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The Indian Journal of Genetics and Molecular Research (Half yearly) will publish high-quality, original research papers, short reports and reviews in the rapidly expanding field of human genetics. The Journal considers contributions that present the results of original research in genetics, evolution and related scientific disciplines. The molecular basis of human genetic disease developmental genetics neurogenetics chromosome structure and function molecular aspects of cancer genetics gene therapy biochemical genetics major advances in gene mapping understanding of genome organization.

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Medical Biotechnology and the Challenge of Chronic disease

B.S. Ramakrishna
Christian Medical College, Vellore

The field of Medical Biotechnology has changed our lives in the 21st century to a great extent in understanding our genome, proteome, metabolome, microbiome, pharmacogenomics, nutrigenomics, nanomedicine, regenerative medicine and so on and so forth. Though the cutting edge technologies are available for diagnostics and therapy, chronic diseases remain a challenging. In India chronic diseases are projected to account for 53% of all deaths. World Health Organization (WHO) projects that in India over the next 10 years more than 60 million people will die from a chronic disease and deaths from infectious diseases, maternal and perinatal conditions, and nutritional deficiencies combined will decrease by 15%. However, the deaths from chronic diseases will increase by 18%.

One such chronic disease is Crohn's disease which causes chronic inflammation of the intestine. It is caused by an abnormal innate immune reaction to intestinal bacteria in an individual of the appropriate genetic makeup. At Christian Medical College, Vellore, over a period of two decades number of cases diagnosed with Crohn's disease is comparatively higher than that of TB.

TLRs and NOD receptor are two receptor families determine the balance between tolerance and inflammation. NOD2 mutations are associated with ileal crohn's disease in Western countries. Whereas those common NOD2 mutations (R702W, G908R, F1007S) are lacking in Indian patients. Also, known Single Nucleotide Polymorphism (SNPs) in Heat Shock Protein 70, Tumor Necrosis Factor alpha promoter, IL23 receptors are not found in Indian patients with Crohn's disease. Hence, based on the prior experience in immunopathology of the disease, our group has been working on identifying a unique immune footprint for Crohn's disease in Indians.

Using the model of inflammatory bowel disease in Indians, this talk attempts to show how focused research may provide clues that can be translated to therapy or to public health measures that treat or prevent diseases.

Stem Cell Therapy - Liver Disease and Regenerative Medicine

Rosy Vennila
Stanley Medical College, Chennai

The Liver is a complex organ performing several vital functions in man. In acute disease and following surgical resections, the liver can regenerate, wherein the hepatocytes enter the cell cycle and divide repeatedly till the hepatic mass is restored. In the event of chronic injury, the liver loses this capacity to regenerate resulting in Hepatic Failure. Liver Transplantation is the treatment in this situation.

There is a growing interest in the field of Regenerative Medicine, Tissue engineering and cell therapy. This has triggered research into search for such options to replace Liver transplantation. Stem cells and progenitor cells belonging to various lineages have been identified in Adult Liver. Possibilities exist in the future for use of hepatocytes, stem cells or liver progenitors to treat decompensated Liver diseases.

The Insights provided by such research, as well as the feasibility and hurdles in clinical translation in Hepatology will be discussed.

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Angiogenesis: The formation of Blood Vessels

Suvro Chatterjee
AU-KBC Research Centre, Anna University, Chennai

Angiogenesis is a key cellular event essential during growth and development. It involves in the growth of blood vessels by sprouting and intussusception. The interplay maintains a ratio in forming vessels. Shift in the balance leads to an unchecked vessel leads to vascular diseases. Formation of blood vessels follows two morphologically distinct patterns 1) Sprouting and 2) Intussusceptive angiogenesis. Sprouting angiogenesis is the formation of new blood vessel from pre-existing one. Intussusception, an alternative to sprouting mode of angiogenesis, is new blood vessel formation by splitting off existing ones. It is characterized by the formation of intraluminal pillars within blood vessels and subsequently fuse, which result in vessel expansion and remodeling. The intussusceptive angiogenesis permits rapid expansion of the capillary plexus by creating a hierarchical tree that leads to modification of the branching geometry of supplying vessels. Our work shows that nitric oxide signaling leads to changes in the rate of sprouting and intussusceptive angiogenesis. We aim to elaborate the specific molecular mechanism of nitric oxide signaling in defining the ratio of sprouting and intussuscpetive angiogenesis in health and diseases.

Microarrays: Bidding Adieu?

V.L. Ram Prasad
Spinco Biotech, Chennai

A microarray is an analytical device that comprises an array of molecules (oligonucleotides, cDNAs, clones, PCR products, polypeptides, antibodies, and others) or tissue sections immobilized at discrete ordered or nonordered micrometer-to-millimeter-sized locations on the surface of a porous or nonporous insoluble solid support. These devices have been highly effective for the simultaneous detection of large numbers of SNPs/transcripts in a sample, and microarrays have become important analytical tools in many branches of the biological sciences. A microarray-based analytical strategy is quicker and more convenient than serial testing for each analyte, and it has been successfully applied to both RNA and DNA-based assays. The current scope of microarray applications that includes resequencing, mutation detection, copy number variation, comparative genome hybridization, drug discovery, expression analysis, is shrinking due to the advancements in the Next generation sequencing. Having said that Microarrays continue to play an important role in validation and Diagnostics.

