



On the Trail of Genomic Pioneers



Meet Rama Shankar Verma
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1) Can you tell us a bit about your research interests?

• In the area of Immunotoxins

Immunotoxins are chimeric proteins with a cytotoxic protein linked to a cell-targeting moiety such as a cytokine or monoclonal antibody. Immunotoxins offer means for selective targeting and elimination of cancer cells over-expressing specific cell surface receptors. After binding to target cells, these proteins are internalized by receptor-mediated endocytosis and cell death is induced by the toxin moiety. Our objectives are:

- (i) To produce a diphtheria toxin based chimeric protein for targeting adenocarcinomas over-expressing LHRH (leutinizing hormone releasing hormone) receptor.
- (ii) To alter the above protein by mutating its furin cleavage site to cleavage site for urokinase plasminogen activator so that the protein becomes dual specific for LHRHR and UPAR.
- (iii) To produce a humanized immunotoxin with GMCSF as the targeting moiety and human DNA fragmentation factor 40 as the effector molecule for targeted therapy of AML.

Targeted anticancer therapy with recombinant fungal immunotoxins:

Restrictocin constructs Immunotoxins are chimeric molecules in which cell binding ligands are coupled to toxins or their subunits. In our studies we are targeting c-kit

receptors, which is overexpressed on many cancer cell types like small cell lung carcinoma, GISTs, pancreatic cancer etc by fusing genetically kit ligand with a fungal toxin restrictocin. Restrictocin is highly specific ribonucleases, which cleaves a single phosphodiester, bond between G4325 and A4326 in a purine rich conserved domain in 28Sr RNA and thus inhibit protein synthesis.

Another peptide, a 12mer molecule, is reported to preferentially bind and internalize into HNSCC cells. The immunotoxin RT-HN1, an extended restrictocin is also a part of the study.

Objectives:

- a) To construct restrictocin based chimeric toxin RT-SCF by directly fusing genes for SCF with restrictocin and RT- HN1, extended restrictocin with HN1 peptide.
- b) Expression studies and purification of the recombinant Immunotoxins.
- c) To test the efficacy on cell lines expressing corresponding receptors

• Fanconi anemia

Fanconi anemia (FA) is a rare autosomal recessive /X-linked disease characterized by multiple congenital abnormalities, bone marrow (BM) failure, aplastic anemia, cancer/leukemia susceptibility. While the underlying molecular basis of the disease has been identified the molecular basis of the clinical features or symptoms of FA has not yet been completely elucidated.

Our research objectives are as follows:

- (i) Gene expression profiling of Fanconi anemia Patients and Carriers.
- (ii) Identifying marker genes that could be used



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for screening Fanconi anemia carriers.

(iii) Understanding the transcriptional regulatory role of FANCC and FANCA proteins in FA cell lines.

(iv) Verify epigenetic mechanisms of regulation (Methylation) that may exist in FA cell lines.

- **Stem cell**

Trans-differentiation of Stem cell into cardiac tissues

To assess the trans-differentiation potential of Adult Stem cell into Cardiomyocyte, this work uses Mouse bone marrow derived stem cells (BMSC) and allows them to differentiate into cells of cardiomyogenic lineage in presence of differentiation inducing factors like 5-Azacytidine (5-azaC) & Niche. We are using a biomaterial scaffold – Cardiogel, to imitate in vivo niche conditions of the Heart. Cardiogel along with 5-azaC has been found to increase differentiation of cells in cardiomyogenic lineage. Cardiogel also shows stress protection ability against Oxidative stress. BMSC growing on cardiogel showed increased proliferation, faster adherence and resistance to enzymatic dissociation. Cardiogel can be used as a delivery material for stem cells in Cardiac regeneration therapy though its tissue engineering abilities is yet to be harnessed. In order to generate Ventricular Cardiomyogenic Cell lines, we have generated Mouse Mesenchymal stem cell lines, which are now being characterized.

Besides these we have also standardized a faster & cost efficient protocol for cardiomyocyte isolation and culture.

Developing patches using biodegradable material and stem cells

Cells when grown in culture adhere to the surface of a treated plastic flask or dish, which is very different from the 3 Dimensional interactive environments in which they reside in vivo. While recreating an exact environment akin to the natural matrix is a considerable feat, a 3 dimensional space can be fabricated that can support proliferation and induce differentiation spontaneously or by directed measures. Scaffolds can be biological or artificial in origin. Biological scaffolds tend to have some advantages such as; being biological in nature they are much more compatible and are usually biodegradable, easily synthesized and fabricated.

