuclear protein that is only present in dividing cells and is expressed during all cell-cycle phases except for the G0-phase. Microscopic screening of about 25% of GH-treated podocytes revealed they were hypertrophic and binucleated. Supplementation of DAPT, an inhibitor of Notch signaling, or AG-490, an inhibitor of GH/JAK2 signaling

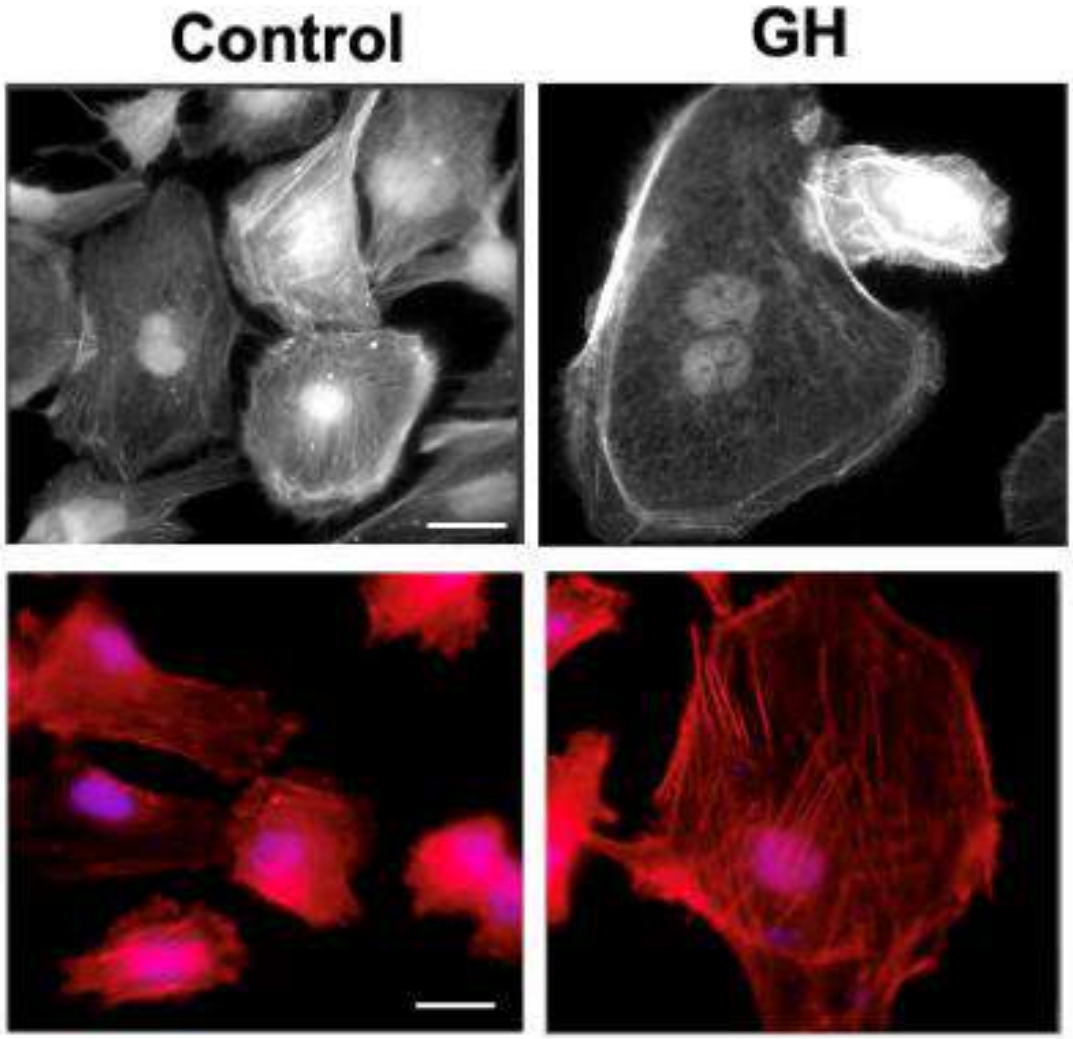

Figure 2: GH (250 ng/ml) treated podocytes showed binucleation (upper panel) and abnormal actin-stress fibers (bottom panel). Exposure to GH result in podocyte hypertrophy.

prevented GH-induced Notch signaling and attenuated cell-cycle reentry of differentiated podocytes (6).

GH-induces karyokinesis but not cytokinesis in podocytes: Podocytes that were exposed to GH showed proliferative phenotype (as evidenced by Ki-67 and CyclinB1) and possess two nuclei (Figure 2). However, there was no absolute increase in the number of cells, suggesting successful karyokinesis but not cytokinesis (6). Therefore, we carefully assessed the expression of markers that represent each phase of the cell cycle, cell cycle regulators, and checkpoints. Podocytes treated with GH showed activation of cyclin D1 and CDK4, key regulators of the G1 phase of the cell cycle. Cyclin D1 levels signal mitogenic stimuli, and when enough CDK4 is activated, G1 progresses. We also observed the expression of proliferating cell nuclear antigen (PCNA) in GH-treated cells. PCNA is present throughout the cell cycle of proliferating cells, except for the G0 phase and it plays a key role in DNA replication and repair. We observed the accumulation of p53 and p21 in GH-treated podocytes. p53 functions at the G1/S and

G2/M checkpoints, pausing the cell cycle at these stages if DNA damage is present, allowing for DNA repair mechanisms to be activated and it also induces the expression of p21, which blocks the activity of cyclin-dependent kinases (CDKs) necessary for cell cycle progression. Together, our data suggests that GH-induced cell-cycle re-entry of otherwise quiescent podocytes.

Since we observed elevated p53 levels in GH-treated podocytes and aberrations in cell-cycle progression, we assessed for DNA damage markers. Interestingly we observed the accumulation of Ku-80, RAD50, and γH2AX selectively in GH-treated podocytes, suggesting the double-strand breaks and activation of DNA repair mechanisms in response to such damages preferably via non-homologous end-joining DNA repair pathways. In addition to activation of DNA damage repair signals, we have also observed increased expression of RhoA in GH-treated podocytes. Although RhoA, a small GTPase protein that primarily regulates the actin cytoskeleton, impacts cell adhesion, migration, and contractility, all of which are important for cell cycle progression, increasing RhoA expression slow down the cell cycle by inhibiting the G1 to S phase transition. Further, RhoA can also inhibit cell cycle progression by involving cleavage furrow formation, an essential step to form two new daughter cells. In our study, we notice abnormalities in cleavage furrow formation in GH-treated cells in addition to elevated RhoA expression. GH-induced cell cycle re-entry cause podocyte cell death: GH enforced quiescent podocytes to undergo cell cycle re-entry, however, these cells failed form two daughter cells. Though there was GH-induced nuclear division, cytokinesis was not accomplished. Often, the failure of cell cycle progression is accompanied by accumulation of DNA damage and association cell death. We then analyzed the activation of cell death pathways in GH-treated podocytes. We noticed increased levels of cleaved forms of PARP and caspase-3, Bax (proapoptotic markers), and suppression of Bcl2 (antiapoptotic marker) in

GH-treated podocytes. Our FACS analysis revealed that the majority of GH-treated podocytes (~40%) are early apoptotic and ~15% are late apoptotic. Our study revealed that in the settings of diabetes elevated GH levels evoked cell cycle activation, but failure of cytokinesis resulted in mitosis-associated cell death, known as mitotic catastrophe (Figure 3). Similar to GH, ectopic expression of the Notch1-intracellular domain also induced mitotic catastrophe. Notch inhibitors or inhibitors for GH-signaling successfully ameliorated GH-induced mitotic catastrophe of podocytes. In addition to apoptosis, GH-treated podocytes also secrete profibrotic TGF-β1 and proinflammatory TNF-α (7,8). We showed that TGF-β1 also activates Notch signaling in podocytes and contributes to mitotic catastrophe. TGF-β1-induced podocyte injuries include reactivation of Notch signaling, hypertrophy, mitotic catastrophe, epithelial-mesenchymal transition, and excess secretion of fibrotic extracellular matrix components. In vivo, GH-induced TGF-β1 is associated with glomerular sclerosis. GH-induced TNF-α exhibited a paracrine effect where it skews

circulatory monocytes and macrophages into the glomerular regions and inflammatory response and glomerular injury. In summary, our studies deciphered cellular and molecular clues for GH-induced podocyte damage and in turn glomerular injury vis-a-vis nephropathy.

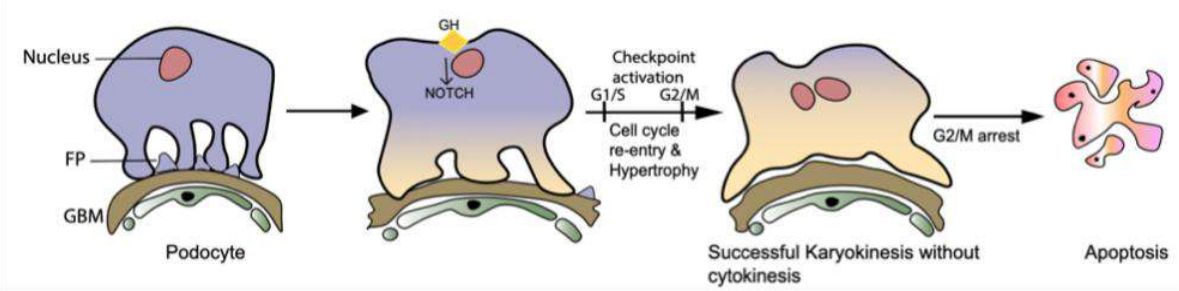

Figure 3: GH-induces mitotic catastrophe in quiescent podocytes. In the diabetic settings elevated GH acts on terminally differentiated podocytes and evoke the re-activation of Notch signaling. As a consequence, podocytes re-enter the cell cycle and become hypertrophic. Aberrations in cell cycle events such as activation of G1/S and G2/M checkpoints results in the mitotic arrest. Owing to these aberrations GH-treated podocytes undergo karyokinesis, but not cytokinesis. Binucleated podocytes and activation of DNA damage repair mechanisms in binucleated podocytes eventually results in podocyte apoptosis. FP-foot processes; GBM-Glomerular basement membrane.

Acknowledgments: I thank Ms. Praghna Reddy of nephlab.com for helping to prepare illustrations. I thank SERB, ICMR, and UoH-IoE for providing funding.

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Securin Overexpression and Precocious Anaphase as Candidate Biomarkers for Early Detection of Areca Nut-Mediated Carcinogenesis: Insights into the Mechanism of Securin Upregulation

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In India, people of the northeastern region, traditionally consume betel quid consisting of raw areca nut (RAN) with a slaked lime wrapped in a small part of a betel leaf without tobacco. This unprocessed RAN consists of higher alkaloids, polyphenols, and tannins compared with the dried one. People often swallow the entire betelquid after chewing, which is believed to contribute to the development of oral, esophageal, and gastric cancers. Heavy RAN chewers experience higher DNA damage, p53 overexpression, and lower GSH levels in peripheral lymphocytes. Such observations prompted us to propose that in addition to cytogenetic parameters, the levels of endogenous GSH and p53 protein could act as effective biomarkers for RAN chewers (1). The mutagenicity and genotoxicity of RAN-alkaloids have been demonstrated in several short-term assays, and it has been suggested that RAN should be considered a human carcinogen since it induces preneoplastic and neoplastic lesions in experimental animals (2,3).

An attempt was made to set a primary culture from the oral and esophageal cancer sample biopsies (although the success rate was very low), where we observed 5 to 30% premature anaphase cell plate and 5 to 10% aneuploid cells. Our subsequent studies demonstrated that higher expression of securin, induction of precocious anaphase (premature separation of sister chromatids) and chromosomal instability are associated with an increased risk of RAN-induced oral, esophageal and gastric cancers in both mice and humans (4,5) (Figure 1). Upregulation of securin and subsequent chromosomal mis-segregation leading to

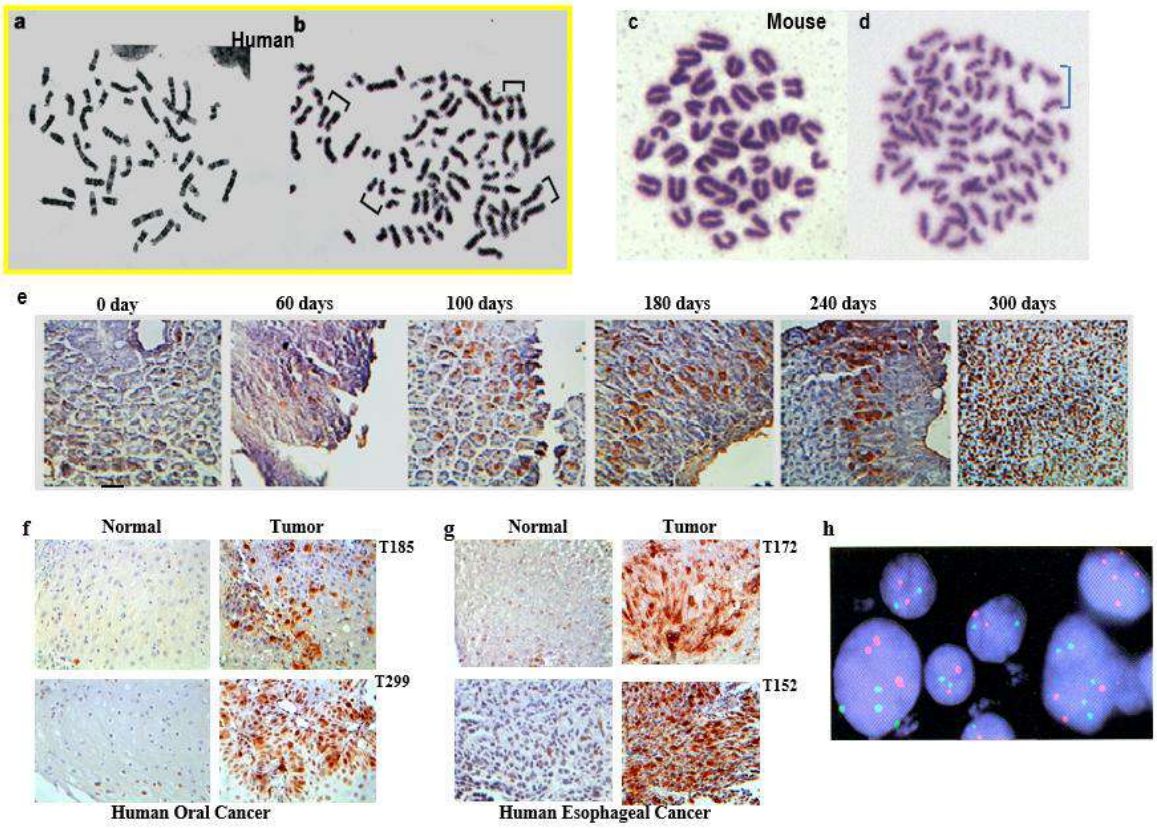

Figure 1: Karyotype analysis of genomic instability and securin upregulation in mouse and human tissues after exposure to RAN with lime. (a) Normal metaphase spread from human lymphocytes, (b) Premature sister-chromatid separation in lymphocytes of RAN heavy chewers, (c) Normal metaphase spread from mouse bone marrow cells, (d) Premature sister-chromatid separation from mouse exposed to RAN + lime. Brackets show sister chromatids lying separated in mitotic figures that show the phenotype. (e) Immunohistochemical images of mouse stomach treated with RAN + lime for various durations. Normal expression of the securin gene in the untreated control and gradual upregulation of securin expression in treated amples for different time periods are shown. Representative images of an immunohistochemical (IHC) analysis of tumour and adjacent normal tissues (f) in oral squamous cell carcinoma and (g) in esophageal squamous cell carcinoma done with anti-Securin antibody. Patients ID numbers are shown on the right side of the images. The magnification of all these images is x40 and the scale bar: 200 μm.

chromosomal instability has been observed in various malignancies, including pituitary, colorectal, thyroid, lung, prostate, oral and esophageal squamous cell carcinoma (6).

Securin, an oncogene originating from rat pituitary tumor cells as pituitary tumor transforming gene 1 (PTTG1), is linked to malignancies. It prevents separin from promoting sister chromatid separation during mitosis. Precocious anaphases are observed in blood lymphocytes of human esophageal cancer patients and in yeast having Mad2 deregulation and in Drosophila with Bub1 checkpoint gene mutation (7). Another study found that spindle inhibitors increased precocious anaphases in

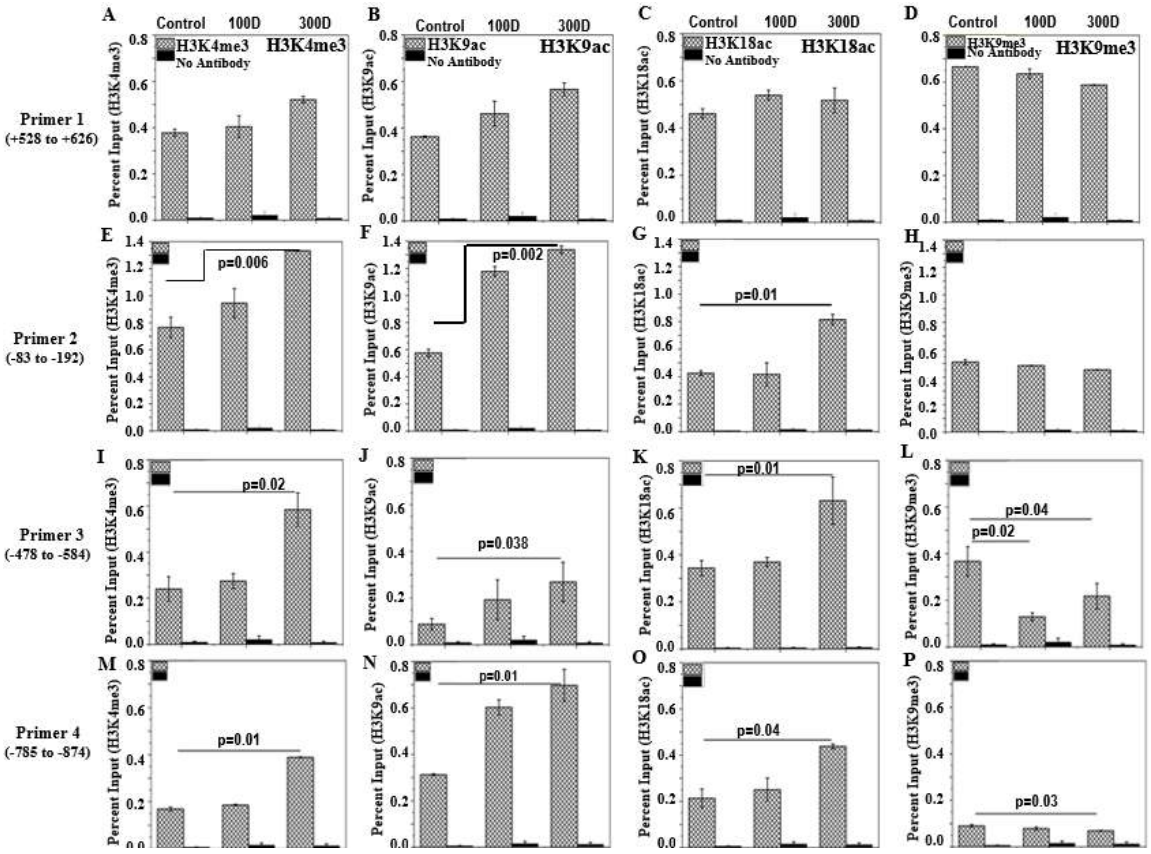

Figure 2: ChIP analysis of histone H3 methylation and acetylation at the region covering upstream to downstream of the promoter of the securing gene. ChIP-qRT-PCR assays for H3K4me3, H3K9ac, H3K18ac and H3K9me3 recruitment to the four regions of the securin gene + 528 to + 626 (upper panel A–D), – 83 to – 192 (E–H), – 478 to – 584 (I–L) and -785 to -874 (lower panel M–P) were analysed by 4 sets of primers in mouse stomach cells treated with RAN + lime for 0, 100 and 300 days. Chromatin was cross-linked, fragmented and immunoprecipitated with no antibody (as a negative control) or anti-H3K4me3, H3K9ac, H318ac and H3K9me3 ChIP-grade antibodies. The purified DNA was used to amplify with four sets of primer pairs covering four regions (+ 528 to + 626 with Primer 1; – 83 to – 192 with Primer 2; – 478 to – 584 with Primer 3 and – 785 to – 874 with Primer 4) of the securin promoter by qPCR. As input, 10% diluted chromatin fragments were retained and used in qPCR for the enrichment analysis. The percentage of input values represents the mean of four different animals ± SEM. Data were analyzed using one-way ANOVA with Tukey's multiple comparison post-tests. P values less than 0.05 are considered significant

Hct116 cells and murine primary embryonic fibroblasts after partial loss of the Mad2 gene, while chromosome mis-segregation events increased without these agents (8). Significant differences in chromosome numbers were identified between wild-type and Hct-MAD2+/- cells using interphase fluorescence in situ hybridization with chromosome-specific anti-centromeric probes (Figure 1). The important issue that came out of all these studies is the induction of precocious anaphases along with overexpression of securin as well as deregulation of mitotic checkpoint genes during the early days of exposure to RAN and lime with and without tobacco, potentially leading to chromosome mis-segregation, and abnormal cell production with aneuploidy. Securin upregulation leads to aneuploidy in multiple

cancers. Therefore, a gradual and significant increase in the frequency of precocious anaphases in RAN heavy chewers, suggests that these parameters can serve as biomarkers for the cancer risk.

Extensive research on this gene has been performed due to its clinical importance, however, the precise mechanisms by which securin induces its oncogenic function and the mechanisms that regulate its overexpression remain unknown. To evaluate the mechanism of securin upregulation, a sequencing scan in human pituitary adenoma biopsies failed to identify any promoter mutations, of the securin gene and no loss of heterozygosity was reported for the region mapping the PTTG1/securin locus. In another study, the methylation status in a CpG island at the proximal promoter region of the PTTG1/securin gene was evaluated and no methylation was observed in either healthy tissues or differentiated thyroid carcinoma samples, or prostate cancer cell lines regardless of the expression status of PTTG1/securin gene.

On the other hand, it was demonstrated that exposure to RAN with lime creates the more relaxed structure of chromatin in the mouse system which could be a causative factor for the transcriptional enhancement of key gene(s) relevant to carcinogenesis (9). It is now well-documented that the structure and integrity of the genome can be altered by disrupting epigenetic control mechanism, which ultimately alters the expression of genes that are critically involved in tumorigenesis. Chromatin remodeling occurs through the posttranslational modifications of basic amino acid residues of histone tails, which either cause activation or repression of gene expression. Therefore, the epigenetic histone modification patterns in the promoter region of the securin gene were evaluated because such modifications do have profound effects on gene promoter activity. Immunohistochemistry data revealed hyperphosphorylation of Rb and upregulation of E2F1 in the RAN-treated samples. Increased trimethylation of H3 lysine 4 and acetylation of H3 lysine 9 and 18 both globally and in the promoter region of the securin gene were observed by increasing the levels of lysine-N-methyltransferase 2A, lysine-acetyltrans ferase, EP-300 and PCAF after RAN treatment. ChIP-qPCR data revealed that the quantity of DNA fragments retrieved from the immunoprecipitated samples was maximum in the -83 to -192 region than further upstream and downstream of the promoter for H3K4Me3, H3K9ac, H3K18ac and H3K9me3 [10] (Figure 2).

The diagrammatic representation (Figure 3) illustrates the changes in chromatin structure and Histone H3 modifications following exposure to RAN and lime. Therefore, the results of this study have led to the hypothesis that RAN + lime exposure relaxes the chromatin, changes the epigenetic landscape, and deregulates the Rb–E2F1 circuit which might be involved in the upregulation of securin and some other proto-oncogenes that might play an important role in the initial phases of RAN + lime mediated carcinogenesis.

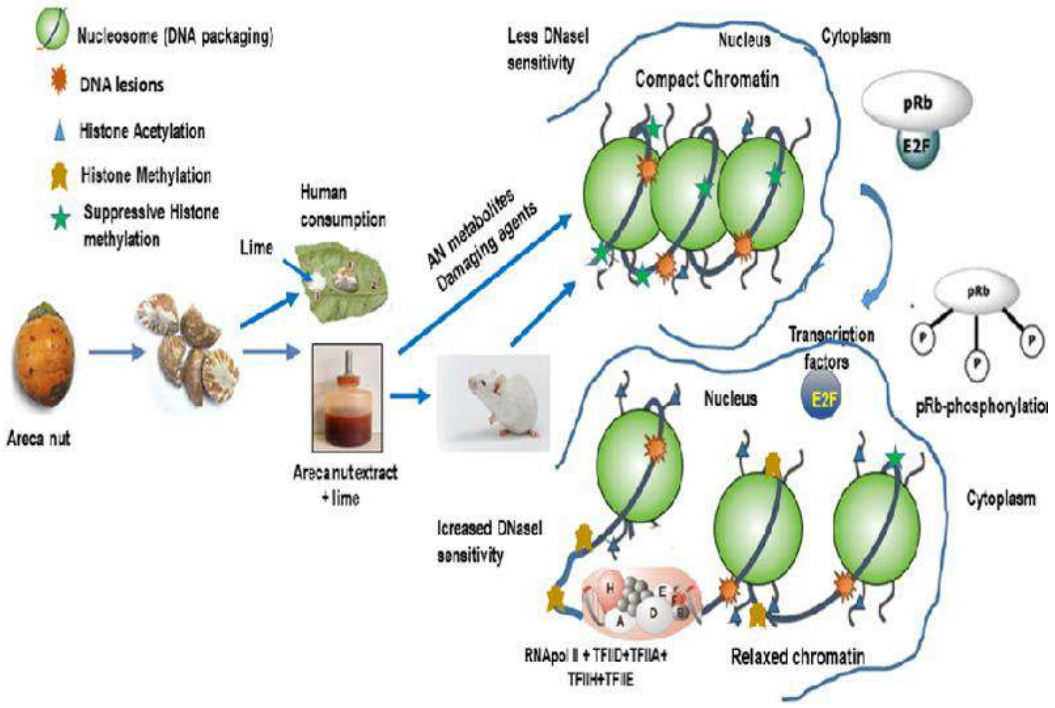

Figure 3: Diagrammatic representation of alteration of chromatin structure and Histone H3 modifications after areca nut and lime consumption. Histone H3 tail lysine residues, frequently subject to posttranslational modifications (PTMs), are indicated along the left side. The typical distribution of these H3 PTMs is also indicated along the length of the chromatin strand as shaded blocks. RAN and lime exposure induces DNA lesions are also indicated on the chromatin strand. The illustration also depicts that RAN and lime phosphorylates pRb and therefore E2F is released and enters into the nucleus and facilitates transcription of the target genes by assembling RNA pol II and several transcription factors like TFIID, TFIIA, TFIIE, TFIIH, and E2F.

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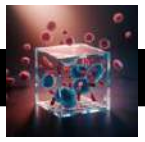

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The creation via destruction: Caspase-activated DNase causes DNA strand breaks to regulate macrophage differentiation


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In 1882, Élie Metchnikoff discovered phagocytosis in starfish larval cells that engulf foreign particles later named macrophages (1). Subsequent studies revealed that macrophages regulate development, tissue repair, homeostasis, and innate immunity in metazoans (2,3). The tissue-resident macrophages originate from the yolk sac and embryonic erythro-myeloid progenitors that occupy every organ before birth and self-renew lifelong. After birth, hematopoietic stem cells also generate short-lived monocyte-mediated macrophages required for a few tissue-resident macrophage pools. Macrophages and the phagocytic process have remained essentially conserved throughout evolution from single-celled creatures to highly sophisticated vertebrates (4). The mechanisms of these versatile heterogeneous phagocytic macrophage differentiation processes remain unknown.

The fruit fly *Drosophila melanogaster* is an excellent genetic model for understanding the development of macrophages and innate immune responses. Its blood system contains only myeloid-type cells, consisting of 90–95% macrophage-type cells developed from multiple origins, similar to mammals. In the first wave, macrophages originate from the embryonic head mesoderm, whereas the subsequent wave begins during the late embryonic phase in the cariogenic mesoderm, which then becomes a larval hematopoietic organ called the lymph gland. The multipotent progenitor cells in the lymph gland develop until the late third instar larval stage and then disintegrate during

metamorphosis and contribute to adult macrophages (5, 6).