Application of Next Generation Sequencing in Fundamental and Clinical Sciences

Karthi Sivaraman

Next generation Genomics Facility, C-CAMP, Bangalore

Next Generation Sequencing (NGS) has opened the floodgates on biological research in the recent years. Given its ability to interrogate entirely novel genomes, and the ability to aid novel discoveries in well-studied novel systems, NGS has rightly replaced microarrays as the preferred tool for high throughput biological discovery. There are many instances where high throughput sequencing has aided the both fundamental discovery as well as applied sciences. For instance, high throughput sequencing has allowed scientists to understand host specific evolution of uropathogenic *Escherichia coli*. Similarly, genomics has been used to understand the spread and evolution of the *Vibrio cholerae*. In the first part of my talk, we will see examples of how NGS facilitates the understanding varied biological questions: from plants, to non-model animals, to disease causing pathogens. In the second part, we see how NGS provides a way to understand regulatory networks in pathogens. Last, but not least, NGS technologies provide a window into the fascinating world of cancers. Cancers are diseases of the genome; a case of what happens when genomes go awry. NGS technologies let us have a glimpse of changes that happen within the cancer genome. In the third part of the talk, we will see examples of how NGS technologies lead to better cancer treatment.

Molecular Pathogenesis of Immune System Disorders

Madhumathi

Scientist, Stem cell and Molecular Biology Lab, IIT- Madras

Immune system is a highly organized and complex network of tissues, cells and molecules which safeguard the body. The major purpose of the immune system is to defend the body from any infection, foreign molecules or external injury. But if the very system meant to protect falls sick, how would the body respond? The system gets confused and goes haywire resulting in erratic and adverse symptoms. The immune system may become either over-reactive, under reactive or dysregulated. The molecular mechanisms of few of such diseases like Hyper IgE syndrome (genetic disorder), Lymphatic filariasis (parasitic infection) and Lambert–Eaton myasthenic syndrome (autoimmune disease) will be discussed in the talk. Breaching of self and non-self distinction due to genetic anomaly will result in hyper IgE syndrome characterized by susceptibility to bacterial infection and skin diseases. Similarly when parasitic multi-cellular worms selfishly down regulate immune response of the host to avoid long-term detection, there is again the component of induced anarchy in immune molecular mechanisms. Finally when immune system is auto reactive to neuro receptors there would be progressive loss of muscle weakness attributed to cross-reactive antibodies in the neuro-muscular junction. In conclusion, the talk would attempt to shed light on the existing molecular mechanisms to understand immune disease process for developing viable medical intervention for long-term disease management.

Biology of Angiogenesis

Suvro Chatterjee
AU-KBC Research Centre, Anna University, Chennai

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Application of Next Generation Sequencing in Fundamental and Clinical Sciences

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Study on CD40L mimetic molecules on Dendritic cells

S. Sivagami, M.Yogalakshmi, and M. Sivanandham

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Dendritic cells are among the most effective antigen presenting cells which are initially immature and have to mature for the induction of an efficient immune response. An important signal for initiating maturation of DCs is through the CD40-CD40L signaling pathway. Activating dendritic cell through CD40L can enhance the immune response. Herein we are introducing Ammonium benzoyl trimethylchloride (ABTC), N Benzhydrylbenzamide (NBB), 3-(Dimethylamino) propiophenone hydrochloride (3-DPH) are CD40L mimetics that was designed using LigBuilder which is a structure based drug design program. These molecules are shown to bind to its cognate receptor CD40 by computational docking.

We utilized dendritic cells from peripheral blood monocytes (PBMC) using GM-CSF and IL-4 were treated with varying concentrations of these compounds and observed for antigen processing and presentation. Mimetics can stimulate dendritic cell and thereby lead to maturation. The antigen processing capacities of dendritic cells were observed by treating the cells with FITC- Dextran. Although these mimetic molecules are efficient, among these 3-DPH shows good antigen processing capacity in a concentration of 8 μM. The differentiation and maturation induction capacity of these organic compounds provides scope for developing it as an alternative to the recombinantCD40L molecules which are prone to degradation.

Stem Cells: A Promising Candidate for cardiovascular Diseases

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[1]Dept.of Biotechnology, Dr.MGR Educational Research Institute, Chennai, [2]Dept. of Biotechnology, D.G Vaishnav College, Chennai

Despite the development of new therapeutic strategies and surgical treatments, heart attacks and congestive heart failure remain among the Nation's most prominent health challenges despite many breakthroughs in cardiovascular medicine. Various experimental studies prove that the infusion of stem cells may improve cardiac regeneration in several ways. Moreover, predominantly bone marrow derived cells were shown to restore blood flow, thereby providing a novel therapeutic option for the prevention and/or treatment of heart failure. Recent research is providing early evidence that adult and embryonic stem cells may be able to replace damaged heart muscle cells and establish new blood vessels to supply them. Thus the adult stem cell therapy today holds the promise of replacing lost heart muscle and enhancing cardiovascular revascularization, thereby treating the cardiovascular diseases promisingly. This is due to plasticity of adult stem cells.

