Cell Biology Newsletter

ENDOMETRIOSIS

Table of content

Neurospora deficiencies: The long and short of it.

Durgadas P. Kasbekar

Centre for DNA Fingerprinting and Diagnostics, Hyderabad 500001

Email: kas@cdfd.org.in.

1. Short deficiencies: Gene knockouts. The mycelium of the filamentous fungus Neurospora is essentially one large multinucleate cell [1]. Individual Neurospora genes have been knocked out by transformation of strains disabled for non-homologous end joining. In such strains transformation occurs only via homologous recombination, provided the transforming DNA sequences flanking the selectable marker (eg, the *hph* hygromycin-resistance gene) share homology to sequences flanking a gene to be knocked-out (*gko*). One can then select for the replacement of *gko* by *gko::hph* on hygromycin-medium. Generally, the transformations are done in vegetative spores (conidia) that typically contain 3-10 nuclei. Thus the primary transformant is usually a heterokaryon in which only one nucleus is transformed, from which one can then isolate homokaryotic conidia that contain nuclei of the [*gko::hph*] genotype. However, if the *gko* gene is essential, then [*gko::hph*] homokaryotic conidia are not obtainable from the [(*gko::hph*) + (*gko$^+$*)] heterokaryon. In a few cases knockouts were not obtained despite repeated attempts for reasons that were not clear [2]. Could it be that some of these recalcitrant genes have nucleus-limited function, because of which nuclei bearing a null allele (*Δ*) fail to be rescued by wild type nuclei in a [*WT* + *Δ*] heterokaryon? In a twist worthy of Lewis Carroll, no nucleus-limited genes have actually been reported in the literature, but the phenotype of some fungal mutants suggests they might indeed exist [3].

2. Long deficiencies: The challenge. In 2012, I accepted the offer of the first ″Haldane Chair″ at the Centre for DNA Fingerprinting and Diagnostics. This is a prestigious position, named after the legendary geneticist J. B. S. Haldane, and correspondingly, my research had to meet dizzyingly raised expectations. I decided to explore the construction of heterokaryons in which the deleted nucleus was deficient for a chromosome segment bearing hundreds of genes. Such heterokaryons are easy to visualize in one′s mind′s eye, but they were never previously made in any system. By constructing heterokaryons in which in one nuclear type has a deficiency for a

large chromosome segment one can ask whether that segment includes any nucleus-limited genes.

In *Neurospora crassa* deficiencies of large chromosome segments (*Df*) can be obtained in the progeny of *T* x *N* crosses, where "*T* " is a strain with an insertional translocation, and "*N*" a strain of normal sequence [4]. Insertional translocations transfer a donor chromosome segment into a recipient chromosome without any reciprocal exchange. In *T* x *N* crosses, alternate segregation (ALT) generates asci with eight parental type ascospores (4 *T* + 4 *N*), whereas adjacent-1 segregation (ADJ) produces four viable ascospores with a duplication and four inviable ones with the complementary deficiency (4 *Dp* + 4 *Df*) (Figure 1). Viable ascospores become pigmented (black, B) whereas inviable ones remain unpigmented (white, W). Since ALT and ADJ are equally likely, the two ascus types are made in equal numbers (i.e., 8B:0W = 4B:4W), as are the three viable progeny types (*T* = *N* = *Dp*). The challenge was to construct heterokaryons with [*N* + *Df*] or [*T* + *Df*] or [*Dp* + *Df*] genotypes.
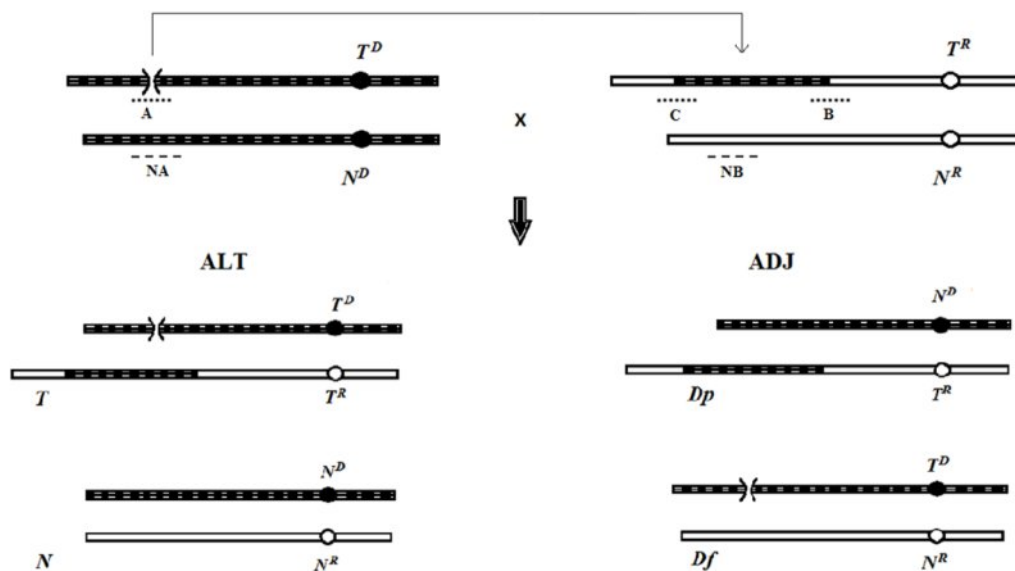
Figure 1. Alternate (ALT) and adjacent-1 (ADJ) segregation in a *T* x *N* cross. T^D and T^R are the donor and recipient chromosomes of the *T* and N^D and N^R are their *N*-derived homologues. Dotted lines indicate the A, B, and C breakpoint junctions, and dashed lines the NA and NB segments in the normal sequence homologues disrupted by the translocation. In ALT, T^D and T^R segregate to one spindle pole, and N^D and N^R to the other. Subsequently, meiosis II and post-meiotic mitosis generate eight parental-type nuclei, viz. 4 *T* + 4 *N*. In ADJ, N^D and T^R segregate to one pole and T^D and N^R to the other, to eventually produce eight non-parental nuclei, 4 *Dp* + 4 *Df*. The *T*, *N*, and *Dp* types are viable, whereas the *Df* type is inviable. *T* progeny contain A, B, and C, *Dp* contain B and C but not A, and *N* contain none.

3. Enter *N. tetrasperma*! I had done my Ph. D. under the supervision of Eugene Katz, and Katz had done his Ph. D. under the supervision of Sydney Brenner. Brenner championed the nematode *Caenorhabditis elegans*, a self-fertilizing hermaphrodite, as a genetic system. My academic lineage prepared me well enough to see that *N. tetrasperma*, a less well studied Neurospora species, shares certain genetic characteristics with *C. elegans* and those are useful to construct [*Dp* + *Df*] strains.

In a Neurospora sexual cross, the haploid parental nuclei of *mat A* and *mat a* mating type fuse to produce a diploid zygote nucleus that generates eight haploid progeny nuclei (4 *mat A* + 4 *mat a*) following meiosis and a post-meiotic mitosis. In *N. crassa* the progeny nuclei are partitioned into the eight initially uninucleate ascospores, whereas in *N. tetrasperma* they go into four initially binucleate ascospores, each ascospore receiving a non-sister pair (1 *mat A* + 1 *mat a*). Thus, *N. crassa* produces only homokaryotic ascospores that upon germination generate self-sterile mycelia, of *mat A* or *mat a* mating type, which can mate with opposite mating type mycelium derived from another ascospore, whereas *N. tetrasperma* produces dikaryotic [*mat A* + *mat a*] ascospores that upon germination generate mycelia that can undergo a self-cross. A subset of conidia (vegetative spores) from the dikaryotic *N. tetrasperma* mycelia can be homokaryotic by chance. Also, occasionally a pair of smaller homokaryotic ascospores (1 *mat A* + 1 *mat a*) can replace a dikaryotic ascospore, and the dominant *Eight-spore* (*E*) mutant increases the frequency of such replacement, and can produce asci with upto eight homokaryotic ascospores, as in *N. crassa*. Although mycelia from homokaryotic ascospores and conidia are self-sterile, they can out-cross with like mycelia of the opposite mating type.

It follows that ALT and ADJ segregation in a *N. tetrasperma T* x *N* cross should produce asci with, respectively, four viable [*T* + *N*] or four viable [*Dp* + *Df*] heterokaryotic ascospores. Self-cross of either heterokaryon type should again generate [*T* + *N*] and [*Dp* + *Df*] progeny. The [*T* + *N*] heterokaryon can produce homokaryotic conidial derivatives of either mating type, whereas [*Dp* + *Df*] produces homokaryotic conidia of only the mating type of the *Dp* nucleus, since *Df* homokaryons are inviable. Both heterokaryons contain the same genes therefore any phenotypic difference between them would flag the possibility that the *Df* nucleus is deleted for a nucleus-limited gene.

3

There was, however, one problem. No insertional translocations were available in *N. tetrasperma*. The fact that both ALT and ADJ generate 4B:0W asci, makes it difficult to detect translocations in this species.

4. Heaving translocations across species boundaries. Several insertional translocations were identified in *N. crassa* based on the fact that *T* x *N* crosses show 8B:0W = 4B:4W [4]. I decided to introgress the *N. crassa T*s into *N. tetrasperma*. Introgression is the transfer of genes and genome segments from one species into another by hybridization and recurrent backcrosses. Fortunately, my laboratory was uniquely placed to do this. Insertional translocations are defined by three breakpoint junctions, ″A″ on the donor chromosome and ″B″ and ″C″ (proximal and distal) on the recipient chromosome, and the breakpoint junctions of several *N. crassa T*s were determined by my Ph. D. student Parmit Singh. This made it possible for another Ph. D. student, Dev Ashish Giri, to use PCR with breakpoint junction-specific primers to identify the *T* progeny from one backcross in order to set up the next backcross. Giri recurrently backcrossed the *N. tetrasperma* 85 strain (*N* type) with successive generations of *T* progeny until he obtained self-fertile [*T* + *N*] progeny, whose self-cross yielded both [*T* + *N*] and [*Dp* + *Df*] heterokaryons [5]. To the best of my knowledge this was the first systematic transfer of chromosome rearrangements from one species to another.

5. More name-dropping: Bateson, Dobzhansky, Muller, Metzenberg, … Homokaryotic derivatives obtained from the[*T* + *N*] heterokaryons containing the translocation were designated as T^{Nt}, because their genome was nominally from *N. tetrasperma* except at the *N. crassa*-derived translocation breakpoint junctions, but, in fact, ~ 8-16 % of their genome was *N. crassa*-derived. Therefore it was conceivable that the phenotype of the T^{Nt} strains might be affected by Bateson-Dobzhansky-Muller incompatibility (BDMI) of residual *N. crassa* genes in the *N. tetrasperma* background. When two lineages (e.g. *N. crassa* and *N. tetrasperma*) derived from a common ancestor become reproductively isolated, genes that undergo mutation and adaptive evolution in one lineage can become dysfunctional when transferred into the other, since other genes have undergone mutation and evolution in the second lineage, and the derived alleles were never ″tested″ together prior to hybrid formation. BDMI between the derived alleles can make the hybrid lethal, sterile, or display some other detriment [6]. Another possibility was that a misalignment between *N. crassa*- and *N. tetrasperma*-derived homologous genome segments in

meiosis could trigger meiotic silencing by unpaired DNA (MSUD), an RNAi-mediated gene silencing process discovered by Robert Metzenberg and colleagues [7]. Misalignment induces transcription of 'aberrant RNA', which is made double-stranded, and then processed into single-stranded MSUD-associated small interfering RNA (masiRNA) used by a silencing complex to identify and degrade complementary mRNA.

We found that crosses of the T^{Nt} strains with wild-type *N. tetrasperma* showed two novel ascus dysgenesis phenotypes [8]. One was the production of heterokaryotic ascospores in eight-spored asci. Ascospores in *N. crassa* and *N. tetrasperma* 8B:0W asci delimit one each of the eight nuclei generated following the post-meiotic mitosis. However, in crosses of the T^{Nt} strains with strain 85 we found that 8B:0W asci also included heterokaryotic ascospores, which suggested that ascospore delimitation and the post-meiotic mitosis are not as strictly coupled in these crosses. The second ascus dysgenesis phenotype was a transmission ratio distortion (TRD) that apparently disfavored the homokaryotic ascospores formed following ALT segregation (i.e., *Dp* $\gg N$ and T^{Nt}). The replacement of heterokaryotic ascospores by homokaryotic pairs increases viable ascospore numbers only in the post-ALT asci, therefore we hypothesized that BDMI or MSUD triggered by a *N. crassa*-derived gene results in an insufficiency for an ascospore maturation factor and this creates a "tragedy of the commons" that jeopardizes ascospore maturation in asci with >4 viable ascospores. In ongoing research we are testing whether these latter phenotypes are caused by MSUD.

6. Summary. We have introgressed insertional translocations from one Neurospora species into another, and made heterokaryons containing complementary duplications and deficiencies in their constituent nuclei. Additionally, we found that ascospore delimitation can be uncoupled from the post-meiotic mitosis, and discovered a novel type of transmission ratio distortion.

Literature cited.

[1]   Griffiths, AJS. 2013 Neurospora: the organism, its genes, and its genome. In : Neurospora: Genomics and Molecular Biology. Edited by D. P. Kasbekar and K. McCluskey, Caister Academic Press, Norfolk, UK. Pages 1-8.

[2]   Colot HV, Park G, Turner GE, Ringelberg C, Crew CM, Litvinkova L, Weiss RL, Borkovich KA, and Dunlap JC. 2006 A high-throughput gene knockout procedure for Neurospora reveals functions for multiple transcription factors. Proc Natl Acad Sci U S A. 103: 10352-10357.

[3]   Kasbekar, DP. 2014 Are any fungal genes nucleus-limited? J. Biosci. 39: 341–346.

[4]   Perkins, DD. 1997 Chromosome rearrangements in Neurospora and other filamentous fungi. Adv. Genet. 36: 239−398.

 [5]   Giri, DA, Rekha, S, and Kasbekar, DP. 2015 Neurospora heterokaryons with complementary duplications and deficiencies in their constituent nuclei provide an approach to identify nucleus-limited genes. G3 5: 1263-1272.

[6]   Coyne, JA 2016 Theodosius Dobzhansky on hybrid sterility and speciation. Genetics 202: 5-7.

[7]   Shiu, PKT, Raju, NB, Zickler, D, and Metzenberg, RL. 2001 Meiotic silencing by unpaired DNA. Cell 107: 905−916.

[8]   Giri, DA, Rekha, S, and Kasbekar, DP. 2016 Crosses heterozygous for hybrid Neurospora translocation strains show transmission ratio distortion disfavoring homokaryotic ascospores made following alternate segregation. G3 6: 2593-2600.

Proteases in inflammation and cancers

Prof. Snehasikta Swarnakar

CSIR-Indian Institute of Chemical Biology, Kolkata-700032

Email: sneha

Proteases are critical player in cellular homeostasis and systemic circuitry. Most of them are involved in cellular differentiation, proliferation, apoptosis and angiogenesis. The specific class of protease namely matrix metalloprotease (MMP)s are important regulator in inflammation and tumor growth both at primary and remote site. They are a family of different subgroups of zinc-dependent endopeptidases (**Table 1**) that degrade most of the components of extra cellular matrix (ECM) as well as few non-ECM components.

Table 1. An overview of MMP family and it subtypes.