During lymph gland progenitor differentiation, intermediate progenitor cells show DNA strand breaks investigated by immunostaining of DNA damage response (DDR) marker γH2Av (Figure 1B). These DNA breaks are induced by active *Drosophila* effector caspases (Drice), which were investigated by immunostaining and also

We used the lymph gland as a model system for macrophage development (Figure 1A).

using effector caspase reporter lines (Figure 1B-C). Lineage tracing of these caspase/DDR cells implies that they survive and develop into macrophages (Figure 1D). We confirmed the role of caspase causing DNA damage and macrophage development as effector caspase (Drice) and initiator caspase

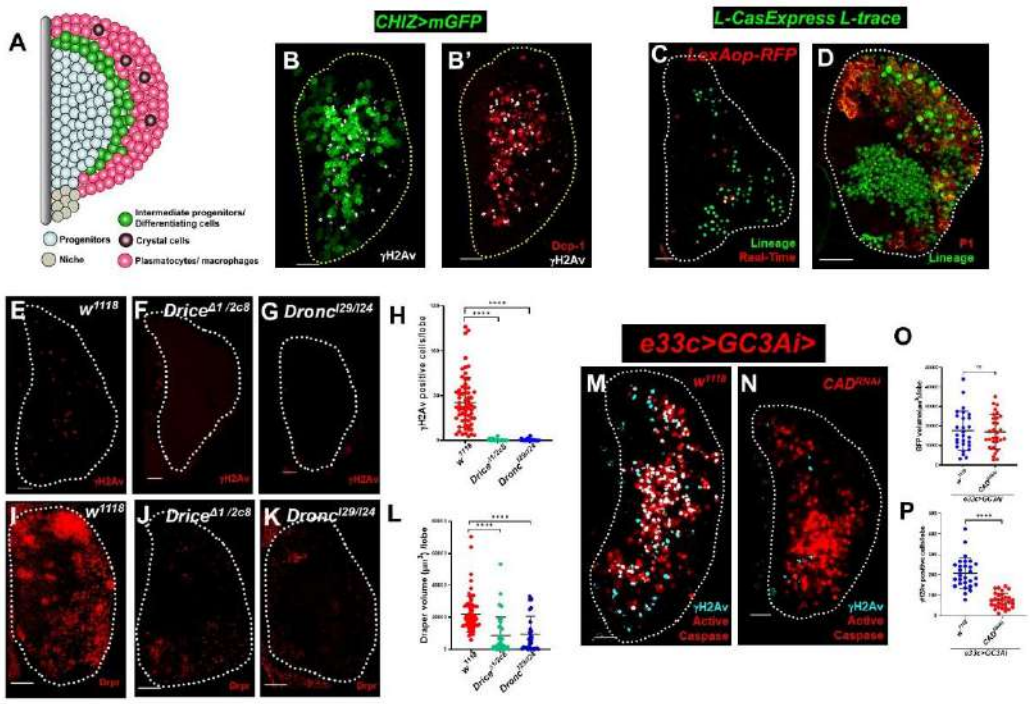

Figure 1: Caspase-mediated DNA breaks promote macrophage differentiation: The model represents cell types in the primary lobe of the third instar lymph gland (A). The lymph gland intermediate zone cells (CHIZ-positive, green) show γH2Av staining (white) (B) and co-staining of γH2Av and Dcp-1 (red) (B'). Real-time (red) (C) and caspase lineage-positive cells (green) were found in the lymph gland where caspase lineage cells were positive with macrophage marker P1 (D). γH2Av positive cells decrease in Drice (F) and Dronc (G) transheterozygote mutants compared to control (E) shown in graph (H). Similarly, Draper (marks macrophage) is also reduced in mutants (I-L). Knockdown of CAD in the whole lymph gland (e33c>GC3Ai) doesn't change caspase activity (red) but γH2Av positive cells (cyan) reduced significantly compared to control (M-P).

(Dronc) mutants' lymph glands lack of DNA damage and significantly decreased

macrophage differentiation (Figure 1E-L). Is caspase directly regulating the macrophage

differentiation or through the DNA damage? Caspase-activated DNase (CAD) inhibited by inhibitor ICAD (inhibitor of caspase-activated DNase). However, caspase-mediated cleavage of ICAD, free CAD causes DNA strand breaks (7). Depletion of CAD by RNA interference reduced the DNA damage and macrophage differentiation in the lymph gland; however, the level of caspase activity remains the same, suggesting the DNA strand breaks regulate the macrophage differentiation (Figure 1M-P).

Further, we studied the mechanistic regulation of the caspase activation where we found that the InR/PI3K/Akt signaling maintained the sub-lethal level of Apoptotic signal-regulating kinase 1 (Ask1) activity. The Ask1 downstream activates a low level of the JNK signaling in the differentiating progenitors that causes transient caspase activity in differentiating progenitors required for macrophage differentiation (Schematic model in Figure 2A). Caspase also regulates the embryonic macrophage's proper differentiation and activity (8).

Caspases have various non-apoptotic roles, including the monocyte to macrophage differentiation (9); therefore, we were curious to know the evolutionary conservation of caspase and DNA damage-mediated macrophage differentiation. We found that the conservation of protein sequences of effector caspases, Drice, and Dcp-1 in five *Drosophila* species like *D. ananassae*, *D. malerkotliana*, *D. bipectinata*, and *D. biarmipes*, which are phylogenetically close to *D. melanogaster*. Further, we found that the effector caspase activity and DNA damage response in the lymph glands of all five *Drosophila* species indicated the conservation of the mechanism for macrophage differentiation (Figure 2B-D)) (Maurya & Mondal, 2024, submitted). Caspase activation plays non-lethal roles in *Drosophila* and other organisms during development and disease (10). Also, programmed DNA breaks coordinate gene expression changes without cell death in several types of cell differentiation (11). Single-cell transcriptomics on lymph glands revealed a group of cells (1.2%) called cluster X enriched in DDR, Myb, and cell cycle genes (12). Given their similar location and cell counts within the lymph gland, these cells are most likely the CAD-mediated DNA-damaged cells that we are reporting here.

Studies showed that caspase-mediated CAD activation is necessary for skeletal muscle cell differentiation and changing gene expression (13). CAD-mediated DNA breaks primarily take near CTCF sites (chromatin insulators), directly affecting a promoter or impacting promoter-enhancer interactions for gene regulation (14). Our findings suggest that an evolutionarily conserved mechanism of caspase/CAD-mediated DNA breaks in differentiating myeloid progenitors may influence the specification of versatile macrophage fate by regulating chromatin landscape and gene expression, which provide advantages them for various types of infection and injury in the future, preparing macrophages for trained immunity.

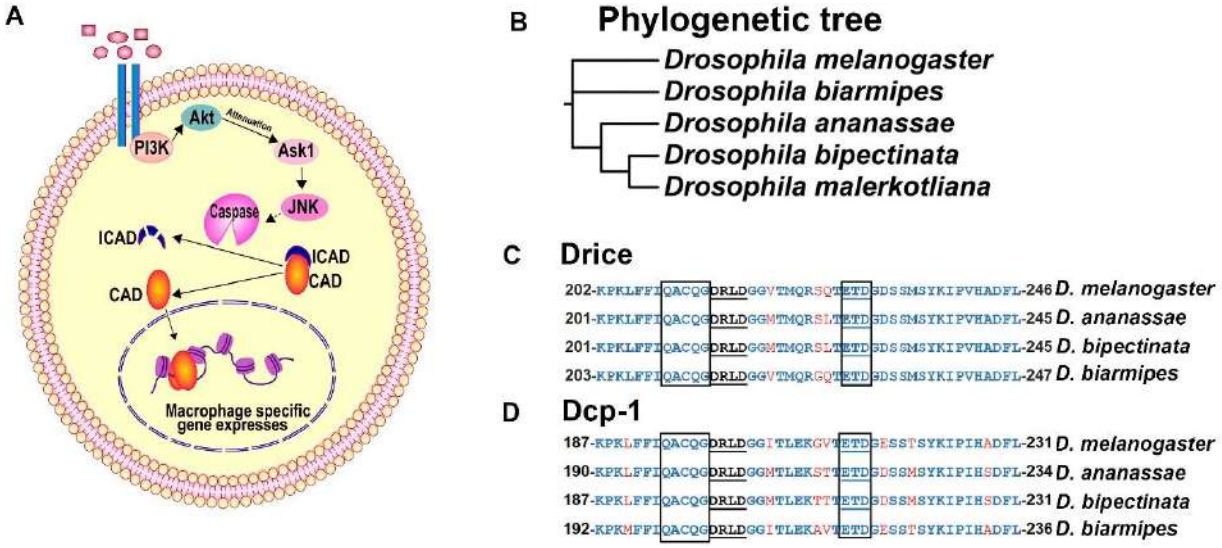

Figure 2: Mechanism of caspase-mediated DNA damage and their evolutionary relationship. For macrophage differentiation, a model showing InR/PI3K/Akt/Ask1 mediated caspase activity and CAD-mediated DNA breaks that possibly activate the expression of macrophage specific genes (A). Phylogeny of five *Drosophila* species: *D. melanogaster*, *D. ananassae*, *D. malerkotliana*, *D. bipectinata*, and *D. biarmipes* shows evolutionary relationship (B). Multiple sequence alignment of the c-terminal region of caspases Drice (C) and Dcp-1 (D) of *D. melanogaster*, *D. ananassae*, *D. bipectinata*, and *D. biarmipes*. The blue highlighted area shows the identical sequences, cleavage tripeptide region (ETD), active pentapeptide region shown in a rectangular box, and the alternative cleaved site shown in black underline.

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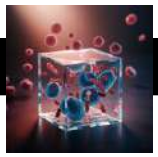

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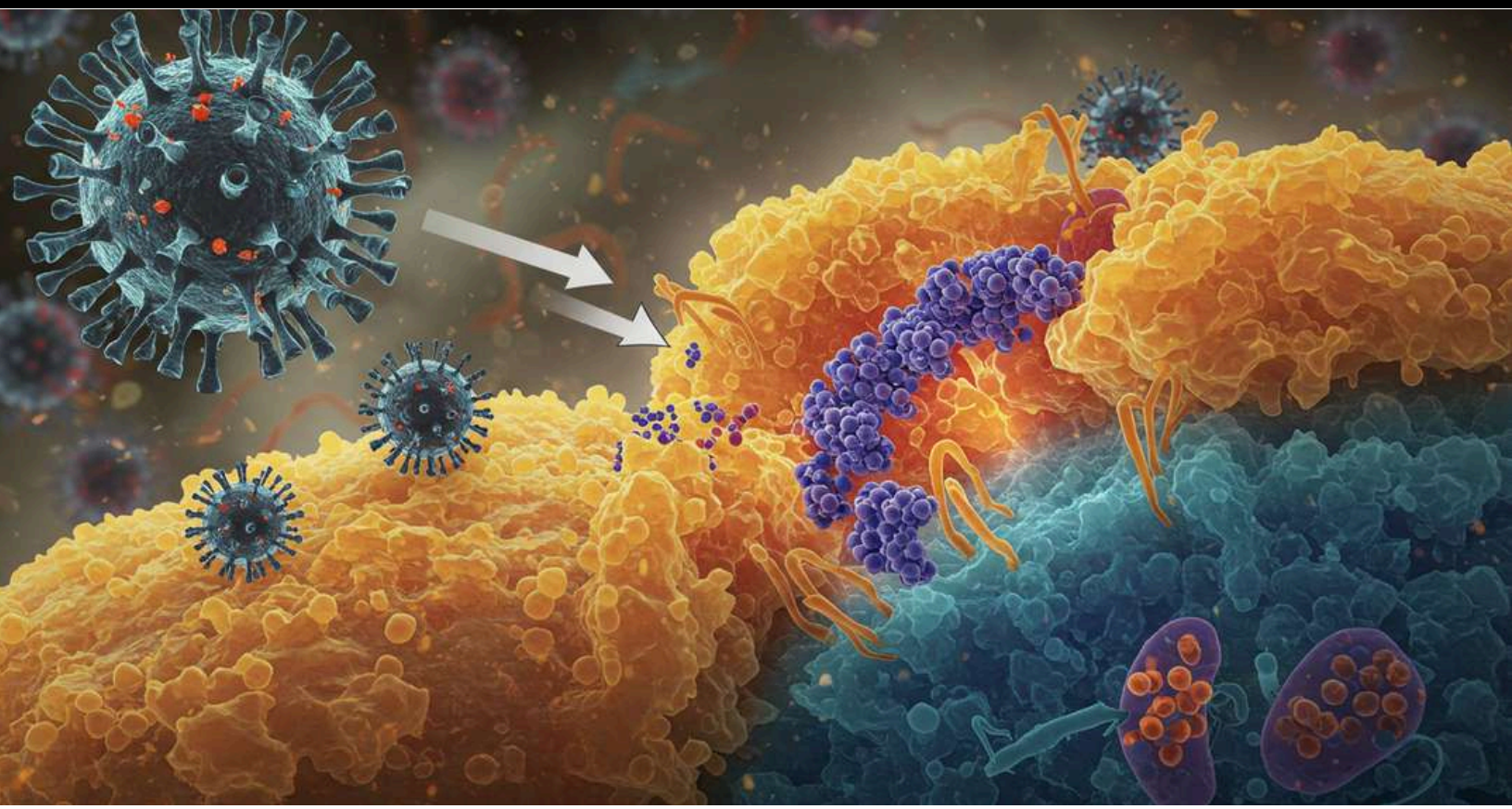
Research Article - Section 2

VIRAL PATHOGENESIS AND INFECTION



Viruses employ diverse strategies to subvert host cellular processes, enabling their replication, persistence, and pathogenicity. This section delves into how viruses hijack host cellular machinery to promote infection and disease progression. Amit Tuli and colleagues reveal how the SARS-CoV-2 accessory protein ORF3a manipulates lysosomal functions by disrupting Rab7 cycling, aiding viral replication and egress. Jayasri Das Sarma's team investigates the human β-coronavirus OC43, showing that it impairs cell-to-cell communication by downregulating connexin 43 and disrupting the Golgi apparatus. Anand Prakash explores how host cellular factors, particularly BRN3A and WWOX, contribute to HPV-induced cervical cancer, emphasizing the interplay between viral oncogenes and host regulatory pathways. Together, these studies offer critical insights into virus-host interactions shaping disease outcomes.

SARS-CoV-2 virulence factor ORF3a modulates lysosomal functions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Introduction

The pandemic of Coronavirus disease 2019 (COVID-19) caused by the emergence of novel Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) has claimed millions of human lives throughout the world. The adaptive mutations acquired by SARS-CoV-2 is the reason behind the fatality rate of COVID-19 is very high when compared to other virus infection. These mutation in SARS-CoV-2 has led to the emergence of multiple natural variants-of-concern with different characteristics in comparison to its wild-type strain.

SARS-CoV-2 is a beta-coronavirus (β-CoV), with a (+) sense single-stranded RNA as genetic material. The 5' end of SARS-CoV-2 genome makes up more than two-thirds of the genome and encodes for ORF1ab polyproteins, whereas the 3' end contains genes which encodes for structural proteins such as nucleocapsid (N), envelope (E), membrane (M), and spike (S). This region of the virus genome also contains genes which encodes for six accessory proteins ORF3a, ORF6, ORF7a, ORF7b, ORF8, and ORF10. Conventionally, these viral accessory proteins are involved in the disruption of host cellular pathways; escaping immune evasion and increasing infectivity. Among these accessory proteins, the largest protein encoded is ORF3a and interestingly, the SARS-CoV-2 ORF3a is approximately 90% similar and 72% identical in nucleotide sequence with ORF3a of the SARS-

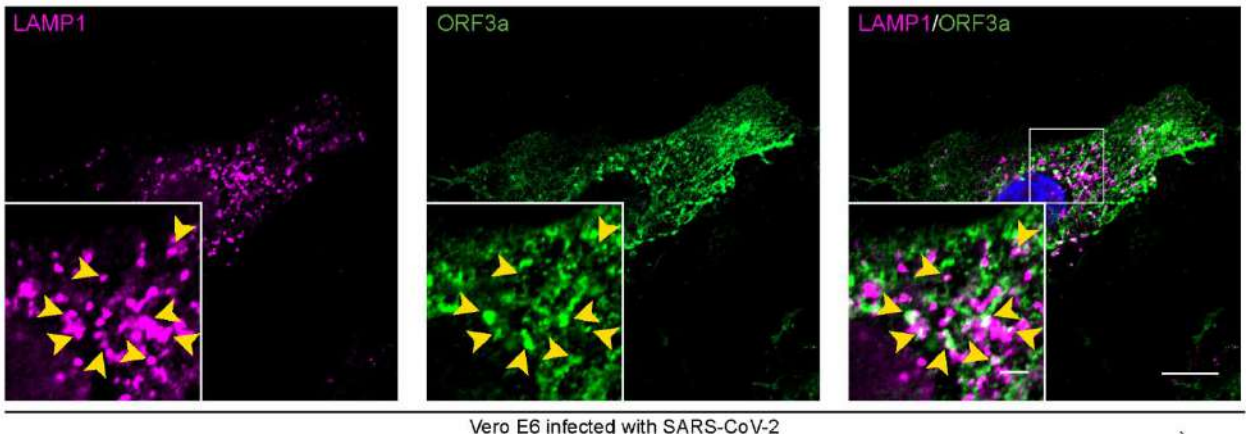

Figure 1: Representative confocal images showing that SARS-CoV-2 ORF3a localizes to lysosomes in SARS-CoV-2-infected Vero E6 cells immunostained for LAMP1 (lysosomal marker) and ORF3a (Credit: Walia K. et al, Nat Commun 15, 2053 (2024)).

CoV-2 ORF3a protein contains three transmembrane domains and a cytosolic domain.

The intracellular vesicular trafficking and recycling pathway form physiologically functional arms of the host cell endo-lysosomal system. Many pathogens, from bacteria to viruses, manipulate these sorting pathways for their pathogenesis. To this end, there is a growing interest in understanding the molecular mechanisms underlying these cellular processes that are manipulated by various pathogens. It is really exciting to understand that diverse host cell factors together are responsible for functional establishment of the host endo-lysosomal system. Among these host factors, the small G-proteins, which consistently cycle between GTP-bound/active and GDP-bound/inactive state, and tethering complex/proteins are responsible for regulating multiple cellular processes, including intracellular cargo trafficking from one compartment to another. The current study hypothesized a key role of SARS-CoV-2 accessory protein ORF3a as a virulence factor and we tried to investigate the mechanism how ORF3a aids the virus in pathogenesis.

Results

Our study demonstrated that, SARS-CoV-2 ORF3a localizes to lysosomes and leads to disruption of both endocytic as well as autophagic degradative pathways via hijacking host cell factor, Vps39, a subunit of the tethering factor HOPS complex (lysosomal tethering factor) (Figure 1). ORF3a interacts with Vps39 and abrogate the interaction of Vps39 with PLEKHM1 (lysosomal adaptor), crucial for endosome/autophagosome fusion to lysosome, thus, blocking HOPS-mediated fusion of late endosome (LEs) and autophagosome with degradative lysosomes. The sequestration of Vps39 by ORF3a further led to the disruption in the GTP hydrolysis

pathway and thus causing the hyperactivation of small G protein, Rab7. Mechanistically, ORF3a by sequestering Vps39 promotes the formation of the Vps39-TBC1D5 complex and concurrently disrupting the interaction of Rab7 with its GTPase Activating Protein (GAP), TBC1D5. This interaction of ORF3a with Vps39 is crucial for the disruption in the Rab7-TBC1D5 complex and thus elevating the levels of GTP-bound Rab7 in the cell.

Next, we confirmed our findings in SARS-CoV-2-infected cells and found that expression of ORF3a was crucial for hyperactivation of Rab7 in infected cells. Furthermore, we found that ORF3a-induced hyperactivation of Rab7 actually resulted in the increased pathogenesis of SARS-CoV-2 in the host cells, indicating a key role of ORF3a to promote Rab7 activation, which in turn is required for viral replication. The ORF3a-induced disruption of Rab7 GTPase cycle also affected the other Rab7-related pathway like retrograde transport pathway. We found that, the expression of ORF3a blocked the retrieval of CI-M6PR from Rab7-positive endosomes to the trans-Golgi network (TGN) and, this leads to the disruption in the transport of newly synthesized lysosomal hydrolases from the TGN to lysosomes. Also, ORF3a expression and SARS-CoV-2 infection impaired the fusion of Rab7-positive compartments with Arl8b-positive compartments that eventually mediate virus egress from host cells. Another recent study had shown that, other than classical biosynthetic pathway, β-coronavirus utilises deacidified lysosomes for its egress.

Taken together, our results suggest that SARS-CoV-2, through its accessory protein ORF3a, blocks Rab7 GTP-GDP cycling, disrupting the transport of lysosomal hydrolases to lysosomes and also blocking lysosome fusion with other compartments which further facilitates the egress of the virus via lysosomal exocytosis (Figure 2).

Conclusions

Taken together, our findings show that ORF3a is a major virulence factor for SARS-CoV-2 that modulates and disrupts the properties and function of host late endocytic compartments by altering the GTPase cycle of Rab7, an indispensable regulator of late endosomes, and lysosomal positioning and function. ORF3a impairs fusion of late endosomes with lysosome and promotes the egress of the virus via lysosomal exocytosis.

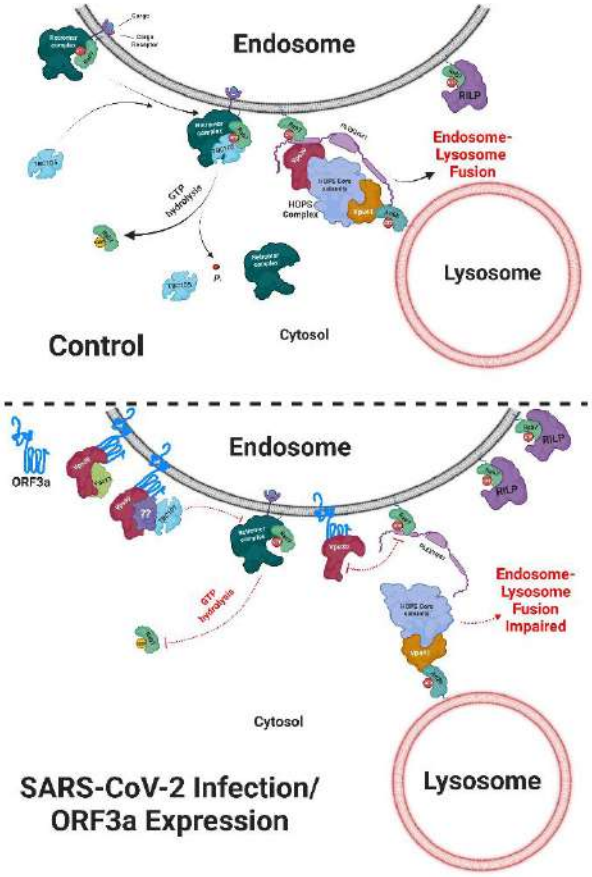

Figure 2: Graphical abstract showing the impact of the interaction between SARS-CoV-2 ORF3a and Vps39 in blocking Rab7 GTP-GDP cycling (Credit: Walia K. et al, Nat Commun 15, 2053 (2024)).

Article published in:Nature Communications, Volume 15
Article number: 2053 (2024)
DOI: https://doi.org/10.1038/s41467-024-46417-2

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Human β-Coronavirus OC43 Alters Cell-to-Cell Communication and Cell Adhesion



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The recent global outbreak of COVID-19 was caused by SARS-CoV-2, a member of the β-coronavirus genus. Research is hindered with SARS-CoV-2 due to high risk, severe symptoms, and the requirement for BSL3 facilities. On the contrary, a human coronavirus HCoV-OC43, belonging to the same genus as SARS-CoV-2, can be an excellent model for understanding pathobiology as it causes mild symptoms and can be worked in a BSL2 facility (Figure 1). Despite their differences, OC43 and SARS-CoV-2 share many similarities. Both have genomes of about 30 kb, with a striking 53.1% identity. They also share comparable transmission methods, symptoms, and pathogenic characteristics. While their life cycles are quite similar, the key difference lies in how they infect host cells: SARS-CoV-2 binds to ACE2 receptors, while OC43 attaches to 9-O-acetylated sialic acids (1). Our laboratory has previously investigated the intracellular trafficking of spike proteins from these viruses. We reported distinct localization patterns, with particular attention to the functions of the cytoplasmic tail of the spike protein (2). However, these techniques have made a host of undeclared assumptions and thereby raising questions on the fidelity of their G4 landscape description. Formaldehyde can covalently cross-link DNA-protein complexes, not nascent DNA.

Researchers from around the globe are trying to use different inhibitors and target host proteins to restrict viral replication and spread. Connexin 43 (Cx43), a gap junction protein family member, can be a good target host protein because its modulation has been reported in various infections such as MHV-A549(3–6), Adenovirus (7), Zika virus (8), and SARS-CoV-2 (9). Connexin 43, when synthesized, exits from the endoplasmic reticulum to the Golgi

apparatus, and in the trans-Golgi, six connexin molecules combine to form a connexon, which is transported to the cell surface in vesicles with the help of microtubules. Once the connexons reach the cell surface, they form hemichannels that help the cell communicate with the outside environment. When the hemichannel aligns with the hemichannel from its neighboring cell, it forms a functional gap junction. Hundreds to thousands of such gap junctions combine to form a gap junction plaque (Figure 2). These hemichannels and gap junctions allow the passage of small molecules less than 1kDa. The Golgi apparatus plays a crucial role in Cx43 assembly and trafficking.

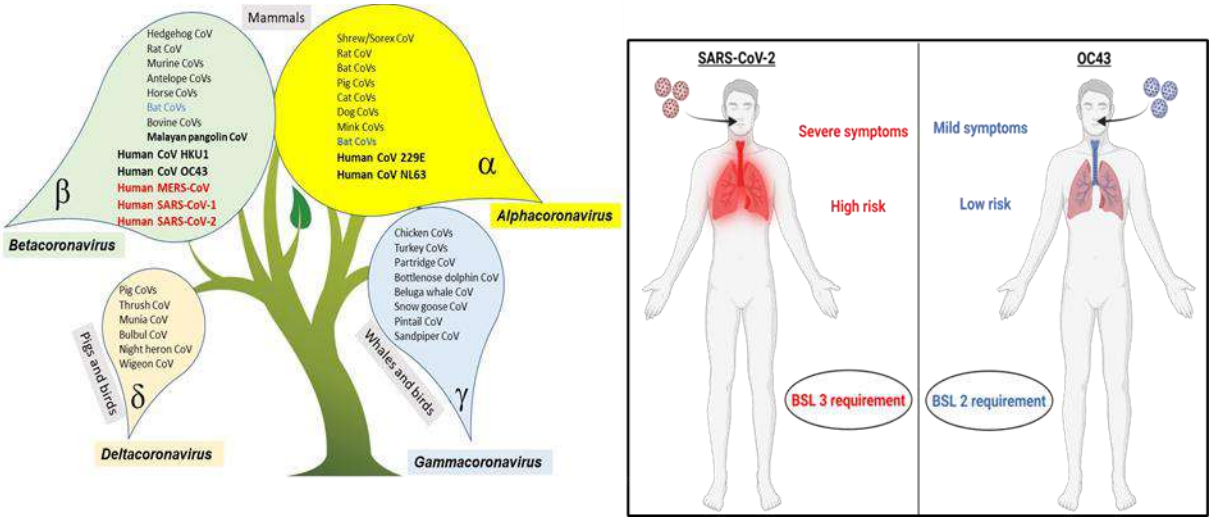

Figure 1: (A) Phylogenetic tree depicting that both viruses belong to the β-coronavirus genus. (B) OC43 can be a good model for studying SARS-CoV-2 pathogenesis because it poses a low risk, has mild symptoms, and can be worked on in a BSL2 facility. (A) adapted from Shors, Teri. "Coronavirus." AccessScience, McGraw-Hill Education, Nov. 2020, and (B) made in BioRender.

A recent study from our laboratory employs HCoV-OC43 to explore its effect on gap junction-mediated cell-to-cell communication in A549 human lung epithelial cells (10). Using A549 cells infected with OC43, we observed reduced expression of Cx43 at protein levels, increased ER stress markers such as HSP70, HSF1, and ERp29, and altered Cx43 localization. This alteration significantly affects the formation of functional gap junctions and hemichannels, impairing cell-to-cell communication. In the context of viral infections, such as with the human β-coronavirus OC43, the Golgi apparatus undergoes significant disruptions (Figure 3). OC43 infection leads to the impairment of Golgi function and hampers the trafficking of essential proteins, including Cx43. This discovery of a novel mechanism of the Golgi apparatus disruption is associated with virus-induced alterations in cellular communication pathways, particularly GJIC. This impairment prevents the proper formation of functional hemichannels and gap junctions, which are crucial for maintaining cellular

homeostasis (10). Therefore, this disruption in the Golgi apparatus contributes to the pathogenic effects of the virus, highlighting its importance in the overall cellular response to β-coronavirus infections

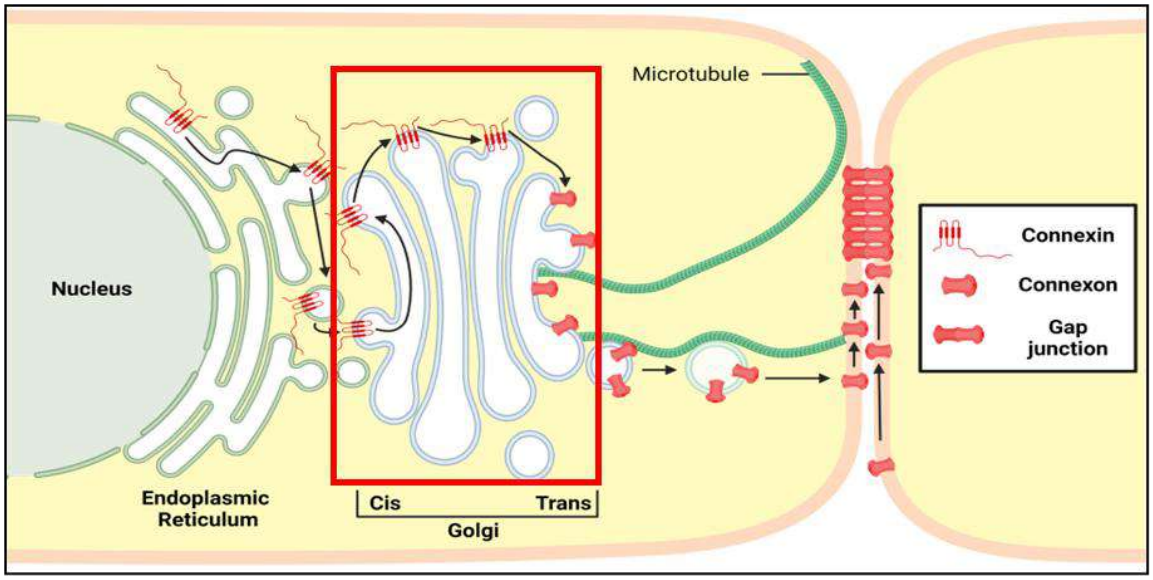

Figure 2: An illustration showing Cx43 protein expression, assembly, and trafficking to the cell surface. The Golgi apparatus plays a crucial role in Cx43 assembly and trafficking. Figure made in BioRender.

Further studies with HCoV-OC43 shed light on the expression of other junctional proteins. Both tight junction proteins such as Occludin and ZO-1 and adherens junction protein E-cadherin expressions are reduced post-infection in A549 cells. As reported earlier by our laboratory in a recent publication (10), one of the possible mechanisms can be due to virus-induced disruption of the Golgi apparatus. Ongoing research focuses on other pathways such as p65 (RelA), a key member of the NF-κB pathway, following OC43 infection, which might contribute to the alteration in tight junction protein expression.

Exploring these mechanisms provides opportunities for developing effective antiviral strategies from the host standpoint. Ongoing initiatives aim to design therapeutic approaches against β-coronavirus infections, and further exploration of human coronaviruses will strengthen our preparedness for potential future human β-coronaviral threats. The modulation of junctional proteins emerges as a promising avenue for the development of potent medications to combat upcoming coronavirus infections effectively.