Keywords: Stem cells; Cardiac regeneration, Cardiovascular revascularization; Plasticity.

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Applications of Tissue Microarray (TMA) platform for the Molecular characterization of Cancers

Siddhartha Roy, K.N.C. Murthy, Chandra Rao Juvva

Triesta Reference Laboratory, HealthCare Global Enterprises, Bangalore 560027

Tissue Microarray (TMA) facilitates rapid high throughput analysis and profiling of molecular targets on multiple tissue samples simultaneously. The multifold approach helps parallel reactions to happen at the same time, reducing run-time and minimizing tissue loss or damage. Construction of TMAs comprises of making cylindrical core specimens from 100s of FFPE tissue samples and arranging them in the form of arrays on a TMA block. Each of these blocks can be subjected to molecular reactions at the DNA, RNA or the protein levels. A single TMA procedure can evaluate 100s of samples at the same time for a single target, contrary to conventional practices where only one sample can be characterized at a time. Most of the applications of TMA have been in the field of cancer research and diagnostics. Examples include qualitative and quantitative evaluation of molecular alterations in tumor samples, analysis of prognostic and predictive markers, search for newer drug targets in the molecular level and understanding of tumor staging and progression. The presentation would comprise of application of TMAs in cancer research, drug development & validation and cross-tissue gene expression studies. We would like to discuss some limitations of TMA platforms, the way it can automated and with the help of dedicated softwares, the process can be quite fast and sensitive.

Stem cell transplantation and Cancer Treatment: Our experience from a Super-specialty Oncology Hospital

Siddhartha Roy, E. Venkataswamy and Vidya Harini Veldore

Triesta Reference Laboratory, HealthCare Global Enterprises, Bangalore - 560027

Stem cell transplantation remains the most favored modality in cases of refractory disease in cancer. Chemotherapy and/or radiation therapy at higher doses have a debilitating effect on the regenerative capacity of bone marrow, so Bone Marrow Transplantation and Peripheral Blood Stem Cell Transplantation help in restoring the stem cells. There are three prevalent modes of transplantation: Autologous (Patient's own stem cells isolated and replaced back), Allogeneic (Compatible donor's stem cells isolated and introduced in the recipient) and Syngeneic (Transplantation among Twins). The motive behind transplanting healthy Stem Cells back into the patient after high doses of chemo and radiotherapies is to rejuvenate bone marrow to regenerate. The whole process takes real-time, round-the-clock monitoring, and decision making on part of the whole team, through number of days. The stem cells are isolated, accumulated, frozen and then transfused after thawing. Molecular biology techniques like HLA typing, CD34 enumeration by immunophenotyping etc has to be employed for the completion of a successful transplantation procedure. The emergence of Cancer Stem Cells (CSCs) pose a threat as they have properties similar to the native stem cells, so differentiating hematopoietic stem cells from the leukemic stem cells by biomarker characterization is crucial. One approach involves targeting the cancer stem cells by incorporating the fact that hematopoietic stem cells express *Thy-1* and *c-kit* whereas leukemic stem cells express *IL-3* (interleukin-3) receptor á-chain. We would be presenting the salient features of the three approaches, their advantages and limitations and would share our experience from a cancer super-specialty centre.

Interaction studies of active ingredients of *Withania somnifera (Ashwagandha) with* Beta-1 adrenergic receptor: A target for Cardiovascular Disease

Nitika Sharma, Vidhyalakshmi H, Rekha Ravindran, Sujata Roy*

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Cardiovascular Diseases (CVD) remain one of the leading causes of deaths despite several advancements in the medical interventions. The synthetic drugs that constitute the current pharmacological armamentarium are themselves effective in managing the condition by targeting different receptor proteins but not without setbacks. For cardiovascular diseases, herbal treatments have been used in patients with congestive heart failure, systolic hypertension, angina pectoris, atherosclerosis, cerebral insufficiency, venous insufficiency, and arrhythmia. However, many herbal remedies used today have not undergone careful scientific assessment. With the high prevalence of herbal use in the United States/India today, clinicians must inquire about such health practices for cardiac disease and be informed about the potential for benefit and harm. Continuing research is necessary to elucidate the pharmacological activities of the many herbal remedies which are now being used to treat cardiovascular diseases. Around 80 different validated protein/ receptor targets have been identified for CVD's which are associated with different pathways like Calcium signaling pathway, Arachidonic acid metabolism and Metabolic pathways etc. Beta-1 adrenergic receptor is one of the promising targets for cardiovascular disease. Many available drugs are targeting this receptor molecule, example: Acebutolol; Sotalol; Practolol; Levobetaxolol. But those are having some adverse side-effects. So we need molecule with fewer side effects. It has been experimentally established that Withania somnifera is beneficial for the heart, but the exact molecular mechanism is not known. The Docking studies revealed that Witheferin-A binds to the active site of Beta-1 adrenergic receptor with significant affinity and its interaction energy is better than Levobetaxolol

PknE, a Serine / Threonine kinase from *Mycobacterium tuberculosis* modulates apoptosis and arginase signalling in macrophages

Dinesh Kumar P and Sujatha Narayanan
Department of Immunology, National Institute for Research in Tuberculosis (ICMR), Chennai

Introduction

Pathogenic microbes evade host innate immunity using virulence factors enabling survival and persistence. The mechanisms of immune subversion are of great interest as it provides insights in to the pathogenesis and aids in the development of new therapeutics. *Mycobacterium tuberculosis* (MTB), the causative organism of tuberculosis was found to survive inside the macrophages through the inhibition of apoptosis. The aim of the present study was to identify the apoptotic phenotypes suppressed by PknE of MTB.