Sub-groups	Collagenases	Gelatinases	Stromelysins	Matrilysins	Membrane-type MMPs	Other MMPs
MMPs	MMP-1, -8, -13	MMP-2, -9	MMP-3, -10, -11	MMP-7, -26	MMP-14, -15, -16, -17, -24, -25	MMP-12, -19, -20, -23, -26, -28
Substrate	Collagen I, II, III, VII, VIII, X and gelatin	Collagen IV, V, VII, X, XI, XIV and gelatin	Collagen II, IV, IX, X, gelatin, α-casein, and β-casein	Collagen I, II, III, IV, V, X and casein	Gelatin, fibronectin, laminin, fibrinogen and fibrin	Collagen I, IV, elastin and gelatin

Wound formation and its healing are dynamic processes of ECM remodeling that are mainly influenced by MMPs and tissue inhibitors of metalloproteinases (TIMPs). Exploring microRNA profiling and gene-gene interaction is other major interest in understanding the disease manifestation. Although, molecular changes due to different environmental cues is another important issues. The goal is to identify novel molecular target and development of target based medicine to control disease development. Dr Swarnakar's laboratory has been engaged in working on the role of MMPs in gastric injury, ovarian cancer and endometriosis that become a new paradigm shift in the subject (**Fig 1**). The remarkable feature of her research is that it ranges from the basic to applied sciences. Her extensive research in understanding the association of MMPs in gastric disorder and endometriosis in human is a seminal contribution in the field of

inflammation biology. Role of MMPs in other diseases that includes alcohol induced liver and lung injury, hepatic carcinoma, neuronal disorder and periodontitis is under investigation.
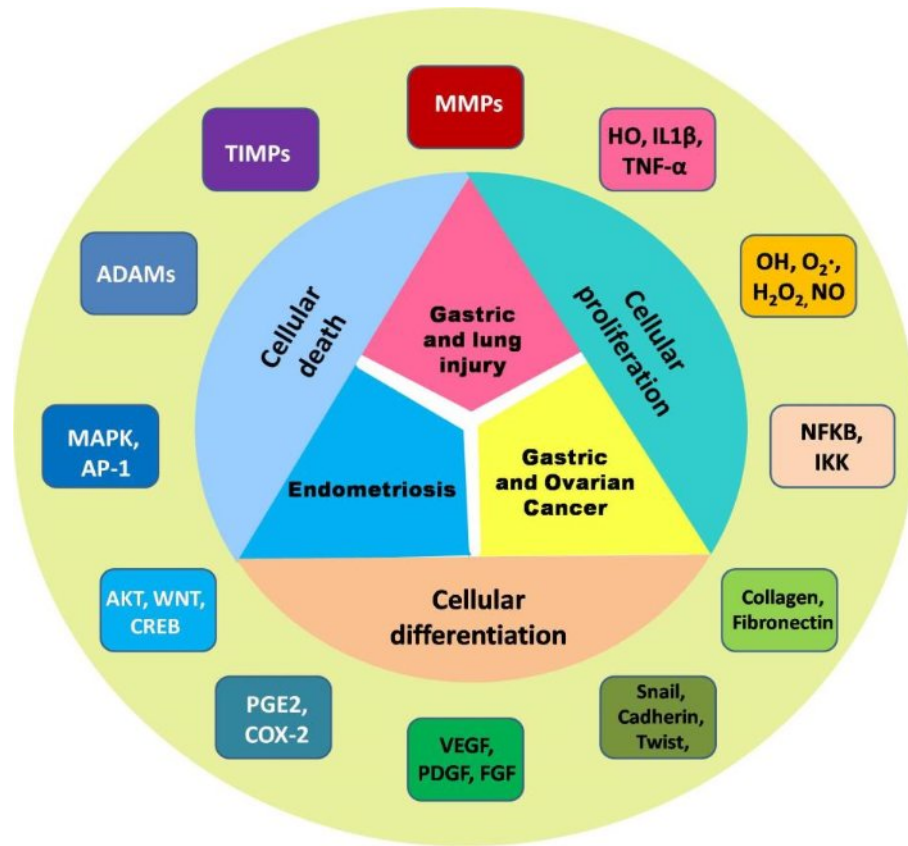
Figure 1. Cellular and molecular responses in different diseases.

MMPs and gastro-intestinal inflammation

Inflammation is a complex series of cell/ matrix interaction involving cellular proliferation, migration, and differentiation. The evidence for MMP9 as major player in gastric ulcer comes from animal and cell culture studies in our laboratory. Gastric ulcer model using non-steroidal anti-inflammatory drugs (NSAIDs), ethanol, physical stress and infection by *H. pylori* has been developed. Her research highlighted the importance of MMP9 and 3 in gastric ulceration involving mucosal inflammation which are important factors in facilitating lymphocyte infiltration in gastric tissues. Our studies indicated that ulcerated gastric mucosal tissue of human exhibit significant increase of MMP9 activity and moderate reduction of MMP2 (pro and active) activity than that of normal tissue. While that MMP2 participate in the physiological turnover of

the gastric ECM, MMP9 play key role in the early inflammatory phase of gastric ulcers. Thus, MMP9 and/or 3 inhibitors could be used for therapeutic application to control gastric inflammation. Moreover, MMP9 inactivation by TIMP1 is alternative regulatory mechanism via protease antiprotease balancing to arrest gastric inflammation. Dr. Swarnakar's lab demonstrated that MMP3 and 9 activities augmented in NSAID-induced and ethanol-induced gastric inflammation. She for the first time established MMP-dependent pathway for cag^{+ve} and cag^{-ve} H. Pylori infection and identified MMP9 dependent pathway as an alternative mechanism for cag^{-ve} Hp infection in gastric ulceration. Her group made pivotal contribution in highlighting gastro-protective roles of antioxidants via regulation of MMPs. She has elucidated that curcumin (an active constituent of turmeric) inhibits MMP9 while preventing *H. pylori* infected and NSAID-induced gastric ulcer. Eradication of *H. pylori* infection and remodeling of gastric tissues by curcumin through modulation of MMP9 and 3 was reported by her group [1,2]. She demonstrated the anti-ulcer activity of curcumin through dual mechanisms: (1) inhibition of MMP9 mediated inflammatory responses and (2) activation of MMP2 to promote angiogenesis during gastric ulcer healing. Her work demonstrated that famotidine, nanocapsulated quercetin, melatonin (N-Acetyl-5-hydroxy tryptamine), but not omeprazole arrest gastric ulcer via MMP9 mediated pathway [3,4]. She also found that tight binding of melatonin in the active site might be involved in reducing the catalytic activity of MMP9 [5]. This finding could provide a novel approach to physical docking of biomolecules to the catalytic site of MMPs. She established that diabetic rats are more susceptible to gastric damage compared to non-diabetic ones due to excess MMP13 production leading to excess gastric damage. She documented that melatonin arrests diabetic gastric ulcer by down regulation of MMP13 and halts the progression of the disease [6]. Furthermore, ethanol induced liver injury is associated to MMP9 and NFkB upregulation that inhibited significantly by melatonin [7].

Role of MMPs in gastric and ovarian cancer

Since persistent gastric ulceration and inflammation leads to gastric cancer, hence, genomic approaches have been initiated to address the issues of cancer progression. Her group looked into whether presence of single nucleotide polymorphisms (SNP) at the promoter sites of different MMPs influences gastric cancer development. Dr. Swarnakar reported the link between promoter polymorphism of MMP1, 3, 9, 7 and risk of gastric cancer in eastern Indian population. Functional polymorphism of MMP1 -422T/A was found to be linked with lower stomach tumor

in eastern Indian population. Furthermore, MMP1 2519 A/G polymorphism displayed poor cellular differentiation attributing a higher risk of cancer progression [8]. Her group showed that MMP3 -707 G/G and -1612 5A/6A polymorphisms are potential predictor of gastric cancer risk [9]. Her group also found that MMP9 -1562 C/T polymorphisms are potential predictor of gastric cancer risk [10]. The SNP at position -1562C/T of MMP9 may results in the modulation for binding of transcription factors to the MMP9 gene promoter and thereby causes differences in protein expression and enzymatic activity. Knowledge on mechanisms of MMP9 up regulation during gastric cancer may provide new paradigm in diagnostics and therapeutics. She recently documented the association of MMP7 –181 A/G promoter polymorphism and gastric cancer risk while nicotine enhances cAMP-response element-binding protein (CREB) phosphorylation that effectively binds to MMP7 promoter with G allele carrier thus show additive effect over polymorphism in the cancer susceptibility [11]. The meta-analysis study revealed that tobacco enhanced the risk for cancer more markedly in AG and GG carriers. Her group found greater transcriptional activity towards A to G transition under basal/nicotine-induced/ (CREB) overexpressed conditions in gastric adenocarcinoma cells [11]. Both MMP7 and 9 transcriptional regulation during gastric cancer development remains poorly known. Her laboratory has also developed chemical induced gastric cancer models, which are used to identify specific MMPs that are responsible for increased invasiveness and metastatic responses in murine model. Moreover, small molecules and dietary antioxidants are being tested in the murine cancer models to understand their protective efficacy against tumor development and metastatic responses [12]. A hospital-based study was done in east Indian population of epithelial ovarian cancer (EOC) patients that indicate significant changes in serum MMP7 activity in pre and post treated patients thus indicating serum MMP7 as a prognostic marker for survival and recurrence of EOC.

Role of MMPs in Progression of Endometriosis

For last one decade, Dr. Swarnakar's lab has illuminated the in depth mechanism of endometriosis in the context of regulation of MMP activity (**Fig 2**). Endometriosis is a gynecological disease, where endometrium-like structure develops outside the uterus. It is an inflammatory and progressive disease that affects 10-15% of reproductive women. Her group identified the roles of MMP9 and MMP3 in endometriosis and found that the ratio of MMP9 vs TIMP1 could be a potential prognostic marker to evaluate the progression and severity of

endometriosis. Furthermore, studies from her laboratory have revealed the importance of MMP3 on apoptotic pathway in endometriosis [13]. Oxidative stress acted as the initial trigger for c-fos expression leading to MMP-3 upregulation during the onset of endometriosis, which further influences more inflammation through MMP9 pathway.
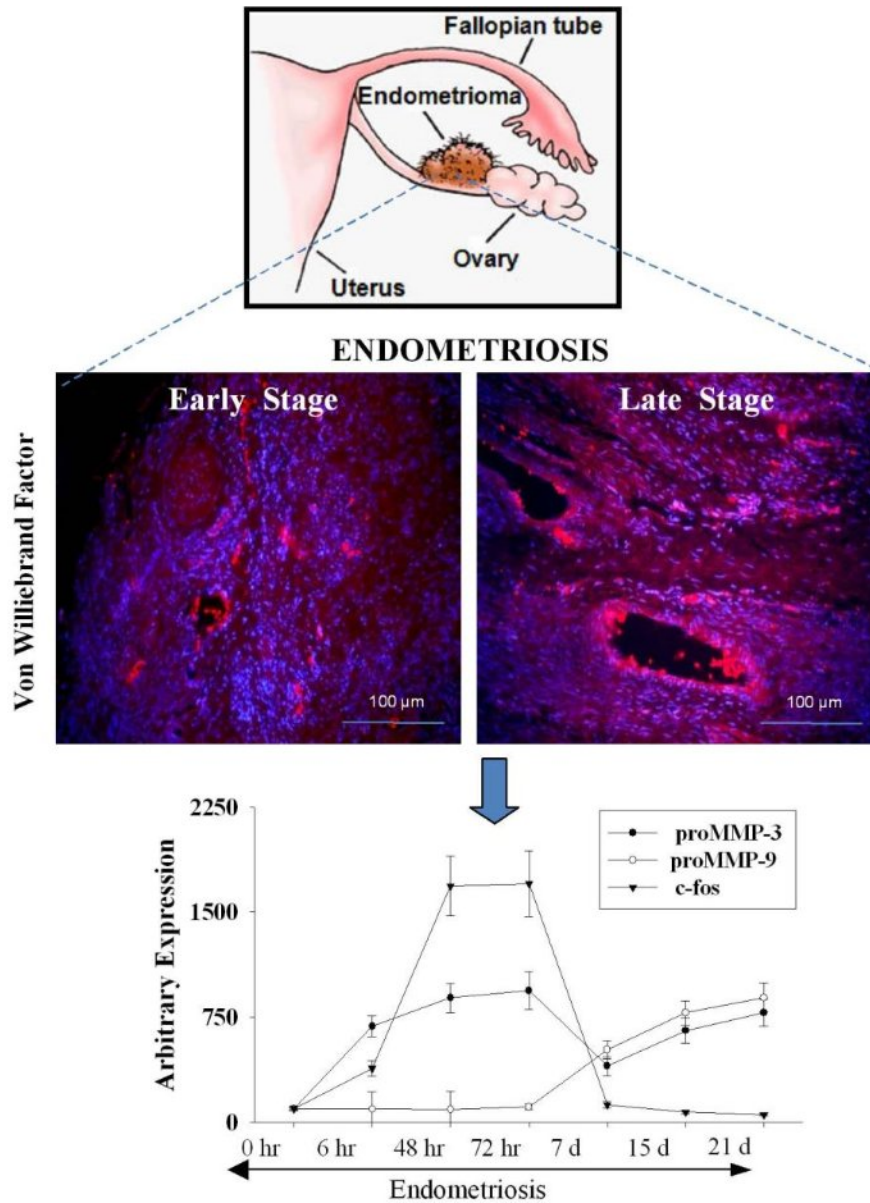
Figure 2. *Mechanism for pathogenesis of endometriosis*. Endometriosis develops outside uterus with typical histological features. Figure 2 shows Von Willebrand factor staining for angiogenesis makers in early and lates stages of endometriosis. Expression profiles of different MMPs during progression of endometriosis.

11

Dr. Swarnakar's group identified the importance of an intrinsic anti-oxidant, melatonin in arresting peritoneal endometriosis in mice model via down regulation of MMP9 and MMP3 activities. In addition, melatonin triggered apoptosis while regressing endometriosis through caspase3 mediated pathways. Moreover, her group identified the novel role of curcumin as a potent anti endometriotic compound by inhibiting MMP3 expression. Curcumin accelerated apoptosis in endometriomas predominantly via cytochrome-c mediated mitochondrial pathway [13]. Her group also showed the inhibitory action of curcumin on MMP2 and angiogenesis during the early phage of endometriosis development. Recent studies from her group established the importance of MMP2 activity via COX-2-PGE2-pAKT axis in promoting angiogenesis during endometriosis progression [14].

Studying with the human ectopic ovarian endometrioma it has been found that elevated MMP2 activation mediate through increased MT1MMP and decreased TIMP2 expressions. *In vitro* study using human endothelial cells showed that prostaglandin E2 (PGE2) significantly increased MMP-2 activity as well as angiogenesis. Inhibition of COX2 and/or phosphorylated AKT suppressed MMP2 activity reducing angiogenesis. Her recent interests are focused on different cellular events that transforming ovarian endometriosis into cancerous condition and how genetic polymorphisms of MMPs can influence the propensity of endometriosis development on Indian population.

References

1. Kundu, P., De R., Pal, I, Saha, D., Mukhopadhyay, K A., and Swarnakar, S*. (2011) *Plos One*. 6(1):e16306. Curcumin alleviates Matrix metalloproteinase-3 and -9 activities during eradication of Helicobacter pylori infection in cultured cells and mice.

2. Sharma, A V., Ganguly, K., Paul, S., Maulik, N. and Swarnakar, S*. (2012) *Anti Redox Signal* 16(4):351-62. Curcumin heals indomethacin-induced gastric ulceration by stimulation of angiogenesis and restitution of collagen fibres via VEGF and MMP-2 mediated signaling.

3. Ganguly, K., Swarnakar, S*. (2012). *Biochimie.* 94(12):2687-98. Chronic gastric ulceration causes matrix metalloproteinases-9 and -3 augmentation: alleviation by melatonin.