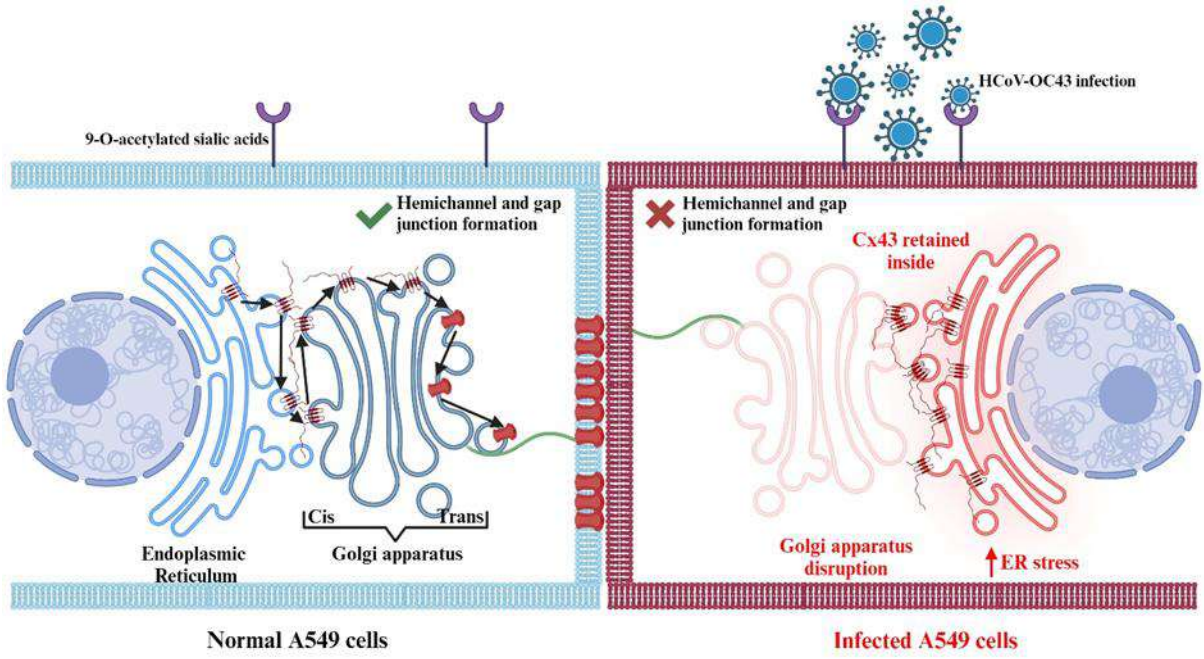

Figure 3: Infection with HCoV-OC43 leads to reduced Connexin 43 expression. HCoV-OC43 disrupts the Golgi apparatus, which modulates the Cx43 localization and impairs the formation of hemichannels and gap junctions. Figure made in BioRender

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Human Papilloma Virus induced Cervix Cancer: Involvement of cellular factors

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According to WHO cancer report 2018, with incidence rate approximately 5.6 lakh and more than 3 lakh death annually, uterine cervix cancer has been marked rank 3rd among the most frequent occurring gynaecological cancer. In Asia, with more than 50% incidence cases it is a leading cause of female morbidity and motility, considered as a major challenge seeking comprehensive attention worldwide (1). Several risk factors linked with uterine cervix cancer such as weak defence mechanism, smoking, multiple sexual partners, early marriages, low socio-economic background, obesity, parity, Human Papilloma Virus (HPV) infection, etc. (2) however, HPV is the most important carcinogen of uterine cervix cancer responsible for 99% of cervical cancer (3,4). Its natural host tissues are the skin and mucosal epithelia of the body where HPV known to cause warts, condylomas and number of cancers (5). According to the transformation capability, HPV are classified into low-risk, intermediate-risk and high-risk. The low-risk HPV types are (6, 11, 40, 42, 43, 44, 54 etc.), are mainly related with benign lesions and low-grade dysplasia but not with cancer. Intermediate (26, 53, 66) and high-risk HPVs (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 68, 70), are associated with condyloma and invasive carcinoma. Among the high-risk HPVs, HPV type-16 and -18 are the two main contributors of cervical cancer (6). Genotyping study in Korean women although showed high-risk HPV types 16, 52 are the two most common HPVs whereas in the western countries HPV-16 and 18 are the most the prevalent HPV types has been identifying (7). In India, it is estimated that HPV type 16 and 18 are associated with nearly 76.7% of total cervical cancer occurs (8). In a study investigating the prevalence HPV in Eastern Uttar Pradesh women by (9) and (2), approximately 9.9% of asymptomatic women were infected with HPV, with the 63.7% most prevalent genotype being HPV-16, further in malignant cases where HPV

infection increased with lesion grade HPV 16 was also found predominant.

HPV is a double-stranded circular DNA virus of around 8kb genomic size comprised of three different regions which are early transcribed region (E), and late transcribed region (L) and upstream regulatory region (URR) (Figure 1). During HPV integration, E2 is often disrupted. E2

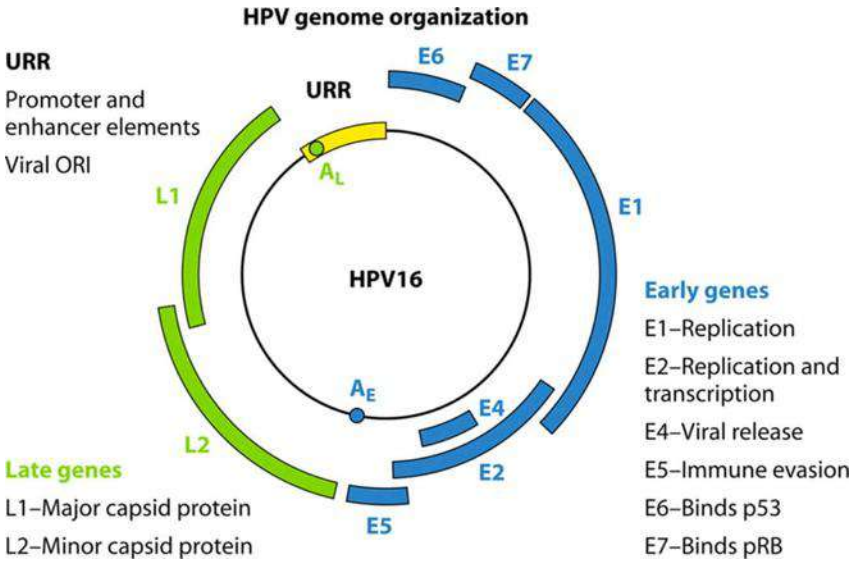

Figure 1: Genome Structure of Human Papillomavirus showing orientation of Early gene (E), Late gene (L) and up-stream regulatory region (URR) (12).

plays a protective role in viral latency and controlled replication by suppressing E6/E7 gene expression (Figure 2) (11, 12). Epidemiological evidence reveals the complexity of cancer development, with host cellular factors playing a crucial role (Figure 3). After infection of HPV, cellular factors involved in various biological processes being disturbed, which results into a development of cervical cancer (13, 14). Disruption and integration of HPV-DNA into the human genome is an essential etiological step in HPV-associated carcinogenesis (15). HPV integrate to the host genome targeting the common fragile sites such as FRA3B (located at 3p14.2, and associated with the FHIT gene), FRA16D (located at 16q23.2 and linked to the WWOX gene) and FRA6E found at 6q26 etc. Several studies have demonstrated FRA16D is among the most active common human fragile site later known as WW domain-containing oxidoreductase (WWOX) gene linked with various cancers (16, 17). This gene encodes a 46.6 kD protein with two N-terminal WW domains, a SDR (short-chain dehydrogenase) domain, and a NLS (nuclear localization signal) domain. It is shown that 2-fold upregulated full length WWOX transcript in approximately 54.7 % uterine cervix cancer tissues but reduced expression of full-length WWOX protein with the increase in malignancy. Cervical cancer samples showed reduced WWOX protein expression but high

WWOX transcript expression. The study also found nuclear localization of WWOX mRNA, suggesting nuclear retention of the full length WWOX transcript in cervical carcinoma samples. This suggests that the full length WWOX transcript is relatively stable. The same phenomenon was observed in the SiHa cell line, where low protein expression with high mRNA transcript levels. This suggests a defect in the mRNA export mechanism, leading to reduced WWOX protein expression. So, the nuclear retention of WWOX transcript in aggressive cervical carcinoma tissues

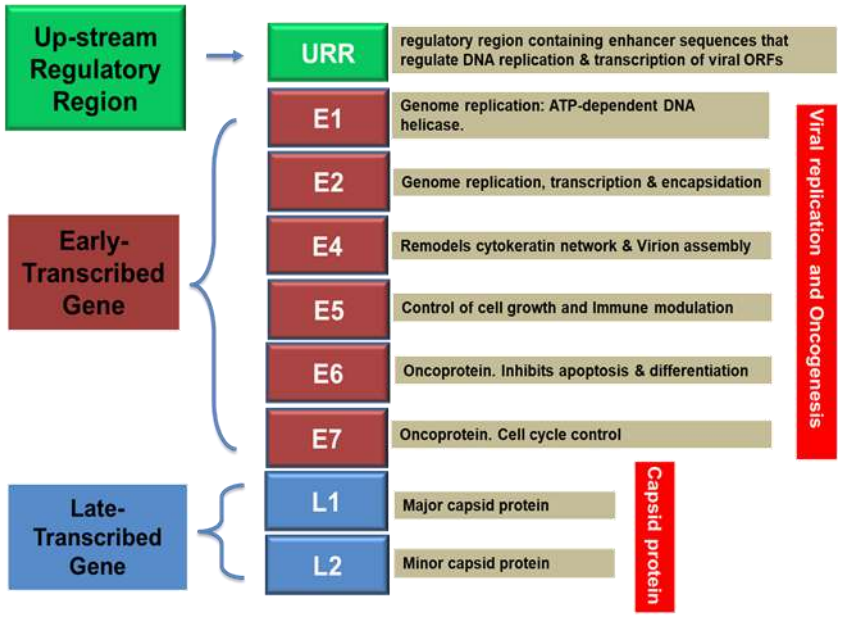

Figure 2: Structure of Early gene (E), Late gene (L) and up-stream regulatory region (URR) showing their function.

suggestive of its post-transcriptional regulation and reduction of WWOX protein expression functioning as a tumour suppressor in cervical cancer (18).

In cancer cases, it is observed that the expression of early transcribed genes, E6 and E7 and HPV-URR play a pivotal role where E6 with the help of E6AP ubiquitin ligase degrades p53 oncogene of the host and E7 inactivates host tumour-suppressor retinoblastoma (pRb) protein which is a master regulator of genomic stability, cell growth, cell cycle, cell differentiation, replication, and apoptosis (6, 19,20). HPV-URR is made up of 800-1000 bp, and harbour binding sites for several transcription factors, enhancer and silencer sequences of the host such as SP-1, TEF-2, AP-1, NF-1, Oct-1 and BRN3A. These cellular factors interact with HPV-URR during several stage of infection and establishment of HPV into the host tissue (21). Among these multiple cellular factors, BRN3A binds at the URR of high-risk type HPV 16 and 18 but not with the low-risk HPVs and help in augmenting tumorigenic transformation of the uterine cervix tissue (22). BRN3A is a homeodomain family of transcription factor (TF) additional POU (shows homology

with pit-1, oct-1/2, unc-86) domain and thus included under the POU family transcription factor (23). Theil et al. in 1993 proposed that BRN3A high expression is enough to transform murine fibroblasts primary cultures. BRN3A involvement was reported in many cancers (24). BRN3A's upregulation in uterine cervix CIN3 lesions activates HPV-16 and -18 oncogene expression, leading to uterine cervix cancers (22). BRN3A during cancer development, interacts physically with several host factors. BRN3A is an antiapoptotic transcription factor in neuronal cells that activates Bcl-2 and Bcl-xl promoters and supress Bax, protecting apoptosis (25,26).

Several studies demonstrated the involvement of p73, and Stress-activated c-Jun N-terminal kinases (JNKs) in cervical cancer. P73 activated in response to DNA damage and genotoxicity however JNK involved in anti-apoptosis, cell proliferation, and autophagy related pathways (27-30). Das Purkayastha and Roy, 2011 (31) demonstrated that p73 co-localizes with BRN3A in cervical cancer SiHa cells, indicating its characteristic of a transcriptional activator. Physical interaction of BRN3A with active form of JNK in cervical cancer also supports the existence of physical interaction between the two proteins in cervical cancer. Further, HIPK2 (Homeodomain-interacting protein kinase 2) is a well known co-repressor of BRN3A (23).

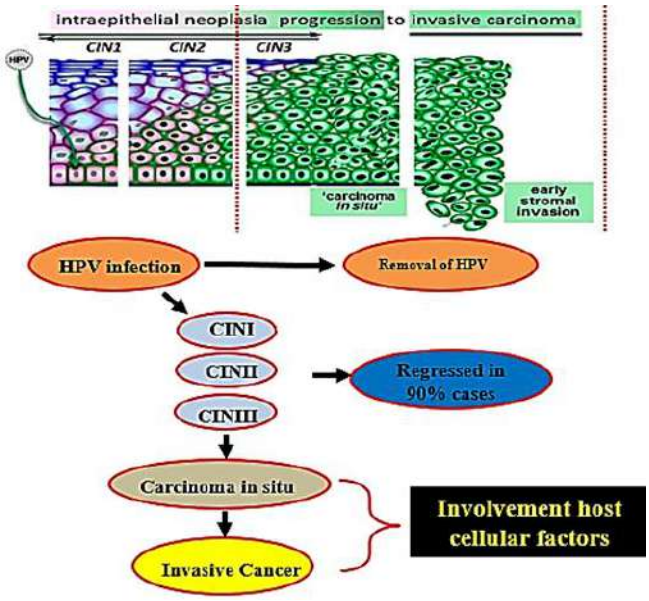

Figure 3: Image showing stages of progression of neoplasia to the cancer stage. During infection, HPV requires a support form host cellular factors to develop neoplasia into an invasive carcinoma.

However, in cervical cancer, cells treated with genotoxic drugs like cisplatin activates HIPK2 although BRN3A expression remains unchanged suggesting its possible role in chemoresistance (31).

BRN3A expression is auto-regulated by an enhancer region located 5-9 kilobases upstream of the transcription start site (TSS). A novel variant (A>G) present at 5692 bases upstream of the TSS, rs1555813 (Figure 4). Genotyping studies showed a 1.32-fold higher allele

frequency in cancer cases compared to normal control subjects and odds ratio (OR) analysis proposes a as a risk-factor for developing cancer with the SNP (OR=2.60, $p \leq 0.004$) in homozygous (GG) but not in heterozygous conditions. This SNP creates a functional binding site for progesterone receptor β, increasing luciferase gene activity in SiHa cells (32). Further findings speculated BRN3A could be a potential modulators of other cellular processes like angiogenesis and neoplastic metabolism (33).

Thus, in conclusion, only HPV may not be a sufficient factor to develop an infected cell into a malignant form until host factors, like WWOX or BRN3A, of the infected cell itself provide essential support to dysregulate the biological processes maintaining the cellular homeostasis.

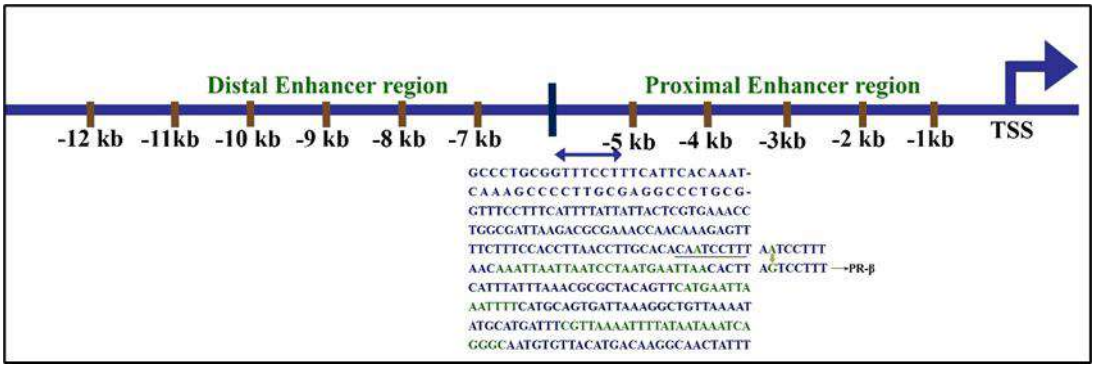

Figure 4: Structure of BRN3A regulatory region showing variation in the BRN3A proximal enhancer region. Sequences depicting multiple BRN3A protein binding sites (green colour) and novel variation (A>G) present at 5692 bases upstream of the TSS (underlined nucleotides) turning into progesterone receptor-β (PR-β) binding site.

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ISCB's Prestigious Awards

Professor S P Ray Chaudhuri 75th Birthday Endowment Lecture

The Indian Society of Cell Biology created an endowment fund on the 75th birthday of Professor S P Ray-Chaudhuri and initiated the above lecture series. Prof. S P Ray-Chaudhuri was the first President of the Indian Society of Cell Biology when it formally came into being in 1976. He was doyen among the cytologists of that time in India. His contributions to teaching and research are beautifully summarized by Prof. Rajiva Raman (Sachi Prasad Ray-Chaudhuri: The First President of the ISCB) in the December 2017 issue of Cell Biology Newsletter. Having trained under Prof. H. J. Muller in Edinburgh, he initiated research in the areas of radiation genetics and comparative cytogenetics at Calcutta University. He moved to Varanasi as the Head of the Department of Zoology at Banaras Hindu University. After his retirement, he returned to Calcutta University and continued working till mid 1980s. He breathed his last in the year 1994 at 87 years of age.

Since its inception there has been 19 lectures till date including the first lecture by Dr. Obaid Siddiqi (1984). The complete list is available on the website of the society.

Professor Rita Mulherkar Lecture Award

Prof. Rita Mulherkar has contributed extensively to the area of cellular and molecular biology of cancer. She worked as a Senior Scientist at the erstwhile Cancer Research Institute, now ACTREC, Tata Memorial Centre until January 2014. She specialized in the area of oncology, working mainly on head and neck cancers using genetic and genomic approaches, and she was also interested in gene therapy. She made the first transgenic mouse in the country with a distinct phenotype and is still used as a model to study hair follicles and skin carcinogenesis. She has also actively contributed to the growth of ISCB, being a member of its executive committee on several occasions and its President from 2009 to 2011. Her students instituted the Professor Rita Mulherkar Lecture Award as a mark of their respect for her and recognizing her immense contributions to the Cellular and Molecular Biology of Cancer. The first lecture was delivered by Dr. Amit Mishra in 2016.

20TH PROF. S. P. RAY-CHAUDHURI 75TH BIRTHDAY ENDOWMENT LECTURE AWARD

PROF. ARUN KUMAR SHUKLA

Department of Biological Sciences and Bioengineering at the Indian Institute of Technology, Kanpur

Professor Arun Kumar Shukla is a distinguished scientist, mentor, and teacher, who has made fundamental contributions in the field of G protein-coupled receptors (GPCRs) with an emphasis on their activation, signaling, and regulatory paradigms. After completing M.Sc. in Biotechnology from Jawaharlal Nehru University in New Delhi, he obtained Ph.D. under the supervision of the Nobel Laureate, Professor Hartmut Michel, Max Planck Institute of Biophysics Frankfurt, Germany. Subsequently, Professor Shukla carried out his post-doctoral research at Duke University Medical Centre in Durham, North Carolina, USA with Professor Robert J. Lefkowitz (Nobel Laureate, 2012) in a very close collaboration with Professor Brian Kobilka (Nobel Laureate, 2012) at Stanford University, California, USA. He joined the Department of Biological Sciences and Bioengineering at the Indian Institute of Technology, Kanpur in 2014, where he currently serves as a Professor and holds Sonu Agrawal Memorial Chair.

Professor Shukla's research has elucidated the molecular mechanisms underlying the action of several blockbuster drugs through their cognate GPCRs, and thereby, paved the way for designing improved therapeutics with minimized side-effects. His has also discovered previously unappreciated mechanisms utilized by GPCRs to receive the information on the outside of the cells and relay the message across the cell membrane through structural and conformational changes. Professor Shukla's laboratory has recently discovered a new class of seven transmembrane receptors, now referred to as Arrestin-Coupled Receptors (ACRs), a close parallel to GPCRs, which signals exclusively through arrestins but not through G-proteins. His laboratory has also established and leveraged a synthetic antibody technology platform to design novel, first-in-class inhibitors of GPCR endocytosis by targeting protein-protein interactions, sensors of GPCR activation by targeting active receptor conformations, and allosteric modulators of GPCR trafficking by stabilizing distinct GPCR signaling complexes.

Professor Shukla's research contributions have been recognized with several awards including the OPPI Scientist of the Year Award 2024, Infosys Prize in Life Sciences 2023, Shanti Swarup Bhatnagar Prize 2021, Khosla National Award 2021, Sun Pharma Research Award 2019, Rajib Goyal Prize in

Life Sciences 2019, National Bioscience Award, 2018 Swarnajayanti fellowship 2018, B.M. Birla Science Prize 2018, CDRI Award for Excellence in Drug Research 2018, EMBO Young Investigator Award 2018, and the fellowship of all the three science academies of India. He also serves on the editorial boards of several international journals including Molecular Cell, Science Signaling, and Journal of Biological Chemistry. Professor Shukla is a life member of the Indian Society of Cell Biology, and his work was also recognized by the Professor Rita Mulherkar Lecture Award in 2018.

Professor Shukla has pioneered the field of membrane protein structural biology in India through his exemplary research program, which in turn has established our country at the global arena in this research domain. In addition to his scientific contributions to Indian Science, Professor Shukla's constant advocacy at multiple levels has also been instrumental in garnering funding and infrastructure support in this research area. His outstanding research performance from the Indian soil has inspired many younger scientists working abroad to return to India, and his leadership efforts, inspiration to the next generation, and contribution towards improving the research infrastructure in the country over the past several years is truly commendable. He takes immense pride in mentoring younger generation, and several of his students and post-doctoral fellows have now become independent scientists in academia and industry in India and abroad.

In recognition of his outstanding contributions to biological sciences and cell biology, in particular, the Indian Society of Cell Biology takes pride in conferring the 20th Prof. S. P. Ray-Chaudhuri 75th Birthday Endowment Lecture Award on Professor Arun Kumar Shukla.

5TH PROF. RITA MULHERKAR AWARD LECTURE

DR. SANDEEP M. ESWARAPPA

Associate Professor,
Indian Institute of Science, Bangalore

Dr. Sandeep M. Eswarappa, a clinician-turned-molecular biologist, is an Associate Professor in the Department of Biochemistry at the Indian Institute of Science (IISc), Bengaluru, and serves as the Chair of the Office of Research Grants at the institute. His research focuses on translational readthrough, a phenomenon involving the recoding of stop codons to produce extended protein isoforms. Remarkably, his team at IISc identified five out of the sixteen mammalian genes known to exhibit translational readthrough. They also discovered a novel role of microRNAs in stop codon recoding

Building on these fundamental insights, his lab has developed and patented two innovative strategies for therapeutic induction of translational readthrough to address diseases caused by premature stop codons. Additionally, his team was the first to demonstrate active translation in mammalian erythrocytes, challenging a long-standing paradigm that translation does not occur in these anucleate cells.

Dr. Eswarappa's work has led to numerous publications in prestigious journals such as EMBO Journal, EMBO Reports, Journal of Cell Science, Journal of Biological Chemistry, and Molecular Biology of the Cell. His contributions have earned international recognition, including the ASBMB's Herbert Tabor Early Career Investigator Award and the EMBO Global Investigator Award. He is also a member of the editorial boards of Journal of Biological Chemistry and eLife. He has received Swarnajayanti Fellowship from the Department of Science and Technology (DST), the C. V. Raman Young Scientist Award from the Karnataka Government, the Vishishta Seva Puraskar from Mysore Medical College, and membership in the prestigious Guha Research Conference.

In recognition of his outstanding contributions to biological sciences and cell biology, the Indian Society of Cell Biology confers the 5th Prof. Rita Mulherkar Award Lecture on Professor Sandeep M. Eswarappa.

The 47th All India Cell Biology Conference and International Symposium with the theme of "Cell in Action": Key Moments and Highlights

By: Dr. Debasmita Pankaj Alone

A group picture of the 47th AICBC and International Symposium on "Cell in Action" participants and delegates

"If I have seen further, it is by standing on the shoulders of giants," Isaac Newton once said — a reminder that scientific progress is never an isolated endeavor but rather thrives with collaboration and guidance. The Indian Society of Cell Biology (ISCB) organizes the All India Cell Biology Conference to bring together brilliant minds from across India, each building on the work of those before them in cell biology. The 47th All India Cell Biology Conference and International Symposium with the theme of "Cell in Action" was successfully hosted by the Indian Society of Cell Biology (ISCB) and the School of Biological Sciences, NISER, at the Pathani Samanta Auditorium, NISER, Bhubaneswar. It was held from December 16th to 18th, 2024, to provide an inclusive and dynamic platform for global leaders, scientists, young investigators, research scholars, and students to share their work and facilitate the flow of ideas in modern cell biology.

Conference Structure and Participants

The three-day event featured an enriching program, including one endowment lecture, one award lecture, ten scientific sessions, three student oral presentation sessions, and two poster presentation sessions. Centered on the theme "Cell in Action," ten scientific sessions were organized to encompass a wide range of topics. Each session was chaired by domain experts, ensuring high-quality discussions. A 5-minute question-and-answer session was held after each presentation to facilitate interaction and meaningful discussion among participants.

The conference featured talks on a diverse array of topics, from cell biology in therapeutics to disease biology, integrative biology, plant sciences, and the physics of life. A total of 21 invited talks

and nine faculty presentations were delivered by an illustrious group of speakers, including pioneering researchers and experts. Participants from over 70 institutes and Universities nationwide registered for the event, showcasing 162 abstracts for student oral and poster presentations. The conference was crafted in an interactive format that encouraged participants to connect with each other and exchange ideas effectively.

Inaugural Session

The conference was inaugurated with the lighting of the lamp and the unveiling of the *Book of Abstracts* by prominent dignitaries, including Prof. H.N. Ghosh, Director, NISER; Prof. Surendra Ghaskadbi, President, ISCB; Prof. Sathees C. Raghavan, Secretary, ISCB; Dr. Pankaj V. Alone, Chairperson, School of Biological Sciences; Prof. Chandan Goswami, Co-convener,47th AICBC; and Dr. Debasmita Pankaj Alone, Convener,47th AICBC.

After the presidential address from Prof. Surendra Ghaskadbi, President, ISCB, the keynote lecture was delivered by Prof. Thomas C.G. Bosch from the Zoological Institute, University of Kiel, Germany, chaired by Prof. Surendra Ghaskadbi. Prof. Bosch in his talk titled "A microbial perspective on animal development and behavior" emphasized the crucial evolutionary link between animals and microbes, stating that co-evolution significantly impacts development and behavior. He explained that lab animals may survive without microbes but fail to thrive naturally. Highlighting that much of Earth's undiscovered biodiversity is microbial, he asserted that any study of animal development without considering microbes is incomplete. Using Hydra as a model, he showed that microbes provide essential signals and shape phenotypes, paving the way for new research in cell and developmental biology.

Inauguration of the 47th AICBC and unveiling of the abstract book

Keynote address by Prof. Thomas C.G. Bosch, Zoological Institute, University of Kiel, Germany

Scientific Sessions

Conference Day 1 Highlights

After the tea interval, Dr. Subhash C. Lakhotia chaired the opening scientific session focusing on "Cell Signaling and Cellular Trafficking." Prof. Ashim Mukherjee from Banaras Hindu University, Varanasi, discussed his research showing how the Multiple Ankyrin repeat single KH domain-containing protein (Mask) interacts with Notch-ICD to enhance Notch signaling by preventing its breakdown in lysosomes, which helps maintain Notch target gene activity. Then, Dr. Shravan Kumar Mishra, IISER Mohali, explained his work on the role of deubiquitinating enzyme (DUB) Ubp5 localized at the Golgi in *Schizosaccharomyces pombe*. His findings revealed that Ubp5, along with Ftp105, helps ensure the glucose transporter Ght5 reaches the plasma membrane rather than being degraded. Without these proteins, Ght5 fails to reach its proper location and is broken down in lysosomes.

The second scientific session with the theme "Cell Functions and Disease Biology" featured three compelling talks. Chaired by Prof. Chandan Goswami, the first talk was delivered by Dr. S. Ganesh from IIT Kanpur, who hypothesized that neurodegenerative diseases may represent accelerated aging. Using mouse models, he showed that the gene expression profile in younger brains of affected mice mirrors that of older brains in healthy mice, supporting the idea of accelerated aging and its systemic consequences. Dr. Indrani Datta from NIMHANS, Bengaluru, discussed the role of extracellular α-synuclein-aggregates in modulating astrocytic function in Parkinson's disease. The study highlighted how these aggregates impact glutathione machinery, glutamate metabolism, and intracellular calcium homeostasis, ultimately disrupting neuron-glia communication and contributing to neurodegeneration. Dr. Shantibhusan Senapati from the Institute of Life Sciences, Bhubaneshwar, explored the molecular and functional heterogeneity of cancer-associated fibroblasts (CAFs) in Pancreatic Ductal Adenocarcinoma (PDAC). His study identified significant molecular diversity among pancreatic fibroblast cells, revealing their potential role in modulating the tumor microenvironment and paving the way for targeted therapies.

During the first poster presentation session, around 70 participants displayed their research work through posters. It sparked engaging discussions as attendees moved between displays, exchanging insights and forming new research connections. Next, the first student oral presentation session was held, chaired by Dr. P.K. Singh, and seven students from various institutions presented their work enthusiastically. The session provided an excellent platform for budding scientists to share their research findings and receive valuable feedback from senior researchers.