Material and methods

The THP-1 cellline was differentiated into macrophages and infected with the strains *M. tuberculosis* H_{37}Rv (wild-type), *M. tuberculosis* H_{37}RvÄPknE (mutant) and complemented H_{37}RvÄPknE. On day 5 post infection microarray analysis was carried out. Subsequently the genes involved in apoptosis, arginase pathway and immune responses, were validated using oligoGE array and qReal-Time PCR.

Results

The deletion mutant of PknE shows a highly activated macrophage transcriptional program. The mutant showed increased pro-apoptotic molecules like BAX, BID of intrinsic pathway of apoptosis with decreased pro-inflammatory cytokines. The decrease in pro-inflammatory response correlated with increased SOCS expression that negatively regulates cytokine signalling. Interestingly, the expression of arginaseI, arginosuccinate lyase and iNOS were decreased.

Conclusion

PknE enhances survival of *M. tuberculosis* inside the macrophages by inhibiting signals that activate apoptosis.

Ä

Effects of overexpression of signal recognition particle receptor (FtsY) in Mycobacteria

Malini Veerasamy and Sujatha Narayanan

Department of Immunology, Tuberculosis Research Centre (ICMR), Chennai – 31

Introduction

A successful pathogen depends on various factors like cell wall components, secreted proteins and enzymes to survive and persist within the host. Understanding the molecular mechanism that enables the bacteria to sustain harsh and stringent environmental conditions is important to design more effective drugs and vaccines. Studies in prokaryotes have shown that FtsY, a component of signal recognition pathway is involved in membrane protein biogenesis, protein secretion and sporulation. Our aim is to investigate the role of *ftsY* in the growth and survival of *M.tuberculosis* under various conditions.

Methods

In order to understand the physiological role of FtsY in mycobacterium we used chemically inducible recombinant *M.smegmatis* mc^2155 to conditionally overexpress FtsY and subjected them to various invitro stress conditions.

RESULTS AND CONCLUSION

Overexpression of FtsY retards growth and sliding motility and altered colony morphology. Localization studies by differential centrifugation showed the presence of the protein in the membrane fraction. Attempts to delete *ftsY* failed in producing any knockouts indicating its essentiality. The observed changes warrant further investigation Experiments are being done to confirm its essentiality and future experiments include macrophage assays using the recombinant *M.tuberculosis* overexpressing FtsY.

Development of Recombinant BCG Based Epitope Vaccine Candidate for Tuberculosis

Aparna Christy, Karthika K.D, P. Kannan and Sujatha Narayanan

Department of Immunology, Tuberculosis Research Centre, Chennai -31

Introduction

Tuberculosis (TB) remains a global health challenge. BCG, the only available vaccine for TB provides variable efficacy in protection against adult pulmonary TB. Developing better vaccines using novel approaches is a major goal for the TB research community. Epitope-based vaccines designed to induce T cell responses specific for *M.tb* antigens are being developed as one of the means of improving vaccine potential. The aim of our study was to construct a recombinant BCG (rBCG) based epitope vaccine for TB and prove its immunogenicity in a mouse model.

Materials and Methods

Epitope grafting was done by Splicing by Overlap Extension (SOE) PCR. Expression of the chimeric antigens in BCG was proved by Western blotting. For the immunogenicity experiments, Balb/c mice were immunized subcutaneously with BCG or individual rBCGs. Cell - mediated immune response to specific mycobacterial antigens was studied by evaluation of in vitro splenocyte proliferation (MTT assay) and cytokine estimation (ELISA). Humoral immune response was studied by measure of serum antibody titre.

Results

Immunodominant epitopes were chosen from three well defined *M.tb* antigens (CFP-10, FBP and INV2). rBCGs (BCG::Cfp, BCG::Fbp and BCG::Inv) expressing the above epitopes on the *M.tb* Chaperonin 10 back ground of the epitope delivery system was constructed. Expression of chimeric antigens in BCG was verified. Immunogenicity studies show that, compared to BCG vaccinated group the splenocytes derived from rBCG vaccinated groups showed greater antigen specific proliferation, characterized with higher IFN-gamma response and reduced IL-4 secretion. Also rBCG vaccination was able to induce specific humoral immune response with an enhanced IgG2a/IgG1 ratio.

Conclusion

Our results indicate that the rBCGs favour a Th1 type response, which is known to be important for mycobacterial immunity and are thus promising TB vaccine candidates.