4. Chakrobarty, S., Stalin, S., Das, N., Thakur Choudhury, S., Ghosh, S. and Swarnakar, S*. (2012) *Biomaterials,* 33(10): 2991-3001. The use of nano-quercetin to arrest mitochondrial damage and MMP-9 upregulation during prevention of gastric inflammation induced by ethanol in rat.

5. Rudra DS, Pal U, Maiti NC, Reiter RJ, Swarnakar S*. (2013) *J Pineal Res*. 54: 398-405. Melatonin inhibits matrix metalloproteinase-9 activity by binding to its active site.

6. Singh, L. P., Sharma, A. V and Swarnakar, S*. (2011) *J. Pineal Res*. 51: 61-74 Upregulation of Collagenase –1 and –3 in indomethacin-induced gastric ulcer in diabetic rat: role of melatonin.

7. Mishra, A., Paul, S. and Swarnakar, S*. (2011) *Biochimie,* 93: 854-66. Downregulation of matrix metalloproteinase-9 by melatonin during prevention of alcohol-induced liver injury in mice.

8. Dey S, Ghosh N, Saha D, Kesh K, Gupta A and Swarnakar S*. (2014) *PLoS One.* 5;9(2):e88040. Matrix Metalloproteinase 1 (MMP1) Transcriptional Polymorphisms are Well Linked with Lower Stomach Tumor Formation in Eastern Indian Population.

9. Dey S, Stalin S, Gupta A, Saha D, Kesh K, Swarnakar S* (2012) *Mol Carcinog* 51 Suppl 1, E42-53 doi: 10.1002/mc.21837. Matrix metalloproteinase-3 gene polymorphisms and their haplotypes are associated with gastric cancer risk in eastern Indian population.

10. Verma, S., Kesh, K., Gupta, A.and Swarnakar, S. (2015) **Asian Pac J Cancer Prev**.16 (17):7393-400. An Overview of Matrix Metalloproteinase 9 Polymorphism and Gastric Cancer Risk.

11. Kesh K, Subramanian L, Ghosh N, Gupta V, Gupta A, Bhattacharya S, Mahapatra NR, Swarnakar S*. (2015) **J Biol Chem,** 290(23):14391-406. Association of MMP7 -181A→G Promoter Polymorphism with Gastric Cancer Risk: influence of nicotine in differential allele-specific transcription via increased phosphorylation of camp-response element-binding protein (CR12]

12. Verma S, Kesh K, Ganguly N, Jana S, Swarnakar S. (2014) *World J Biol Chem*. 26;5(3):355-76. doi: 10.4331/wjbc.v5.i3.355. Review. Matrix metalloproteinases and gastrointestinal cancers: Impacts of dietary antioxidants.

13. Jana, S., Paul, S. and Swarnakar, S* (2012) *Biochem Pharmacol*. 83(6):797-804. Curcumin as anti-endometriotic agent: Implication of MMP-3 and intrinsic apoptotic pathway.

14. Jana, S. Chatterjee, K, Ray AK, Mahapatra, PD and Swarnakar, S* (2016) *PLOS One* 2016 Oct 3;11(10):e0163540. doi: 10.1371/journal.pone.0163540 Regulation of matrix metalloproteinase 2 activity by COX-2/ PGE2/ pAKT axis promotes angiogenesis in endometriosis.

Transplantation of Bone Marrow Stem Cells: A Promising Therapy for Hemophilia A Disease

Neelam Yadav

Department of Biochemistry, Dr. Ram Manohar Lohia Avadh University,
Faizabad-224001, Uttar Pradesh, India.
Email : neelam2k4@gmail.com

Hemophilia A (HA) is caused by mutations within the factor VIII (FVIII) gene, which leads to depleted protein production and inefficient blood clotting. Current therapies include fixes-dose FVIII prophylaxis, factor VIII replacement therapies, and most recently, gene therapy. Several attempts at gene therapy have failed for various reasons-including immune rejections. Liver is the primary site of FVIII synthesis; however, the specific cell types responsible for its synthesis remain controversial. Several reports have demonstrated the capacity of bone marrow stem cells (BMSCs) to transdifferentiate into hepatocytes and liver sinusoidal endothelial cells (LSECs). These findings created enormous interest because they uncovered a new property of BMCs and opened the possibility that these cells could be used in the treatment of liver injury and acute or chronic liver failure. We propose that the severity of the bleeding disorder could be ameliorated by partial replacement of mutated liver cells with healthy cells in HA mice. Our study showed that BM-derived hepatocytes and endothelial cells can synthesize FVIII in liver and correct bleeding phenotype in HA mice. Thus, BM- stem cell therapy is a potential alternative approach to managing HA.

Hemophilia A (HA) is an X-linked recessive bleeding disorder that results from a deficiency of antihemophilic factor VIII (FVIII). The incidence of HA is 1 in 5,000 males, constituting about 80% of all hemophilia cases, and manifests in mild to severe disease, depending on the relative expression of functional FVIII. Severe HA patients have 1% or less of normal plasma FVIII activity and spontaneously bleed. Patients with 1-5% of normal activity have less severe bleeding, and patients with 5-25% of normal activity usually bleed only with surgery or trauma. The clinical manifestation of this disease is unpredictable, recurrent, and spontaneous bleeding in various areas, including soft tissues, major joints and occasionally in internal organs. The standard treatment options for HA are either on-demand or prophylactic therapy with plasma-derived or recombinant human FVIII. The therapeutic use of this purified factor can be a

potential biohazard due to blood-borne pathogens, and is ineffective due to the formation of inhibitors. Moreover, the life-long requirement for replacement therapy can have a significant economic impact on patients.

Present treatment and limitations

Hemophilias are genetic bleeding disorders for which there are still no cures. Three primary types of treatment methods are reported for patients with hemophilias A and B: plasma-derived products, recombinant factors, and gene therapy, as well as the therapies for patients who develop inhibitors: activated Prothrombin complex concentrates, recombinant factor VIIa, and immunosuppressive or immuno-tolerance inducing treatments.

Replacement therapy using plasma-derived concentrates or recombinant FVIII administered after bleeding episodes is the current mode of therapy for HA. Although prophylaxis with protein has been shown to significantly reduce spontaneous bleeds, treatment efficacy is limited by the short half-life of FVIII *in vivo*, high production costs, repeated intravenous administrations, and development of host antibodies to the therapeutic protein. The choice between the two most common therapies, plasma-derived products or recombinant FVIII or FIX, is still a dilemma for clinicians involved in the care of patients with hemophilia.

Gene therapy appears promising in treating hemophilia as the disease is caused by a single gene defect and a small increase in gene products could essentially transform a severe form of hemophilia into a mild one [1]. Clinical studies show that about 20% of HA patients develop inhibitors to treatment [2] and that these patients are difficult to treat [3]. The main concern of gene therapy is safety, immune response, germ line transmission, and insertional oncogenesis.

Liver transplantation cures HA, demonstrating that the liver is a major site of FVIII synthesis. Hepatocytes, LSECs or both have been proposed as site of FVIII synthesis. The main limiting factor for liver or hepatocyte transplantation is the availability of donor liver and immune-rejection. Further, sometimes repeated transplantation is required to maintain the therapeutic effects of the foreign tissue, which may augment the problem of unavailability of liver/hepatocytes.

Cell based therapy

Cell-based therapy using isolated primary hepatocytes [4, 5] or LSECs [6-8] is suggested to treat clotting disorders. Factor VIII or IX are synthesized in the liver before it enters into circulation. Cell therapy is an achievable goal for HA because replacement of even 5% FVIII activity converts the disease to a mild form. Second, insights have been obtained in transplanting healthy cells by relevant methods into appropriate organs. Cell therapy rather than whole organ transplantation has advantages because cells from a single organ could be used for multiple individuals, isolated cells could be expanded, and cells could be derived from stem cells. Moreover, outcomes of cell therapy in HA could be readily determined by FVIII activity assays in blood samples. Third, exciting progress has been made in understanding where FVIII is made in the body.

Recent evidence showed that liver transplantation corrected HA; liver is recognized as the primary site of FVIII synthesis with immunohistochemical staining showing FVIII expression in both hepatocytes and LSECs. Transplantation of LSECs in peritoneal cavity corrected the phenotype of HA mice [7], indicating LSECs are capable of synthesizing functional FVIII protein in the liver, whereas transplantation of hepatocytes did not correct murine hemophilia (Fig. 1). We hypothesized that the partial replacement of mutated liver cells by healthy cells in HA mice could manage the severity of the bleeding disorder. We have shown that lineage depleted (Lin$^{-)}$ BMCs can correct the HA phenotype in mice by producing active FVIII protein [9,10]. Recently, Follenzi and colleagues demonstrate that transplantation of BMCs into HA mice partially restore FVIII production and protected HA mice from bleeding challenge [11]. Whole bone- marrow cells were freshly isolated from wild-type mice and transplanted into recipient HA mice (Fig. 1). A majority of the treated mice (~ 70%) had average 8% to 12% circulating FVIII activity and survived bleeding challenge with correction of hemophilia. In recipient mice, the donor BM-derived mononuclear cells and mesenchymal stromal cells (MSCs) contributed to major FVIII gene expression and activity. Subsequently, it was also demonstrated that transplantation of Kuffer cells (KCs) or mesenchymal stem cells respectively isolated from wild-type mice (Fig. 1) diminished the mortality of recipient HA mice from excessive bleeding, indicating that these cells indeed produce a significant amount of functional FVIII.

Furthermore, previous *ex vivo* gene therapy studies using gene transfer vectors targeting HSCs, megakaryocytes, endothelial progenitor cells, and iPS cells achieved therapeutic levels of FVIII gene expression [12] (Fig. 1). Identification of BM-derived cell types that contribute to the production of biologically functional FVIII will increase the therapeutic potential of the BM cells in adoptive cell therapy as well as *ex vivo* and *in vivo* gene therapy for the treatment of hemophilia.
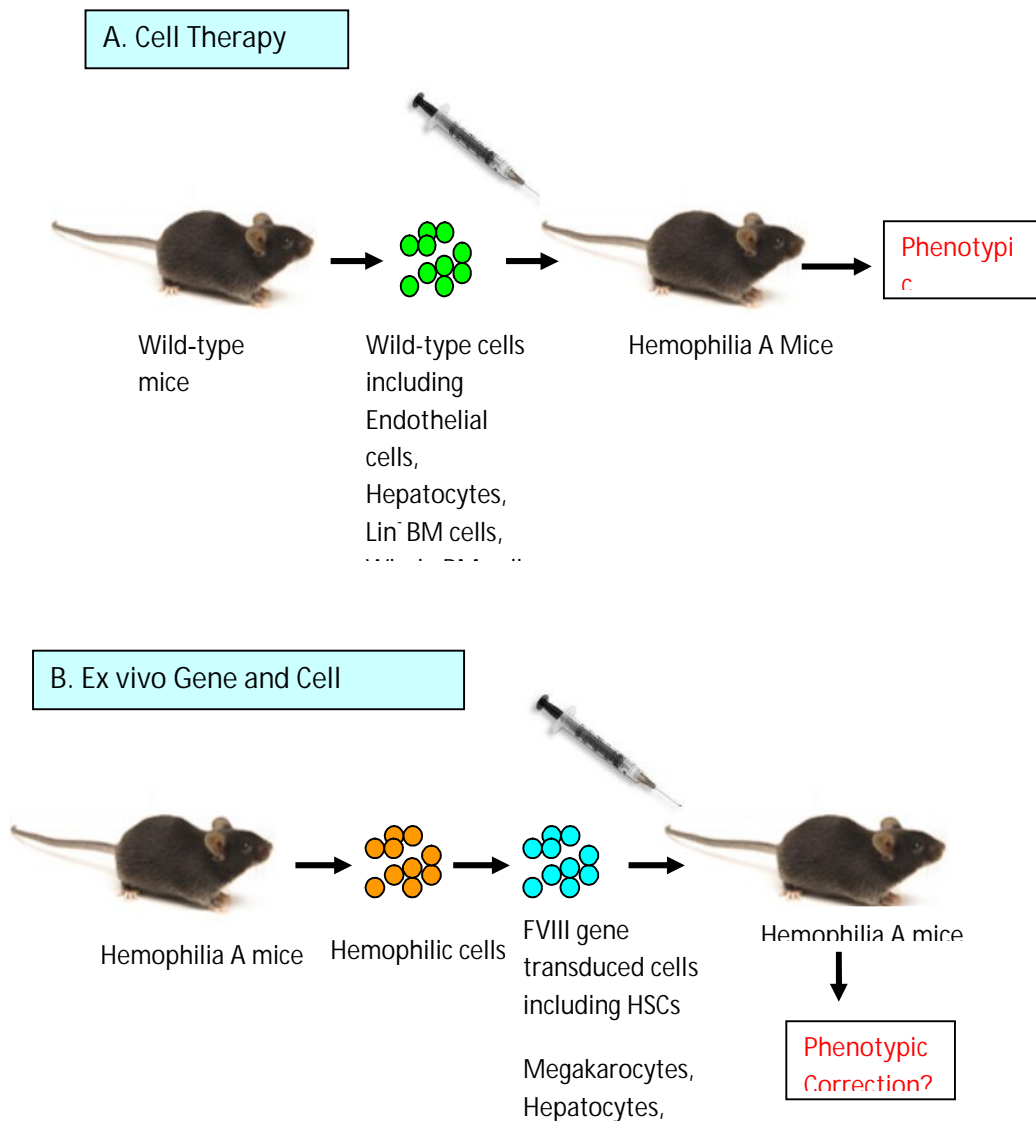
Fig. 1: Phenotypic correction of hemophilia A in mice after adoptive transfer of cells isolated from wild-type mice or FVIII gene-transduced cells isolated from hemophilia A mice. (A) Illustration of adoptive cell therapy. (B) Illustration of *ex vivo* gene and cell therapy.

Preclinical studies for hemophilia

Gene therapy can be use for the treatment of hemophilia A and B diseases, as stable expression of coagulation FVIII and IX may correct the bleeding diathesis. Small and large animal models of HA and HB diseases are available for preclinical testing. Different Phase I clinical trials were initiated for the treatment of haemophilia by gene transfer [13-15]. Several different gene delivery systems were used in these trials, including a retroviral vector, an adenoviral vector, two different adeno-associated viral vectors, and a non-viral gene delivery method. Vanden Driessche *et al.* (1999) also investigated an alternative approach to target dividing hepatocytes to infuse retroviral vectors into neonates, whose hepatocytes are naturally undergoing rapid cell division to fully correct FVIII deficiency in a murine model of HA [16]. This approach was successfully extended to the canine model of HB by [17] achieved up to 3·5% of normal FIX activity levels following retroviral vector transduction of neonatal HB dogs. Long-term expression of clotting factors has been successfully achieved in large animal models of haemophilia using multiple gene transfer strategies, but these findings have not yet been translated into success in patients.