Engaging Discussions: Q&A at the 47th AICBC

Deep Dive into Science: Poster Session at the 47th AICBC

After the snack break, the third scientific session, "Autophagy and Programmed Cell Death," chaired by Dr. Pritha Ray, commenced. Dr. Shweta Saran from JNU, New Delhi, presented her research on TPC2, an intracellular calcium-permeable ion channel, in *Dictyostelium discoideum*. Her study demonstrated that TPC2 regulates lysosomal calcium release through NAADP, impacting autophagy and cellular processes. Dr. Sachin Kumar from CSIR-CDRI, Lucknow, explored pyroptotic death in neutrophils and its association with IL-1β secretion. His findings highlighted unique caspase roles, cytoskeletal reorganization, and nuclear rounding during pyroptosis, providing novel insights into inflammatory disease mechanisms. Dr. Lolitika Mandal from IISER Mohali examined how microRNAs (miRNAs) regulate gene expression in response to nutrient availability. Her study identified an autophagy-mediated pathway that controls miRNA-induced silencing complex turnover, revealing a conserved mechanism crucial for hematopoietic growth and proliferation.

The day ended with the general body meeting of the ISCB, led by President Prof. Surendra Ghaskadbi and Secretary Prof. Sathees C. Raghavan, covering financial plans, research updates, and society initiatives before everyone gathered for dinner. The collaborative atmosphere of the meeting encouraged constructive dialogue about the society's future direction, with members actively contributing ideas for enhancement.

Scientific Dialogue: Poster Session at the 47th AICBC

Prof. S. Ganesh, IIT Kanpur, delivering his talk

Conference Day 2 Highlights

The second day of the conference opened with a breakfast gathering, setting the stage for an insightful keynote session. Prof. Benjamin White, representing the National Institute of Mental Health (NIMH), U.S., delivered the address, with Prof. Jagat K. Roy presiding over the session. During his talk, Prof. White discussed the complexity of the brain, highlighting the vast diversity of cell types and their functions, which are shaped by connectivity, activity states, and hormonal influences. He emphasized recent advances in genetic tools that have allowed his lab and others worldwide better to understand brain circuits and behavior in *Drosophila melanogaster*.

The fourth scientific session on "Plant Sciences" was chaired by Dr. Alok Sinha and featured four presentations delivered by distinguished researchers. Dr. Prabodh Kumar Trivedi, CSIR-CIMAP, Lucknow, depicted miRNA-encoded peptides (miPEPs) as key gene expression regulators, previously dismissed as transcriptional junk. His study identified miPEP858a, which directly regulates MIR858, influencing flavonoid biosynthesis and plant development, with potential applications in crop improvement. Dr. Manoj Majee, BRIC-NIPGR, New Delhi, introduced the AtSKIP31-JAZ-ABI5 regulatory module in Arabidopsis, which controls seed maturation and desiccation tolerance. His research highlighted how F box protein AtSKIP31 degrades JAZ proteins, relieving repression on ABI5 to activate downstream signaling essential for seed survival and vigor. Dr. Y. Sreelakshmi, University of Hyderabad, explored folate biofortification in tomatoes. Her study identified high-folate line PKM-1 (Periyakulam-1) and low-folate line AV (Arka Vikas) accessions and discovered a *trifoliate* (tf5) mutant with enhanced folate levels, offering promising strategies for improving dietary folate intake through staple crops. Dr. Debabrata Laha, IISc Bangalore, talk explored the role of inositol pyrophosphate (PP-InsP) messengers in plant signaling, focusing on their impact on growth, hormone regulation, and nutrient sensing. It highlighted gaps in understanding their molecular mechanisms and the reliance on *Arabidopsis thaliana* studies. He also covered recent research on PP-InsP functions and their conservation across land plants.

After the tea break, the conference resumed with the second session of student oral presentations, overseen by Dr. Abhijit De as the session chair. Seven students from various institutions shared their research findings. Post-lunch, the conference continued with the second round of poster presentations, where seventy-two participants displayed their research through visually engaging posters. Dr. Arun K. Shukla delivered the 20th Prof. S.P. Ray Chaudhuri's 75th Birthday Endowment Lecture, chaired by Prof. Surendra Ghaskadbi and Prof. Sathees C. Raghavan. His talk on GPCRs explored drug interactions, signaling mechanisms, and synthetic antibody fragments for monitoring GPCR activity.

Keynote address by Prof. Benjamin White, National Institute of Mental Health (NIMH), U.S.

Young Minds in Action: Student Oral Presentation at the 47th AICBC

The fifth scientific session in the thematic area of "Developmental Biology" was chaired by Dr. Ritu Trivedi and included two presentations. The first presenter, Dr. Girish Ratnaparkhi, IISER Pune, discussed the role of Caspar, the *Drosophila* equivalent of human FAF1, in primordial germ cells (PGCs) specification. Loss of Caspar or TER94 led to embryonic defects, affecting Oskar protein regulation and PGC fate. Dr. P.C. Reddy from Shiv Nadar University explored neuropeptide diversity in *Hydra*, identifying novel peptides using an *in silico* pipeline. Functional analysis of LW-peptide revealed its role in longitudinal contraction circuits, highlighting early neuronal evolution and therapeutic potential.

Following a brief snack break, the sixth scientific session, "Cell Biology in Therapeutics and Medicine" chaired by Dr. Debasis Dash, featured two compelling presentations exploring genetic therapies and RNA modifications. First, Dr. Bikash Ranjan Pattnaik from the University of Wisconsin, USA, shared research addressing visual impairment from *KCNJ13* mutations using gene therapy and CRISPR techniques, successfully restoring protein function in stem cell and animal models. Next, Dr. Ruthrotha Selvi Bharathavikru from IISER Berhampur explained how RNA modifications, particularly the interaction between WT1 and YTHDC, influence Diabetic Nephropathy progression, offering new diagnostic and therapeutic possibilities for kidney disease. The session concluded with a sponsorship talk from Cactus Global showcasing their scientific communication services designed to help researchers effectively share their findings with the broader scientific community. The day

ended with a mesmerizing Odissi dance showcase by acclaimed performer Padma Shri Aruna Mohanty's troop, followed by a dinner event where attendees mingled and reflected on the day's scientific accomplishments.

Dr. Prabodh Kumar Trivedi, CSIR-CAMP, Lucknow delivering his talk

Cultural program at 47th AICBC

Conference Day 3 Highlights

Day 3 of the conference began with the seventh scientific session, "Physics of Life," chaired by Prof. V. Nanjundiah, ICTS Bengaluru, featuring five diverse presentations. Dr. Vijay Kumar Krishnamurthy, ICTS Bengaluru, explored cell shape dynamics during division, developing theoretical models of cortical stress patterns in *C. elegans* embryos. Dr. Namrata Gundiah, IISc Bengaluru, demonstrated how sustained shear stress triggers fibroblast-to-myofibroblast transformation through gene expression and cellular mechanics changes, with implications for fibrosis treatment. Next, Dr. Gautam Menon, Ashoka University, Sonipat, presented computational models of axonal transport, revealing how cells prevent traffic jams and regulate cargo movement through directional changes and diffusion. Dr. Pramod Pullarkat, RRI Bengaluru, examined axonal resilience under stretch deformation, identifying spectrin tetramers and microtubules as critical protective structures against neurological injuries. Dr. Maithreyi Narasimha, TIFR Mumbai, investigated epithelial sheet deformation in tissue development, focusing on interactions between genetic patterns and mechanical forces in *Drosophila melanogaster* embryos.

The conference continued with student presentations chaired by Dr. Ramanujam Srinivasan, where six researchers presented their work. The post-lunch session, chaired by Dr. Bupendra N. Singh and Prof. Sathees C. Raghavan, featured the 5th Prof. Rita Mulherkar Award Lecture delivered by Dr. Sandeep M. Eswarappa. He presented his work on stop codon readthrough mechanisms that enable extended protein production with applications for treating genetic disorders like beta-thalassemia. He was honored by the chair for his scientific endeavors.

Dr. A.J. Rachel chaired the eighth session titled "Host-Pathogen Interaction" and invited Dr. Asima Bhattacharya, NISER Bhubaneswar, who revealed how CEACAM6 regulates Helicobacter pylori attachment during oxygen fluctuations in gastric cells. Dr. Dibyendu Samanta, IIT Kharagpur, delivered the second talk in this session. He discussed his discovery of a novel heterophilic

interaction between E-cadherin and nectin-4 that plays a crucial role in cell adhesion during early embryogenesis. He also revealed a new interaction between *Mycobacterium tuberculosis* lipoprotein LpqH and E-cadherin, providing insights into pathogen-host cell recognition mechanisms. The last talk of the session was delivered by Dr. Sunil K. Raghav, ILS Bhubaneswar, wherein he demonstrated NCoR1's role in energy regulation during tuberculosis infection.

Dr. Gautam Menon, Ashoka University, Sonipat, delivering his talk

Dr. Namrata Gundiah, IISc Bangalore, sharing her work

The ninth session, themed "Integrative Biology," was chaired by Dr. Rupasri Ain and consisted of three impactful presentations. The first lecture was delivered by Dr. Inderjeet Kaur, LVPEI Hyderabad, wherein she discussed her work on dysregulated lipid metabolism in retinopathy of prematurity (ROP), identifying key pathways as potential targets to prevent vision loss in preterm infants. Dr. Sonal Nagarkar-Jaiswal from CCMB Hyderabad showed how the *Insep* gene maintains neural stem cell balance through vesicle dynamics. Dr. Ramakrishna Kancha, Osmania University Hyderabad, delivered the session's final talk. He validated alternative models using Daphnia and snail larvae for screening cancer drug cardiotoxicity.

The last session of the conference, chaired by Dr. Raj Kamal Tripathi, consisted of three impactful lectures from young budding researchers. Dr. Swapnil R. Shinde, IIT Bombay, linked endosulfan exposure to developmental defects through Hedgehog pathway disruption. During the next talk, Dr. Sujit Kumar Bhutia from NIT Rourkela shared his work wherein he characterized MTP18's function in mitochondrial quality control via LC3 interaction. In the final talk of the conference, Dr. Rakesh Kumar Majhi, IIT Kanpur, described how TRPML1 deficiency alters NK cell metabolism while preserving anti-cancer activity.

Closing Ceremony

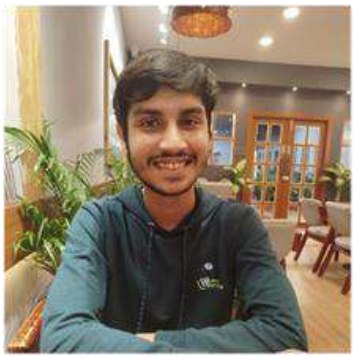
The conference concluded with the valedictory function, where thirteen poster awards and four oral presentation prizes were announced. Following remarks from Prof. H.N. Ghosh, Director of the National Institute of Science Education and Research (NISER), and Prof. Surendra Ghaskadbi,

President of ISCB, Dr. Debasmita P. Alone delivered the vote of thanks. The 47th All India Cell Biology Conference and International Symposium on "Cell in Action" 47th concluded on a high note with researchers exchanging contact information and discussing potential collaborations amid the warm atmosphere of the gala dinner, creating lasting professional connections that will undoubtedly advance the field of cell biology in the coming years.

Interactive Insights: Q&A Session at the 47th AICBC

Celebrating Excellence: Award-Winning Students at the 47th AICBC

DR.A.S. MUKHERJEE MEMORIAL PRIZE



Development of LINE-1 Transposon-Based Sensors and Switches for Breast Cancer Theranostic

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Long Interspersed Nuclear Elements (LINE1 or L1) are the only autonomously replicating transposons in humans, constituting approximately 17% of the human genome. While these mobile genetic elements are usually silenced in healthy somatic cells, they are known to become derepressed in several human cancers, including breast cancers. Despite their prevalence in cancer, they have not been utilised as diagnostic or therapeutic biomarkers.

Recently, mRNA-based Toehold switches have been employed as RNA sensors for diagnostic purposes. This eukaryotic toehold (eToehold) switches manipulate gene translation through structural alterations in the Internal Ribosome Entry Site (IRES). Building on this, we created eToehold switches with IRES sequences sensitive to L1 mRNA, a key player in multiple cancers.

We introduced these L1-sensing switches containing RNA in different cell lines and found them to be translated specifically in cancer cells only. The designed switches displayed substantial fold changes and, hence, are a great platform for detecting cancer cells. We further designed bicistronic constructs in which the second cistron was under the control of the L1-sensing switch, whereas the first cistron was uncontrolled. By encoding viral antigens for seasonal infectious viruses like Influenza in the first cistron, our L1-based diagnostic RNA can be coupled with a seasonal mRNA vaccine - thus enabling easy, regular and large-scale cancer diagnosis.

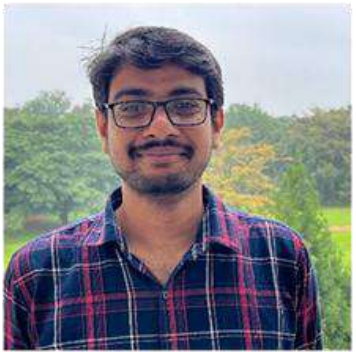
After having proven the diagnostics capability of our L1 RNA Sensor, we further modified the bicistronic construct to create a failsafe therapeutic RNA. We added another ribo switch to mitigate the leaky expression in the absence of L1 and encoded hBax in the first cistron and Bcl2 in the second cistron. This failsafe single RNA circuit gets toggled by L1 mRNA to switch from Bcl2 expression to hBax expression - thus killing the cells in the presence of L1.

We believe that this L1 RNA-based senor approach demonstrates a potential diagnostic and therapeutic strategy for breast cancer. Since it can be coupled with any mRNA vaccine, it allows for a regular diagnosis at a large scale. The same frame work could potentially be extended for diagnostic applications in other cancers,
such as ovarian cancer, where elevated L1 protein and mRNA expression are common.

Keywords: Cancer Theranostic, Long Interspersed Nuclear Elements1, RNA-Sensor, RNA-Switch

Voices of Discovery: Celebrating Oral Presentation Award Winners

ISCB–CACTUS AWARD



Decoding flow-induced molecular mechanisms in collateral artery development

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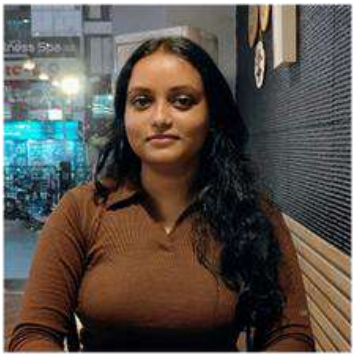
Pial collaterals are artery-to-artery anastomoses in brain which reroute blood flow downstream to an occlusion and improve prognosis in ischemic stroke. Despite high clinical relevance, the precise mechanism of their development remains unknown. Using mouse genetics, intravital and whole-brain imaging of embryos, at cellular resolution, we showed that, pial collaterals develop via artery tip extensions along predetermined micro vascular tracks, in a VegfR2-dependent manner. We next investigated if physical forces like blood-flow can regulate collateral formation. To this end, we have assessed the role of a flow- responsive transcription factor, *Dach1*, responsible for capillary to artery fate conversions (i.e. arterialization). Our *in vivo* data shows a negative correlation between shear stress and *Dach1* expression in brain vasculature. Loss or gain-of-function experiments reveal that, although dispensable during embryogenesis, capillary-specific overexpression of *Dach1* increases collateral network via ectopic induction of arterialization. This is a promising therapeutic avenue which could improve vascularization during ischemic diseases.

Keywords: arterialization, blood flow, endothelial cell, intravital imaging, pial collateral artery

ISCB–CACTUS AWARD



Investigating the molecular mechanism of non-AUG codon-based translation initiation in yeast

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Establishing an Open Reading Frame (ORF) is crucial for decoding the genetic code and subsequent translation into protein. While most mRNAs utilize the AUG codon as the start codon for translation, there are instances where non-AUG codons, such as UUG, CUG or GUG, can also initiate translation. In yeast, one such example is the α-ketoglutarate dehydrogenase (KGD4) gene, which is translated into two isoforms: one using the conventional AUG start codon and the other utilizing an upstream UUG codon, both from a single mRNA. The translation initiation process is typically stringent in its selection of start codons. However, non-AUG codons like UUG can still be chosen as the start codon, which might not be mediated by the conventional translation initiation machinery. It is hypothesized that this UUG codon- based initiation might involve cis-acting mRNA elements or the trans-acting factors associated with the ribosome during non-AUG codon selection. To explore further, mRNA-Tethered Ribosome Interactome Profiling (mTRIP) was conducted to identify trans-acting factors on the 48S ribosomal complex associated with KGD4 mRNA. Remarkably, we identified non-canonical translation initiation factors associated with the 40S ribosomal complex on KGD4 mRNA; however, these factors were absent on the actin mRNA. This finding suggests that the selection of a non-AUG codon as the translation start site may involve an alternative translation initiation mechanism distinct from the canonical one. The 48S complex facilitating non-AUG codon selection may differ from the canonical 48S complex, resulting in ribosome heterogeneity within the cell. Further investigation aims to uncover other mRNAs where this mechanism governs translation initiation and elucidate how these ribosomes are recruited to the mRNA and, particularly, to the non-AUG start site.

Keywords: Non-AUG codon, Trans-acting factor, Ribosome heterogeneity, Start codon selection, mTRIP

ISCB–JCS AWARD

MAPping Microtubule PTMs: Setting the Cellular GPS for Lysosomal Commute

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Intra cellular transport and positioning of organelles are critical for cellular function. Motorproteins, kinesin and dynein, steer long-range, bidirectional transport of organelles on microtubule (MT) highways. Microtubule post-translational modifications (Mt-PTMs) and microtubule-associated proteins (MAPs) are known to differentially regulate motor proteins. In-vitro studies prove the tubulin code hypothesis, suggesting that MT-PTMs generate functionally distinct MT subsets that interact with motors and MAPs to regulate intracellular transport. Therefore, it compels us to propose the existence of a complex regulatory interplay between Mt-PTMs and MAPs, bringing forth an array of molecular traffic rules that precisely control organellar transport and positioning in cells. However, in cells, the MtPTM-MAP interactions are known to be transient and nanoscopic, making them extremely difficult to resolve using conventional diffraction-limited microscopy approach. Therefore, we aim to study the regulation of MtPTM-MAP interactions in cellular systems using super-resolution imaging methods to deduce the exact mechanism by which MT-PTMs and MAPs act in unison to regulate organelle functions. Probing the interactions between MAPs and MT-PTMs in the cellular environment, we observe a distinctive spatial distribution of MAP7, MAP4, Kinesin-1, and Kinesin-3 along tyrosinated, detyrosinated, and acetylated MTs. Investigation into the underlying mechanism reveals that the preferential binding of MAP4 to tyrosinated MTs is independent of its expression levels and structural constraints. Domain-wise analysis of MAP4 shows that the projection (PJ) domain, rather than the microtubule-binding domain (MTBD), determines its binding preference in a kinesin-1-dependent manner. Additionally, we utilize density-based cluster analysis to quantify the nanoscopic spatial distribution of MAPs and their impact on the regulation of lysosomal motility, positioning, size, and number. Our results suggest that the differential distribution of MAPs and motors on different MT subsets establishes an intricate regulatory mechanism to orchestrate lysosomal organization in cells.

Keywords: Microtubules (MT), Microtubule-Associated Proteins (MAPs), Microtubule post-translational modifications (Mt-PTMs), Lysosomes

DR.V.C. SHAH PRIZE

Role of DUBs-Activated Intron-Specific Splicing Regulator in Trafficking of Glucose Transporters

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Schizosaccharomycespombe Ubp5 and its paralog Ubp15 are ubiquitin-specific protease (USP) domain-containing deubiquitination enzymes (DUB) similar to human USP7, harboring meprinand TRAF homology (MATH) domain at their N-termini and five tandem ubiquitin-likefolds at their C-termini. Besides canonical ubiquitin, Ubp5 and Ubp15 also cleave the distant ubiquitin-fold of Sde2 and produce activated Sde2-C. An intron-specific pre-mRNA splicing regulator, Sde2-C, assists spliceosome excise introns with longer distances between their branchpoint and 3' splice site (ss). One of the splicing targets of Sde2-C is *ftp105* pre-mRNA. Ftp105 protein tethers Ubp5 to the Golgi. Glucose transporter Ght5 and Ght2, are among the ubiquitinated substrate of this DUB module. Cells lacking Ftp105 and Ubp5 are sensitive to glucose deprivation, poorly sort Ght5 to the plasma membrane, and degrade the hyper-ubiquitinated transporter in the lysosome. Thus, Ubp5/Ubp15-mediated activation of Sde2 in the nucleus, leading to splicing of *ftp105* pre-mRNA, which in turn regulates the trafficking of glucose transporters.

DR.S.R.V. RAO PRIZE

Homocysteine-dependent Protein Aggregationin Pseudoexfoliation: Interplay between Cytoskeletal Destabilization and Impaired Lysosomal Clearance

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Dysfunction in autophagy is emerging as a key contributor to age-related and neurodegenerative diseases. Such dysfunction results in impaired cellular clearance pathways, leading to the accumulation of misfolded proteins and aggregates. Pseudoexfoliation (PEX) syndrome, an age-related progressive disorder characterized by extracellular fibrillar deposits and often associated with glaucoma, is marked by abnormal protein aggregation. We propose that alterations in cellular clearance pathways in PEX could stem from epigenetically driven changes in metabolic signatures. Elevated homocysteine levels linked to PEX, and we hypothesize that increased homocysteine may act as a risk factor, contributing to abnormal protein aggregate formation by disrupting autophagy through epigenetic modifications. Plasma, lens capsule (LC), and conjunctival tissue samples were collected from donors diagnosed with pseudoexfoliation syndrome and glaucoma, as well as from age-matched controls. MicroRNA was isolated from these samples, and the expression levels were assessed using real-time PCR. Computational algorithms were employed to identify potential target genes of the micro RNA candidates. Protein expression was examined through immunoblotting and immunofluorescence techniques. MicroRNA modulation was carried out using antagomiRs and mimics, and drug treatments were performed on HLE- B3 cell lines. The study demonstrated a reduction in lysosomal function in LC and conjunctival samples, particularly in pseudoexfoliation glaucoma (PEXG) samples. HLE-B3 cells treated with homocysteine showed markedly diminished activity of lysosomal proteins and proteases. Additionally, changes in cytoskeletal dynamics were observed, accompanied by an increase in protein aggregation signals as indicated by immunofluorescence. Elevated homocysteine levels led to significant downregulation of microRNAs involved in the autophagy pathway, suggesting a complex regulatory interplay between homocysteine and microRNA expression in PEX. Our findings highlight the dysregulation of lysosomal surface proteins and proteases in PEXG. Notably, elevated homocysteine levels promote protein aggregation in HLE-B3 cells and induce changes in cytoskeletal organization. Furthermore, we identified dysregulation in microRNAs targeting autophagy-related genes under elevated homocysteine conditions, consistent with observations in patient samples with PEX. This suggests that increased homocysteine levels may impair the autophagic pathway by modulating miRNA regulation.

Keywords: Autophagy, Hyperhomocysteinemia, Lysosomes, microRNAs, Pseudoexfoliation.

PROF B.R. SESHACHAR MEMORIAL PRIZE

m6A epitranscriptome modification mediated regulation of renal injury: reveals a role for YTH domain-containing reader proteins

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Renal Homeostasis is essential for normal physiology and is controlled by a complex and coordinated array of molecular pathways including transcription factors like WT1 (Wilms' tumour gene 1), epigenetic mediators and signalling cascades such as Wnt (Wingless and Int-1).Among the different epigenetic mediators, RNA-mediated epigenetic regulation or epitranscriptomics refers to the post-transcriptional modification of RNA bases in which m6A modification is the most abundant and conserved in eukaryotes and regulates RNA-associated functions like RNA processing, stability, translation, and splicing. Recent studies have reported the role of m6A modifiers as renal repair genes in podocyte injury which is one of the important features of diabetic nephropathy (DN) which is a leading cause of end-stage renal diseases (ESRD). We are investigating the role of m6A modification in renal injury and the associated stress response using renal cell lines and a chemical DN mouse model. Our data demonstrate the differential expression of m6A modifiers across different kidney cell types under metabolic stress (High glucose) and the DN mouse model. Interestingly, we observe the downregulation of m6A reader proteins like YTHDC1 (YTH domain-containing protein 1) and YTHDF2 (YTH domain-containing family protein2) during the onset of renal injury, which are responsible for determining mRNA stability or decay and thus can play a role in the renal stress response physiology. Loss of function mutant cell lines were generated using genome editing which were characterized to understand the mechanistic details associated with the renal cellular stress response, implicating RNA modification associated with post-transcriptional regulation as a key mediator in the renal stress response especially in the pathophysiology of DN.

Keywords: Diabetic nephropathy, m6A modification, m6A reader proteins, Renal homeostasis.

DR.MANASI RAM MEMORIAL PRIZE

Direct reprogramming of human fibroblasts into pancreatic β- cells using pancreatic-specific transcription factors in recombinant protein form

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Direct cell reprogramming, also called transdifferentiation, allows for the reprogramming of one somatic cell type directly into another, without the need to transition through an induced pluripotent state has emerged as a strategy to develop clinically relevant cell types. So far, the generation of β-like cells has been achieved from closely related endodermal cell types through the induction of developmental transcription factors. However, the conversion of more distantly related lineages such as fibroblasts into β-like cells remains elusive. Few studies have reported success in generating insulin-producing cells (IPCs) from Human Dermal Fibroblasts (HDF) cells by genetic manipulation has recently revealed a new therapeutic potential for diabetes. However, clinical application has been hampered by the viral genome integration and the risk of insertion mutagenesis that are entailed. A useful and safe way to generate pancreatic β-cells has not been developed. We describe how the timed-introduction of four developmental transcription factors in recombinant protein form promotes conversion of fibroblasts toward a β-cell fate. Protein transduction domains (PTDs), such as the HIV1-TAT peptide, have been generated and used to promote the uptake of proteins into a range of cell types, including stem cells. Transcription factors having the PTD- domain attached to the terminal end of the protein have been purified and it was proved to be functionally active. The generated pancreatic β-cells, free of integration, are clinically safe and can be utilized as a personalized, cell-based therapy for diabetic patients.

Keywords: Transdifferentiation, TAT-Peptide, Transcription factors, Recombinant proteins, β-like cells.

ISCB SOCIETY AWARD

LncRNA *JINR-1* Promotes Tumor Growth in Head and Neck Squamous Cell Carcinoma (HNSCC)

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HNSCC is a highly aggressive cancer caused by the consumption of tobacco, alcohol, betelquid, or infection with human papillomavirus (HPV). Long non-coding RNAs (lncRNAs) have emerged as important therapeutic targets for multiple cancers, including HNSCC. We show that lncRNA *JINR- 1* expression is significantly higher in HNSCC tumor samples. Transforming growth factor-β (TGF-β) induces *JINR-1* expression in HNSCC cells. Depletion of JINR-1 in HNSCC cells reduces cell proliferation, migration, invasion, and cisplatin resistance. *JINR-1* acts as a competitive endogenous RNA (ceRNA) to sponge *miR-216b-5p*, causing upregulation of its target gene GRP78. Our results suggest that TGF-β promotes *JINR-1* expression, which further regulates *miR-216b-5p*/GRP78 axis to promote invasion in HNSCC.

Keywords: ceRNAs, EMT, HNSCC, lncRNAs, TGF-β.

ISCB SOCIETY AWARD

Understanding the Mechanism of Radioprotection by Caffeine

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Human genome is subjected to constant damage by several endogenous and exogenous DNA damaging agents. Among various exogenous sources, radiation is the most commonly encountered and a significant contributor of DNA damage in cells. Caffeine, one of the most widely consumed stimulants across the world, has been the most extensively studied methylxanthine till date. Its role as an antioxidant has been investigated over the past several years. Since free radicals and reactive oxygen species form one of the important intermediates in radiation-induced toxicity within cells, we aimed to explore whether caffeine could impart protection against ionizing radiation induced DNA damage owing to its antioxidant property. In the present study, we assessed the impact of caffeine on radiation-induced DNA damage and the results indicated that caffeine imparted reduced radiosensitivity against the DNA damaging effects of radiation. In-depth analysis revealed that the observed protection is due to reduced induction of single and double DNA strand breaks upon exposure to ionizing radiation. We have also demonstrated that caffeine can efficiently quench the reactive oxygen species (ROS) generated via H_2O_2 treatment or IR exposure in mammalian cells which further validates its antioxidant property, which might be responsible for imparting radioprotective property to caffeine and thereby reducing the effect of radiation induced toxicity. We also observed that lower concentration of caffeine protects the cell from the cytotoxic effect post irradiation owing to reduction of DNA strand breaks formation. Using in vivo model system, we also demonstrated that caffeine treatment in mice extended their lifespan, as compared to untreated control upon exposure to irradiation. Besides ROS, ionizing radiation also generates Reactive Nitrogen Species (RNS), a process mediated by Nitric oxide synthase 2 (NOS2) protein. RNS can also cause structural alterations in DNA and are mediators of radiation induced cellular toxicity. The results of our study revealed that caffeine treatment in NOS2 KO mice exhibited a significantly enhanced survival as compared to corresponding WT strain (C57BI/6) post irradiation, highlighting the role of NOS2 and subsequently, RNS, in radiation-induced toxicity. Besides, transcriptome analysis revealed the upregulation of some of the key antioxidant genes (Gpx3, Gpx7, Gpx4, Idh1 etc.) involved in playing major role for the ROS homeostasis in caffeine treated mice group followed by exposure to IR as compared to control group. Moreover, in NOS2 KO background, the expression of these antioxidant genes was further upregulated in caffeine treated mice group upon IR exposure which explains the enhanced survival of these mice upon caffeine administration against the cytotoxic effects of IR. Thus, the results from our study suggest that caffeine reduces the level of DNA damage induction upon IR and therefore imparts protection to our genome.

Keywords: Caffeine, DNA damage, Ionizing Radiation, ROS

ISCB SOCIETY AWARD

Canonical cell cycle regulators control variant cell cycle in cortex glia of Drosophila nervous system

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Cortex glia in the *Drosophila* central nervous system form a niche around neural stem cells (NSCs) and their progeny to establish crosstalk with their surroundings. Cortex glia cells are unique in their growth as they form a dense network of cytoplasmic extensions around neural cells. Instead of simply undergoing mitosis, they follow endocycle and acytokinetic mitosis to increase their nuclei number. In addition, one cortex glia fuses with another to form cortex glia units that share their cytoplasmic contents. Although they are essential for the development and function of the nervous system, how these cells grow and form extensive and intricate membrane networks remains largely unknown. Here, we show that although cortex glia undergo a variant cell cycle, they are still regulated by canonical cell cycle regulators. Various cyclin and CDK complexes are involved in regulating ploidy and nuclear division of cortex glia to form syncytial cells. Loss and gain of canonical G1/S/G2 regulators severely affect nuclear ploidy and division, as well as cell growth and network formation. Our work provides crucial insights into how the central nervous system develops and functions, which would help in understanding several neurological disorders related to changes in ploidy and genome instability.