Study of Chick Chorioallantoic membrane to test anti-angiogenesis Activity

D. Shanmugapriya, M.Ramya, D. Nithila, R. Jayasree
Rajalakshmi Engineering College, Thandalam

Angiogenesis is the term which describes the sequence of events involved in the formation of new blood vessels by damaged tissues. However, a large amount of angiogenesis factors leads to excessive angiogenesis. This is the key factor responsible for the onset of the deadly disease, Cancer. Various anti-angiogenesis therapies are being developed to inhibit the process of angiogenesis, thus, depriving the cancer cells of essential nutrients and finally leading to their death. One such therapy involves the use of plant extracts with anti-angiogenesis properties as an angiogenesis inhibitor. Research is being carried out to improve the efficiency of this therapy. Plants can be regarded as a chemical cocktail composed of diverse chemical components with various biological activities. In order to improve the effectiveness of the therapy and to reduce the cytotoxic effects of the plant products, the different components of the plant extract can be isolated and various combinations of these components can be tested for their anti-angiogenesis property. Our work involves the study of the chick chorioallantoic membrane of the egg to analyze the anti-angiogenesis activity of test substances. The anti-angiogenesis property of the formulations composed of the different parts of Allium cepa (onion), Fabaceae (Flame of the forest), Mentha (mint) and Calotropis were studied.

Key words: Anti-angiogenesis, plant extracts, Formulation, Chick chorioallantoic membrane, Cancer.

Effects of Oxidative stress in Patients with Rheumatoid Arthritis

D. Vijayakumar
Arunai College of Engineering, Tiruvannamalai

The main objective of the study was to assess the oxidative stress in plasma and erythrocytes of rheumatoid arthritis patients by measuring the levels of thiobarbituric acid reactive substances (TBARS), non- enzymatic antioxidants (Vitamin E, C & reduced glutathione) and enzymatic antioxidants [(Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPX)]. This study has been conducted on twenty adult female rheumatoid arthritis patients and an equal number of healthy subjects. The levels of TBARS, non-enzymatic antioxidant and enzymatic antioxidants activities were measured using colorimetric methods. In the present study, elevated lipid peroxidation and multidirectional changes in the antioxidant defence system were noticed in patients with rheumatoid arthritis. The enhanced lipid peroxidation accompanied by disturbance in antioxidant status indicates that rheumatoid arthritis patients are more prone to free radical mediated oxidative damage.

Modulatory effect of PPAR-gamma agonist pioglitazone on pre-osteoclast cells of Type2 diabetic subjects

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Background: Type2 diabetes mellitus (T2DM) have an increased fracture risk due to anti-diabetic medication use despite a higher bone mineral density (BMD) and increased risk of falls. Pioglitazone is currently the commercially (Actos) available anti-diabetic medicine, a class of Thiozolidinediones (TZDs). PPAR-gamma agonist pioglitazone may cause an increase in osteoclastogenesis in type2 diabetics resulting in reduced bone formation.

Aim: To find the effect of PPAR-gamma agonist pioglitazone on osteoclast cell formation derived from T2DM subjects.

Materials & Methods: Study subjects included (Group I) healthy subjects (n =10; M/F: 5/5), Group II T2DM (n=12; M/F: 7/5) and Group III (group II + pioglitazone treatment). Mononuclear cells isolated from peripheral blood from all the study subjects were cultured in an alpha MEM medium containing 10% FBS, 2mM glutamine, 25ng/ml M-CSF, 40ng/ml RANKL, 10mM dexamethasone in the presence of different concentrations of pioglitazone (2,4,6,8,10 μM) for 14 days. Cultured cells were stained for TRAP activity using a biochemical kit (Sigma).

Results & Conclusion: Pioglitazone stimulated PBMCs from type2 diabetics exhibited a significant increase in number of TRAP positive multinucleated osteoclast cells as compared to untreated cells.

Association of Monocyte Chemoattractant Protein (MCP) 1 gene 2518 A/G Polymorphism with Type2 Diabetic Nephropathy in South Indian Population

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A promoter polymorphism (-2518 A/G) of Monocyte Chemoattractant Protein (MCP)-1 gene located on chromosome 17 have been studied in different ethnic population in various disease conditions including type 2 diabetes and its complication. The main aim of the present study was to examine the association of G allele frequency of A/G polymorphism of MCP1 gene in South Indian subjects with or without type2 diabetic nephropathy (DN). This study includes 75 diabetic subjects, 75 diabetic subjects with nephropathy and 85 healthy volunteers as control subjects. Genotyping of A/G polymorphism was performed by PCR-RFLP method. The significant difference was found in age, body mass index, total cholesterol, high density lipoprotein cholesterol, triglyceride, urea and creatinine level, between the case and control subjects. GG genotypic frequency of A/G polymorphism was significantly higher in T2DM diabetic nephropathy subjects (26.67 % *vs*. 8.24%; *P*=0.001) than in T2DM s (13.33 % *vs*. 8.24 %; *P*=0.198) and control subjects. G allele frequency was higher in diabetic nephropathy group (49.33 %) than in control subjects (21.18 %). The result obtained in this study shows an increase in the frequency of G-A substitution in MCP-1 gene at position (-2580) among south Indian type 2 diabetic nephropathy subjects.