Earlier, we have reported therapeutic potential of BM-derived hepatocytes in phenotype correction of FVIII$^{-/-}$ mice. Uncommitted BM cells (Lin$^-$) were transplanted in the recipients whose liver were perturbed by administration of acetaminophen. Quantitative analyses of donor-derived hepatic and endothelial cells were carried out by flowcytometry. About 85% of GFP$^+$ cells expressed albumin, whereas vWF was expressed in 4 to 8% of donor-derived cells [9]. Combining results of flowcytometry and immuno-histochemistry suggested that engrafted BM cells can differentiate into hepatocytes and LSECs, as shown in **Fig. 2** [9,10]. The phenotypic correction in HA mice was determined by activated prothromboplastin time (aPTT) assay and tail-clip challenge experiment. The plasma FVIII activity in FVIII$^{-/-}$ mice was $0.7 \pm 0.25\%$ of wild type mouse, which was increased to $15.7 \pm 3.2\%$, $26.4 \pm 9.4\%$, and $19.1 \pm 5.1\%$ in transplanted mice after 5, 12, and 18 months of transplantation, respectively. The survival rate in transplanted mice was increased from 23 to 80% in tail-clip challenge experiment. In contrast, the majority of FVIII$^{-/-}$ mice did not survive more than 20 h of tail-clip [9]. Most interestingly, 9 of the 9 transplanted mice examined between 5 to 18 months of the study confirmed that BM cell therapy did not cause the formation of FVIII inhibitors. Overall, these findings were in

agreement with the maintenance of secretory function of BM-derived liver cells and the ability of these cells to supply active FVIII in the diseased mice.

Summary

Hemophilia treatments are readily available in developed countries, however it is estimated that about 70% of the people with this disease worldwide are undiagnosed or under-treated. Treatment of hemophilias is difficult because patients need repeated infusion of missing coagulation factors, some patients develop inhibitors to the infused factors, and gene therapy is still not suitable for treatment. Cell-based therapies using isolated primary hepatocytes or LSECs are suggested to treat clotting disorders. Most recently, BM-derived liver cells, monocytes/macrophages and mesenchymal stromal cells can synthesize FVIII and correct bleeding phenotype in HA.
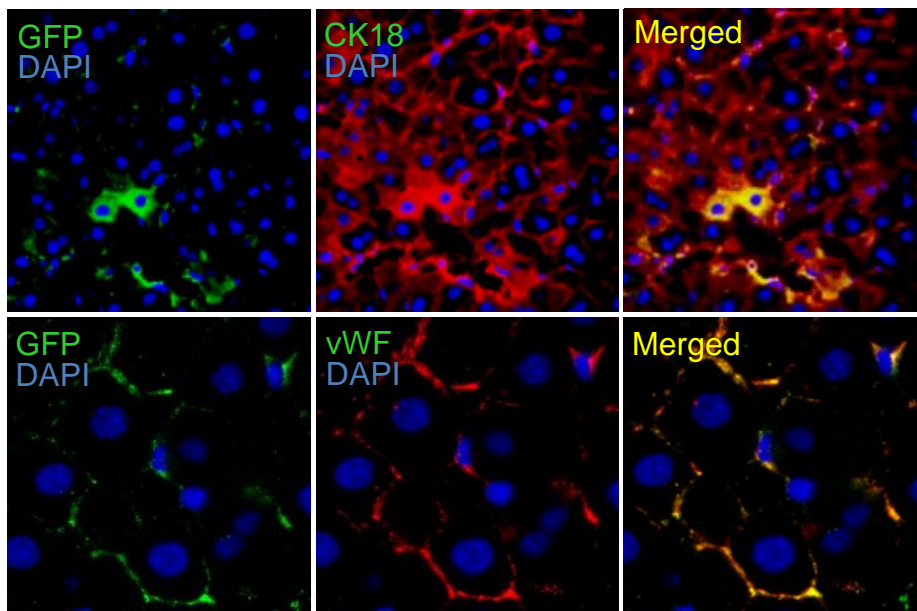
Fig. 2: Transdifferentiation of BM-derived stem cells into hepatocytes and liver sinusoidal endothelial cells in FVIII-KO mouse liver. Liver was perturbed by administering acetaminophen prior to the transplantation of Lin⁻ BM cells. Five months after transplantation, liver cryosections were stained with either anti-GFP and anti-CK18, or anti-GFP and anti-vWF antibodies. Representative images show expression of CK18 (hepatic marker) or vWF (endothelial marker) in BM-derived (GFP⁺) cells. Magnifications: ×200 (top panel); ×600 (bottom panel).

References

1. Gan, Uin, S., Lian, K.O., and Roy, C. 2006. Genetic engineering for haemophilia A. Exp. Opin. Biol. Ther. 6: 1023-1030.

2. High, K.A. 2006. The leak stops here: platelets as delivery vehicles for coagulation factors. J. Clin. Invest. 116: 1840-1842.

3. Mannucci, P.M. 2003. Hemophilia: treatment options in the twenty-first century. J. Thromb. Haemost. 1: 1349-1355.

4. Ohashi, K., Waugh, J.M., Dake, M.D., Yokoyama, T., Kuge, H., Nakajima, Y., Yamanouchi, M., Naka, H., Yoshioka, A. and Kay, M.A. 2005. Liver tissue engineering at extrahepatic sites in mice as a potential new therapy for genetic liver diseases. Hepatology 41: 132-140.

5. Tatsumi, K., Ohashi, K., Shima, M., Nakajima, Y., Okano, T., and Yoshioka, A. 2008. Therapeutic effects of hepatocyte transplantation on hemophilia B. Transplantation 86: 167-170.

6. Do, H., Healey, J.F., Waller, E.K., and Lollar, P. 1999. Expression of factor VIII by murine liver sinusoidal endothelial cells. J. Biol. Chem. 274: 19587-19592.

7. Kumaran, V., Benten, D., Follenzi, A., Joseph, B., Sarkar, R., and Gupta, S. 2005. Transplantation of endothelial cells corrects the phenotype in hemophilia A mice. J Thromb. Haemost. 3: 2022-2031.

8. Follenzi, A., Benten, D., Novikoff, P., Faulkner, L., Raut, S., and Gupta, S. 2008. Transplanted endothelial cells repopulate the liver endothelium and correct the phenotype of hemophilia A mice. J. Clin. Invest. 118: 935-945.

9. Yadav, N., Kanjirakkuzhiyil, S., Kumar, S., Jain, M., Halder, A., Saxena, R., and Mukhopadhyay 1 A. 2009. The therapeutic effect of bone marrow derived liver cells in the phenotypic correction of murine hemophilia A. Blood 114: 4552-4556.

10. Yadav, N., Kanjirakkuzhiyil, S., Ramakrishnan, M., Das, T.K., and Mukhopadhyay A. 2012. Factor VIII Can Be Synthesized in Hemophilia A Mice Liver by Bone Marrow Progenitor Cell-Derived Hepatocytes and Sinusoidal Endothelial Cells. Stem Cells Dev. 21: 110-120.

11. Follenzi, A., Raut, S., Merlin, S., Sarkar, R., and Gupta, S. 2012. Role of bone marrow transplantation for correcting hemophilia A in mice. Blood 119: 5532-5542.

12. Petrus, I., Chuah, M., and VandenDriessche, T. 2010. Gene therapy strategies for hemophilia: benefits versus risks. J. Gene Med. 12: 797-809.

13. Roth, D.A., Tawa, N.E., Jr, O'Brien J.M., Treco, D.A., and Selden, R.F. 2001. Nonviral transfer of the gene encoding coagulation factor VIII in patients with severe hemophilia A. New Engl. J. Med. 344: 1735–1742.

14. Manno, C.S., Chew, A.J., Hutchison, S., Larson, P.J., Herzog, R.W., Arruda, V.R., Tai, S.J., Ragni, M.V., Thompson, A., Ozelo, M., Couto, L.B., Leonard, D.G., Johnson, F.A., McClelland, A., Scallan, C., Skarsgard, E., Flake, A.W., Kay, M.A., High, K.A., and Glader, B. 2003. AAV-mediated factor IX gene transfer to skeletal muscle in patients with severe hemophilia B. Blood 101: 2963-2972.

15. Powell, J.S., Ragni, M.V., White, G.C., 2nd., Lusher, J.M., Hillman- Wiseman, C., Moon, T.E., Cole, V., Ramanathan-Girish, S., Roehl, H., Sajjadi, N., Jolly, D.J., and Hurst, D. 2003. Phase 1 trial of FVIII gene transfer for severe hemophilia A using a retroviral construct administered by peripheral intravenous infusion. Blood 102: 2038–2045.

16. VandenDriessche, T., Vanslembrouck, V., Goovaerts, I., Zwinnen, H., Vanderhaeghen, M.L., Collen, D., and Chuah M.K. 1999. Long-term expression of human coagulation factor VIII and correction of hemophilia A after in vivo retroviral gene transfer in factor VIII-deficient mice. PNAS 96: 10379–10384.

17. Xu, L., Gao, C., Sands, M.S., Cai, S.R., Nichols, T.C., Bellinger, D.A., Raymer, R.A., McCorquodale, S., and Ponder, K.P. 2003. Neonatal or hepatocyte growth factor-potentiated adult gene therapy with a retroviral vector results in therapeutic levels of canine factor IX for hemophilia B. Blood 101: 3924–3932.

Biography

Neelam Yadav is Assistant Professor in Department of Biochemistry, at Dr. R.M.L. Avadh University, Faizabad, India. She was Visiting Scientist in Department of Medicine, Albert Einstein College of Medicine, New York, USA, under Raman Fellowship Programme of University Grant Commission (UGC) New Delhi, Government of India.

# Investigating two Hallmarks of Cancer – Genome Instability and Tumor Promoting Inflammation

Libi Anandi, Vaishali Chakravarty and Mayurika Lahiri*

Indian Institute of Science Education and Research, Dr Homi Bhabha Road, Pashan, Pune 411008

*email: mayurika.lahiri@iiserpune.ac.in

According to the GLOBACON report of 2012, India has the highest incidence as well as highest mortality in breast cancer among women [1, 2]. A closer look at the statistics reveals that one in every two women diagnosed with breast cancer die of the disease. Furthermore, it also reveals that there has been a shift in the age of occurrence of breast cancer among Indian women with the average age at diagnosis being between 45-50 years, in contrast to above 60 years in the western countries. In addition to this, these cancers, especially in the young population are more aggressive as compared to the Caucasian population [3, 4]. Interestingly, it has been observed that only 15% cases of breast cancer are due to inherited mutations whereas the remaining 85% cases are sporadic in nature [4]. This fact draws our attention to the large proportion of sporadic cases and the various factors which can play a vital role in initiation of breast cancer as well its progression.

Hanahan and Weinberg in 2011 proposed certain capabilities acquired by the cell during tumor development termed as "Hallmarks of Cancer" as depicted in figure 1. According to them, 'genomic instability' and 'tumor promoting inflammation' are two characteristics, which aid in acquiring the hallmark capabilities. Various environmental factors such as radiations, environmental pollutants, cigarette smoke as well as chemotherapy itself, are potential agents which can induce genomic instability and hence act as "enabling factors" to acquire cancerous phenotype. On the other hand inflammation is capable of accumulating cells of the immune system in the microenvironment thereby resulting in secretion of certain biomolecules like reactive oxygen species (ROS), cytokines and phospholipid mediators. These are potential signaling molecules and thus convert the microenvironment into an "active state" and probably contribute to initiation or progression of the multistep process of tumorigenesis. (summarized in figure 1).
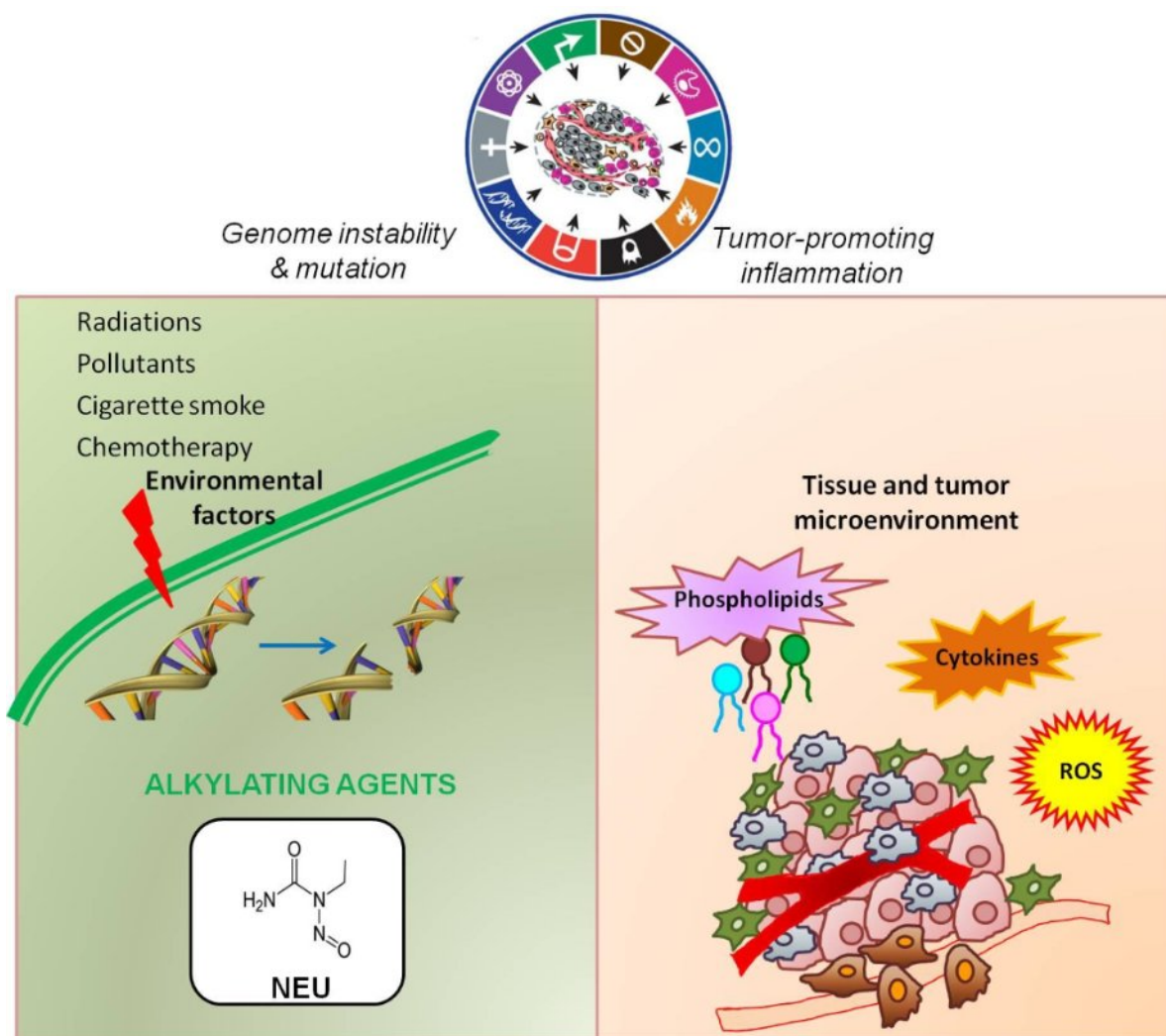
Figure 1: Schematic depicting the "enabling characteristics" that cell acquires to become cancerous.

Alkylating agents, one of the major components of cigarette smoke and cancer chemotherapy, are well known DNA damaging agents as well as known or suspected carcinogens. It is well known that, exposure to such genotoxic agents results in DNA breaks which in turn activates ATM and ATR kinases, key proteins in the DNA Damage Response (DDR) pathway which further phosphorylates their downstream targets, Chk2 and Chk1, respectively [5]. In our recent study, using N-nitroso-N-ethylurea (NEU), a simple monofunctional S_N1 type-DNA ethylating agent, we investigated the activation of DDR kinases following DNA damage induced by NEU in human cancer cell lines [6]. In our study, NEU damage led to phosphorylation of Chk1 and Chk2 in a dose-dependent as well as temporal manner. In contrast to earlier reports where

alkylation damage was detectable only after 1-2 cell divisions [7], our study revealed the activation of Chk2 and Chk1 in 10 and 20 minutes, respectively. Contrary to other reports where γH2AX activation was observed after 24hrs of DNA damage [8], in our study, γH2AX foci were detectable within 2 hrs of NEU damage, which further supported the activation of Chk2 kinase within 10 minutes. Given that alkylating agents cannot directly induce double strand breaks, our results called for further investigations to understand how NEU-induced alkylation damage was being processed to form DSBs within 10 minutes.