Keywords: cortex glia, *Drosophila*, ploidy, syncytiu

ISCB–CACTUS AWARD

Role of Autophagy in the Pathogenesis of Diabetic Retinopathy

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Diabetic retinopathy (DR) is a retinal vascular disease with a prevalence of 18% in India. Hypoxic insult due to prolonged hyperglycaemia leads to increased cell death and hence accumulation of cellular debris. These are cleared off by glial cells to maintain retinal homeostasis. However, failure to do so causes prolonged neuro inflammation and neurodegeneration in the retina. Therefore, this study focuses to explore the role of microglia-mediated autophagy in DR.

Bulk transcriptome analysis of blood was performed for different categories of DR (Proliferative DR and Non-Proliferative DR) vs Diabetic individuals without DR (DM) and normal controls (n=3 each). Validation of crucial autophagy genes/pathways in a larger cohort, retinal tissue samples (Diabetic vs control) and hyperglycaemia induced retinal primary cultures was performed using SYBR green chemistry. Statistical analyses were performed using the student's t-test. Cell death/Autophagy triggering factors such as collagen deposition and ROS were also checked using specific assays.

Most of the autophagy associated genes including *CATSB, ATG4b, P62, LC3II, LAMP1, LAMPII* (to list a few) were found to be significantly (p-value<0.05) downregulated in the DM and NPDR samples while the significant opposite trend was seen in the PDR category against normal controls. The above genes were also found to be downregulated (though not significant) in the retinal tissues from individuals with diabetes. Collagen deposition and ROS accumulation was significantly observed in DM retinas and PDR vitreous respectively.

Impaired/reduced autophagy potentially contributes to the development of early DR while a further continued microglial activation leading to an aberrant autophagy might drive the retina to proliferative stage of DR.

Keywords: Autophagy, Microglial cells, Diabetic Retinopathy, Transcriptomics

ISCB–CACTUS AWARD

Dynamics of DNA methylation during male gametogenesis in *Hydra*

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DNA methylation plays a critical role during germ cell development and gametogenesis. The dynamics of DNA methylation during germ cell development are very crucial, any kind of aberrations in this process can perturb the germ cell development and chromatin packing during male gametogenesis resulting in infertility. However, the precise regulatory mechanism of DNA methylation and the effect of altered DNA methylation during gametogenesis is not well understood. The evolutionary origin of DNA methylation and its role in gametogenesis is also not clear. Therefore, we choose to study early metazoan *Hydra* (a cnidarian) in which pluripotent I-cells give rise to primordial germ cells (PGCs) by induction and develop into gametes similar to mammals. Here, using the homology search, we found that the homologs of *dnmt1*and *dnmt3* genes are conserved in *Hydra*. The expression analysis of the *dnmt1* gene using in situ hybridization shows higher expression in males compared to asexual and female polyps. Whereas *dnmt3* is expressed exclusively in male polyps. The elevated expression of the dnmt3 gene in males suggests its *de novo* methylation role during important for the condensation of chromatin in the male genome to protect the sperm genetic material during the successive round of DNA replication at the time of spermatogenesis. Immunofluorescence analysis of DNA 5-methylcytosine (5mC) indicates that DNA methylation marks are increased in the PGCs, migrating to the mature gonads of *Hydra*. Our results demonstrate the conserved mechanism of DNA methylation during the development of PGCs and male gametogenesis in Hydra except for the intermittent loss of DNA methylation in early PGC determination. Currently, we are studying the effect of loss of 5mC on PGC development and transgenerational epigenetic inheritance.

ISCB–CACTUS AWARD

GLP1R agonist attenuates HFD-induced insulin resistance by blocking TRIM mediated degradation of insulin receptor

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Metabolic disorders associated with obesity, such as Type 2 diabetes (T2DM) and non-alcoholic fatty liver disease (NAFLD), are closely correlated with impaired insulin receptor signaling. Nevertheless, little is known about the precise processes underlying decreased insulin receptor (INSR) signaling in obesity brought on by a high-fat diet. Here, we discovered that, in diet-induced obesity, the insulin receptor (INSR) is the target of tripartite motif-containing protein (TRIM), an E3 ubiquitin ligase that ubiquitinates and degrades it by proteasomal means. Diets high in fat cause SREBP1c (Sterol Regulatory Element-Binding Protein 1c) to enter the nucleus, which raises TRIM levels. Diet Induced Obesity (DIO) mice developed significant insulin resistance and fat buildup in their livers as a result of TRIM's ubiquitylation of INSR and facilitation of its proteasomal degradation. On the other hand, lowering TRIM in the liver decreases hepatic fat accumulation, increases INSR expression, and decreases hepatic insulin resistance. Additionally, we discovered that by decreasing TRIM expression, PK2, a small molecule agonist of the Glucagon-Like Peptide-1 Receptor (GLP1R), raises the biological activity of the hepatic insulin receptors in DIO mice. Mechanistically, a high-fat diet reduces AMPK signaling in DIO mice, which increases TRIM expression by encouraging SREBP1c to shift nucleus, which in turn decreases the amount of INSR on the cell surface. All things considered, these results illuminate the function of TRIM in insulin signaling and suggest that it may be used as a therapeutic target to address insulin resistance.

Keywords: GLP1R agonist, Insulin resistance, NAFLD, Obesity, TRIM

ISCB–CACTUS AWARD

Enzymatic and structural roles of *Candida albicans* Rev1 in DNA damage response and disseminated candidiasis

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Translesion DNA synthesis (TLS) is a fundamental biological process that enables DNA replication through Various lesions to ensure timely duplication of genetic information and to prevent cell death due to replication fork collapse. *Saccharomyces cerevisiae* possesses eight DNA Pols and five of them were found to carry out TLS efficiently. Rev1, a member of Y-family DNA Pols, functions in concert with a B- family enzyme Polζ in promoting TLS through UV lesions, abasic sites and through damaged bases; implicating their involvement in mutagenic TLS. Interestingly, for such a function the catalytic activity of Rev1 seems to be dispensable. Unlike Polζ that possesses robust DNA polymerase activity, biochemical assays suggest that Rev1 predominantly incorporates a C opposite any templating residues, but the biologically relevant of this activity of Rev1 remains elusive. In this study, we have characterized Rev1 from a pathogenic yeast *Candida albicans* and analyzed various truncated and site directed mutants of Rev1 to delineate its structural and catalytic roles during various lesion by pass. In addition, we also determined the mode by which Rev1 gets recruited to the stalled replication fork. Since systemic candidiasis due to *C. albicansis* one of the leading cause of death in immunosuppressed individuals, role of Rev1 in fungal pathogenesis was evaluated. The obtained results will be discussed in greater details.

Keywords: *Candida albicans*, Candidiasis, PCNA, Rev1, TLS.

ISCB–JCS AWARD

Role of Inorganic Polyphosphate in Mammalian Granule Biology

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Inorganic polyphosphate (polyP) is a polymer of orthophosphate residues linked by high energy phosphoanhydride bonds. This bio-polymer is found ubiquitously in all taxonomic kingdoms, and its abundance is highest in prokaryotes and unicellular eukaryotes. In Bacteria, Protista and Fungi, polyP synthesis, function and storage are well explored and understood. In mammals, polyp is stored in lysosome-related organelles (LROs) including platelet dense granule but its synthesis machinery and its regulation remain enigmatic. PolyP synthesis in budding yeast is allosterically regulated by diphosphoinositolpentakisphosphate ($InsP_7$). Our lab has shown that mice lacking the $InsP_7$ synthesizing enzyme inositol hexakisphosphate kinase 1 (IP6K1), have low platelet polyP levels compared to wild-type mice. To further study the relationship between IP6K1 and polyP, we selected a rat mast cell (RBL 2H3) model where polyP is exclusively stored in serotonin-containing granules. Organelle fractionation and subcellular localisation studies suggest that polyP is synthesised inside the granules instead of ER/Golgi. Furthermore, we found that polyp synthesis in isolated intact mast cell granules is an ATP-dependent process and exogenous supply of $InsP_7$ enhances polyP signals. An intact potential difference across the granule membrane is pre-requisite for polyP synthesis. Treatment of isolated granule with the V-ATPase inhibitor, bafilomycinA1, disrupts polyp synthesis. In addition, depletion of IP6K1 causes reduced serotonin and polyp levels and loss of granule acidification in RBL 2H3 cells. The crosstalk between $InsP_7$, granule stability and polyP synthesis is yet to be understood. Our aim is to uncover the mechanism underlying this crosstalk. Our ongoing analysis of the polyp interactome in granule may shed some light on this phenomenon.

Keywords: PolyP, IP6K1, $InsP_7$, Granule, Acidification

ISCB–CACTUS AWARD

Novel Approach to Rescue Post-transplant Diabetogenic Effect of Calcineurin Inhibitors by Meddling with Proteasome

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The emergence of new-onset diabetes via post-transplantation poses a formidable challenge in solid organ transplantation, primarily due to the administration of immunosuppressive clacineurin inhibitors such as CsA and FK506. These agents target the Cn-NFATc pathway, leading to age-dependent diabetes. Cn-NFATc signalling plays a crucial role by regulating multiple pancreatogenesis factors; thus, even a minor disruption in the homeostasis of this pathway can significantly impair β-cell functionality. Several of these pathways are meticulously regulated at the post-translational level by the ubiquitin-proteasome system(UPS). Despite this understanding, the interplay between Cn-NFATc signalling and UPS inβ-cell differentiation and preserving its function remains an unexplored area. Hence, our study identified the inhibiting of Cn-NFAT signalling to hamper endodermal differentiation, whereas blocking proteasomal activity enhanced this differentiation from pluripotent stem cells (PSCs). Mechanistic investigation through a comprehensive screening approach identified PSMD2, part of the 19S regulatory subunit of proteasome, to be significantly downregulated during endodermal differentiation. Small molecule- mediated inhibition of signaling pathways and luciferase assays revealed PSMD2 to be a direct downstream target of the Cn-NFATc pathway, highlighting novel crosstalk between the Cn-NFATc pathway and the UPS in endoderm specification. The functional analysis by PSMD2 knockdown and over-expression constructs confirmed that the downregulation of PSMD2 is essential for facilitating endodermal differentiation. Additionally, the downstream mechanistic insights from Co-IP and ChIP assays revealed that the PSMD2-Nanog-Gata4/6 axis plays a pivotal role in determining the endoderm fate towards pancreatic β-cell differentiation. In essence, the innovation of this study lies in unraveling the novel mechanism of pancreatic β-cell development by screening for specific UPS-associated genes that serve as downstream targets of the Cn-NFATc pathway, which essentially performs its role in endoderm cell fate via Nanog regulated pathway at the downstream level. Ultimately, we aim to leverage this knowledge to reconstruct human islets, paving the way for advancements in diabetes treatment.

Keywords: Cn-NFATc pathway, Diabetes, Embryonic Stem Cells, Proteasome, Pancreatic β-cells

CACTUS Global x All India Cell Biology Society: Advancing Scientific Communication - 47th All India Cell Biology Conference

Dr. Smita Jain presenting at the 47th All India Cell Biology Conference

As part of our ongoing partnership with CACTUS Global, we were delighted to host them at the 47th All India Cell Biology Conference at the National Institute of Science Education and Research (NISER), Bhubaneswar, from December 16-18, 2024. The event brought together over 300 researchers from India and abroad, creating an engaging platform for discussions on advancing scientific communication. Representing CACTUS Global was Dr. Smita Jain, (Director, Partnerships – India), who presented the company's global initiatives in empowering scientists for over 22 years, which generated enthusiastic responses from the attendees. Researchers showed great interest in their offerings, particularly in manuscript writing, editing, and AI-powered research solutions, with many expressing a desire to adopt these tools for their labs.

A key highlight was the recognition of CACTUS Global as an Indian company with a diverse suite of products and services for researchers. Senior researchers from various institutions expressed keen interest in future collaborations, leading to promising discussions.

In continuation of our partnership, in January, CACTUS Global conducted a two-part webinar themed "Optimizing Research: Structuring Manuscripts & Leveraging AI." The first session, led by Hema Thakur, focused on crafting manuscripts that capture attention and facilitate publication. The second, by Dr. Sunaina Singh, showcased practical AI tools that streamline the research workflow from draft to submission. With 280+ attendees, this webinar received commendations for its insightful exploration of AI in research and its practical tips for enhancing academic writing. We are happy to collaborate with a leading science communication and technology company like CACTUS Global and look forward to strengthening this impactful partnership to drive excellence in scientific communication.

Alisha Fernandes (Marketing Manager, Cactus Communications) at the CACTUS booth

Dr. Smita Jain (center) with Dr. Debasmita Alone (left) and Prof. Sathees Raghavan (right)

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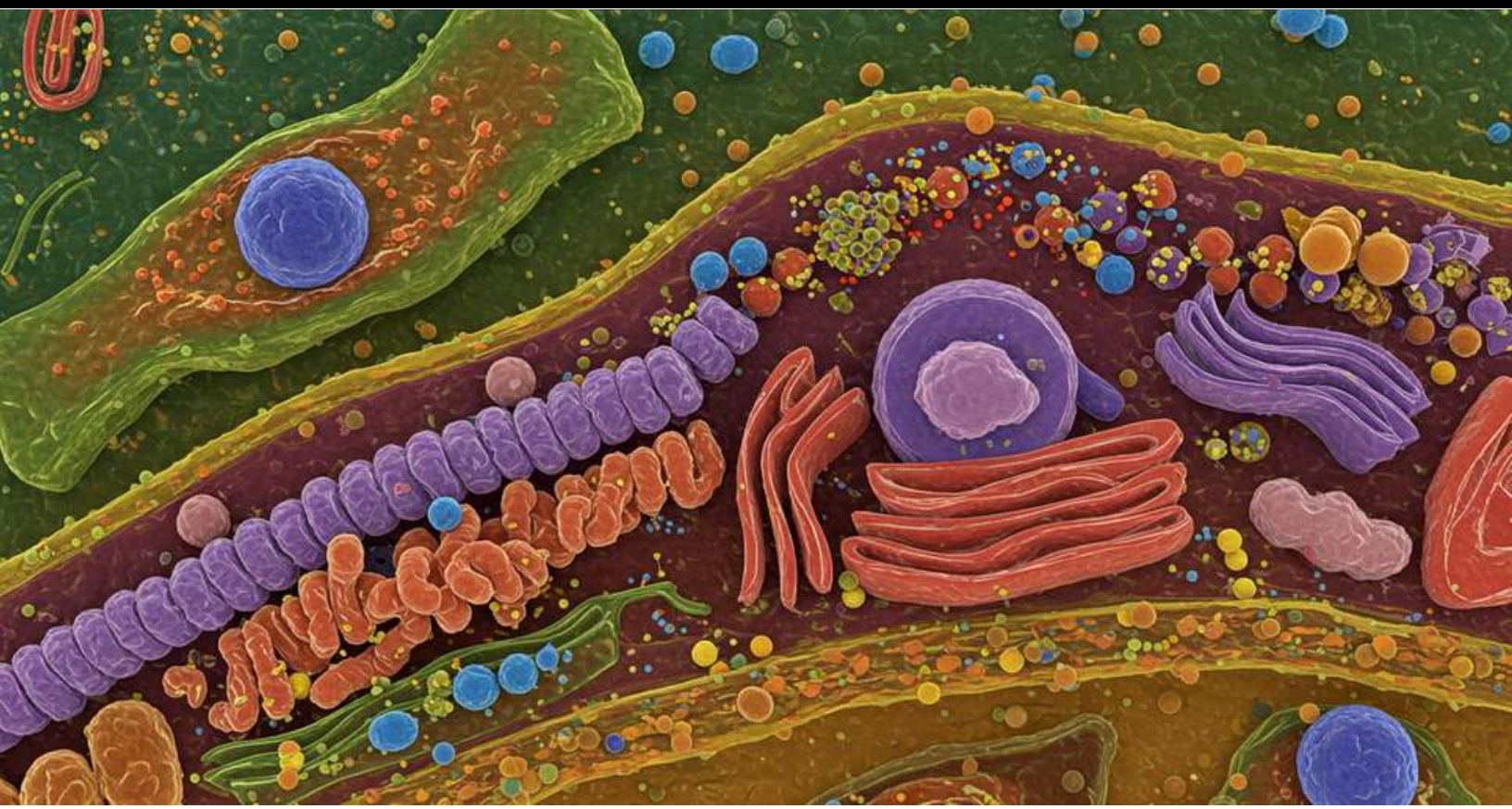
Research Article - Section 3

CELLULAR ORGANIZATION AND DYNAMICS



Cellular function relies on precise spatial and temporal organization, ensuring that biochemical reactions occur in the right place at the right time. This section explores diverse mechanisms of cellular architecture and dynamics. Neelabh Datta examines how cells use biomolecular condensates—membraneless organelles formed via phase separation. Shankar Prasad Das and team introduce techniques to overcome the staining challenges of Malassezia, uncovering, its nuclear organization and cell cycle dynamics. Devrath Rath and colleagues investigate the mycobacterial protein MSMEG_0311, revealing its critical role in maintaining cell wall integrity, drug sensitivity, and polar localization during growth. Together, these studies showcase cutting-edge approaches to decipher cellular complexity across diverse systems.

How Do Cells Organize Their Chaos?

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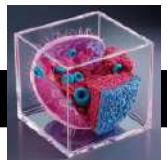
As you all know that cells are like bustling cities, teeming with activity; the question that comes to mind is, how do they maintain order amidst such chaos? The answer lies in compartmentalization, the division of labor within the cell through specialized structures, both with and without membranes. Among these structures are intriguing entities known as biomolecular condensates. Unlike traditional, membrane-bound organelles, these condensates assemble and disassemble freely, concentrating specific biomolecules to carry out precise functions. So, what make these tiny, membranes-less assemblies such efficient organizers, and why do they matter?

Biomolecular condensates are spatial hubs where various biochemical activities unfold. They ensure that processes like transcription, RNA processing, and translation—key steps in gene expression—occur with clockwork precision. Interestingly, recent research shows that these condensates form through a process called phase separation, where certain molecules separate out from the surrounding cell fluid to form their own microenvironments. These tiny compartments, composed of proteins and RNA, form distinct compositions that behave differently from the surrounding cellular soup. The internal structure of condensates is driven by weak interactions between molecules, especially through parts of proteins known as intrinsically disordered regions (IDRs), which help gather the right components in the right place at the right time.

Think of it like a crowded room where people are drawn into groups based on shared interests. These interactions are fleeting, constantly forming and breaking, but they give each group a unique identity, much like how different condensates serve different cellular functions. Some concentrate enzymes and substrates to accelerate reactions, while others work by sequestering molecules, like hiding key players from the action until they're needed. These functional hubs allow cells to organize their complex biochemistry, but the mechanisms underlying how condensates control specific processes remain elusive.



The material properties of condensates also play a crucial role in their function. Condensates aren't solid, nor are they completely fluid—they're viscoelastic. Imagine a blob of jelly—sometimes it stretches, sometimes it resists. This physical flexibility allows condensates to perform a variety of tasks. For instance, more solid-like condensates are better suited for sequestering molecules, keeping them locked in place. On the other hand, more fluid-like condensates excel at promoting interactions between molecules, acting as reaction centers. The ability of condensates to switch between these different states suggests that their material properties could be fine-tuned to suit the cell's needs at any given time.

Research has found that when these properties go awry, it can lead to diseases, including cancer. For instance, condensates can become too rigid, impairing their ability to sequester molecules or facilitate reactions properly. Understanding the physical characteristics of condensates might not only help decode how they function but also open up new avenues for treating diseases linked to faulty condensate behavior. To study how condensates influence cellular processes, scientists have often turned to purified systems—lab-created versions of condensates. These models have shown that condensates can either accelerate or suppress biochemical reactions, depending on their composition and material properties. For example, when certain enzymes and their substrates are packed into condensates, their reaction rates can skyrocket. On the flip side, some condensates can inhibit translation, the process where mRNA is turned into proteins, by sequestering key translation factors.

However, studying condensates in living cells presents a bigger challenge. Knocking out phase-separating proteins can lead to a slew of unintended consequences, making it difficult to pinpoint the exact role of condensates. Enter optogenetics—a cutting-edge tool that allows scientists to control the formation of condensates with light. By using light to trigger the assembly of condensates, researchers can precisely control when and where condensates form, enabling them to explore the immediate effects of these structures on cellular processes.

In a recent study by Lee et al. 2024, scientists used optogenetics to control the condensation of mRNA molecules within cells. They found that when mRNAs were sequestered into these light-induced condensates, the translation of the mRNAs was inhibited. What's fascinating is that this inhibition occurred without changing the sequence of the mRNA or the levels of regulatory proteins. Simply by altering the phase behavior of the mRNA's environment, translation was suppressed. The more solid-like the condensates became, the less mobile the resident molecules were, and the stronger the translation inhibition. Interestingly, the repression of translation was not as dramatic as one might expect based on how much mRNA was sequestered. This suggests that individual mRNAs experience a kind of molecular tug-of-war—sometimes they get trapped in the condensates, while other times they manage to escape and engage in translation. The transient nature of these interactions adds another layer

of complexity to how condensates control cellular processes.

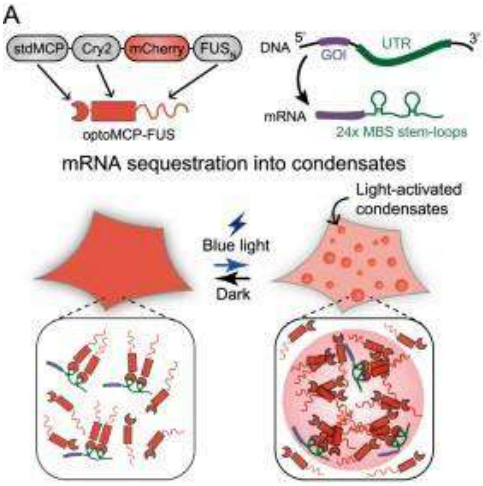
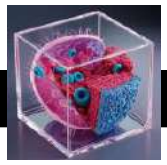


Figure 1: The image illustrates the design of a light-activated system for RNA sequestration into condensates using optoMCP-FUS, a fusion protein comprising mCherry, FUS, and a light-sensitive Cryptochrome 2 (Cry2). This system enables controlled recruitment of mRNAs with specific stem-loop structures (MS2-binding sites) into condensates upon blue light activation, simulating and studying RNA compartmentalization in response to light-controlled phase transitions (1).

RNA plays a central role in organizing these condensates, participating in a variety of RNA-protein and RNA-RNA interactions. However, not all mRNA interactions with condensates result in translational inhibition. Some condensates, called "translation factories," actually promote the translation of specific mRNAs. For instance, during spermiogenesis, a phase separation event involving FXR1 protein is crucial for activating the translation of mRNAs necessary for sperm development. In contrast, the phase separation of another related protein, FMRP, results in translational repression. This dichotomy suggests that the composition of the condensate, and possibly post-translational modifications of its components, can drastically influence its function.

Moreover, some condensates once thought to be inactive, like stress granules, have been found to harbor translationally active mRNAs. This raises an intriguing possibility—condensates may not be as functionally uniform as once believed. The same condensate could serve multiple roles depending on its molecular makeup, physical properties, and the specific needs of the cell.

The ability to fine-tune the material properties of condensates opens up exciting possibilities for research and therapeutic applications. Could the manipulation of condensate properties become a new frontier in drug discovery? By understanding how condensates modulate translation and other biochemical processes,



scientists might be able to design therapies that target specific condensates or even create synthetic condensates for therapeutic purposes. As researchers continue to develop tools like optogenetics to explore the dynamic nature of these microenvironments, one is left to wonder: could manipulating the physical properties of these condensates be the key to unlocking new ways to control cellular behavior and treat disease?

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Breaking Barriers: Innovative approaches to unveil the nuclear dynamics and cell cycle of the mysterious fungi *Malassezia*




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Nuclear staining is a cornerstone of cell biology, offering a powerful tool to explore the inner workings of cells. Researchers can illuminate cell nuclei by using dyes like DAPI and Hoechst, providing valuable insights into cellular functions, division, and genetic material. These techniques are widely applied in cancer research, neurobiology, and developmental biology, helps to study cell proliferation, DNA damage, and gene expression with cell cycle. However, the human commensal *Malassezia*, present unique structural challenges that resist conventional staining methods. We tried to overcome the complexities of *Malassezia*'s cellular structure, aiming to develop new strategies for nuclear staining and unlocking its potential for further research on fungal biology, infections, and its possible role in certain diseases associated with humans (1).

Malassezia is known as skin-resident commensal fungus reliant on host lipids for survival, utilizing enzymes like lipases and phospholipases to degrade sebum (2). Under certain conditions, it transitions to a pathogenic form, producing virulence factors such as indoles, reactive oxygen species, azelaic acid, and biofilms, contributing to diseases like Pityriasis versicolor, Seborrheic dermatitis, and Atopic dermatitis (3). Recent studies suggest that *Malassezia* colonizes beyond the skin and associates with diseases in the gut (e.g., Crohn's, colorectal, and pancreatic cancer) (4), lungs (e.g., cystic fibrosis, lung cancer) (5), and brain (e.g., Alzheimer's, Parkinson's diseases) (6). However, its exact role in these diseases remains unclear due to challenges like its lipid-rich cell wall, slow growth, and specific nutritional needs, which hinder deeper investigation.

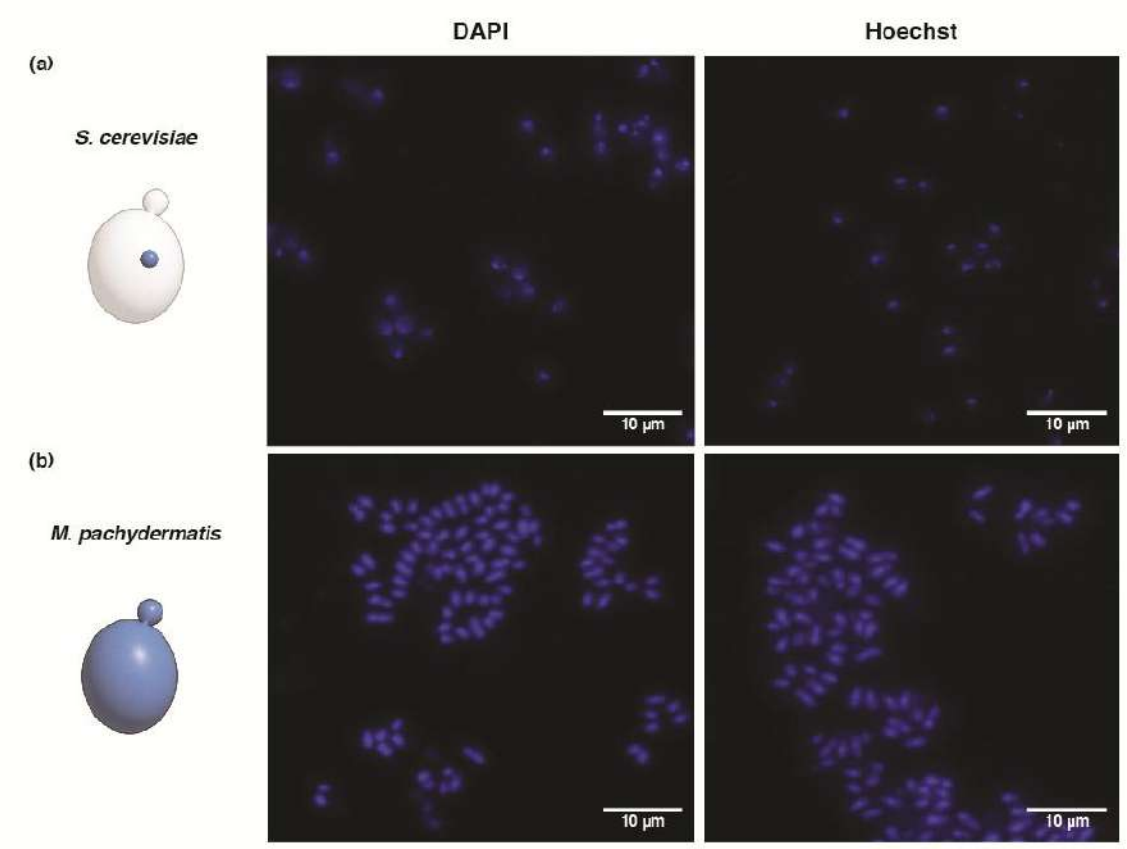


Figure 1: Comparison of nuclear staining profiles of yeasts using traditional methodology. Nucleus as observed in (a) S. cerevisiae and (b) *M. pachydermatis* using the stains DAPI and Hoechst

.A major factor hindering nuclear staining in *Malassezia* is its rigid, lipid-rich cell wall. Earlier studies highlighted that staining the *Malassezia* nucleus is particularly challenging with standard agents like DAPI (7). We encountered similar challenges when attempting to stain the nucleus of *Malassezia* pachydermatis. DAPI/Hoechst molecules appeared to bind non-specifically to their cell wall, resulting in non-specific fluorescence and whole-cell staining (Figure 1).