Regulatory Role of PPAR-gamma Agonist Pioglitazone in Osteoclastogenesis of Type2 Diabetic Postmenopausal Women

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Pioglitazone, a class of Thiazolinediones (TZDs) is an effective oral drug used for management of type2 diabetes mellitus. Pioglitazone exerts its action through the activation of peroxisome-proliferator activator receptor-ã (PPAR-gamma) and turning on gene transcription. Activation of PPARg by TZDs play an important role in bone metabolism by promoting hematopoietic stem cells to differentiate into osteoclast, thus increasing bone resorption. Many observational studies have suggested the effect of TZDs on bone loss and high fracture incidence in diabetic postmenopausal women; few studies have shown the role of TZDs in estrogen synthesis. The effect of pioglitazone on osteoclastogenesis which may involve the suppression of estrogen function in type2 diabetic postmenopausal women has not been previously studied. In this connection, we have identified osteoclasts in type2 diabetic postmenopausal women using an invitro technique from peripheral blood monocytes in the presence of macrophage colony stimulating factor (M-CSF) and soluble RANKL.

The study were divided into three groups, healthy control postmenopausal women (n=13) (mean age 44.61±17.10), type 2 diabetic postmenopausal women (n=13) (mean age 48.87±16.98) and type 2 diabetic premenopausal women (mean age 32.12 ± 7.10) (n=13). PBMCs were treated with different concentrations of pioglitazone (2-10µM) and 17 beta estradiol (0.1-1nM) for 7-15 days. TRAP-positive multinucleated cells containing three or more nuclei were considered as osteoclasts. Pioglitazone treated cells showed a significant increase in osteoclasts as compared with untreated cells from healthy and diabetic pre and postmenopausal women .Reduction in osteoclast cells were observed in estradiol treated cells as compared to untreated cells.

Association of Endothelial nitric oxide synthase gene Glu298Asp polymorphism in Type2 diabetic foot ulcer among south Indian population

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[6]Department of Biochemistry, VRR Institute of Biomedical Science (Affiliated to University of Madras), Kattupakkam, Chennai-600 056, India, [4] Vellore Diabetes Clinic, Vellore, Tamilnadu, India, [5]St John's Medical College Hospital, Bangalore, Karnataka, India

Background: Diabetic foot complications has become one of the most common causes of nontraumatic lower extremity amputations in the world. Diabetic foot ulcer is a pathological condition where a decreased nitric oxide may present. In recent years, eNOS gene polymorphism (Glu298Asp) has gained enormous attention due to their association with diabetes mellitus regulation.

Materials & Methods: The study group consisted of 133 type2 diabetic foot ulcer subjects and 147 healthy volunteers as controls.500µl of blood sample was used to isolate genomic DNA by Phenol-chloroform method. PCR amplification was carried out with suitable forward 5′ TCC CTG AGG GCA TGAGGC T 3′ and reverse 5′ TGA GGG TCACAC AGG TTC CT 3′primers, followed by *BanII* restriction digestion enzyme and the products (137bp,320bp,457bp) were analysed using 2% agarose gel electrophoresis.

Results: Allele and genotype frequencies in both groups were analysed by student's t-test. 101 of 133 diabetic subjects had GG genotype, 29 had GT genotype and 3 had TT genotype. 133 of 147 controls showed GG genotype, 13 showed GT genotype and 1 showed TT genotype. The genotypic frequencies for Glu298Glu, Glu298Asp, Asp298Asp were 0.904(n=133), 0.088(n=13), and 0.06(n=1) in control group and 0.759(n=101), 0.218(n=29) and 0.022(n=3) in type2 diabetic foot ulcer group. The positivity for Asp allele was significantly increased in type2 diabetic foot ulcer than in controls (P=0.008).

Conclusion: The present study finds that there was a significant association of the Glu298Asp variant polymorphism of the eNOS gene with diabetic foot ulcer.

Study on finding the role of Peroxisome Proliferator – Activated Receptor -g (PPAR-gamma) agonist Pioglitazone in A549 Lung Adenocarcinoma Cell line

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Background: Pioglitazone is a synthetic ligand of nuclear receptor peroxisome proliferator-activated receptor PPAR-gamma that is approved for the treatment of type2 diabetes mellitus. PPAR-gamma has been associated with anticancer activities in a variety of cancer cell lines through inhibition of proliferation and promotion of apoptosis. The exact mechanism of PPAR-gamma to cancer growth inhibition remains unclear.

Aim: The present study is aimed to find the anti-proliferative and apoptotic effect of pioglitazone on lung adenocarcinoma cell lines (A549.).

Materials and Methods: A549 Lung adeno carcinoma cell line was used for the study. Cells were treated with different concentrations of pioglitazone (5, 25, 50,100 μM). The cytotoxic effect was assessed by Tryphan blue assay and apoptosis by DNA fragmentation assay and protein expression of PPAR-gamma and MMP 9 by western blot technique.