Reports suggested that post ionizing radiation (IR) treatment, phosphorylation of Chk2 precedes Chk1 activation and as phosphorylation of Chk2 decreases, Chk1 activation gradually increases indicating the presence of crosstalk between ATM and ATR pathways [9]. Since both the checkpoint kinase activations were observed in our study, it was intriguing to check the presence of a crosstalk between the two canonical pathways. We observed absence of any possible crosstalk between the two pathways following damage by NEU. Furthermore, alkylating agents have been shown to alkylate "N" and "O" positions of DNA leading to the formation of 12 different DNA adducts which in turn may result in A:T to T:A transversions or G:C to A:T transition mutations [10, 11]. The DNA adducts formed by these agents are generally recognised by the mismatch repair (MMR) proteins, namely Msh2-Msh6 and Mlh1-Pms2 heterodimers during the first round of replication. Further, it has been observed that p53, Chk1, Chk2 as well as Cdc25A are activated in a MMR dependent manner [7, 12], thus implying that MMR proteins played an important role in the DDR pathway. To investigate the role of MMR proteins in NEU induced DNA damage, a MMR deficient cell line was exposed to NEU damage. Surprisingly, activation of Chk1 as well as Chk2 was observed, thus clearly indicating at the dose and time of damage, mismatch repair proteins did not play any significant role in NEU induced DNA damage.

Apart from the DNA damaging effects, alkylating agents have been demonstrated to cause mammary tumors in rodent models [13, 14]. Rat mammary epithelial cells, following 30 days post NEU exposure (at different doses) underwent point mutations which as an accumulative effect provided the cells the ability to form multilayered colonies. They also acquired the ability to form colonies on soft agar and could produce noticeable sized tumors when grafted into

female athymic nude mice [15]. These results pointed towards neoplastic transformation due to alkylation damage. Following exposure to NEU, we investigated the ability of NEU to induce transformation using three-dimensional (3D) cultures of non-transformed MCF0A epithelial cells. 3D cultures aid in distinguishing transformed cells from the non-transformed cells by changes in morphology of the acinar structures formed when these cells are grown on extracellular matrix. Such acini resemble the *in vivo* breast acini structurally and functionally. In our study we have shown the characteristic features of epithelial-mesenchymal (EMT) where loss of polarity, establishment of mesenchymal characteristics and down-regulation of epithelial like characters were observed in MCF10A cells treated with NEU at the single cell stage. □-6 integrin, which marks the basal region, showed mislocalization. E-cadherin and □-catenin, both cell-cell junction markers [16], showed aberrant staining patterns, with E-cadherin showing a loss phenotype while □-catenin showed cytoplasmic localization. Vimentin, which is a characteristic mesenchymal marker showed strong expression in the NEU-treated acini. These phenotypes were distinct at all doses of NEU, that is, at 2, 3 and 5 mM NEU and also confirmed that a EMT-like phenotype was acquired by breast epithelial cells upon NEU treatment. These results gave strong implications of transformation occurring in breast epithelial cells on exposure of an alkylating agent, NEU.

Apart from the various environmental factors which influence the induction and progression of cancers, bioactive molecules present in the tissue microenvironment as well as tumor milieu can contribute to the occurrence and progression of the disease. Phospholipid mediators such as lysophosphatadic acid, prostaglandins, platelet activating factor and platelet activating factor-like molecules are secreted by cells of the immune system and are present in the microenvironment. Under chronic inflammatory conditions the proportion of these molecules increases in the adjoining tissues. Given the contribution of chronic inflammation in various cancers, the possibility of these molecules behaving as potential factors for cancer initiation and progression has increased multifold. Level of LPA has been shown to be high in various cell lines as well as in ovarian cancer patients and has been demonstrated to play a vital role in tumor angiogenesis, migration, proliferation, invasion and metastasis [17-19]. Similarly, platelet activating factor (PAF), acting through PAF-R (Platelet activating factor receptor, a G protein-coupled receptor) has been demonstrated to play important roles in various cancers. Apart from the well-known

roles of PAF in inflammation, platelet aggregation various groups have demonstrated the contributing role of PAF in cancer progression. PAF has been shown to play a role in neo-angiogenesis mediated by bFGF (basic fibroblast growth factor) and VEGF (vascular endothelial growth factor) as well as angiogenesis mediated by thrombopoietin, TNF-α (Tumor Necrosis Factor- α), CD-40 and hepatocyte growth factor (HGF). In addition to this, PAF induces motility of HUVECs, endothelial cells [20], peripheral blood lymphocytes[21] eosinophils, [22] as well as breast cancer cells. Our recent study, demonstrated the ability of PAF to enhance migration in MDA-MB 231 cells (invasive breast cancer cells) corroborates with these published reports. Crucial role played by MAPK pathway (JNK, ERK and p38) in regulating cell movement is well known. ERK is known to phosphorylate myosin light chain kinase, FAK or calpain to regulate cell migration [23]. Apart from this, ERK has been shown to be involved in the migration of breast cancer cells exhibiting lung metastasis [24]. In contrast to published reports, we observed that PAF-induced enhanced motility was independent of ERK activation. However inhibition of JNK pathway reduced motility of PAF-stimulated and un-stimulated cells suggesting that JNK pathway was involved in both PAF-induced as well as in the inherent motility of breast cancer cells. PI3K pathway, another pathway well known to play a vital role in cell motility, was investigated to understand its role in motility of PAF-stimulated cells. Surprisingly, we observed that inhibition of this pathway though reduced inherent motility of cells, it could only partially rescue increased motility, thus implying the role of PI3K pathway in inherent motility of cells and a possible role in PAF-induced motility. Further studies are being performed to decipher the pathway through which PAF elicits motility in breast cancer cells. Literature suggests various pathways activated upon exposure to PAF suggesting that in different cell types, PAF may stimulate different signal transduction pathways to elicit various responses. In HUVECs, PAF exposure was found to induce transformation while in neurons, it induced apoptosis [25, 26]. Role of PAF in various cancers has been well studied; however the precise role in breast cancer initiation remains to be elucidated. PAF has been found to be present in breast tissue of patients suffering from breast cancer and has also been shown to correlate with lymph node metastasis [27, 28]. Accumulation of PAF could occur either due to enhanced production and secretion or due to abnormal degradation. In support to this, Kispert *et al* reported the inhibitory effect of cigarette smoke on platelet PAF-acetyl hydrolase (PAF-AH, enzyme inactivating PAF), thus resulting in accumulation of PAF, which enhanced motility of breast cancer cells as well as

increased adherence to lung endothelium [29]. However, the possibility of PAF inducing transformation in breast epithelial cells has not yet been explored. We observed that in non-transformed breast epithelial cells, PAF induced formation of abnormal acinar structures when cells were grown as 3D cultures under constant stimulation of PAF. Apart from the increase in the number of cells in the acini, indicative of proliferation or evasion of apoptosis, the acinar structures also showed presence of protrusion-like structures indicative of EMT-like phenotype. Taken together, we demonstrated the ability of PAF to induce transformation of non-transformed breast epithelial cells. These results appeal for further investigations to delineate the pathway and identify novel targets to design novel therapeutics.

References

1.   Ferlay J SI, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray, F.: GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11. In.; 2012.
2.   International Agency for Research on Cancer [http://globocan.iarc.fr]
3.   Three year report of the population based cancer registries 2009-2011: Report of 25 PBCRs. In: *National Cancer Registry Programme.* India: Indian Council Medical Research.
4.   Leong SP, Shen ZZ, Liu TJ, Agarwal G, Tajima T, Paik NS, Sandelin K, Derossis A, Cody H, Foulkes WD: Is breast cancer the same disease in Asian and Western countries? *World journal of surgery* 2010, 34(10):2308-2324.
5.   Abraham RT: PI 3-kinase related kinases: 'big' players in stress-induced signaling pathways. *DNA repair* 2004, 3(8-9):883-887.
6.   Bodakuntla S, Libi AV, Sural S, Trivedi P, Lahiri M: N-nitroso-N-ethylurea activates DNA damage surveillance pathways and induces transformation in mammalian cells. *BMC cancer* 2014, 14:287.
7.   Stojic L, Cejka P, Jiricny J: High doses of SN1 type methylating agents activate DNA damage signaling cascades that are largely independent of mismatch repair. *Cell Cycle* 2005, 4(3):473-477.
8.   Bakkenist CJ, Kastan MB: DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. *Nature* 2003, 421(6922):499-506.
9.   Shiotani B, Zou L: Single-stranded DNA orchestrates an ATM-to-ATR switch at DNA breaks. *Molecular cell* 2009, 33(5):547-558.
10.   Engelbergs J, Thomale J, Rajewsky MF: Role of DNA repair in carcinogen-induced ras mutation. *Mutation Research* 2000, 450(1-2):139-153.
11.   Barbaric I WS, Russ A, Dear TN: Spectrum of ENU-induced mutations in phenotype-driven and gene-driven screens in the mouse. *Environ Mol Mutagen* 2007, 48(2):124-142.
12.   Wang Y, Qin J: MSH2 and ATR form a signaling module and regulate two branches of the damage response to DNA methylation. *Proceedings of the National Academy of Sciences of the United States of America* 2003, 100(26):15387-15392.
13.   Stoica G, Koestner A, Capen CC: Characterization of N-ethyl-N-nitrosourea-induced mammary tumors in the rat. *Am J Pathol* 1983, 110(0002-9440 (Print)):161-169.

14. Givelber HM, DiPaolo JA: Teratogenic effects of N-ethyl-N-nitrosourea in the Syrian hamster. *Cancer Research* 1969, 29(0008-5472 (Print)):1151-1155.

15. Stoica G, Jacobs R, Koestner A, O'Leary M, Welsch C: ENU-induced in vitro neoplastic transformation of rat mammary epithelial cells. *Anticancer research* 1991, 11(5):1783-1792.

16. Kim K, Daniels KJ, Hay ED: Tissue-specific expression of beta-catenin in normal mesenchyme and uveal melanomas and its effect on invasiveness. *Experimental cell research* 1998, 245(1):79-90.

17. Shida D, Kitayama J, Yamaguchi H, Okaji Y, Tsuno NH, Watanabe T, Takuwa Y, Nagawa H: Lysophosphatidic acid (LPA) enhances the metastatic potential of human colon carcinoma DLD1 cells through LPA1. *Cancer research* 2003, 63(7):1706-1711.

18. Yu X, Zhang Y, Chen H: [Lysophosphatidic acid (LPA) stimulates invasion and metastatic colonization of ovarian cancer cells through Rac activation]. *Zhonghua zhong liu za zhi [Chinese journal of oncology]* 2015, 37(2):95-100.

19. Goldsmith ZG, Ha JH, Jayaraman M, Dhanasekaran DN: Lysophosphatidic Acid Stimulates the Proliferation of Ovarian Cancer Cells via the gep Proto-Oncogene Galpha(12). *Genes & cancer* 2011, 2(5):563-575.

20. Montrucchio G, Lupia E, Battaglia E, Del Sorbo L, Boccellino M, Biancone L, Emanuelli G, Camussi G: Platelet-activating factor enhances vascular endothelial growth factor-induced endothelial cell motility and neoangiogenesis in a murine matrigel model. *Arteriosclerosis, thrombosis, and vascular biology* 2000, 20(1):80-88.

21. McFadden RG, Bishop MA, Caveney AN, Fraher LJ: Effect of platelet activating factor (PAF) on the migration of human lymphocytes. *Thorax* 1995, 50(3):265-269.

22. Schweizer RC, van Kessel-Welmers BA, Warringa RA, Maikoe T, Raaijmakers JA, Lammers JW, Koenderman L: Mechanisms involved in eosinophil migration. Platelet-activating factor-induced chemotaxis and interleukin-5-induced chemokinesis are mediated by different signals. *J Leukoc Biol* 1996, 59(3):347-356.

23. Huang C, Jacobson K, Schaller MD: MAP kinases and cell migration. *Journal of cell science* 2004, 117(Pt 20):4619-4628.

24. Choi C, Helfman DM: The Ras-ERK pathway modulates cytoskeleton organization, cell motility and lung metastasis signature genes in MDA-MB-231 LM2. *Oncogene* 2014, 33(28):3668-3676.

25. Ryan SD, Harris CS, Mo F, Lee H, Hou ST, Bazan NG, Haddad PS, Arnason JT, Bennett SA: Platelet activating factor-induced neuronal apoptosis is initiated independently of its G-protein coupled PAF receptor and is inhibited by the benzoate orsellinic acid. *Journal of neurochemistry* 2007, 103(1):88-97.

26. Axelrad TW, Deo DD, Ottino P, Van Kirk J, Bazan NG, Bazan HE, Hunt JD: Platelet-activating factor (PAF) induces activation of matrix metalloproteinase 2 activity and vascular endothelial cell invasion and migration. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 2004, 18(3):568-570.

27. Pitton C, Lanson M, Besson P, Fetissoff F, Lansac J, Benveniste J, Bougnoux P: Presence of PAF-acether in human breast carcinoma: relation to axillary lymph node metastasis. *Journal of the National Cancer Institute* 1989, 81(17):1298-1302.

28. Montrucchio G, Sapino A, Bussolati B, Ghisolfi G, Rizea-Savu S, Silvestro L, Lupia E, Camussi G: Potential angiogenic role of platelet-activating factor in human breast cancer. *The American journal of pathology* 1998, 153(5):1589-1596.