The focus of our study is achieving differentiation of stem cells in vitro and maintaining a considerably sized culture of the same for cell-based therapy.

Our research objectives are as follows:

- (i) To study the proliferation and maintenance of stem cells over PLA.
- (ii) To investigate the possibility of the creation of niche like structures inside these cell clumps. Stem cell 'niche' development and identification/characterization.
- (iii) To study the differentiation of bone marrow derived Mesenchymal stem cells into specific lineages by loading microspheres with specific differentiation agents such as 5- Azacytidine.



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(iv) Refining and standardizing protocols for use of these microspheres as in vitro scaffolds for the maintenance of potency and proliferation of stem cells.

Hepatic transdifferentiation of human bone marrow mesenchymal stem cells

Human bone marrow derived mesenchymal stem cells (BMSCs) have got tremendous potential to differentiate into many cell kinds of the mesodermal, ectodermal and endodermal lineages. Hepatic transdifferentiation of hBMSCs by various hepatogenic growth factors, serum components as well as by genetic manipulations have been reported both in vitro and in vivo. A culture system containing cholestatic serum (obtained from obstructed bile duct) is an innovative way of proliferating mesenchymal stem cells and differentiating them selectively towards hepatocyte like cells. In such a hepatogenic environment, BMSCs are fine tuned towards hepatic lineage due to presence of liver specific activating factors, as is happening in vivo in liver injury conditions to maintain hepatic homeostasis. The primary objective of this project is to evaluate the effectiveness of cholestatic serum of a unique kind (obtained from heart failure patients having etiology of ischemic hepatitis or cardiac jaundice) in inducing hepatic transdifferentiation of hBMSCs on various scaffolds of natural origin. Human BMSCs derived can be used as a novel in vitro model for hepatotoxicity testing. Also, in future, it can be used as effective cell source for noninvasive

therapeutic transplantation in treating injured livers. However, a more detailed biochemical profiling and molecular characterization of cholestatic serum obtained from heart failure patients is needed before taking it for clinical applications.

Development and Characterisation of Tissue Engineered Xenografts

Acellular Tissue-engineered or decellularized xenografts and homografts have already been implanted in humans or are currently approaching the clinical setting. Efficiently decellularized, tissue-engineered homografts might prolong durability by reducing recipient inflammation, fibrous scarring, and calcification, ultimately decreasing the number of patients requiring reconstructive cardiac surgery. The postulated proof of immune reactions towards acellular xenografts and growth potential being translated to humans lacks any scientific evidence. The objective of this project is to examine host immune response toward acellular Tissue engineered xenografts and further examine the effects of cell seeding on acellular scaffold.

- **Understating Role of Transcription Factor**

Neural retinal leucine zipper (Nrl): Nrl Neural retinal leucine zipper is transcription factor of Maf-subfamily. Nrl is conserved in vertebrates and is specifically expressed in photoreceptor and pineal gland. Nrl acts synergistically and antagonistically with transcription factors to regulate expression of rhodopsin and other rod specific genes. Mutation in Nrl are associated



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with retinal degenerative disease in humans. We are characterising biophysically Nrl DNA interaction.

- **Folate Receptor a Model for Targeted Approach for Cancer**

Methotrexate has been used as an effective anticancer drug for a long time. Conceptually it is accepted that methotrexate (MTX) and folic acid (FA) are transported by folate receptors in cancerous cells, but the exact mechanism of MTX uptake in human leukemia is unknown. The objective of this study was to investigate different transport systems for FA and MTX, and to delineate their uptake mechanism in MOLT4, K562, Hut78 leukemia cells and normal human T cells. In MOLT4, uptake of MTX was higher than FA, similar to that of K562, Hut78 and normal T cells. In MOLT4 cells MTX uptake was maximum at pH 7.4 where as FA uptake was maximum at pH 4.5. Uptake of FA and MTX was significantly inhibited by anions, suggesting anion dependent transport system. FA Uptake was found to be energy dependent where as MTX uptake was energy independent. RT-PCR and Immunofluorescence results demonstrated the presence of reduced folate carrier as well as proton coupled folate transporter and absence of folate receptor in MOLT4 and normal T cells. These data suggest the existence of two separate and independent carrier-mediated transport systems for the uptake of FA and MTX in normal and leukemic human T cells.