Results: On treatment with pioglitazone, the morphology of A549 cells was changed in a time and dose dependant manner. At 100μM concentration, the change in cell morphology was observed immediately. The percentage of viability showed significant ($p<0.05$) reduction of viable cells at 100 μM concentration at 24h and 48h and more significant ($p<0.01$) at 72h. At 25 and 50 μM concentration the significance ($p<0.05$) was observed from 48hrs. Treated cells showed significant increase in PPAR-gamma expression with respect to the increasing dose, but MMP 9 expression was decreased with respect to higher dose. The DNA fragmentation results showed multiples of 180 bp, and produced band width of 1440 and 720 bp at the concentration 100 μM.

Conclusion: It is concluded that pioglitazone causes change in cell morphology, reduction in cell viability, DNA fragmentation and inhibition of MMP 9 expression by activation of PPAR-gamma expression.

In Silico Structure Based Designing of Potent Peptide Inhibitors for Renin and Angiotensin Converting Enzymes

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The enzymes Renin and Angiotensin Converting Enzymes (ACE'S) are associated with hypertension, congestive heart failure and diabetic nephropathy. Renin is a protease and ACE's are carboxy peptidases. There are three kinds of ACE's in humans (evidence from databases). Renin is a prohormone acting on free floating angiotensinogen. All the three ACE's are membrane bound , one of the receptors of insulin participating in signal transduction and involved in the conversion of Angiotensin I to Angiotensin II, a potent vaso constrictor involved in raising blood pressure. These ACE's and renin are crucial key targets for development of drugs. The so- called anti hypertensive drugs used for treating hypertension are having their plasma half life less than that of their target enzymes. So this study was proposed with the aim to design peptide aptamers for all these enzymes. Computational tools such as Swiss model and Thematics server were used do design the 3D structure of all the enzymes and for identification of target sites. The small peptide aptamers were designed using the molecular builder tool of the Argus lab software and the target sequences were built into small peptide chains and then both the targets and aptamers were converted into PDB format. Docking results on this peptide using Hex software indicated that the peptide has potency to bind to the target sites on the enzymes. The proposed, small peptide has shown all the desirable features of a potent inhibitor and hence it may be a potential lead compound.

Omega-3 Fatty acids from marine microbes

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SynkroMax Biotech Pvt Ltd, Chennai 600116

Omega-3 fatty acids regularly make headlines for their potential to prevent cardiovascular diseases, asthma, depression etc. Evidently, n-3 fatty acids reduce blood triglycerides level and regular intake reduces the risk of secondary and primary heart attack. Long chain EPA/ DHA omega-3 fatty acids supplementation can be co-preventative and co-therapeutic. Current research suggests increasing accumulated long chain omega-3 fatty acids for health benefits and natural medicine in several major diseases, hence global fisheries are generally acknowledged to be threatened indicating a requirement for new and sustainable sources of n-3 oils. New sources of these oils would reduce pressure on declining fish resources worldwide.

This presentation deals with the molecular effects of n-3 fatty acids on coronary heart disease, cancer, atherosclerosis, type II diabetes and need for alternative mean for the synthesis of beneficial polyunsaturated fatty acids considering of aquatic population. Marine microorganisms have been isolated and are found to be efficient producers of EPA and DHA. Identifying and characterizing molecules in the omega-3 fatty acid pathway will help the pharmaceutical industry to explore drugs that mimic these compounds.

Screening of Nattokinase Enzyme Producing Microorganisms

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Nattokinase is a potent fibrinolytic enzyme, which belongs to the group of alkaline serine protease. Nattokinase was first derived from *Bacillus subtilise* var *natto*, isolated from traditional Japanese soybean food, Natto. This enzyme offers a completely natural means of preventing and dissolving blood clots. It closely resembles plasmin and actually enhances the production of plasmin. It is a potent cardiovascular drug and the enzyme activity is enhanced in the plasma for a longer half-life with oral administration. Compared with conventional clot dissolving drugs, Nattokinase has several advantages, such as, safety, convenience, oral administration, confirmed efficacy, prolonged effects, preventive effect, low cost and stability in gastrointestinal tract and these characters make Nattokinase a promising oral medicine for thrombolytic therapy.

Nattokinase is truly a multidimensional nutrient supplement and can play a key role in treating hypertension and hypercoagulation. It has a 4-fold greater thrombus dissolving ability than plasmin and very efficiently initiates endogenous fibrinolysis by cleavage and inactivation of plasminogen activator inhibitor-1. This ultimately leads to efficient lysis of detrimental coagulation of blood in the body. This enzyme is classified under Nutraceuticals and available as an OTC product. Best Nattokinase, New Nattokinase, Nattokinase X-tra, Nattozymeetc are some of the brand names available in the market..

In this research work, as a first step, massive isolation work was carried out in order to screen for Nattokinase producing microorganisms. Seventy cultures were isolated from various sources and preliminary plate assay method (hydrolysis of casein) was carried out. Out of these seventy cultures, thirty three were shortlisted, which were very much positive in the preliminary plate assay method. *Bacillus sp*, which exhibited high enzyme activity at pH 8.0 and above and at a temperature of 37°Cwas selected for further studies. The physiochemical properties of these enzymes have been characterized and their effectiveness in thrombolysis *in vitro* has been further studied.