29.   Kispert S, Marentette J, McHowat J: Cigarette smoke induces cell motility via platelet-activating factor accumulation in breast cancer cells: a potential mechanism for metastatic disease. *Physiological reports* 2015, 3(3).
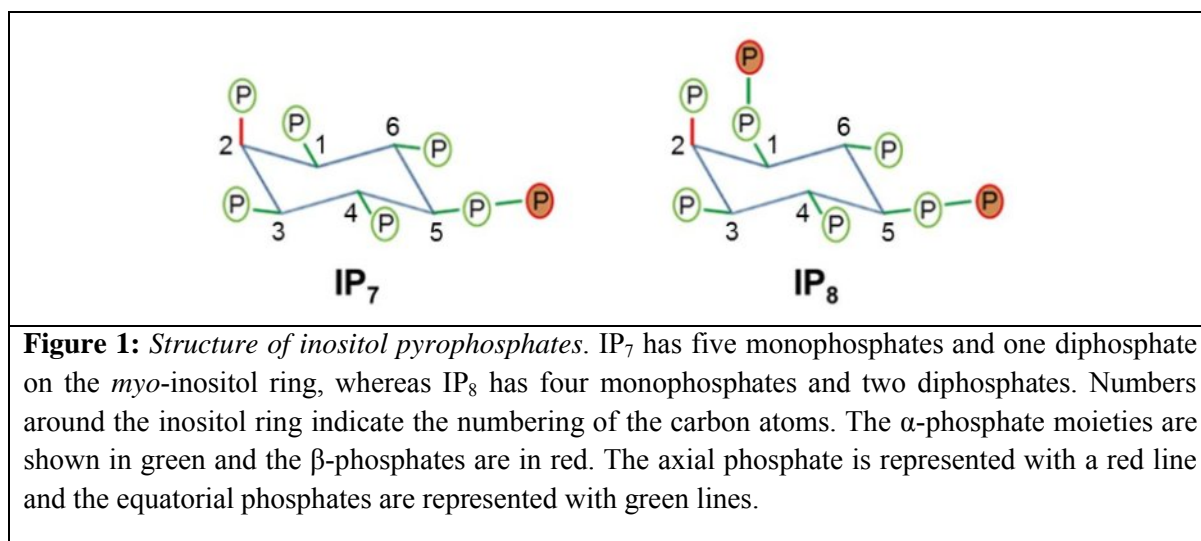
Protein pyrophosphorylation by inositol pyrophosphates

Manasa Chanduri and Rashna Bhandari

Laboratory of Cell Signalling, Centre for DNA Fingerprinting and Diagnostics (CDFD),
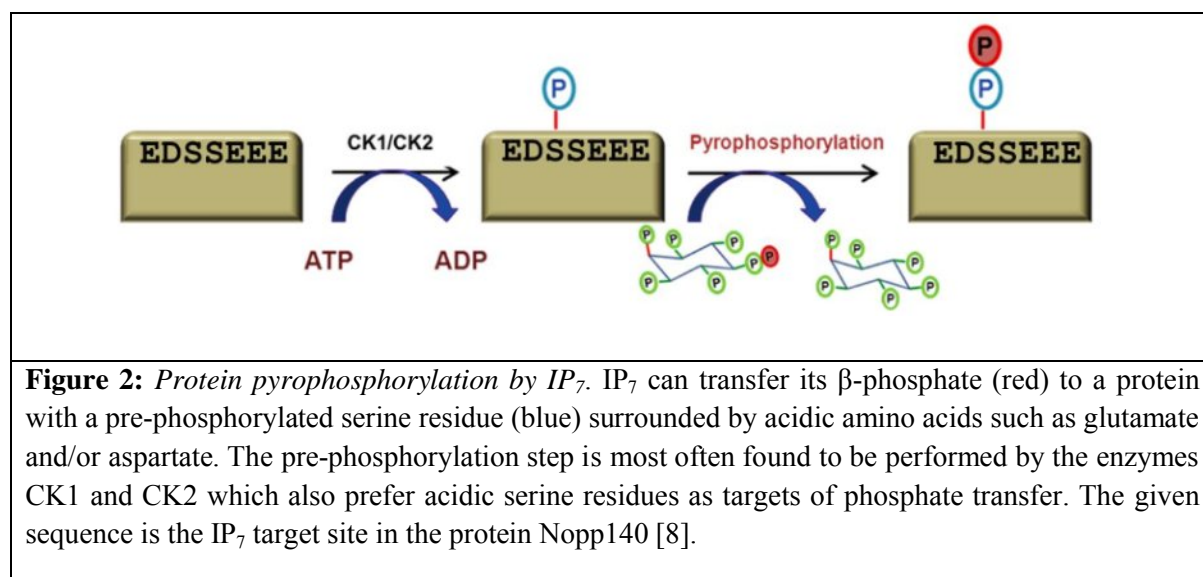
Hyderabad, India

Inositol pyrophosphates

Inositol pyrophosphates are a class of soluble inositol phosphates that possess high-energy pyrophosphate bonds (**Figure 1**) [1, 2]. 5-diphosphoinositol pentakisphosphate (IP_7) and 1, 5-bis-diphosphoinositol tetrakisphosphate (IP_8) are the most well-studied inositol pyrophosphates (3). Generated predominantly by inositol hexakisphosphate kinases (IP6Ks), IP_7, at a concentration of 0.5-1.3 μM, is the most abundant inositol pyrophosphate in mammalian cells [3, 4]. Over two decades of research has brought forth numerous pathways regulated by these small molecules, including insulin release, vesicular trafficking, energy metabolism and DNA repair [5]. IP_7 has been shown to modulate protein function by means of direct binding or by protein pyrophosphorylation [3, 5].

Figure 1: *Structure of inositol pyrophosphates.* IP_7 has five monophosphates and one diphosphate on the *myo*-inositol ring, whereas IP_8 has four monophosphates and two diphosphates. Numbers around the inositol ring indicate the numbering of the carbon atoms. The α-phosphate moieties are shown in green and the β-phosphates are in red. The axial phosphate is represented with a red line and the equatorial phosphates are represented with green lines.

Protein pyrophosphorylation

Protein pyrophosphorylation is a regulation mechanism unique to inositol pyrophosphates, in which the β-phosphate of IP_7 or IP_8 is transferred to proteins in an enzyme independent manner (**Figure 2**). Owing to the presence of high energy pyrophosphate bonds, inositol pyrophosphates were always envisaged as phosphorylating agents [2, 6]. Two studies

successfully demonstrated that IP$_7$ can transfer its phosphate to proteins in eukaryotic cell lysates, and identified the pre-requisites for such an enzyme-independent phosphotransfer [7, 8]. Mg^{2+} was recognised as an essential cofactor and phosphoserine residues surrounded by acidic amino acids (such as glutamate and aspartate) in the target protein seemed to form an agreeable consensus for IP$_7$ mediated phosphotransfer (**Figure 2**). As IP$_7$ donates its β-phosphate to a pre-phosphorylated serine residue on a protein to generate pyrophosphoserine, this reaction is termed as 'protein pyrophosphorylation'. Since CK1 and CK2 target acidic serine residues for phosphorylation, these enzymes are the priming kinases that phosphorylate sites which are subsequently targeted by IP$_7$. Our analysis of known pyrophosphorylation substrate sequences suggests that phosphoserines present in intrinsically disordered regions of the target protein are more prone to pyrophosphorylation [9, 10]. Although the exact mechanism is not well understood, it is speculated that the disordered target protein folds around the Mg^{2+}-IP$_7$ complex, providing a catalytic pocket for the phosphotransfer (Bihani and Bhandari unpublished).

Figure 2: *Protein pyrophosphorylation by IP$_7$.* IP$_7$ can transfer its β-phosphate (red) to a protein with a pre-phosphorylated serine residue (blue) surrounded by acidic amino acids such as glutamate and/or aspartate. The pre-phosphorylation step is most often found to be performed by the enzymes CK1 and CK2 which also prefer acidic serine residues as targets of phosphate transfer. The given sequence is the IP$_7$ target site in the protein Nopp140 [8].

Protein pyrophosphorylation is a reversible reaction as *in vitro* studies have suggested that the pyrophosphate moiety on the protein is acid labile [8] and can be cleaved by alkaline phosphatases [11]. This suggests that like protein phosphorylation, IP$_7$-mediated pyrophosphorylation may act as a signal to regulate protein function.

Biological implication of protein pyrophosphorylation

Three yeast proteins viz. nuclear localization sequence-binding protein (NSR1), suppressor protein SRP40 (SRP40) and YGR130C, and two mammalian proteins viz. mammalian homologue of SRP40 (Nopp140) and Treacher Collins–Franceschetti syndrome 1 (TCOF1) were the first IP_7 substrates identified [8]. However, the role of pyrophosphorylation in regulating the function of these proteins remains elusive. Studies from Adolfo Saiardi's group and from our laboratory have demonstrated the role of protein pyrophosphorylation in two critical cellular processes, energy homeostasis and vesicular transport.

Regulation of energy homeostasis

Studies involving *Saccharomyces cerevisiae* (budding yeast) lacking inositol pyrophosphates ($kcs1\Delta$) revealed a role for these molecules in metabolic processes such as glycolysis and ribosome synthesis [9, 12]. Owing to rapid turnover of inositol pyrophosphates, a considerable amount of ATP is spent in maintaining their cellular levels [1, 3]. To understand the role of inositol pyrophosphates in energy homeostasis, the Saiardi laboratory used yeast mutants and assessed the effect of altered inositol pyrophosphate levels on ATP metabolism [12]. They observed that concentrations of ATP and IP_7 are inversely related as $kcs1\Delta$ yeast have increased ATP levels. A detailed molecular analysis revealed that the glycolytic transcription factor, GCR1, was pyrophosphorylated by IP_7, inhibiting its binding to GCR2, thereby shutting down the transcription of glycolytic genes. Conversely, mitochondrial function was reduced in both yeast and mammalian cells with lower levels of inositol pyrophosphates Thus, IP_7-mediated pyrophosphorylation of GCR1 regulates the glycolytic/mitochondrial metabolic ratio to maintain cellular ATP levels. Based on these observations, inositol pyrophosphates are now classified as metabolic messengers and energy sensors [13]. In fact, the kinetic properties of IP6Ks supports the classification of their product as an energy sensor. The K_m of IP6Ks for ATP is almost equivalent to cellular ATP concentrations i.e.,~ 1 mM. Therefore, the synthesis of IP_7 is tremendously influenced by the rise and fall of intracellular ATP levels.

Recent observations in our laboratory suggest that inositol pyrophosphates regulate rDNA transcription, affecting ribosome biogenesis in *S. cerevisiae* [9]. $kcs1\Delta$ yeast are sensitive

to translation inhibitors due to a decrease in the number of both 40S and 60 ribosome subunits, suggesting a defect in ribosome biogenesis. Three components of the RNA polymerase I complex (Pol I), RPA190, RPA43 and RPA34.5, which participate in rDNA transcription, were found to undergo IP_7-mediated pyrophosphorylation. Pol I recruitment to the rDNA promoter was unaltered in cells lacking inositol pyrophosphates, but the rate of transcription elongation was reduced, implying a defect in transcription of rRNA, which in turn results in low ribosome levels [9]. Cellular energy status is known to dictate the regulation of Pol I mediated rRNA synthesis. Our studies suggest that IP_7 may act as 'conduit' to relay changes in intracellular ATP levels to energy demanding processes such as ribosome biogenesis, thus contributing to energy homeostasis.

Regulation of intracellular vesicular transport

The biological significance of protein pyrophosphorylation was first demonstrated in the process of vesicular transport. The β-subunit of the adaptor protein complex-3 (AP3B1) involved in vesicle formation and targeting, was found to be pyrophosphorylated by IP_7, preventing its interaction with the kinesin motor Kif3A [14]. Inhibition of this interaction leads to decreased viral particle release from mammalian cells, suggesting a role for IP_7 in regulating viral protein transport. Pyrophosphorylation may act as a positive signal to restrict the spread of virus from one cell to another by regulating protein transport.

Recently, we have identified the dynein intermediate chain (IC), a non-catalytic subunit of the dynein motor complex, as a substrate for IP_7 [10]. The dynein motor is responsible for vesicle movement on microtubule tracks towards the cell interior. Pyrophosphorylation of IC by IP_7 was found to positively regulate its interaction with p150^{Glued}, a subunit of the dynactin complex, ensuring optimal movement of vesicles in the cell. Several dynein-dependent processes such as endosomal sorting and Golgi structure maintenance are disturbed in cells with decreased inositol pyrophosphate levels. As dynein-dynactin interaction promotes motor attachment to the vesicle membrane, we propose that pyrophosphorylation of IC may act as a regulatory switch that favours dynein-dependent vesicle transport.

These studies suggest that protein pyrophosphorylation may primarily act by altering protein-protein interactions either positively or negatively. Microtubule-dependent motors viz.

kinesin and dynein are regulated by inositol pyrophosphates either directly or indirectly via protein pyrophosphorylation. As motor-dependent protein transport is an energy-driven process, IP_7 may signal motor proteins to control the rate of protein transport depending on cellular energy levels.

Future perspectives

Protein pyrophosphorylation was discovered less than a decade ago, and its role in cellular physiology is being parsed out gradually. The major limitation in the identification of protein targets of IP_7 is the lack of a sensitive detection method for *in vivo* pyrophosphorylation. Current methods rely on the use of radioactive assays to detect pyrophosphorylation *in vitro* and semi *in vivo* [8-10, 12, 14]. Development of sensitive techniques such as mass spectrometry faces a challenge due to the lability of the pyrophosphate modification and inability of the technique to distinguish between a bisphosphorylated and a diphosphorylated peptide. However, promising tools and methods developed in the Fiedler laboratory are enabling the detection and identification of pyrophosphorylated peptides [15]. In our laboratory, we are trying to develop an antibody to specifically detect the pyrophosphoserine on proteins to enable an affordable and easy method to analyse an IP_7 target. If these attempts are successful, one can hope to map the pyrophosphoproteome of a cell in the coming decade, and widen the scope for the study of protein pyrophosphorylation to every cell biologist.

Authors: Manasa Chanduri is a research scholar and Rashna Bhandari is a Staff Scientist at the Centre for DNA Fingerprinting and Diagnostics (CDFD), Hyderabad, India.
Email: rashna@cdfd.org.in
Lab page: http://www.cdfd.org.in/labpages/dr_rashna_bhandari.html

References

1. Menniti FS, Miller RN, Putney JW, Jr., Shears SB. Turnover of inositol polyphosphate pyrophosphates in pancreatoma cells. J Biol Chem 1993; 268: 3850-3856.
2. Stephens L, Radenberg T, Thiel U, Vogel G, Khoo KH, Dell A, *et al*. The detection, purification, structural characterization, and metabolism of diphosphoinositol pentakisphosphate(s) and bisdiphosphoinositol tetrakisphosphate(s). J Biol Chem 1993; 268: 4009-4015.

3. Wundenberg T, Mayr GW. Synthesis and biological actions of diphosphoinositol phosphates (inositol pyrophosphates), regulators of cell homeostasis. Biol Chem 2012; 393: 979-998.
4. Saiardi A, Erdjument-Bromage H, Snowman AM, Tempst P, Snyder SH. Synthesis of diphosphoinositol pentakisphosphate by a newly identified family of higher inositol polyphosphate kinases. Curr Biol 1999; 9: 1323-1326.
5. Thota SG, Bhandari R. The emerging roles of inositol pyrophosphates in eukaryotic cell physiology. J Biosci 2015; 40: 593-605.
6. Laussmann T, Eujen R, Weisshuhn CM, Thiel U, Vogel G. Structures of diphospho-myo-inositol pentakisphosphate and bisdiphospho-myo-inositol tetrakisphosphate from *Dictyostelium* resolved by NMR analysis. Biochem J 1996; 315: 715-720.
7. Saiardi A, Bhandari R, Resnick AC, Snowman AM, Snyder SH. Phosphorylation of proteins by inositol pyrophosphates. Science 2004; 306: 2101-2105.
8. Bhandari R, Saiardi A, Ahmadibeni Y, Snowman AM, Resnick AC, Kristiansen TZ, *et al*. Protein pyrophosphorylation by inositol pyrophosphates is a posttranslational event. Proc Natl Acad Sci U S A 2007; 104: 15305-15310.
9. Thota SG, Unnikannan CP, Thampatty SR, Manorama R, Bhandari R. Inositol pyrophosphates regulate RNA polymerase I-mediated rRNA transcription in *Saccharomyces cerevisiae*. Biochem J 2015; 466: 105-114.
10. Chanduri M, Rai A, Malla AB, Wu M, Fiedler D, Mallik R, *et al*. Inositol hexakisphosphate kinase 1 (IP6K1) activity is required for cytoplasmic dynein-driven transport. Biochem J 2016; 473: 3031-3047.
11. Yates LM, Fiedler D. Establishing the stability and reversibility of protein pyrophosphorylation with synthetic peptides. Chembiochem 2015; 16: 415-423.
12. Szijgyarto Z, Garedew A, Azevedo C, Saiardi A. Influence of inositol pyrophosphates on cellular energy dynamics. Science 2011; 334: 802-805.
13. Wilson MS, Livermore TM, Saiardi A. Inositol pyrophosphates: between signalling and metabolism. Biochem J 2013; 452: 369-379.
14. Azevedo C, Burton A, Ruiz-Mateos E, Marsh M, Saiardi A. Inositol pyrophosphate mediated pyrophosphorylation of AP3B1 regulates HIV-1 Gag release. Proc Natl Acad Sci U S A 2009; 106: 21161-21166.
15. Conway JH, Fiedler D. An affinity reagent for the recognition of pyrophosphorylated peptides. Angew Chem Int Ed Engl 2015; 54: 3941-3945.