Poly(amidoamine) {PAMAM} dendrimers have been widely used as a drug carriers for the treatment of various cancers. The purpose of this study is to deliver the drug methotrexate (MTX) and doxorubicin (DOX) into HeLa 229 cancer cells, utilizing the fourth generation, amine terminated PAMAM dendrimer as the drug carrier. In-vitro kinetic studies suggested controlled release of both MTX and DOX in the presence of PAMAM dendrimer. The cytotoxicity studies indicated the improved cell death by dendrimer-drug combination, compared to the control experiments with dendrimer or drug alone. Furthermore, HeLa 229 cells were imaged for the first time utilizing the intrinsic emission from the PAMAM dendrimers and drugs in the absence of conventional fluorophores. Mechanistic studies suggest decreased rate of drug efflux in presence of relatively large sized PAMAM dendrimer, which generates high local concentration of the dendrimer-drug combination inside the cell, providing an easy way to image cell lines utilizing their intrinsic emission properties.

Receptor mediated delivery of siRNA enables silencing of target genes in specific tissues. Folate receptor (FR) is an attractive target for tumor-selective gene delivery. The focus of this study was to deliver the dihydrofolate reductase (DHFR) siRNA expressing plasmid and to silence the DHFR gene in FR positive KB cells, by complexing the plasmid with a folate-polyethylene glycol-polyethylenimine (FOL-PEG-PEI) conjugate, as a gene carrier. A DHFR siRNA sequence was cloned into a pSUPER-RNAi vector and complexed with the FOL-PEG-PEI conjugate. The complex was characterized by particle size



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analyzer, gel retardation and DNase protection assay. The FOL-PEG-PEI/pSUPER-siDHFR complex was transfected to FR overexpressing (KB) and FR negative (A549) cells. The transfection efficiencies and gene inhibition were analyzed by fluorescence microscopy and RT-PCR. The pSUPER-siDHFR/PEI-PEG-FOL complex delivered the siRNA vector and inhibited DHFR gene in KB cells, while A549 cells were unaffected. Lipofectamine mediated transfection of pSUPER-siDHFR, delivered the vector and inhibited the DHFR gene in both KB and A549 cells. FR mediated delivery of siDHFR complexed with PEI-PEG-FOL conjugate inhibits the DHFR expression in FR positive cells alone. This strategy can be extended to deliver a wide range of drugs and post-transcriptional gene silencing therapeutics.

- **Monoclonal antibody production for developing tool for cancer therapy**

Epithelial cell adhesion (EpCAM) has gained interest as a potential therapeutic target and is an attractive candidate tumor-associated antigen (TAA) for antibody-based immunotherapy. The correlation between EpCAM expression and prognosis seems to be tissue specific. Expression of EpCAM is known to be unregulated in a variety of carcinomas including those of the lung and colon. The objective of this study is to generate monoclonal antibody against recombinant EpCAM protein and to evaluate the antibody in normal and retinoblastoma tissue sample by Immunohistochemistry and Flow Cytometric analysis.

2) Which research study or work has strongly influenced your thought and research goals?

1. Tissue regeneration using stem cell
2. Finding a new target or pathways through microarray and gene chip technology
3. Developing new chimeric protein as drug target for cancer treatment

3) What are the unique features of the biotech department?

Please go to Biotech department Web site

4) What are the new initiatives you have taken to incorporate and help early stage researchers and students to develop research temper?

Let choose their project in which our research is going, spent some time to understand the research going in our lab and then decide which area they want to go and pursue their goal by asking some basic questions in particular field.

5) What are your collaborations with the Industry? How do you think Industry can develop better collaborative models with Academic Research efforts?

Yes we have collaborated with two industries and we are focusing understand the role of industry to speedup the target identification by providing the help in using some advanced technology and also to help industry to develop human resources.



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6) Can you tell us about research projects that students or faculties have initiated at your department?

We work on disease related research in our department basically cancer, tissue regeneration using stem cells. etc.

7) What are initiatives taken by the institutes to familiarize the students with the industry?

We have industrial visit of 3rd year students every year and through lecture contributed by scientists and entrepreneurs who work in industry.

8) Anything you want to say to the students that will inspire them to pursue career in biotechnology?

Do the best in your choice of field, and success will come.

9) What are the new genomic tools or technologies you used for your research?

We use microarray, southern hybridization, and subtractive DNA hybridizations techniques in our laboratory

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