This phenomenon may be attributed to interactions between DAPI and membrane lipids, such as phospholipids We hypothesized that the thick cell wall of *Malassezia*, containing 15-20% lipid content, might hinder the penetration of fluorescent dye molecules, compared to the ~3% lipid content in S. cerevisiae. To address this, we employed a combination of chemical and enzymatic treatments to permeabilize the cell wall. We selected zymolyase, an enzyme well-known for its cell wall permeabilization properties, which works by breaking down beta-1,3-glucan, a major structural component of the fungal cell wall, through its glucanase activity. This process effectively creates openings or pores in the cell wall. *Malassezia pachydermatis* cells were treated with zymolyase at various concentrations and time points, followed by

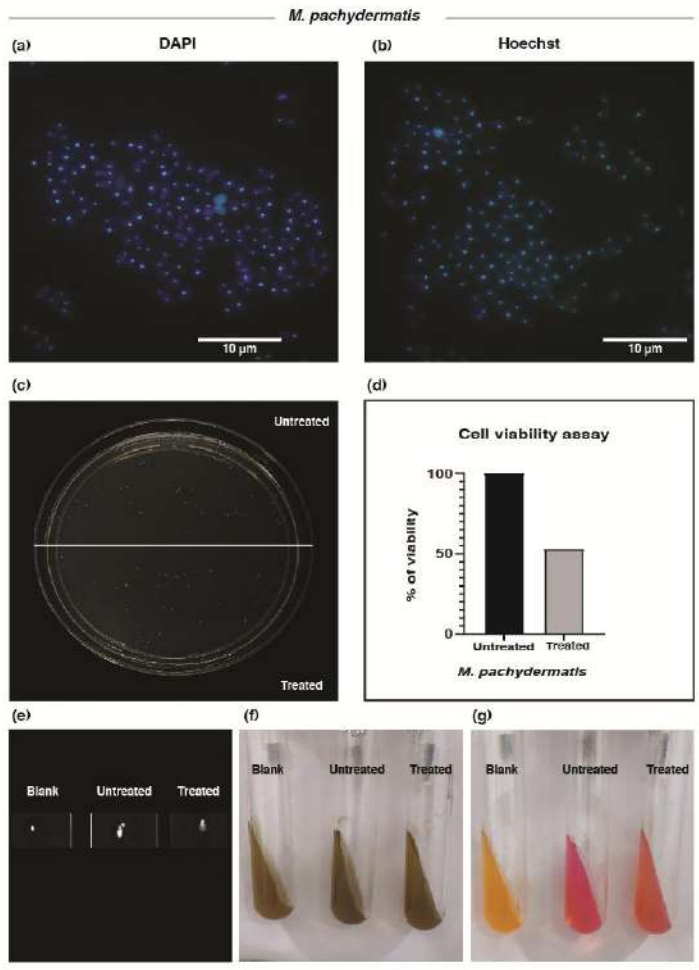
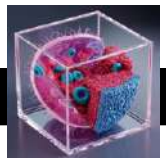


Figure 2: Nuclear staining profiles of M. pachydermatis using the newly developed methodology with (a) DAPI and (b) Hoechst dyes. Viability assessment of treated cells through (c) colony-forming units and (d) its percentage. Metabolic activity evaluation using (e) catalase, (f) bile esculin, and (g) urease assays.

staining with nuclear dyes. Among these, treatment for 2 hours resulted in reduced non-specific staining; however, a distinct nucleus was still not observed. To further enhance permeabilization, we shifted to a chemical-based approach using Triton X-100, a detergent capable of inserting monomers into the lipid membrane to disrupt it and increase cell wall permeability. Like zymolyase, Triton X-100 treatments were performed at multiple time points and concentrations. Treatment for 2 hours with nly Triton X-100 also reduced non-specific staining but did not yield a clear, prominent nucleus. To improve the results, we developed a novel solution, named CP solution (Cell Permeabilization Solution), after multiple rounds of standardization. The CP solution comprised of 40 µl of zymolyase (10 mg/ml), 500 µl of Triton X-100 (diluted 1:9 in Milli-Q water), and 5 µl of β-mercaptoethanol (to enhance permeabilization). Cells were treated with CP solution for 2 hours and then stained with DAPI or Hoechst dyes. This approach successfully revealed clear and distinctly stained



nuclei (Figure 2). This marks the first-ever demonstration of a stained *Malassezia* nucleus. We checked the viability of the treated cells and interestingly, they demonstrated approximately 60% viability and retained metabolic activity, as confirmed by catalase, bile-esculin, and urease assays (Figure 2). These findings indicate that the treated cells remain functional and can be utilized for further research.

We further extended our research to analyze the cell cycle of *Malassezia*, a study that has not been reported previously. Understanding the cell cycle is crucial for investigating microbial growth, replication, and responses to environmental or therapeutic interventions. It aids in identifying cell cycle disruptions caused by antimicrobial agents, assessing virulence factors, and studying pathogenic mechanisms, thereby providing insights into microbial physiology and potential therapeutic targets. However, no prior studies have analyzed the cell cycle of *Malassezia.* Taking on this challenge, we developed a simple and effective staining methodology to study the cell cycle of M. pachydermatis using propidium iodide (PI). Log-phase cells were fixed overnight with 70% ethanol, treated with RNase, and stained with PI. The cells were then analyzed via flow cytometry, using S. cerevisiae as a control. Remarkably, the cell cycle of M. pachydermatis was successfully visualized as distinct peaks corresponding to the G1, S, and G2 phases, similar to the control. With this established methodology, we conducted a cell cycle arrest study in M. pachydermatis using nocodazole. Given the longer doubling time of *Malassezia* (approximately 3 hours per cycle), nocodazole treatment was extended for two cycles. Following treatment, the cells were stained with PI and analyzed through fluorescence microscopy and flow cytometry. The nocodazole-induced arrest was confirmed by the presence of dumbbell-shaped cells observed under the microscope and the arrested G2 peak detected in flow cytometric analysis (Figure 3), represented the first demonstration of cell cycle analysis and arrest in *Malassezia*.

While these approaches may seem like basic research in cell biology, they represent essential and fundamental aspects of scientific inquiry. Today, emerging technologies are expanding the boundaries of knowledge to unprecedented levels. However, many organisms continue to challenge researchers by presenting significant technical barriers that restrict deeper investigation. Addressing these challenges is crucial, and it is the responsibility of researchers to develop innovative solutions to overcome them. In this spirit, we have developed a robust methodology to study the challenging fungus *Malassezia*. This fungus is historically understudied due to its rigid cell wall, lipid-rich membrane, and slow growth, but recent advancements now enable deeper investigation into how the commensal dwells in our skin and other internal organs of the human body. Our robust methodology provides a reliable approach to studying *Malassezia*, opening new avenues in nuclear dynamics, understanding pathogenic mechanisms, and developing antifungal drug. Importantly, this technique is adaptable to other fungi posing the same

challenges, contributing to fungal biology research and targeted therapeutic strategies.

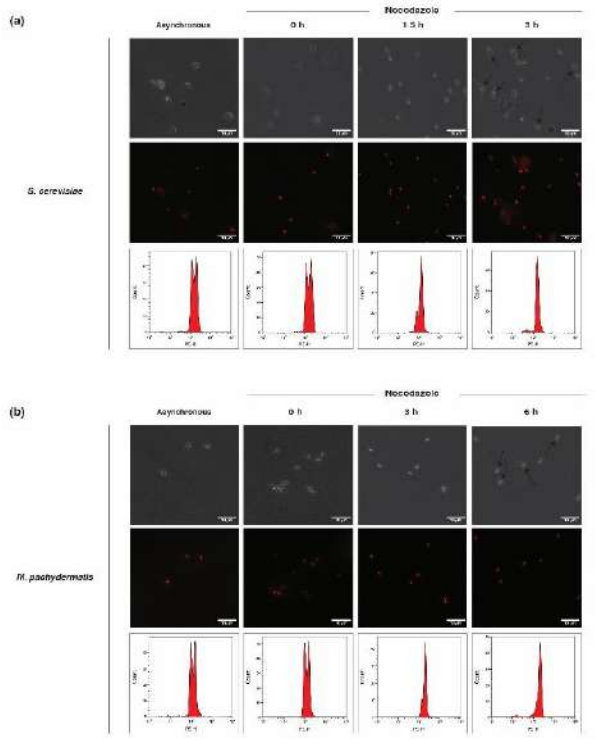


Figure 3: Cell cycle analysis of *Malassezia*. Microscopic images and FACS profiles showing nocodazole-induced cell cycle arrest in (a) S. cerevisiae (asynchronous cells without nocodazole and treated for 0 h, 1.5 h, and 3 h) and (b) M. pachydermatis (asynchronous cells without nocodazole and treated for 0 h, 3 h, and 6 h). Black arrows indicate large budded cells arrested by nocodazole.

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MSMEG_0311: Unravelling its role in Mycobacterial Drug Sensitivity and Cell Wall Dynamics




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Mycobacterium species, including pathogens like *Mycobacterium tuberculosis* and *Mycobacterium leprae*, are famous for their uniquely resilient cell walls, which make them highly resistant to antibiotics (1). Understanding the biology of *Mycobacterium* species has proven vital for developing effective treatments against infections like tuberculosis (TB), where robust cell walls and unique defense mechanisms present therapeutic challenges. The mycobacterial cell envelope is organized into three distinct layers (2) (Figure 1). The outermost capsular layer is enriched with glycans such as mannans, α-glucans, and arabinomannans, which are unique compared to those found in other Gram-positive or Gram-negative bacteria. Beneath this capsule lies the cell wall, featuring a tripartite mAGP complex comprising a mycolic acid layer (mycomembrane), an arabinogalactan layer, and a peptidoglycan layer. Further inward, the periplasmic space encompasses the conventional plasma membrane. Given the complexity of these structural components and their biosynthetic pathways, detailed Investigation into mycobacterial cell wall metabolism is essential. Understanding proteins that influence the cell wall metabolism might unlock new methods for combating mycobacterial infections. We performed a CRISPRi screen in *Mycobacterium* smegmatis to identify novel genes essential for the growth of the organism including genes involved in cell wall metabolism. MSMEG_0311 was a top hit

among several essential genes identified in the screen. The exact function of the gene was unknown, but early clues suggested it might play a significant part in cell wall associated function (3).

We observed that MSMEG_0311 is highly conserved across different mycobacterial species including *M. leprae*. The conservation extending to *M. leprae* was particularly intriguing, as this species is known for its reductive evolution, which has led to the deletion of many genes and the accumulation of numerous pseudogenes (4), indicating that MSMEG_0311 and its homologues have a vital role in cellular functions across mycobacterial species. In-silico analyses indicated that MSMEG_0311 is a cell wall associated protein which contains a glycosyl transferase domain, a feature often associated with enzymes that modify sugars and lipids. We explored the cellular effects of silencing MSMEG_0311 using CRISPRi, in order to gain clues about its function

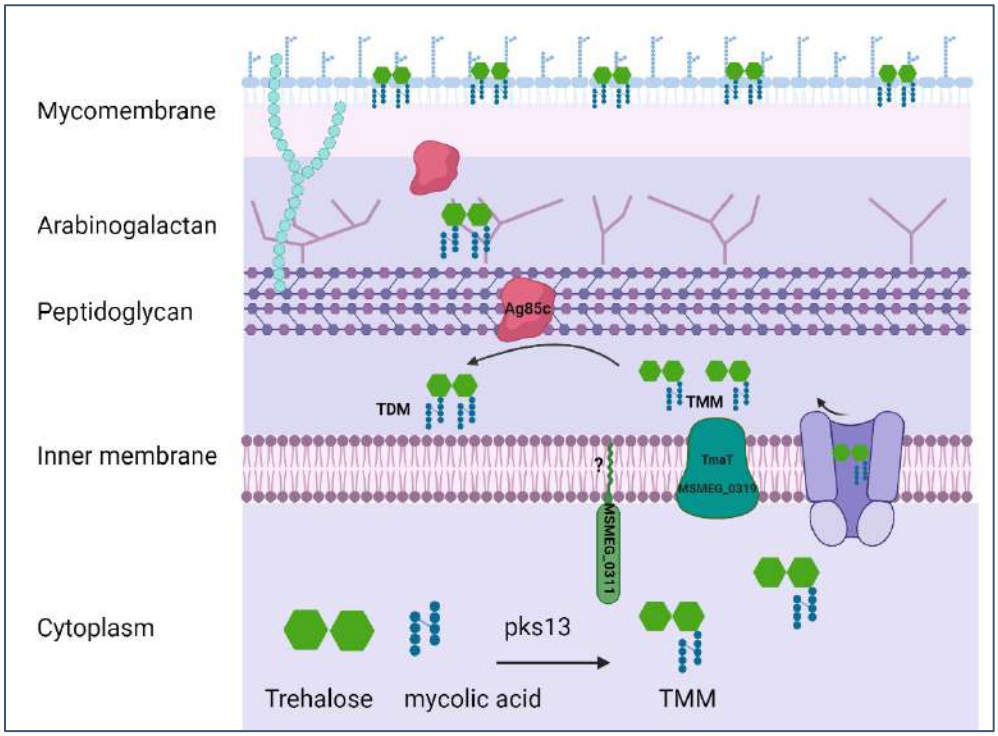

Figure 1: Schematic showing *mycobacterium* cell wall and the transport of trehalose mycolate across the cell membrane

(Figure 2). Knockdown of MSMEG_0311 (MSMEG_0311 K.D) led to a severe growth defect in the strain (Figure 2A), and noticeable changes were observed in colony morphology with reduced cording seen in MSMEG_0311 K.D as compared to control (NTA). The cording feature of MSMEG_0311 K.D is owing to the presence of cord factor called trehalose dimycolate (5), an important part of mycobacterium cell wall (Figure 2B). Further, the cell permeability of the strain was also affected (Figure 2D, E), suggesting its influence on the mycomembrane—a lipid-rich layer unique to mycobacteria. To gain insights into the

pathways affected upon silencing MSMEG_0311 transcriptomic analysis was carried out and differential expression of several cell wall associated genes was observed (highlighted in red boxes in Figure 2C). Interestingly, there was a 17-fold upregulation of iniA (MSMEG_0695) whose role in isoniazid resistance has been implicated earlier. Subsequently, lipids were isolated and a TLC run was performed where it was ascertained that MSMEG_0311 knockdown leads to altered trehalose mycolate levels (Figure 2F). This implied that cells lacking this protein might struggle to maintain adequate levels of TDM thereby leading to loss in cell wall integrity, or the layer containing these glycans might be compromised which are essential to their protective cell wall. These findings suggested that MSMEG_0311 is pivotal to mycobacterial survival, representing a potential target for therapeutic intervention against this otherwise resilient pathogen.

It is interesting to note that unlike most bacteria, mycobacteria add new cell wall material exclusively at the poles rather than along the lateral walls (6). Several polar-localized proteins, such as DivIVA, are critical for recruiting and regulating factors involved in cell division and cell wall synthesis. Given this polar-centric cell wall synthesis, we sought to examine the localization of MSMEG_0311, to further ascertain its potential involvement in cell wall-associated functions. A GFP fused MSMEG_0311 over-expressing strain was constructed to track protein localisation and it was observed to localize specifically at the cell poles (Figure 2G), the primary sites for new cell wall deposition, supporting its role in cell wall biosynthesis. Interestingly, with use of fluorescently red labelled D- amino acid dyes, we found that MSMEG_0311 preferentially accumulates at the older, faster-growing pole (Figure 2G bottom panel).

Building on the observed indications of MSMEG_0311's involvement in cell wall-related functions, we proceeded to evaluate the strain's sensitivity to various drugs targeting the cell wall and also some targeting protein synthesis in disk diffusion assay. Cells with reduced MSMEG_0311 expression showed increased susceptibility to cytoplasmic acting drugs, pointing to higher drug accessibility due to weakened cell wall integrity and a slight resistance to cell wall acting isoniazid drug (Figure 2H). Also, we found that silencing MSMEG_0311 made the cells significantly more sensitive to various antibiotics in broth conditions, including bedaquiline and vancomycin (Figure 2H).

In conclusion, our work shows that the essential protein, MSMEG_0311, plays a crucial role in cell wall integrity, morphology, sensitivity to certain antibiotics, and cell survival. These findings suggest that MSMEG_0311 and its orthologues could represent a critical target for new therapeutic strategies and improve our understanding of mycobacterial multi-layered cell wall metabolism.

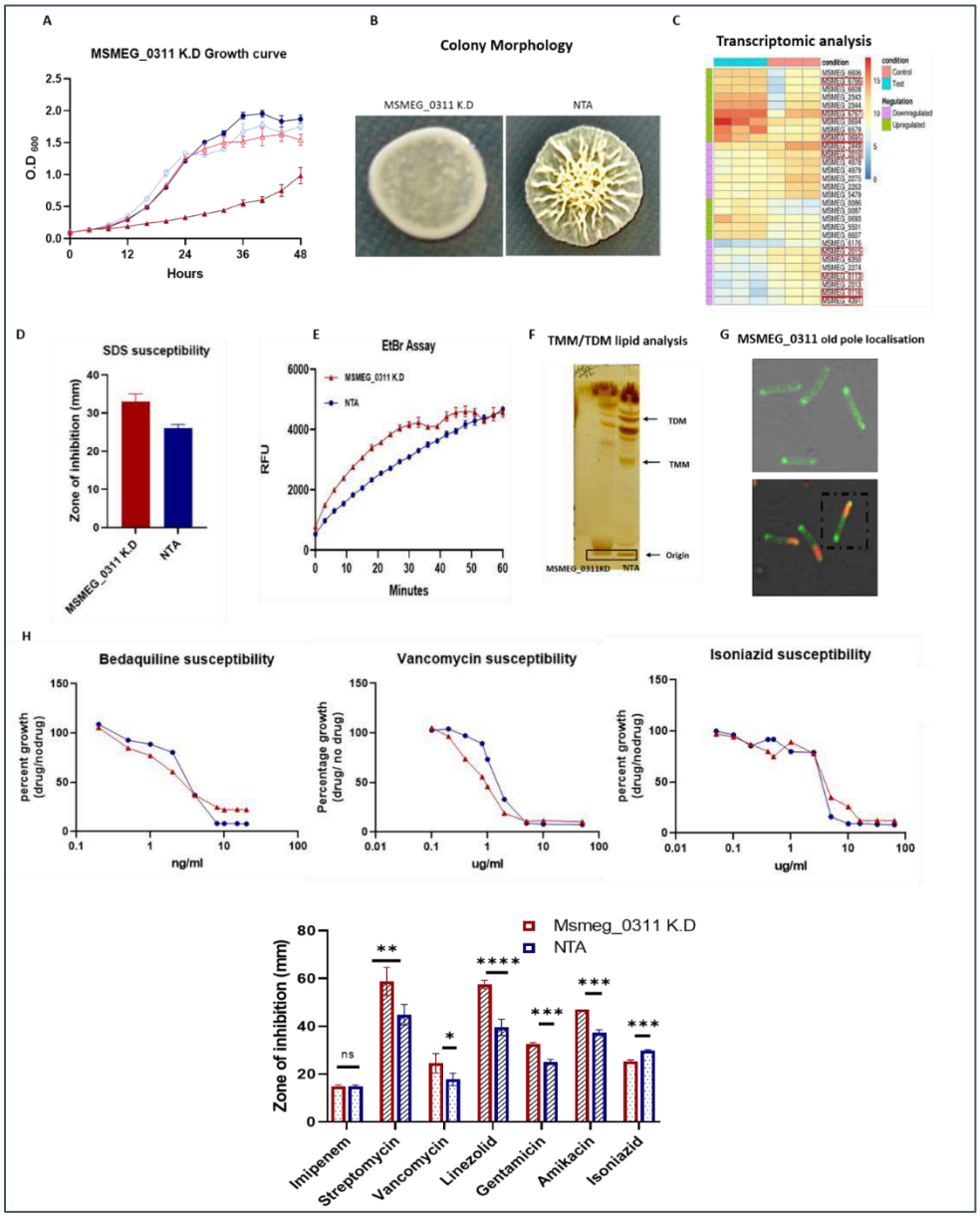

Figure 2: Studies indicating involvement of MSMEG_0311 in cell wall associated function (MSMEG_0311 K.D: knockdown of MSMEG_0311, NTA: control cells). Induction of MSMEG_0311 silencing leads to severe growth defect (highlighted in red) (A), altered colony morphology on solid medium (B), higher surfactant sensitivity in SDS disk diffusion assay (C), increased cell permeability as evidenced by higher accumulation of ethidium bromide (D), differential expression of cell wall associated genes (highlighted in boxes) (E), Depletion of TDM levels (F), GFP-fused MSMEG_0311 shows polar localisation and preferential accumulations towards old pole as evidence by RADA dye assay (G), Differential antibiotic susceptibility observed in MSMEG_0311 knockdown cells in broth and agar conditions (Statistical significance was calculated with Student's t-test; *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001) (H).

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Research Article - Section 4

GENE REGULATION AND DEVELOPMENT

Gene expression and developmental processes are intricately regulated at multiple levels, with precise control ensuring cellular identity and function. This section showcases studies that unravel critical regulators shaping immune responses, neural development, and cancer progression. Sathees C. Raghavan's team uncovers how specific microRNAs—miR-29a, miR-29c, and miR-501—modulate RAG1 expression, offering insights into B-cell development and genomic stability. Richa Arya and colleagues highlight the role of the transcription factor Cut in orchestrating glial cell morphogenesis and polyploidy during central nervous system development in Drosophila. Neeraj Jain’s study identifies the CERS6-AS1/FGFR1 axis as a synthetic vulnerability in mantle cell lymphoma, offering a potential combinatorial therapeutic strategy targeting tumor-stroma interactions. Together, these findings illuminate the dynamic interplay of genetic and epigenetic factors in development and disease.

Understanding microRNA-mediated mechanisms of RAG1 regulation in lymphoid cells


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The discovery of microRNA (miRNA)-mediated regulation of translation in 1993 shed light on one of the potent posttranscriptional regulators of eukaryotic gene expression. Since then, several miRNAs have been identified in mammals, plants, fish, worms, and so on, mostly 21-25 nt in length. MicroRNAs are 18- to 24-nt noncoding RNAs, which play a vital role in the post-transcriptional regulation of several genes by degrading or blocking the translation of respective target mRNAs. In the context of immune system regulation, miR-181a was identified as the first mammalian miRNA regulating the immune response in lymphoid cells. The expression of miR-181a was detected in T and B lymphocytes, suggesting its role in T and B cell development and differentiation.

Mature microRNA sequences are generated from their precursors through carefully regulated steps. Transcription of primary miRNAs by RNA polymerase II and III and subsequent cleavage of the primary RNA by the enzymes Drosha and DiGeorge syndrome critical region 8 (DGCR8)-elements of the microprocessor complex-produces precursor miRNAs (pre-miRNAs). The pre-miRNAs are then exported from the nucleus

to the cytoplasm by exportin 5, where they are cleaved into double-stranded (dsRNA) duplexes by the RNase enzyme Dicer. At this point, loading of the mature miRNA into the RNA-induced silencing complex (RISC) by the Argonaute (Ago) protein and subsequent binding of the seed sequence (6-8 nt) of the miRNA to miRNA-recognition elements (MREs)

within the 3' untranslated region (3'UTR) of target genes ensures the repression of target genes through either mRNA cleavage or translational repression (1).

The regulation of the immune system by various miRNAs is crucial for its normal functioning. While several microRNAs have been identified as regulators of different stages of B and T cell development, such as their development, differentiation and activation, the regulation of recombination-activating genes (RAGs) and, consequently, the V(D)J recombination process by microRNAs has remained largely unexplored. Recombination activating genes (RAGs),

consisting of RAG1 and RAG2, are responsible for generating diverse antigen receptors by initiating V(D)J recombination in developing lymphocytes. Besides its sequence-specific nuclease activity during V(D)J recombination, RAGs can also act as a structure-specific nuclease, leading to chromosomal breaks and genome instability (2). Thus, stringent regulation of RAG expression is essential to maintaining genome stability in lymphoid cells.

We investigated the potential role of miRNAs in the post-transcriptional regulation of RAG1 expression in lymphoid cells. Through in silico, ex vivo, and in vivo approaches, we identified miR-29c as a regulator of RAG1 expression in a B cell stage-specific manner in both mice and humans by binding to its 3'UTR (3). Enrichment of mature miR-29c-3p inside the cells following transfection with pre-miR-29c construct led to reduced RAG1 expression, which was validated by overexpression and inhibition studies (3). An increase in the RAG1 expression upon Dicer knockdown established the role of the miRNA pathway in RAG1 regulation. To determine the direct interaction between miR-29c-3p and RAG1 3'UTR inside the cells, we used CRISPR-Cas9 to mutate the miR-29c-3p binding site at the RAG1 3' UTR in the genomic loci. Mutation of the miR-29c-3p binding site directly affected RAG1 expression at the transcript and protein

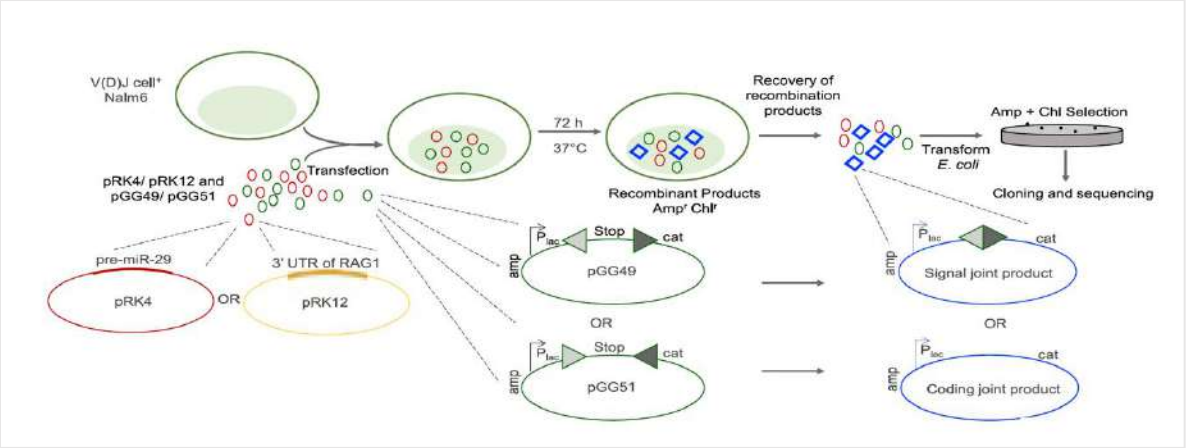

Figure 1: Outline of the extrachromosomal V(D)J recombinase assay. The schematic depicts the transfection of episomes into the pre-B cell line, Nalm6, active for V(D)J recombination. After 72 h, the minichromosomes were harvested, then transformed into *E. coli* for detection of recombinants on ampicillin (A) and chloramphenicol (CA) LB agar plates. The recombination is depicted between a consensus 12 signal (open triangles) and 23 signal (dark green triangles) leading to either coding joint formation (pGG51) or signal joint formation (pGG49). "cat" denotes the chloramphenicol acetyltransferase gene, and "stop" denotes the prokaryotic transcription terminator. The *E. coli* lac promoter is denoted as Plac. The episome, pGG49 or pGG51 (3 μg), was co-transfected with either pRK4 (10 μg) or pRK12 (10 μg), and the recombination efficiency was determined by the formula: (CA/A) * 100.

level within the cells, confirming the involvement of miR-29c-3p in RAG1 regulation (3). This was consistent with our luciferase reporter assay, wherein we observed increased expression of luciferase in cells with the mutated miR-29c-3p binding site at RAG1 3'UTR compared with the WT RAG1 3' UTR miR-29c-3p binding site (3). Furthermore, using an extrachromosomal V(D)J

recombination assay, we observed that overexpression of pre-miR-29c or 3'UTR of RAG1 leads to a change in V(D)J recombination efficiency, the physiological function of RAG1 inside cells (3) (Figure 1). However, there was no significant impact on RAG2 activity and expression on modulation of miR-29c levels. Our results align with the phenotype observed in miR-29c null mice, which exhibit a reduction in mature B cells. We hypothesise that the decrease in the total number of mature B cells could be due to increased DNA breaks generated following upregulation of RAG1 in the knockout mice, this warrants further investigation. Taken together, this study highlights a novel mode of RAG1 regulation by microRNAs, in addition to its regulation at the transcriptional level by transcription factors and enhancer elements.

Unlike miR-29c, miR-29a knockout mice exhibited a stronger impact on immune system regulation. A weakened immune response with an innate defect in B cell activation, germinal center production, and thymic involution, as reported previously in these mice (4), led to a reduced survival span of about seven months after birth. Although the defects in the immune system caused by miR-29a-/- are reported, the putative targets of miR-29a in the context of immunity were not studied. A similar seed sequence among the miR-29 family members and its complementarity to RAG1 3'UTR in both mice and humans prompted us to investigate the regulation of RAG1 by another miR-29 family member, miR-29a, in lymphoid cells. Overexpression and inhibition studies suggested regulation of RAG1 by both the miRNAs (1). We performed Argonaute2-immunoprecipitation and high-throughput sequencing of RNA isolated by crosslinking Immunoprecipitation (HITS-CLIP) studies to establish the interaction of miR-29a-3p, miR-29c-3p and RAG1 with Argonaute proteins (1). Furthermore, miR-29a-3p regulates RAG1 expression in a B- and T-cell-specific manner. To decipher the physiological relevance of this regulation in vivo, we overexpressed pre-miR-29a in mice bone marrow (BM) cells, which led to the generation of mature miR-29a-3p in the BM cells (1). An enhanced expression of miR-29a-3p led to the downregulation of RAG1 protein in mice with no significant alteration in the haematological parameters or liver/kidney function of treated mice (1). Interestingly, we observed a reduction in the V(D)J recombination efficiency in miR-29a overexpressing mice pro-B cells, which explains the specific regulation of RAG1 expression by this miRNA in B cells (1). In summary, these studies highlight the regulation of RAG1 by the miRNAs, 29a and 29c with the potential influence of differential expression patterns and non-seed sequences on target gene regulation (Figure 2).

Our *in-silico* approach to investigate potential miRNAs associated with RAG1 regulation identified miR-501-3p as one of the potential regulators, with two binding sites on the RAG1 3'UTR (3). Through various experimental approaches, we observed a direct interaction of miR-501-3p with the 3'UTR of RAG1 and subsequent changes in RAG1 expression upon modulation of miR-501-3p levels (5). While this

miRNA influenced V(D)J recombination in B cells, it was not regulated in a developmental stage-specific manner, revealing its limited impact in vivo (5).