F-box proteins are care taker of cellular homeostasis and their role in pathogenesis

Parul Dutta and Manas Kumar Santra

National Centre for Cell Science, S.P. Pune University campus, Pune, Maharashtra, India

E-mail:duttaparul5@gmail.com; manas@nccs.res.in

As multicellularity began to emerge in nature there was an urgent requirement of division of labour among various cells all working in harmony with each other to bring out the wellbeing of entire individual. Though different cells in an organism perform different functions they maintain a dynamic equilibrium with each other to maintain homeostasis in the organism, failing which the organism collapses. This dynamic equilibrium is splendidly maintained even at single cell level by precise mechanisms regulating turnover of various cellular entities within the cells through strict feedback and feed forward loops. One of such important homeostatic functions performed by cells is proteostasis, as proteins are the major functional contributors within the cells that can be modified rapidly in spatio-temporal manner. Constant quality control surveillance goes on in cells to monitor proteome required at any particular instance. One of the major systems at the heart of maintaining proteostasis is the Ubiquitin Proteasome System (UPS) [1]. The UPS is involved in rapid post translational modification of selective proteins through conjugation of ubiquitin (Ubiquitylation) and thereby plays crucial role in myriad cellular functions as signal transduction, DNA damage sensing and repair, apoptosis, metabolism, cell cycle regulation and many more. Ubiquitylation of proteins through UPS requires concerted activity of 3 different enzymes [1, 2]. The ubiquitin activating enzyme E1 activates ubiquitin moiety and subsequently transfer to ubiquitin conjugating enzyme E2. Then, E2 transfers the activated ubiquitin to specific substrates selected by E3 ubiquitin ligases. Importantly, E3 ubiquitin ligase determines the specific substrates to be ubiquitylated. The ubiquitylated substrates may either activate the downstream signaling or undergo proteasomal degradation.

E3 ubiquitin ligases as future of therapeutic interventions

Recent reports suggest that human have only 2 E1 enzymes, around 30 E2 enzymes and more than 600 putative E3 ubiquitin ligases [2, 3]. However, deregulation of E1 and E2 is not reported

to be associated to disease conditions but a plethora of reports record the involvement of deregulated E3 ubiquitin ligases in conditions like neuropathology, immunological disorders and cancers. Targeting UPS for management of diseases with altered protein turnover can be very effective because 80% of the total cellular proteins are degraded via UPS. Till now most of the drugs that target protein turnover includes proteasome inhibitors eg. bortezomib are in clinical use but these drugs target variety of substrates resulting in side effects such as neuropathy. As the UPS comprises of more than 1000 proteins, so selecting targets other than proteasome itself would be more specific and effective strategy for therapeutic purpose [2]. Deubiquitylating enzymes (DUBs) and E3 ubiquitin ligases are the most promising druggable target within UPS because of their selective substrate specificity. DUBs may not serve as an efficient druggable target as their targets are shared among a group of 5 proteases and thus challenging their specificity. On the other hand E3 ubiquitin ligases have selective targets and even these selective targets are recognized only upon their specific post-translational modifications. Thus, the specificity of E3 ubiquitin ligases provides an advantage of using these enzymes as tools to regulate protein degradation in a spatio-temporal manner with minimal side effects [2].

Ubiquitin conjugation determines the fate of substrate

Ubiquitin is highly conserved 76 amino acids protein ubiquitously expressed in eukaryotes. Conjugation of ubiquitin moieties to the substrate is a post translation modification essential to various biological relevant processes. Owing to the important contribution of ubiquitin mediated proteostasis in maintaining physiological balance, the discovery of ubiquitin mediated protein degradation was awarded Nobel Prize in chemistry for the year 2004. Generally, ubiquitylation involves covalent attachment of 76th amino acid (glycine) of ubiquitin protein to lysine residue of the selected target protein through an isopeptide bond. This isopeptide bonding is between carboxyl group of ubiquitin and amino group of the substrate. Depending upon the amino acid involved in ubiquitin linkages and number of ubiquitin moieties attached to the substrate, ubiquitination can be categorised into mono-ubiquitination, multi-mono-ubiquitination or polyubiquitination. For example, K-63 linked mono-ubiquitination leads to cellular signalling while multi-mono ubiquitination other than K63 linked ubiquitination is involved in degradation of target proteins. During polyubiquitylation, one of seven lysine residues of ubiquitin is

involved in catenation of ubiquitin moieties. Attachment of 4 or more ubiquitin moieties through K48 or K11-linked catenation leads to proteasomal degradation of proteins [3].

Ubiquitination of proteins is catalysed by E3 ubiquitin ligases. E3 ubiquitin ligases can be allocated into two major categories, RING finger and HECT type E3 ubiquitin ligase. RING finger E3 ligases recruit the target proteins to the vicinity of E2 enzyme to promote their ubiquitination whereas, HECT type E3 ubiquitin ligase can both recruit and ubiquitylate its target substrate independent of E2.

RING finger E3 ubiquitin ligases are the major and most well studied class of E3 ubiquitin ligases. RING finger E3 ubiquitin ligases can work either alone or in conjugation to a multi subunit protein complex such as APC/C complex or SKP1-Cullin 1-F-box protein (SCF) complex. SKP1 is S phase kinase associated protein 1, it acts as an adapter protein for the SCF complex. In mammals there are 8 cullin proteins (cullin 1, cullin 2, cullin 3, cullin 4A cullin 4B, cullin 7 and cullin 9) and cullin 1 functions as scaffold for SCF complex. C- terminal of cullin 1 binds to Rbx1 and facilitates the recruitment of specific E2 enzyme whereas its N-terminal is bound to SKP1, which is further bound to the F-box protein, a variable component of the SCF complex.

F-box proteins are the substrate recognizing component of the SCF complex and they mostly recognise the substrates following specific phosphorylation. Substrate phosphorylation serves as signal for F-box proteins to identify them and recruit them to the SCF complex for their ubiquitination. They have two major functional motifs, a variable substrate recognizing motif mostly at the C-terminal and F-box motif (first identified in FBXO1 or cyclin F) located mostly at the N-terminus facilitating its interaction with SKP1 protein of the SCF complex.

F-box proteins: variable component with stringent function

The human genome encodes around 69 F-box proteins which are divided into 3 broad classes based on the presence of substrate recognition motifs present in the C-terminal [1]. F-box proteins containing WD repeats domains are grouped into FBXW category. This group includes ten F-box proteins of which FBXW1 or β-TRCP1, FBXW11 or β-TRCP2 and FBXW7 are the most thoroughly investigated. The second category include twenty two F-box proteins with leucine rich repeats referred as FBXLs, SKP2 is the best studied ubiquitin ligase belonging to

this category. Rest thirty seven F-box proteins are categorised as FBXOs as these F-box proteins do not have any common substrate interaction domain like the other two classes. The members of this group are not well studied. Till now only a handful of F-box proteins have been characterized. The important biological function of many of the orphan F-box proteins is still not well documented and is a subject of further investigation.

Owing to the high substrate specificity and ability to target specific proteins, F-box proteins are reported to regulate crucial processes involving unidirectional advancements such as circadian rhythm and cell cycle progression. F-box proteins act as ON/OFF switches to maintain unidirectional drive. They play a central role in regulating the turnover of several cell cycle regulatory proteins to allow efficient genome duplication and segregation. Apart from its role in regulating synchrony in cell cycle, F-box proteins are also crucial in maintaining genomic integrity by regulating effective DNA damage response and repair. Based on these roles F-box proteins are further classified as oncogene or tumour suppressors but some of the F-box proteins were found to have dual roles in a context dependent manner. For instance β-TrCP, a member of FBXW family, functions either as tumor suppressor or oncogene in context dependent manner. Human genome has two β-TrCP genes, β-TrCP1 (Fbxw1) and β-TrCP2 (also termed HOS or Fbw11). β-TrCP is involved in the proteasomal degradation of many proteins involved in cellular signalling and cell cycle progression, cell proliferation, circadian clock, oxidative stress and neurodegeneration, and metabolism, inflammatory and numerous stress responses. β-TrCP activates NF-κB signalling through proteasomal degradation of IκBα. β-TrCP is also known to inhibit β-catenin signalling by degrading it in a proteasome dependent manner. β-TrCP plays an important role in the regulation of cell cycle progression by degrading Emi-1, Wee1 and Cdc25A. Emi-1 degradation is important to activate anaphase promoting complex. β-TrCP1 knockout mice develop normally while β-TrCP2 knockout died in utero because of p19 stabilization consequently growth arrest [4].

Many of the F-box proteins are also reported to act as oncogene. The most widely studied F-box protein acting as an oncogene is SKP2 or FBXL1. Exogenous expression of SKP2 in prostate gland of transgenic mice resulted in carcinoma development. Additionally, its overexpression in T-lymphoid lineage resulted in T cell lymphoma. Moreover, SKP2 overexpression is observed in many human cancers as nasopharyngeal, breast, melanoma,

pancreatic cancer, prostate cancer and colorectal cancers. Higher level of SKP2 is associated with increased tumour size, increased histological grades and poor prognosis in hepatocellular, breast cancer, gastric cancers and melanoma. SKP2 has been reported to act as an oncogene by targeting many tumour suppressors such as p21, p27 and p57. SKP2 also targets retinoblastoma like proteins RBL2, FBXO1 and many more [4].

FBXW7 is one of the best studies F-box proteins and is a member of haploinsufficient tumour suppressors (4). It has been reported to be inactivated due to mutations in many types of cancers and around 6% of primary cancers have non-functional FBXW7. FBXW7 is frequently mutated in around 30% of acute lymphoblastic leukaemia, 35% of cholangiocarcinoma, 9% of endometrial and colorectal cancers and 6% of gastric cancers harbour FBXW7 mutations. FBXW7 acts as a tumour suppressor by degrading various oncoproteins like MYC, JUN, NOTCH, mTOR, myeloid cell leukaemia 1(MCL1), SERBP, KLFs, CCAAT/enhancer binding proteins(C/EBPs), MED13 and MED13L. Many other F-box proteins such as FBXW8, FBXL3 FBXO1, FBXO4, FBXO11, FBXO18 and FBXO31 have been reported to act as tumour suppressors [4].

Expanding roles of FBXO31 as a potent tumour suppressor

One of the emerging F-box proteins FBXO31 is reported to act as a tumour suppressor in many cancers; however it is reported to act as an oncogene in lung and oesophageal carcinoma [5 – 7]. FBXO31 is located in 16q24.3 region in human genome [7]. Inactivation of FBXO31 is mostly due to loss of heterozygosity (LOH) in many cancers including breast, ovarian, hepatocellular and prostate carcinoma. Recently it has also been documented that posttranslational level of FBXO31 is regulated by multiple miRNAs in different cancers [8, 9]. FBXO31 is a potent senescence inducer. It gets stabilized upon any sort of genotoxic stress and arrests the cells at G1 phase of the cell cycle through proteasomal degradation of cyclin D1 thus, preventing the partitioning of the damaged genome. Additionally, stabilization of FBXO31 upon genotoxic stresses leads to degradation of MDM2 eventually allowing robust stabilization of p53 [10]. The activated p53 then performs the downstream functions depending upon the extent of DNA damage allowing repair, senescence induction or apoptosis. Patient samples analysis revealed that the absence of FBXO31 leads to increased tumour size, tumour cell infiltration, cancer grade and poor prognosis. Piling literatures on various aspects of FBXO31 showed the involvement of

FBXO31 in cell cycle regulation. FBXO31 maintains the unidirectional progression of cell cycle by promoting proteasomal degradation of an important DNA licensing factor Cdt1 at G2 phase [11]. It also targets a key transcription factor FOXM1 at G2/M boundary to efficiently regulate multiple genes at the same time [12]. In addition to its role in cell cycle progression, it has been documented that FBXO31 regulates in neuronal morphogenesis and migration by targeting polarity protein Par6c for proteasomal degradation [13].

Conclusion and future perspective

Rising evidences suggest close involvement of F-box proteins in maintaining cellular homeostasis. Deregulation of one or more F-box proteins lead to various diseases such as neurodegenerative diseases, cancer and immune deficiency mostly due to altered expression and activity of their targets. Therefore, it is important to understand the cellular targets of F-box proteins and their role in various biological processes.

Among 69 F-box proteins only four F-box proteins (FBXW1, FBXW7, FBXW11 and SKP2) have been studied extensively till date. Knowledge regarding the cellular functions and targets of rest of the 65 F-box proteins is still at the primitive stage. Looking at the expanding functions of even these less studied F-box proteins necessitate a complete and thorough understanding of all the F-box proteins in normal and pathophysiological conditions. Understanding the pathway upstream and downstream of these F-box proteins will help in precise regulation of their substrates.

Recently, FDA has approved proteasome inhibitor Bortezomib for the treatment of multiple myeloma. Ironically, inactivation of proteasome affects all the cellular processes requiring intact proteasome system and thereby resulting in undesired side effects.

Taking this into consideration it would be beneficial to target a particular F-box protein to regulate the turnover of its disease associated cellular targets. This approach was utilized in designing the inhibitor against a well-studied F-box protein SKP2 [4]. Recent advancements in the clinical trials with SKP2 inhibitor showed promising therapeutic outcome as anticancer agent. However, this strategy will also affect the turnover of other targets essential for cellular function indicating that this strategy may lead to the stabilization of other unwanted substrates of the targeted F-box which may diminish its potential to act as an effective drug.

Of note, a better approach will be to design small molecules targeting the interface of F-box protein and its disease associated selected substrate. This strategy will reduce the undesired side effects by affecting the turnover of a particular substrate and at the same time maintaining the normal turnover of its other cellular targets. In order to utilize this therapeutic strategy, it is essential to understand the role of F-box proteins under various biological functions, tissue specificity, redundancy and its crystal structure. So, exploring the roles of various F-box proteins is essential to come up with effective strategies against stubborn ailments involving altered protein turnover.