Calculation of Secondary structure factors for the Medicinal Plant *Cissus quardrangularis*

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We have concentrated in the medicinal plant *Cissus quardrangularis* to study its emerging medicinal value. We have retrieved a particular protein sequence of *Cissus quardrangularis* from NCBI databank. Then the retrieved sequence was subjected to swiss pdb viewer to predict primary structure. We have also concentrated in the secondary structural factors for this plant. The secondary structure factors have been calculated by Expasy server. From these results, we have observed that the composition of structural factors were very high. So, we have concluded that these structural parameters may be useful for the process of drug designing. *Cissus quardrangularis* has the anti fungal and anti bacterial activity.

Targeted drug delivery using niosome: The magic bullet

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Objective

To achieve targeted drug delivery using Niosomes- "The Magic Bullet"

Abstract

Niosomes are microscopic lamellar structure formed on admixture of non-ionic surfactant of the alkyl or dialkyl poly glycerol ether class and cholesterol with subsequent hydration in aqueous medium. Types: 1) SUV-Small Unilamellar Vesicle, 2) MLV-Multi Lamellar Vesicles, 3) LUV-Large Unilamellar Vesicles. Niosomes are designed to concentrate the drug to the tissue of interest as a result drug is localized on the targeted site. Hence, healthy tissues are not affected by the drug. The properties of the vesicles can be changed by varying the composition of vesicles, size, lamellarity, tapped volume, surface change and concentration.

Advantage

Niosomes are the best carrier as they act as a depot releasing the drug in the controlled manner, variety of drugs can be loaded due to hydrophilic, lipophillic nature, exhibits flexibility in structural characteristics, osmotically active and stable, surfactants are biodegradable, biocompatible, and non immunogenic.

Therapeutic applications include (1) Targeting of bioactive agents to Reticulo Endothelial Systems, (2) Delivery of peptide drugs,(3)Carrier for haemoglobin.

Conclusion

Niosomes are the promising targeted drug delivery system since they are cost effective and prepared from uncharged single chain surfactant and cholesterol.

Treatment of spinal cord injury using human bone marrow derived neural stem cell-like cells

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Rajalakshmi Engineering College, Thandalam, Chennai-602 105

Background

Stem cells have enormous potential to differentiate or transform into a diverse range of specialized cells that make humans what they are today. In the face of complications that are known to arise during spinal cord injury treatments like laminectomy and open decompression, one has to device a safer procedure, which brings our focus on stem cell engineering.

Objective

In Stem cell treatment for spinal cord injury, the isolation and culturing of Neural Stem Cells (NSCs) is an arduous process and the alternative and effective method could be trans-differentiation of human adult Bone Marrow Stromal Cells (hMSC) into the Neural Stem Cell-like cells (hmNSCs). This could increase the feasibility of spinal cord injury treatment over existing methods, while improving its economical viability.

Method

Mesenchymal Stem Cells are isolated from adipose tissue, umbilical cord blood, periosteum, synovial membrane, muscle, dermis, blood, bone marrow, trabecular bone. The culture expansion of hMSCs is followed by its conversion into neuroprogenitor-like cells and the osteogenic, chondrogenic adipogenic neurogenic differentiation of the cultured cells are performed by adhering to standard protocols. These cells, after proper analysis like clonal analysis, can be used for spinal cord repair and other related disorders.

Conclusion

Economical feasibility and efficacy of the proposed idea would promote an unbiased chance of undergoing this inexpensive treatment. However, only after conclusive experimental trials the success rate and potential use of this futuristic technology will be deduced.

Treatment for alkaptonuria by gene therapy

B. Vinodhini, M. Swetha, Hemavathy Nagarajan, Millicent Mabel
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Background

Alkaptonuria (AKU), the prototypic inborn error of metabolism, was the first human disease to be interpreted as a mendelian trait. Alkaptonuria is a disorder caused by deficiency of recessive homogentisate 1,2 dioxygenase, an enzyme required for the catabolism of phenylalanine and tyrosine in liver and kidney.

Objective

The defect is caused by mutation in HGD gene that maps to human chromosome 3q21-q23. CCC sequence motif is a mutational hot spot in homogentisate-1,2-dioxygenase gene.

Methodology

A total of 43 single residue substitutions impairing HGO enzyme activity have been identified in AKU patients and model organisms. For mutation analysis, 10 ml EDTA blood samples were obtained from two different group of subjects, one unaffected and the other affected. Genomic DNA was isolated from leukocyte nuclei and the sequence differences were analyzed by single stranded conformational analysis (SSCA). Since, a substitution of the amino acid valine for methionine at position 368 (Met368Val) is the most common HGD mutation. Mutations in the HGD gene probably inactivate the enzyme by changing its structure. Therefore gene therapy has to be implemented by changing the amino acid sequence.

Conclusion

If being diagnosed earlier in foetus, the gene therapy method can easily be implemented. In adults , reduction in intake of phenylalanine rich food can help in reducing the

Accumulation of HGA and thus will reduce the symptoms of alkaptonuria.

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Standard journal article

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