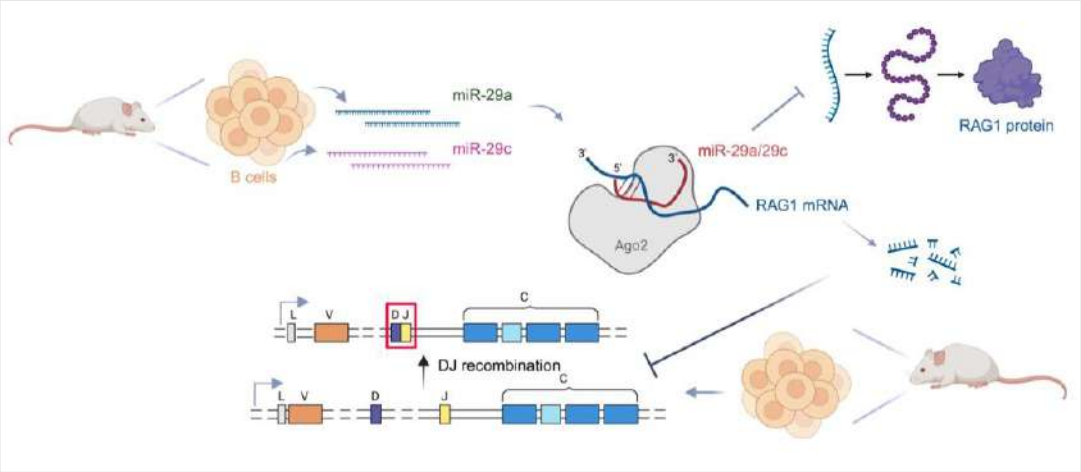

Figure 2: Schematic representation of miR-29a and miR-29c mediated regulation of RAG1 in B cells. The mature B cells in the spleen have high miR-29a-3p and miR-29c-3p expression and, consequently, low RAG1 expression because of the binding of miRNAs to the 3'UTR of RAG1 as part of the RISC complex. This leads to either mRNA degradation or translational repression of RAG1 in the splenic cells. Lack of RAG1 protein expression inhibits V(D)J recombination in mature B cell stages, ensuring B cells' activation and maturation in their respective cellular compartments. A low miR-29a and 29c expression in the developing B (pro/pre- B) cells leads to high RAG1 expression and an efficient V(D)J recombination to express the pre-BCR (pre-BCR) and generate antigen diversity.

Analysis of RNA sequencing (RNA-seq) datasets from T-cell acute lymphoblastic leukemia (T-ALL) and chronic lymphocytic leukemia (CLL) patients revealed differential regulation of RAG1 expression by miR-29a-3p, miR-29c-3p, and miR-501-3p (1, 3, 5). These findings raise the possibility of using these miRNAs as biomarkers for the early detection of B-cell lymphoma, T-cell leukemia, and CLL, warranting further clinical investigation. A key future direction will involve assessing the contribution of these regulatory mechanisms in maintaining genomic stability in lymphoid cells through RAG1 regulation, while also exploring their potential as biomarkers and therapeutic agents.

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Role of Homeodomain transcription factor Cut in the development of glial niche around neural cells in *Drosophila* central nervous system


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Understanding the development and function of complex tissues such as the central nervous system (CNS) is fascinating. We used *Drosophila*, commonly known as fruit flies, as a model organism to explore how cells within the CNS interact and communicate with one another. Glial cells in the CNS provide essential support to neurons and neural stem cells (NSCs). Like the mammalian nervous system, *Drosophila* has various types of glial cells, each serving distinct functions crucial for proper neural functioning. Morphological studies in *Drosophila* have identified three primary categories of glial cells: those associated with the surface, the cortex, and neuropiles (Figure 1A) (1,2).

Interestingly, not all cells in the *Drosophila* CNS are diploid; some glial sub-types are polyploid in nature. Currently, two types of glial cells, subperineural and cortex glia, are known to be polyploid (3-5). This report focuses on *Drosophila* cortex glia, which envelop NSCs and their progeny neurons. In the ventral nerve cord (VNC) of the Drosophila larval nervous system, these cells are primarily located on the ventral and lateral sides. They remain closely associated with neural cells and regulate their development and fate (6). We investigated how these cells develop a complex cellular network (Figure1 B) around neural cells and achieve an increase in ploidy.

An increase in ploidy is a conserved process that involves increased DNA content. Somatic increase in ploidy is a normal developmental process in many organisms, including protists, algae, angiosperms, mollusks, insects, mammals, and humans (7,8). Although under extreme conditions, polyploidy could contribute

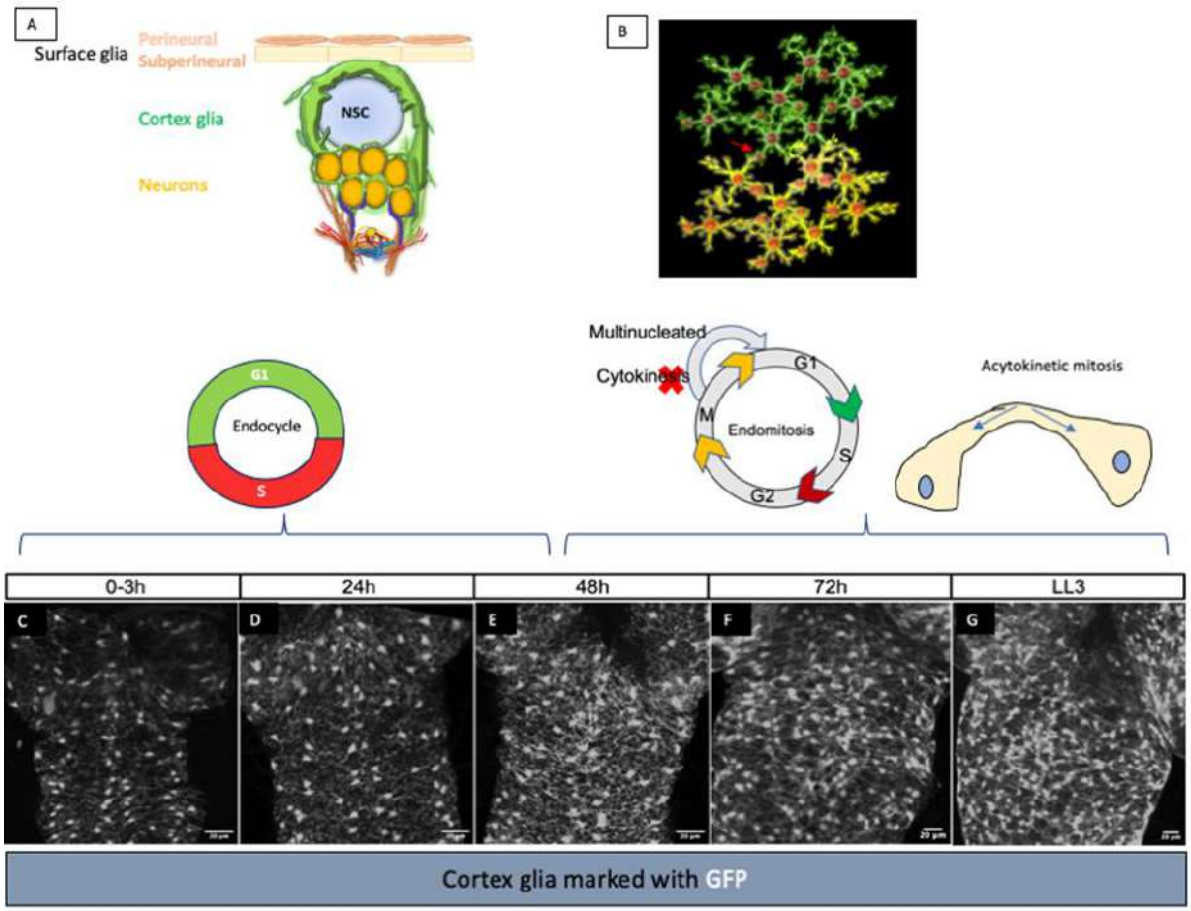

Figure 1: Cortex glia undergoes variant cell cycle A: *Drosophila* has 3 major glial subtypes: surface, cortex and neuropile glia, where cortex glia (green) is known to enwrap the NSC and their progenies B: Complex reticulate network of cortex glia where one multinucleated cortex glia (in green) fuses (red arrow) with the other cortex glia (in yellow) to form syncytium cortex glia units to exchange their cytoplasmic contents. (C-G): An increase in the number of thoracic cortex glia nuclei from 0h ALH to LL3 stage, where first cortex glia increases their DNA content upto 48h after larval life stage and after 48h stage the nuclei increase in number by undergoing endomitosis and acytokinetic mitosis.

to cancer and tumor growth, it is well known to play several beneficial roles, such as aiding in the stress response, wound healing, DNA damage resistance and repair, formation of binucleate alveolar cells in the lactating mammary gland, and development of megakaryocytes.

A cell can achieve a polyploid state via different mechanisms; a brief description is provided below (9):

1. Acytokinetic Mitosis: In this process, cells undergo normal mitosis but fail to complete cytokinesis. This mechanism has been observed in postnatal mammalian hepatocytes and in the syncytial blastoderm of *Drosophila* embryos.

2. Endomitosis Occurs when cells are arrested in metaphase or anaphase and do not complete cytokinesis. Examples include megakaryocytes, which are involved in platelet production (10).

3. Cellular Fusion: This mechanism involves the fusion of two cells to form a larger cell with multiple sets of chromosomes. This process is involved in the formation of placental syncytial trophoblasts and the differentiation of mammalian skeletal muscles.

4.Endocycle/endoreplication/endoreduplication: In this process, cells do not undergo mitosis or cytokinesis but cycle through continuous G and S phases, resulting in a giant cell with an enlarged polyploid nucleus. This mechanism is observed in various organisms, including plants, arthropods (11), and mammals (9).

Here, we emphasize *Drosophila* cortex glia, which achieve increased ploidy by undergoing a variant cell cycle. During the early larval period, the nuclei of cortex glia grew and their DNA content increased by endo-cycling (Figure 1C-E). Later, they increased in number through endomitosis and acytokinetic mitosis, without undergoing traditional cell division (Figure 1E-G) (5,12). Interestingly, these cells develop elaborate morphology owing to the continuous growth of cell extensions and cell fusion events, which enables them to share cytoplasmic material within their interconnected network (Figure 1B) (5,12). To enwrap the newly formed cells during the development of the nervous system, the cortex glia expand their cellular network and increase the number of nuclei without undergoing cell division.

Our recent study, conducted using a combination of genetic, molecular, and cellular biology approaches, demonstrated that the homeodomain transcription factor Cut plays a crucial role in regulating the morphogenesis of cortex glia in several ways. Cut is an evolutionarily and functionally conserved homeodomain transcription factor in *Drosophila*. CUX1 and CUX1/2 have been identified in humans and mice, respectively. It is expressed in several tissues and has diverse functions. In particular, it helps to determine cell specificity and identity in *Drosophila* and mammals. Although Cut plays various roles in cellular processes and gene regulation, researchers are still actively investigating the molecular mechanisms underlying the functional versatility of this protein.

In our study, we found that Cut influences the development of cortex glia at the nuclear level by controlling their ploidy and at the cellular level by regulating the formation of complex membrane networks around neural cells. The loss of Cut disrupts DNA replication, preventing cortex glia nuclei from increasing their DNA content which are ultimately eliminated from the nervous system (Figure 2 B, D). Notably, ectopic levels of Cut in these cells resulted in a multifold increase in DNA content and interfered with nuclear division (Figure 2E). Cut-deficient cortex glia exhibited stunted growth of their cellular network (Figure 2A-B), whereas high levels of Cut promoted longer main branches at the expense of finer extensions that encase individual neural cell bodies (Figure 2C). Therefore, we conclude that, in addition to regulating the ploidy of cortex glia cells, different levels of Cut are essential for the growth and complexity of their cellular networks. Our work highlights a novel function of Cut in regulating

the growth and branching of cortex glia and variant cell cycles.

We hypothesized that cortex glial cells alter their ploidy to accommodate the complex nature of CNS development. Immediate enwrapping of newly added neural cells may be necessary for their protection and communication, and to meet the metabolic demands of the developing nervous system. Standard cell division involves cell rounding, cytoskeletal reorganization, and potential disruption of cell-cell connections (13). These processes can be harmful to cells that perform essential barrier functions. Additionally, cells that have already undergone fusion may not be able to divide in the conventional way. Therefore, by utilizing alternative cell cycle mechanisms, cortical glial cells can increase their nuclear count and genomic content without compromising their cellular architecture.

Programmed increases in ploidy have several advantages; however, unregulated changes in ploidy can lead to genomic instability and diseases such as cancer. Changes in ploidy are also associated with neurodegenerative diseases including Alzheimer's disease, Down syndrome, vascular dementia, Huntington's disease, and amyotrophic lateral sclerosis (ALS) (13). Our study explored the role of Cut in regulating the growth and ploidy of cortical glia. This study provides important insights into the development and function of CNS. More significantly, this may enhance our understanding of various neurological disorders associated with changes in ploidy, and potentially lead to new solutions for these conditions.

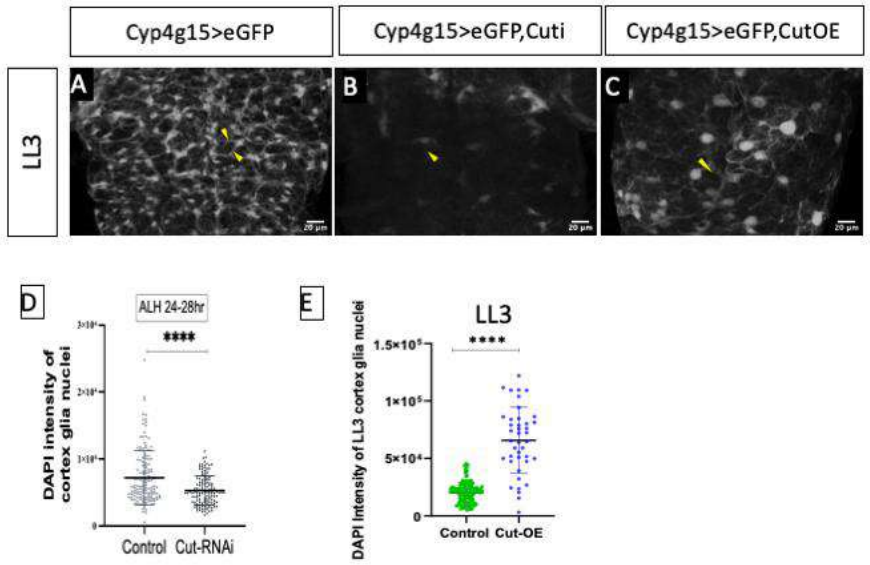

Figure 2: Cut is required for cortex glia growth and branching (A) In the tVNC cortex, glia form a honeycomb structure by extending their processes in multiple directions (yellow arrowhead), which sends out several thinner branches, creating a very fine meshwork to enwrap the individual neural cell bodies. (B) Cut defective cortex glia (Cyp4g15>eGFP, Cut-RNAi) are unable to grow the extensions and thus have one abnormal-looking cytoplasmic extension only (C) Overexpression of Cut in cortex glia enhances growth of cell body, main branches (yellow arrowhead) at the cost of

terminal extensions (D) Quantification of DAPI intensity showing that Cut defective cortex glia (Cyp4g15>eGFP, Cut-RNAi) are unable to increase their DNA content, (E) Quantification of each cortex glia nuclei showing that DNA content increased significantly in the background of Cut overexpression (Cyp4g15>eGFP, Cut-OE).

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Targeting CERS6-AS1/FGFR1 axis as synthetic vulnerability to constrain stromal cells supported proliferation in Mantle cell lymphoma


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Problem with Current Therapies in Mantle Cell Lymphoma

Mantle Cell Lymphoma (MCL) is a subtype of B-cell non-Hodgkin's lymphoma (1). The lymphoma occurs in the mantle zone of the lymph node follicle, and hence the name 'Mantle Cell Lymphoma.' MCL constitutes between 5-10% of all non-Hodgkin's B-cell lymphoma and characterised by the translocation of t(11:14)(q13;q32) and overexpression of Cyclin D1. MCL is an aggressive cancer with a poor prognosis. The most common type of MCL originates from mature B cells and is often found to become unstable and aggressive through accumulating mutations in genes related to cell cycle regulation, such as the DNA damage response pathway. The median survival rate after diagnosis is about 3 years. There are a lot of different ways to treat MCL, such as standard of care(R-CHOP), targeted therapies like Bruton's tyrosine kinase (BTK) inhibitors, and Chimeric antigen receptor T-cell therapy (2,3). But the type of treatment chosen relies on the patient's age, the stage of the disease, and any other health problems they may have. Despite of available therapies, resistance cases emerge rapidly due to various reasons, such as loss or mutation in drug targets like loss of CD19 expression or BTK gene mutation. Nearly 60% of patients presented bone marrow involvement at the time of diagnosis and causes significant morbidity due to relapse disease. The complex network and interaction between the bone marrow microenvironment and MCL cells provide specific niches for lymphoma cells survival. Therefore, discovery of substantial clinically relevant targets and new therapies are needed to eliminate the supportive niche provided by tumor microenvironment (TME), which will possibly enhance the efficacy of existing MCL treatments. Previous research has

shown that the physical contact between MCL and stromal cells has a big effect, showing that it improves survival and makes cells resistant to drugs (4,5).

Our Research Approach and Findings

In this case, we have developed a co-culture model where, we grow MCL cells from six different patients on top of a layer of mouse stromal cells (MS5) (Figure 1A). Our findings revealed a notable increase in MCL cell proliferation and a concomitant loss of response to FDA-approved BTK inhibitors, including both covalent (Ibrutinib) and non-covalent inhibitors (Pirtobrutinib, LOXO-305). We discovered heterogenous population of the tumor cells in the co-culture system, about 10% of the tumor population adhered to the stromal cells forming cobble-stone like colonies while other remains in suspension, to separate out the adherent MCL population from MS5 layer, we employed a slow trypsinization process, which allowed the detachment of MCL cells only without detaching stromal cell adhered layer. Flow cytometry analysis confirmed the purity of separated adherent MCL cells, which displayed high CD45 expression which is present on almost all hematopoietic cells but is absent on MS5 cells. We further noticed these adherent MCLs express a low level of pan B-cell marker CD19 compared to MCLs in suspension, and none of these populations were positive for hematopoietic stem cell marker CD34 or T-cell marker CD3, suggesting that our cultured patient-derived leukemic MCL had predominantly malignant B-cell population. Further analysis revealed that adherent MCL cells expressed significantly higher levels of stemness markers CD44 and Nanog, and were more resistant to BTK inhibitors compared to suspension cells.

To investigate the molecular signatures of these adherent and suspension tumor populations, we performed RNA- sequencing, and found both differentially expressed coding and non-coding RNA, in this study we have focused on long non coding RNAs (lncRNAs). LncRNAs regulates gene expression by influencing chromatin organization, alternative splicing, RNA decay, transcription, and led to tumor progression. From the last decade lncRNA like MALAT-1, ROR1-AS1, FOXP4-AS1, GATA6-AS1, MORT have been linked to MCL proliferation and tumorigenicity. While lncRNAs have demonstrated prognostic value in MCL and could serve as potential therapeutic targets, current studies have not emphasized their role in reprogramming the TME. Among the topmost differentially expressed lncRNAs we have discovered oncogenic CERS6-AS1 is upregulated in Primary MCL tumors (n=98) when compared with heathly B-cells. CERS6-AS1, derived from the CERS6 gene, is consistently upregulated in multiple cancers, including MCL, and is associated with poor clinical outcomes (Figure 1B). It exerts oncogenic effects by sponging miRNAs and interacting with RNA-binding proteins like IGF2BP3, influencing cancer cell proliferation, invasion, apoptosis, and stemness. Our analyses also revealed a significant elevation in CERS6-AS1 expression in co-cultured MCLs compared to mono-cultured ones.

To investigate the functional role of CERS6-AS1 in MCL tetracycline-inducible CERS6-AS1 knockdown clones were generated in Mino cell line using two different shRNAs, achieving approximately 80% knockdown (Figure 1C). Knockdown of CERS6-AS1 resulted in significant decrease in the expression of cancer stem cell markers Oct4, CD44, Myc, and Nanog,

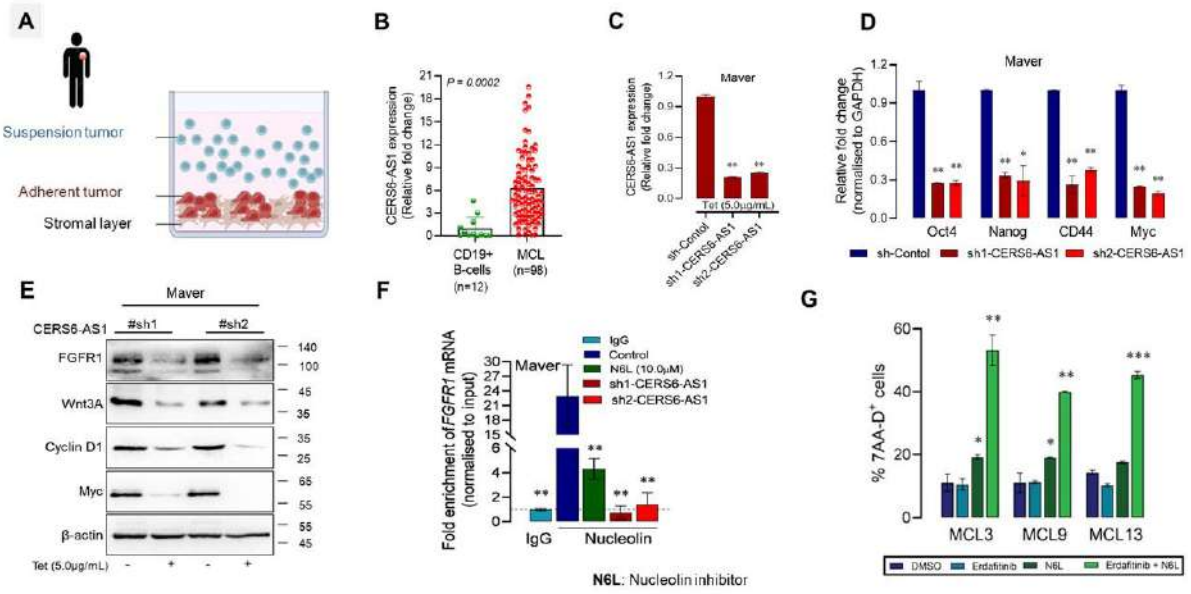

Figure 1: (A) Representative figure showing the separation of MCL-adherent and suspension cells from co-cultured cells. (B) CERS6-AS1 expression was assessed by qRT-PCR in CD19+ B-cells from healthy donors (n=12) and MCL tumor samples (n=98). Statistical analysis was performed using two tallied unpaired Student's t-test. (C)Tetracycline inducible stable knockdown of CERS6-AS1 expression in MCL cells (Mino) by qRT-PCR using two different CERS6-AS1-specific shRNAs. (D) Expression of stem cell markers (CD44, Nanog, Oct4, and Myc) were downregulated in CERS6-AS1 knockdown MCL cells assessed by qRT-PCR. (E) Western blot showing the expression of Wnt signaling proteins: FGFR1, Myc, Cyclin D1 and Wnt3A upon CERS6-AS1 knockdown in 2 MCL cell lines. (F) RIP analysis in the absence or presence of nucleolin targeting Nucant N6L or in CERS6-AS1 knockdown cells demonstrated the co-interaction of CERS6-AS1 and FGFR1 transcript with nucleolin protein. (G)Percentage of necrotic cells demonstrated by 7AA-D staining and flow cytometry in primary MCL (n=3) co-cultured with MS5 cells after N6L or erdafitinib as a single agent or combination (5.0µM each; 72 hours)

as well as decreased ALDH1+ population and increased sensitivity to BTK inhibitors, suggesting that CERS6-AS1 plays a critical role in maintaining cancer stem cell characteristics and contributes to chemotherapy resistance in MCL (Figure 1D). We investigated the oncogenic function of CERS6-AS1 and discovered that CERS6-AS1 altered the expression of FGFR1, a key driver of cancer progression and stem cell-like properties (6). We discovered elevated expression of FGFR1 in primary MCL tumors, and like CERS6-AS1, FGFR1 expression was elevated in MCL cells adhering to the stromal layer. Further investigation revealed that there is decreased expression of FGFR1 in CERS6-AS1 knockdown cells, results in decreased Wnt signalling, further reducing cancer stem cell properties (Figure 1E). These findings suggest that CERS6-AS1 regulates FGFR1 expression and contributes to FGFR1-driven oncogenic signalling in MCL, highlighting its potential as a therapeutic target.

To further understand the mechanistic processing, we performed In-silico analysis and discovered nucleolin, an RNA binding protein that with CERS6-AS1, potentially modulating FGFR1 expression and stability. To validate In-silico analysis, we performed RNA-Immunoprecipitation with Nucleolin as a bait,

validating the CERS6-AS1: nucleolin interaction, which was disrupted by the nucleolin inhibitor N6L. In a similar RIP we discovered that CERS6-AS1 knockdown significantly reduced FGFR1 expression, implicating CERS6-AS1 in stabilizing FGFR1 through nucleolin binding (Figure 1F). These findings demonstrate nucleolin's significance in regulating both CERS6-AS1 and FGFR1, making it a possible therapeutic target in MCL. Given the pivotal role of nucleolin and CERS6-AS1 in maintaining FGFR1 stability, we investigated therapeutic application of combining FGFR1 inhibitor erdafitinib with nucleolin inhibitor N6L in MCL. The combination of both the drug led to synergistically downregulation of cancer stem cell markers (CD44, Myc, Oct4, Nanog), Wnt signaling components (TCF4, Wnt3A), and the expression of FGFR1, nucleolin, and CERS6-AS1, compared to single-agent treatments. In patient-derived MCL cells co-cultured with stromal cells, erdafitinib reduced MCL cell growth, while N6L inhibited tumor cell adhesion to the stromal layer (Figure 1G). Notably, this drug combination did not alter surface expression of chemo-immunotherapy targets (CD19, CD20; target of CAR T-cell therapy and monoclonal antibody rituximab respectively), suggesting combination compatibility with existing treatment modalities for MCL.

Conclusion

We discovered a synergistic impact of combining a nucleolin-targeting agent with erdafitinib, a FGFR inhibitor. This suggests that combinatorial therapeutic strategies targeting multiple components of this axis may offer enhanced efficacy in MCL treatment. In conclusion, our preclinical study highlights the clinical relevance of targeting the nucleolin-CERS6-AS1-FGFR1 axis in MCL.

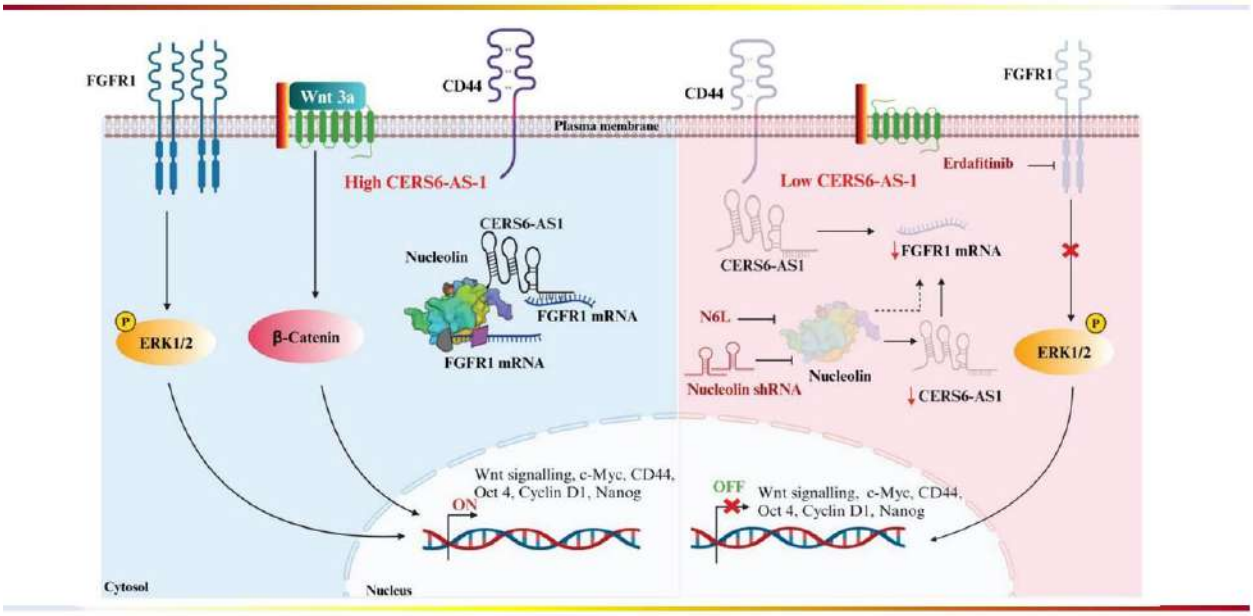

Figure 2: Illustrative representation showing the role of CERS6-AS1/FGFR1 regulating loop in Mantle Cell Lymphoma.

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Unlocking the Symphony: AMPK as the Maestro of RUNX Translational Harmony in Health and Diseases


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RUNX family of transcription factors (RUNX1, RUNX2, and RUNX3) orchestrates a wide array of biological processes, including hematopoiesis, osteogenesis, neurogenesis, and gastrointestinal development. Dysregulation of these factors can lead to serious health issues, making their regulation a critical area of study (1). Recent research from our lab sheds light on the role of AMPK (a key cellular energy sensor) in fine-tuning RUNX activity through phosphorylation (Figure 1A), offering new therapeutic avenues for metabolic disorders and cancer treatment.

Our studies demonstrated that AMPK phosphorylates RUNX2 at Ser118, a modification crucial for promoting osteogenesis while preventing adipogenesis in mesenchymal stem cells (MSCs). High glucose levels disrupt this balance by inhibiting AMPK activation, leading to RUNX2 destabilization, bone fat accumulation (adipogenesis), and reduced bone formation. Treatment with metformin, an antidiabetic drug, restored this balance by activating AMPK, reversing diabetes-induced bone adiposity, and promoting healthy bone architecture in diabetic mice (Figure. 1B) (2). These findings underscore the therapeutic potential of targeting the AMPK-RUNX2 signaling axis for managing metabolic bone disorders. Another study highlighted the impact of glutamine metabolism on RUNX2 activity, revealing that high glutamine levels suppress osteogenesis through mTORC1 activation, which inhibits the mTORC2/AKT/RUNX2 axis, while low glutamine levels enhance osteogenesis by stabilizing RUNX2. Metformin counteracts this effect by inhibiting mTORC1 hyperactivation, thereby stabilizing RUNX2 and promoting bone formation. Additionally, high glucose and glutamine levels were found to hyperactivate mTORC1, driving adipogenesis, whereas metformin rescues RUNX2 expression by inhibiting mTORC1 and activating mTORC2, highlighting its potential therapeutic role in maintaining bone health (3).