Reference

1 Nakayama,K.I., and Nakayama,K. (2006). Ubiquitin ligases: cell-cycle control and cancer. **Nat. Rev. Cancer** 6**,** 369-381.
2 Frescas, D. and Pagano M. (2008). Deregulated proteolysis by the F-box proteins SKP2 and beta-TrCP: tipping the scales of cancer. **Nat. Rev. Cancer.** 8, 438-449.
3 Skaar,J.R., Pagan,J.K., and Pagano,M. (2013). Mechanisms and function of substrate recruitment by F-box proteins. **Nat. Rev. Mol. Cell Biol.**, 14**,** 369-381.
4 Wang, Z., Liu, P., Inuzuka, H. and Wei, W. (2014) Roles of F-box proteins in cancer. **Nat. Rev. Cancer.** 14,233-247.
5 Santra,M.K., Wajapeyee,N., and Green,M.R. (2009). F-box protein FBXO31 mediates cyclin D1 degradation to induce G1 arrest after DNA damage. **Nature** 459**,** 722-725.
6 Huang, H.L., Jiang, Y., Wang, Y.H., Chen, T., He, H.J., Liu, T., Yang, T., Yang, L.W., Chen, J.,Song, Z.Q., Yao, W., Wu, B. and Liu, G. (2015) FBXO31 promotes cell proliferation, metastasis and invasion in lung cancer. **Am J Cancer Res**. 5, 1814-1822.
7 Kumar,R., Neilsen,P.M., Crawford,J., McKirdy,R., Lee,J., Powell,J.A., Saif,Z., Martin,J.M., Lombaerts,M., Cornelisse,C.J., Cleton-Jansen,A.M., and Callen,D.F. (2005a). FBXO31 is the chromosome 16q24.3 senescence gene, a candidate breast tumor suppressor, and a component of an SCF complex. **Cancer Res.**, 65**,** 11304-11313.
8 Liu, D., Xia, H., Wang, F., Chen, C. and Long J. (2016) MicroRNA-210 interacts with FBXO31 to regulate cancer proliferation cell cycle and migration in human breast cancer. **Onco Targets Ther.** 9, 5245-5255.
9 Zhang X, Kong Y, Xu X, Xing H, Zhang Y, Han F, Li W, Yang Q, Zeng J, Jia J, Liu Z. (2014) F-box protein FBXO31 is down-regulated in gastric cancer and negatively regulated by miR-17 and miR-20a. **Oncotarget**, 5,6178-6190.
10 Malonia, S.K., Dutta, P., Santra, M. K. and Green, M. R. (2015) F-box protein FBXO31 directs degradation of MDM2 to facilitate p53-mediated growth arrest following genotoxic stress. **Proc Natl Acad Sci USA**, 112,8632-8637.
11 Johansson, P., Jeffery, J., Al-Ejeh, F., Schulz, R. B., Callen, D. F., Kumar, R. and Khanna, K. K. (2014) SCF-FBXO31 E3 ligase targets DNA replication factor Cdt1 for proteolysis in the G2 phase of cell cycle to prevent re-replication. **J. Biol. Chem.**, 289, 18514-18525.

12 Jeffery, J. M., Kalimutho, M., Johansson, P., Cardenas, D. G., Kumar, R. and Khanna KK. FBXO31 protects against genomic instability by capping FOXM1 levels at the G2/M transition. **Oncogene**, 2016

13 Vadhvani, M., Schwedhelm-Domeyer, N., Mukherjee C., and Stegmüller, J. (2013)The centrosomal E3 ubiquitin ligase FBXO31-SCF regulates neuronal morphogenesis and migration. **PLoS One**, 8, e57530.

Nidhi's opinion

Today, the status of science in India is at rapid pace; it is highly inspiring and promising. We have highly motivated and intellectual young investigators, increased scientific funding opportunities, and curious naïve researchers. Today, our research laboratories make scholars learn much more than just doing experiments. We discuss recent published research, work on novel hypothesis, design and execute independent research projects. We appreciate and critique not just others but our own research work. However, I believe we should have more scientific encounters, such as seminars, journal clubs, scientific workshops and conferences. We should be able to articulate and present our data amongst fellow researchers, as a story that flows and doesn't leave behind the audience misconstrued. Scientific gathering platforms help us develop and grow as researchers.

Science to me is complete freedom of thought, achieving the impossible and serving the society. As researcher, I have realized that one needs to have patience, perseverance and passion to enjoy science. The basic research helps resolve the fundamental problems of the society. The final aim for researchers should be to take the research progress from laboratory to the grass-root levels. We should perform focused research; translate our findings in novel scientific tools and advanced technology development.

Fortunately or unfortunately, the number of publications and impact factor is a major issue for research scholars apart from other research-related issues. One might not know you, but as soon as you publish a high-impact research work, it gets you instant recognition and focus. The majority of scholars believe that publications decide, in which laboratory one will land up for the postdoctoral fellowship, or get job letter from academic and research institutes. However, I have a slightly different opinion; publications give us scientific recognition but it does not necessarily define one's potential as a researcher. We need to understand that the ability to think rational, design novel research ideas, and present our research findings to a layman and how it will benefit us, is important to sustain as a good researcher. Publications might be the best way of presenting our research, but we should be more focused on the research objectives and outcome. Science is also about sharing and caring, which brings me to the second major issue. Today, many of our scientists and researchers believe in sharing research resources and pushing each other towards

progress. The interdisciplinary research approaches provides a comprehensive understanding of a research problem. Therefore, we should motivate this kind of scientific exchange and behavior to embark scientific progress as a community.

Today, India has well-equipped cellular and molecular biology research laboratories performing cutting edge research in science. The increased current cancer incidence highlights the need to investigate the cellular and molecular pathways altered in cancers. As we all know, Immunotherapy as emerged as potential therapeutic drug targets as possible cure to cancer. The progress made so far, motivates us to increase our understanding of both innate and adaptive immune system in cancer. Targeting cancer by altering one's own immune system is a promising medicinal approach and demands further investigation. Additionally, the newly identified genome editing tool, CRISPR technique may also serve as potential therapeutic tool in futuristic cancer treatment approaches. Scientists have already started experiments involving human genome editing using CRISPR. However, the ethical issues related to the CRISPR technique for human use remain unsolved.

About author: Nidhi Sharma is pursuing her Ph.D. in biology in IIT Jodhpur, Rajasthan.

International Congress of Cell Biology 2018

(a tripartite meeting of ISCB, APOCB and IFCB)

Venues for major events: Hyderabad

January 27 – 31, 2018

Title: **The dynamic cell: Molecules and mechanics to form and function**

Indian Society of Cell Biology (ISCB) represents India as a member country of International Federation of Cell Biology (IFCB) and Asian-Pacific Organization of Cell Biology (APOCB). ISCB, IFCB and APOCB have jointly agreed to hold an International Congress of Cell Biology (ICCB) in India. Dr. Vegesna Radha & Dr. Jyotsna Dhawan from Centre for Cellular and Molecular Biology would be organizing secretaries for ICCB.

The IFCB and APOCB are the two primary international organizations and their mission is to foster cell biology research across the world. IFCB and APOCB approached ISCB for holding the tripartite meeting on cell biology in India. ISCB agreed to provide a platform for jointly holding 13th IFCB congress and the 8th APOCB meeting in Hyderabad, India. This meeting also commemorates the 30th anniversary of Asian-Pacific Organization of Cell Biology.

This will be the first-ever joint meeting of these 2 international cell biology organizations as well as ISCB, and will see cell biologists across the world coming together on one platform. This meeting will bring together scientists from diverse disciplines working in various parts of the world but having the common goal of understanding the cellular and molecular biology of the cell and how cells function. We aim to create a platform for cell biologists, to have the opportunity of listening to latest discoveries in the field and, sharing results and ideas. It is hoped that this meeting will bridge people to initiate new collaborations.

Sessions on cell biology education, what a career in cell biology means, scientific writing and ethics are also planned. Latest tools available to cell biologists will also be discussed and showcased. Corporate sector that helps cell biology research by manufacturing/distributing equipment & resources will play an active part in showcasing their latest products. Highlights are

mini symposia, and a special session to commemorate discovery of GPI anchors. It is also proposed to host pre and post-conference satellite meetings in other cities of India.

The conference will include a full range of academic sessions, plenary lectures, and social/cultural events. We plan to have talks from 60-70 invited speakers. Other researchers will get an opportunity to showcase their work through platform/poster presentations. Scientists trained in physical or chemical sciences, but have an interest in understanding the biological cell, are encouraged to participate. We encourage particularly, the participation of young faculties and research scholars during this unique meeting in India.

About the city of Hyderabad

Hyderabad is an ancient city (4th largest in India) located in south- central India. It has a rich history with admixture of diverse cultures. It is famous for its cultural heritage, pearl trading and exotic cuisine. Hyderabad is also known for its IT/ Pharma & Biotech industries. Weather in January will be very pleasant with temps between 15^0C (low) and 28^0C (high).

All the information concerning registration, abstract submission and scientific aspects of the Conference, areas and special topics for scientific sessions will be available shortly on the IFCB/APOCB/ISCB/CCMB webpages.

English will be the official language of the conference.

Invitation for Annual General Body Meeting to all the members of the society

XL All India Cell Biology Conference and annual meeting of Indian Society of Cell Biology will be held in Jiwaji University, Gwalior between November 17-19, 2016. Annual General Body Meeting of the society will be held on Nov. 18, 2016 at 1800h in "Galav Sabhagar", Jiwaji University Campus, Gwalior.

All the members are cordially invited to attend it.

MOHIT K. SAIGAL & CO
CHARTERED ACCOUNTANTS

B-37/122, SAIGAL HOUSE
MAHMOORGANJ, VARANASI
UTTAR PRADESH-221010
PH.0542-2360015, M.No.7275660055

INDIAN SOCIETY OF CELL BIOLOGY

BALANCE SHEET AS ON 31 MARCH,2016

LIABILITIES	AMOUNT	AMOUNT	ASSETS	AMOUNT	AMOUNT
CAPITAL FUND ACCOUNT:			INVESTMENTS		
Opening Balance	24,13,624.05		HDFC FDR's	25,75,000.00	
			UTI Mutual Fund	9,962.17	25,84,962.17
Add: Excess of Income over			CURRENT ASSETS & LOANS &		
Expenditure	1,77,271.00	25,90,895.05	ADVANCES :		
			TDS F/Y 2013-14	21,141.00	
LIFE MEMBERSHIP FEES:			TDS F/Y 2014-15	5,321.00	
			TDS F/Y 2015-16	28,349.00	
Opening Balance	7,14,563.00		Workshop of XXXVIII AICB	1,13,159.00	1,67,970.00
			CASH & BANK BALANCES:		
Add: during the year	1,23,330.00	8,37,893.00	Cash	1,110.10	
			SBI, Varanasi	5,64,225.78	
Audit Fees Payable	2,000.00		Central Bank of India	115520.00	
Accounting Charges Payable	3,000.00	5,000.00			6,80,855.88
TOTAL		34,33,788.05	TOTAL		34,33,788.05

For INDIAN SOCIETY OF CELL BIOLOGY

As Per Audit Report of even date

For MOHIT K SAIGAL & CO.

Mohit K. Saigal
(PARTNER)
M.NO. 016988

PLACE : VARANASI
DATE : 16.10.2016

MOHIT K. SAIGAL & CO
CHARTERED ACCOUNTANTS

CA

B-37/122, SAIGAL HOUSE
MAHMOORGANJ, VARANASI
UTTAR PRADESH-221010
PH.0542-2360015, M.No.7275660055

INDIAN SOCIETY OF CELL BIOLOGY
Receipts & Payment A/c for the period 01.04.2015 to 31.03.2016

	RECEIPTS		AMOUNT		PAYMENT		AMOUNT
To	**Opening Balances:**			By	Investment in FDR (HDFC)		5,00,000.00
	Cash in Hand	23,690.10		By	Audit Fees Payable		3,000.00
	SBI, Varanasi	4,54,524.78		By	Printing & Stationery		2,920.00
	State Bank Of Hydrabad	137.00	4,78,351.88	By	Prise & Award		15,787 00
				By	Seminor & Conference		72,500.00
To	**Membership Fees :**			By	Legal Exps.		3,500 00
	Student & Ordinary	6,087.00		By	Travell Support Exps.		19,195.00
	Life Membership Fees	1,23,330.00	1,29,417 00	By	Bank Charges		712.00
To	Interest on SB A/c		10,311.00				
To	Interest From HDFC		2,55,138.00				
To	HDFC FDR Matured		4,25,000.00				
To	Interest Recevable		252.00				
				By	**Closing Balances:**		
					Cash	1,110.10	
					SBI, Varanasi	5,64,225.78	
					Central Bank of India	1,15,520.00	6,80,855.88
	Total		12,98,469.88		**Total**		12,98,469.88

For INDIAN SOCIETY OF CELL BIOLOGY

As Per Audit Report of even date

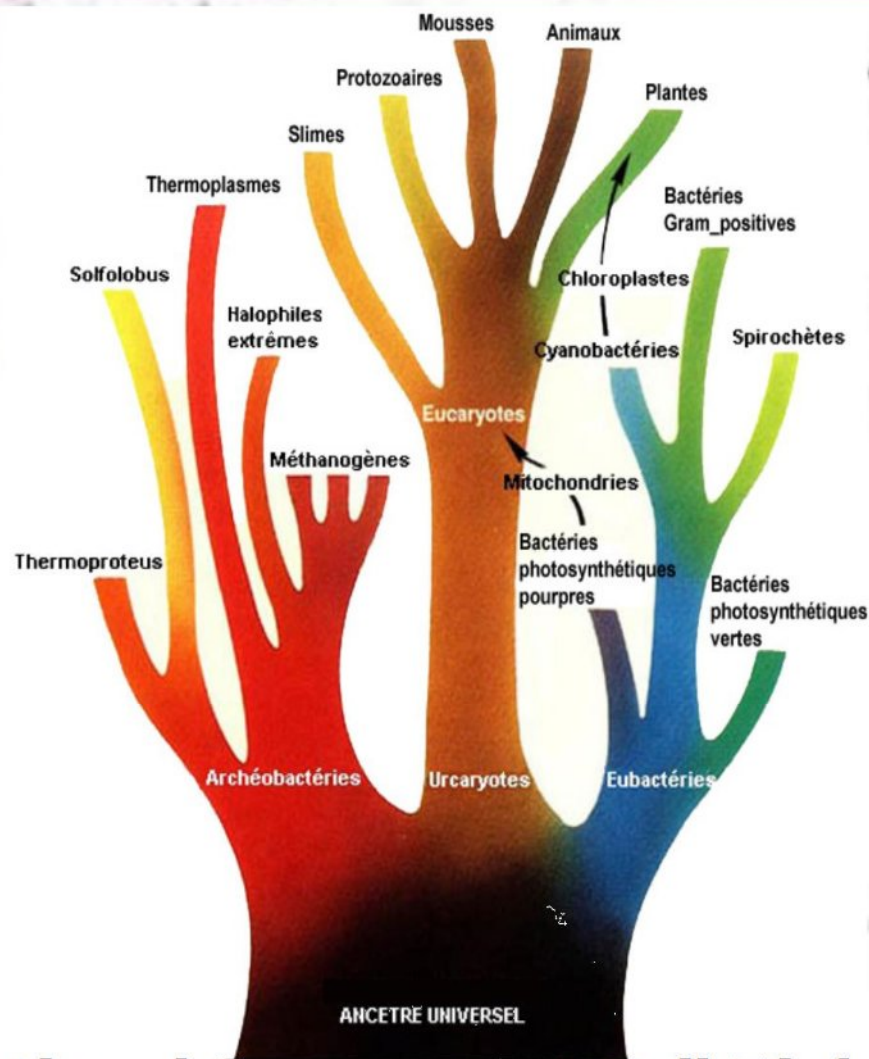For MOHIT K SAIGAL & CO.
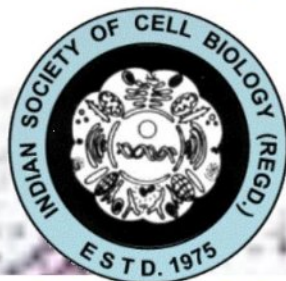(Chartered Accountants)

PLACE : VARANASI
DATE : 16.10.2016

Mohit K. Saigal
(PARTNER)
M.NO. 016988

Mousses
Animaux
Protozoaires
Plantes
Slimes
Thermoplasmes
Bactéries Gram_positives
Solfolobus
Chloroplastes
Halophiles extrémes
Cyanobactéries
Spirochètes
Eucaryotes
Méthanogènes
Mitochondries
Thermoproteus
Bactéries photosynthétiques pourpres
Bactéries photosynthétiques vertes
Archéobactéries
Urcaryotes
Eubactéries
ANCETRE UNIVERSEL

International Congress of Cell Biology: a tripartite meeting of ISCB, IFCB and APOCB January 27-31, 2018, Hyderabad, India

Published by