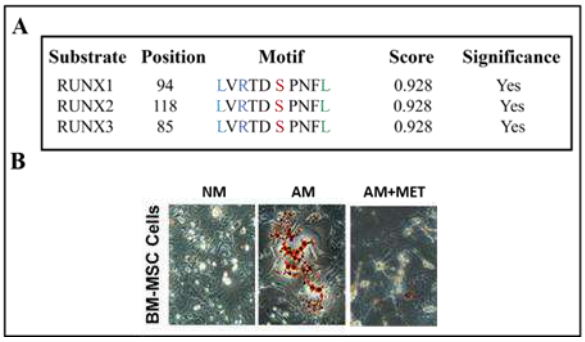

Figure 1: A. The RUNX family serves as substrates for AMPK. B. Pre-activation of AMPK with metformin for 48 hours prior to exposure to adipogenic differentiation medium significantly counteracted the effects of adipogenic inducers in MSCs, as evidenced by the reduced presence of oil droplets (dark brown) compared to the control (NM) and adipogenic medium (AM) alone. Improvised figure from Chava et. al, Cell Death and Disease (2).

In chronic myeloid leukemia (CML), metformin demonstrated efficacy against imatinib-resistant cell lines (4) and patient-derived PBMCs by inhibiting mTORC1 and HIF1-α, thereby reducing glucose uptake, lactate export, and glycolysis, which impaired cancer cell survival (5). Furthermore, metformin enhanced imatinib sensitivity in resistant cells by phosphorylating RUNX1 at Ser 94 via AMPK, suppressing the STAT3 pathway, and reducing tumor-initiating cell markers, highlighting its potential to reduce glucose uptake and lactate production in resistant CML cells and to enhance imatinib sensitivity through RUNX1 phosphorylation and STAT3 inhibition (Figure 2) (6).

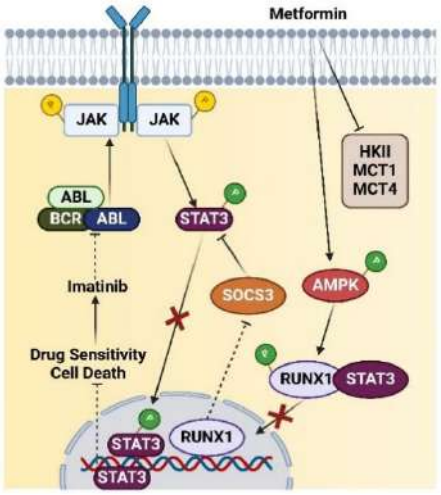

Figure 2: Diagram illustrating how metformin enhances imatinib sensitivity by suppressing STAT3 activation. Improvised figure from Gyatri et. al, Cell Death Discovery (6).

These findings position the AMPK-RUNX axis as a promising target for treating metabolic disorders, osteoporosis, and resistant cancers by demonstrating that targeting AMPK-RUNX2 signaling can prevent bone adiposity and promote osteogenesis in metabolic disorders like diabetes, while metformin's effects on glucose metabolism and transcriptional regulation underscore its potential to overcome drug resistance in leukemia and other cancers. Further research will focus on exploring

metformin's in vivo effects and potential side effects, as well as its broader implications for cancer and metabolic disorder treatments. These studies underscore the transformative potential of the AMPK-RUNX axis, offering hope for innovative therapies in health and diseas

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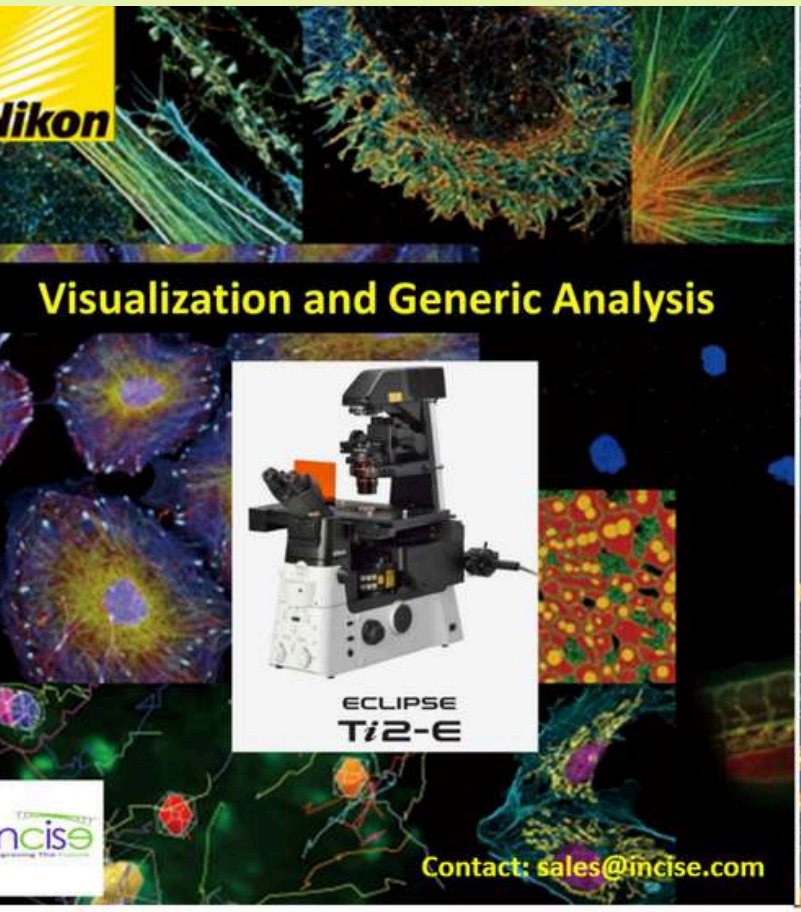

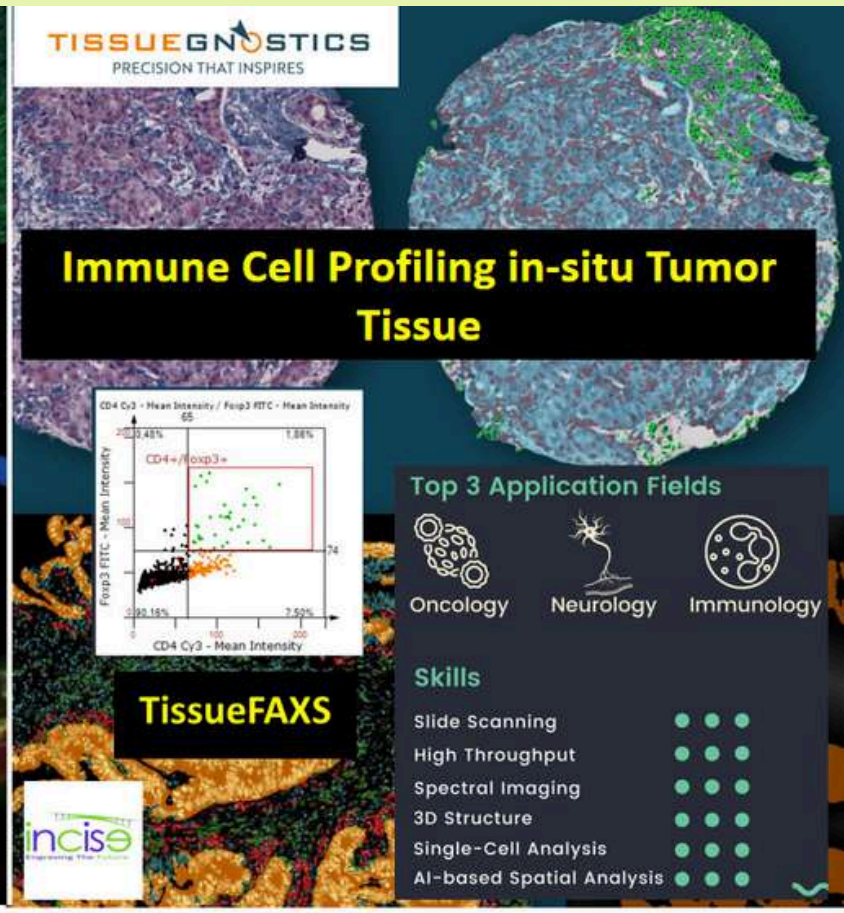

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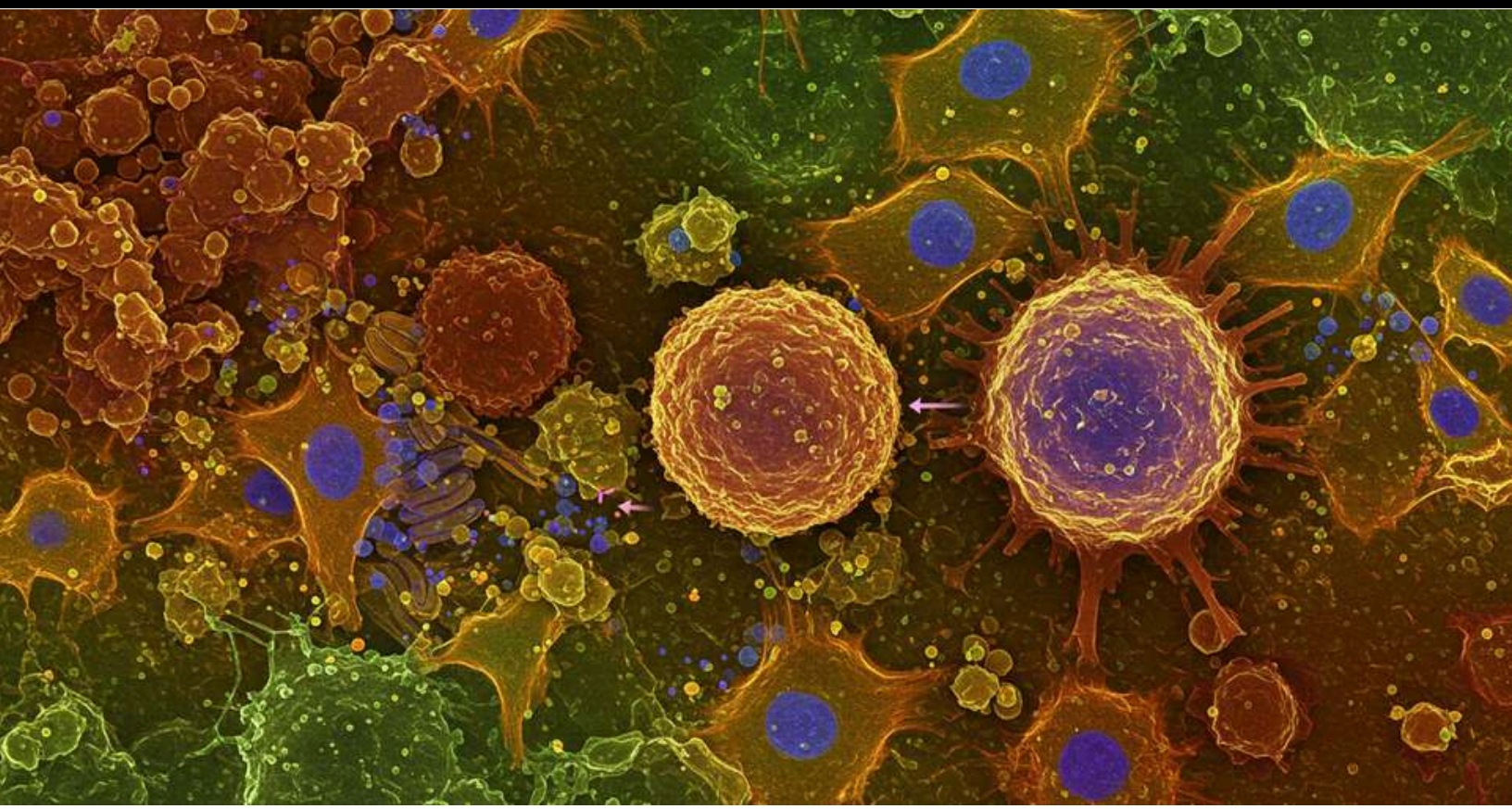
Research Article - Section 5

CELLULAR RESPONSE AND STRESS



Cells are constantly challenged by environmental and metabolic stressors, and their ability to adapt is crucial for survival and function. This section explores distinct stress response mechanisms across biological systems. Ansuman Chattopadhyay and colleagues investigate the oxidative stress induced by fluoride and arsenic in zebrafish, revealing an antagonistic interaction that modulates the Nrf2-Keap1-ARE pathway. Sujit Bhutia's team uncovers a critical survival strategy in oral cancer cells, where prolonged glutamine starvation triggers autophagic lysosome reformation (ALR), a process essential for maintaining lysosomal homeostasis and resisting cell death. Together, these studies shed light on adaptive cellular pathways with implications for toxicity, disease, and therapeutic targeting.

Antagonistic effect of fluoride and arsenic in zebrafish gut: involvement of Nrf2-Keap1-ARE pathway





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Reactive oxygen species (ROS) are by-products of aerobic metabolism, and include species such as hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), and hydroxyl radicals ($OH\cdot$). Owing to their high reactivity, these molecules interact with lipids, proteins, and DNA, causing cellular damage and leading to oxidative stress- an imperative factor linked with the pathogenesis of several diseases (1). Nuclear factor erythroid 2-related factor 2 (Nrf2) is a key transcription factor that has a central role in the cellular defense against oxidative stress. Nrf2 comprises 605 amino acids and has seven functional domains, known as Nrf2-ECH homology (NEH) domains, each responsible for specific regulatory functions (2). Together, these NEH domains allow Nrf2 to act as a master regulator of cellular defense mechanisms, particularly under oxidative stress, by modulating the transcription of a wide array of genes involved in antioxidant and detoxification pathways (3).

Under non-stressed conditions, Nrf2 is primarily sequestered in the cytosol and undergoes regulated degradation to maintain basal antioxidant response element (ARE)-dependent gene expression (4). Two main pathways namely Keap1-mediated degradation and GSK3β-mediated regulation, control Nrf2 availability for nuclear translocation. In Keap1-mediated degradation, Keap1, a cytoplasmic adaptor protein, binds to NEH2 domain of Nrf2 at two specific amino acid motifs (DLG and ETGE). This interaction targets Nrf2 for ubiquitination by the cullin-3/Rbx1 complex, leading to its degradation by the 26S proteasome (5). This pathway is disrupted during oxidative or electrophilic stress, allowing Nrf2 to accumulate in the nucleus (Figure 1).

Nrf2 possesses three nuclear localization signals (NLS1, NLS2, and NLS3) distributed along its structure, which are essential for nuclear import. These signals are recognized by importins, adaptor proteins that facilitate transport of Nrf2 across the nuclear envelope. The importin α5-importin β1 heterodimer forms a complex with Nrf2, guiding it through the nuclear pore complex into the nucleoplasm where it can initiate ARE-dependent gene transcription (6). Through this coordinated regulation, Nrf2 translocates to the nucleus and activates a suite of genes responsible for antioxidant defense, thereby maintaining cellular homeostasis under stress condition. Fluoride (F) and arsenic (As) are mostly noted toxicants coexisting in ecosystem. Individual implementation of oxidative stress in different tissues of animals are largely investigated till date but reports about their combined toxicity is scanty.

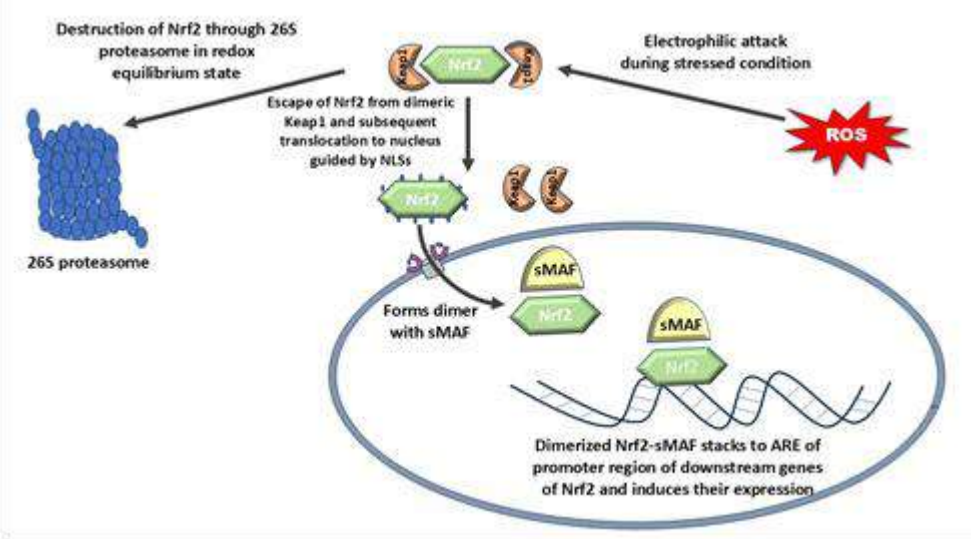

Figure 1: Graphical representation of translocation of Nrf2 inside a cell.

In our study, we examined the production of ROS in the gut tissues of zebrafish exposed to F and As, both individually and in combination. Using confocal microscopy, we observed the maximum green fluorescence signal- indicative of elevated ROS levels- in the gut tissues of zebrafish subjected only to F. This ROS signal was markedly less in tissues from the combined F and As treatment group, implying a potential interaction between F and As that impacts oxidative stress levels. To further comprehend how enteric cells react to oxidative stress, we performed an immunohistochemical analysis to observe the cellular localization of Nrf2. We quantified Nrf2 expression by performing densitometric analysis of the red fluorescence signal representing Nrf2 localization within the nucleus (Figure 2). Our findings displayed nuclear translocation of Nrf2 in all the treated groups, consistent with an activation of

antioxidant defense mechanisms in response to F and/or As exposure. Notably, gut cells from the F group exhibited the highest intensity of nuclear Nrf2 expression. Interestingly, the combined F+As treatment group showed significantly lower Nrf2 nuclear expression, associating with the decreased ROS levels observed in this group compared to F alone. These observations propose an antagonistic interaction between F and As in controlling oxidative stress induced responses in zebrafish gut tissues. The interplay between these two environmental toxins seems to modulate ROS generation and Nrf2 activation, signifying intricate underlying mechanisms that necessitate further investigation to fully elucidate the nature of this antagonistic effect (7).

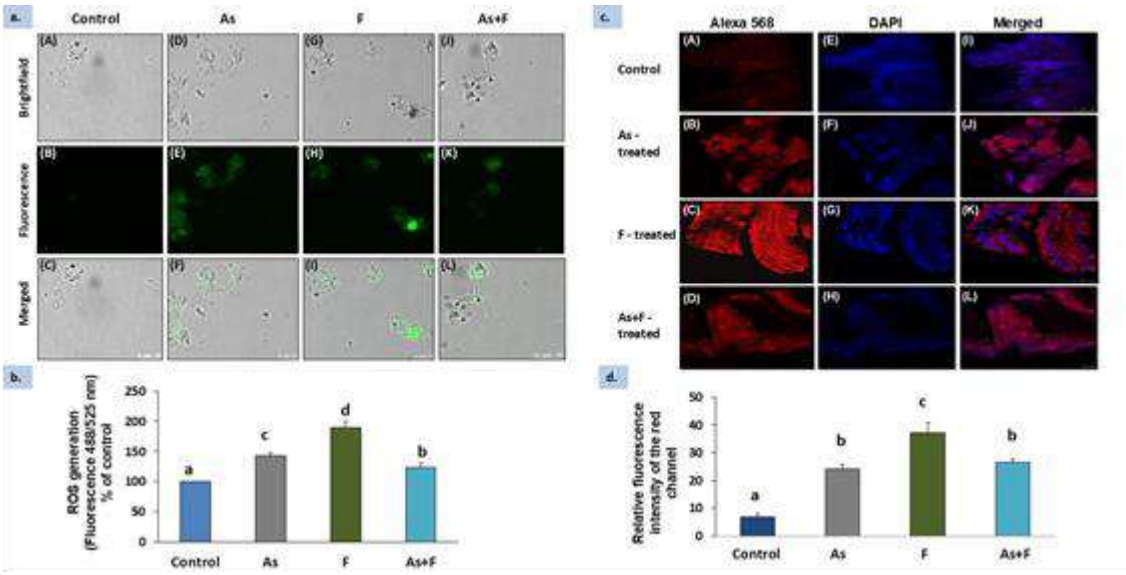

Figure 2: (a) Confocal microscopic images showing ROS generation in gut tissues of the control and treated fish after 60 days of exposure (Magnification = 630×, ZF = 1). ROS was probed with DCFDA (green). (b) is representing data of densitometric analysis of green channels of gut tissue, defining quantities of produced ROS. (c) confocal microscopic images depicting localization of Nrf2 detected by immunofluorescence study where Nrf2 was stained with an anti-Nrf2 antibody and counterstained with secondary antibody conjugated with Alexa 568 (red) (Magnification = 400×, ZF = 1). The nuclei were counterstained with DAPI (blue). In merged images portions with both red and blue dots mark the positioning of Nrf2 protein inside nucleus, (d) densitometric analysis of red channels of gut tissue, defining expression of Nrf2 protein. Values are expressed as mean ± SEM; significance level α = 0.05. Different letters used here denote significantly different means (7).

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Autophagic lysosome reformation: A new paradigm in understanding oral cancer cell survival



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Autophagy is a cytoprotective recycling mechanism triggered by the activation of AMPK and inactivation of mTOR due to cellular stress. It uses the lysosomal pathway to degrade the dysfunctional components in the cell and provide macromolecules to maintain cellular homeostasis. The efflux of these macromolecules from the autolysosome is ample to restore the nutrient pool balance and reactivate mTOR. Concurrently, constant autophagy causes lysosomal pool exhaustion, necessitating cells to maintain lysosomal homeostasis. During autophagy, bulk utilization of lysosomes occurs, therefore, maintaining lysosomal homeostasis is indispensable for cell survival. mTOR reactivation elicits the autophagic lysosome reformation (ALR) in cells by the generation of 'reformation tubules,' which undergo scission, giving rise to nascent lysosomes that later mature into active lysosomes (1). Our study revealed that glutamine deficiency induced a non-linear autophagy, and using confocal microscopy, we unraveled the generation of tubular structures from autolysosomes. The generation of these tubules undertakes major membrane alterations, which depend on mTOR reactivation, RAB7 dissociation, phosphatidyl inositol 3 phosphate (PI3P) dependent-dynamin 2, and clathrin recruitment. Our study established that long-term glutamine deficiency reactivates mTOR, recessing autophagy and commencing ALR, which develops proto-lysosomes from tubules generated from the accumulated autolysosomes to sustain lysosomal homeostasis (2). We verified that suppressing mTOR using rapamycin, a pharmacological inhibitor, decreases lysosomal tubulation. mTOR has been identified to be associated with VPS34, a membrane-trafficking lipid kinase in mammals, for mediating lysosome tubulation during

nutrient deficiency, which assists cell survival (3). Our results demonstrated significant PI3P localization on the autolysosomes and the lysosomal tubules. This suggests that mTOR-activated-VPS34 is crucial for PI3P pool formation over the autolysosome and the reformation of tubules, which recruits dynamin 2 and aids in tubule scission. Further, dynamin2-mediated scission marks the end of ALR, as it leads to the generation of nascent protolysosome, and as emphasized in our study, its suppression leads to the halting of ALR and retaining elongated lysosomal tubules in the cell. Moreover, RAB7, an essential autophagy protein necessary for promoting autophagosome maturation, is known to be related to ALR. Our findings suggest that under glutamine starvation, oral cancer cells recruit RAB7 onto the autolysosome membrane, which later dissociates for the induction of ALR, and its knockdown inhibits the formation of reformation tubules (Figure 1). Additionally, studies have shown Clathrin to be a closely involved protein in ALR regulation (4). In this line, our study showed that although Clathin knockdown at moderate levels did not hinder autophagosome formation, it halts the progression of ALR. These outcomes suggest that RAB7 and Clathrin are essential for tubule elongation under glutamine starvation as their knockdown restricts tubule initiation. We further examined the physiological relevance of ALR in cell survival and found that inhibition of critical proteins of glutamine deficiency-induced ALR enhances cell death in oral cancer cells, suggesting ALR is vital for cell sustenance during stress. Further, in compliance with this, we established that under prolonged glutamine deprivation, oral cancer cells show resilience to cell death but become susceptible when crucial ALR mediators are inhibited (5) (Figure 2).

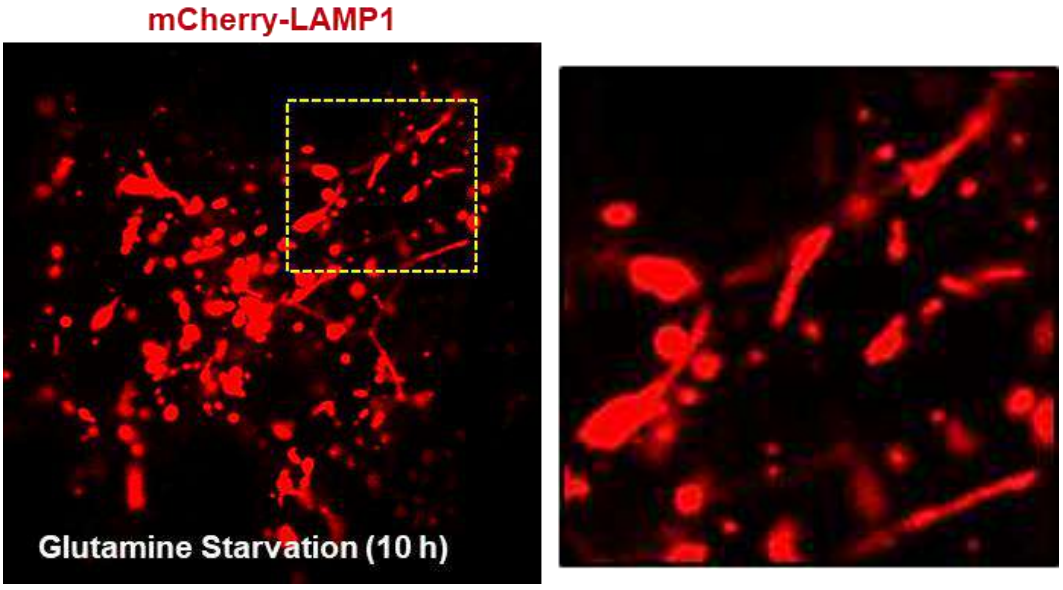

Figure 1: Representative confocal image showing tubular lysosomes in CAL33 cells at 10 h of glutamine starvation

Future impact of the study findings: With the development in the field of ALR research, there is a rapid surge in the discovery of the number of diseases associated with its dysfunctionality. These findings have motivated researchers to screen the major ALR regulatory genes to identify their role in diverse diseases, including cancer. Furthermore, as ALR reinstates lysosome homeostasis during autophagy, learning its dysregulation may open new interventions toward therapy, which will help control the dynamic functioning of lysosomes in cancer and other disorders. Moreover, understanding the structural and functional modifications in ALR regulating genes and their expression may provide new insight into potential therapy for numerous disorders. Given the association of lysosomes and cancer, analyzing more of this pathway may lead to innovative approaches for potential cancer therapeutics. However, since ALR is highly dynamic and a "time constraint" mechanism, there is a great need to identify an appropriate model and an extensive standardization process that will further complement therapy. In conclusion, our findings in oral cancer cells depict the direct association of autophagy and ALR during glutamine starvation along with the mTOR-reactivated modulation of the key ALR mediators for cell survival.

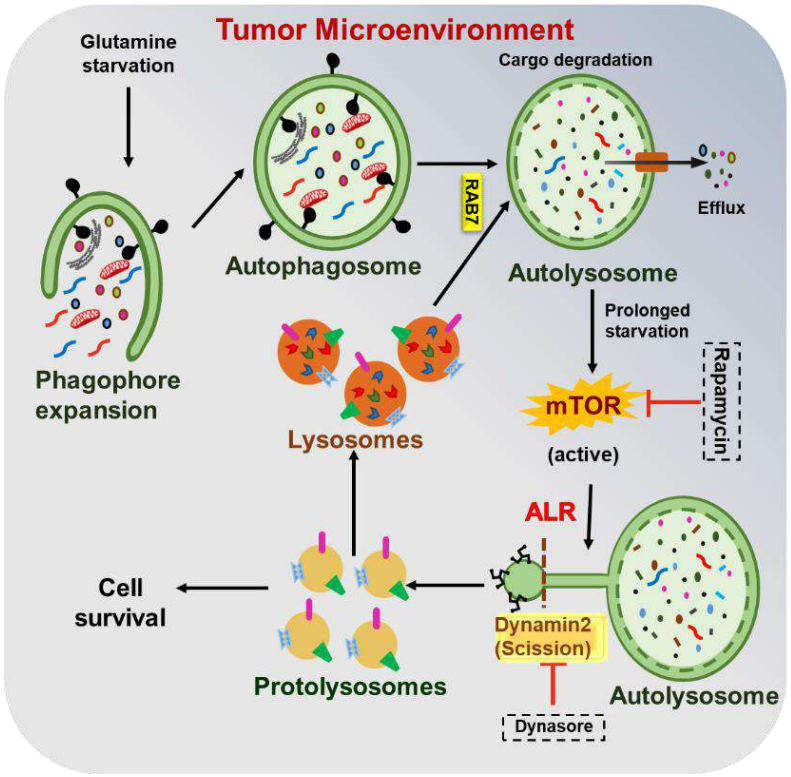

Figure 2: Review of the ALR mechanism in oral cancer cells. Glutamine deficiency in the tumor microenvironment induces autophagy through the formation of a phagophore, which sequesters cytoplasmic cargo before developing into a mature autophagosome. This autophagosome fuses with the lysosome and transforms into an autolysosome that uses lysosomal hydrolases for cargo degradation. However, prolonged starvation depletes the lysosomal pool, reactivating mTOR to

suppress autophagy and triggering the development of reformation tubules from autolysosomes to generate proto-lysosomes. The maturation of these proto-lysosomes generates active lysosomes to maintain vital processes and sustain cell survival.

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