Guest Lectures

Next-generation Sequencing

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INTRODUCTION

The overwhelming majority of deoxyribonucleic acid (DNA) sequence production to date has relied on some Sanger's dideoxy nucleotide triphosphate method or termination method. Over the past 5 years, in the wake of the Human Genome Project, the availability of whole genome assemblies for *Homo sapiens* and all major model organisms has helped us in making a rapid progress in the science of sequencing, as these effectively provide a reference against which short reads can be mapped. Also, a growing variety of molecular methods have been developed, whereby a broad range of biological phenomena can be assessed by high-throughput DNA sequencing (e.g., genetic variation, ribonucleic acid expression, protein–DNA interactions, and chromosome conformation).

Alternative strategies for DNA sequencing can be grouped into several categories. These include (i) microelectrophoretic methods, (ii) sequencing by hybridization, (iii) real-time observation of single molecules, and (iv) cyclic-array sequencing. Here, we use "second generation" in reference to the various implementations of cyclic-array sequencing that have recently been realized in a commercial product. The concept of cyclic-array sequencing can be summarized as the sequencing of a dense array of DNA features by repetitive cycles of enzymatic manipulation and imaging-based data collection.

In cyclic-array sequencing, clonally amplified 28-μm beads generated by emulsion polymerase chain reaction (PCR) serve as sequencing features and are randomly deposited to a microfabricated array of picoliter-scale wells. With pyrosequencing, each cycle consists of the introduction of a single nucleotide species, followed by addition of substrate (luciferin, adenosine 5′-phosphosulfate) to drive light production at wells where polymerase-driven incorporation of that nucleotide took place. This is followed by an apyrase wash to remove unincorporated nucleotide. With Solexa technology, a dense array of clonally amplified sequencing features are generated directly on a surface by bridge PCR (aka cluster PCR). Each sequencing cycle includes the simultaneous addition of a mixture of four modified deoxynucleotide species, each bearing one of four fluorescent labels and a reversibly terminating moiety at the 3′-hydroxyl position. A modified DNA polymerase drives synchronous extension of primed sequencing features. This is followed by imaging in four channels and then cleavage of both the fluorescent labels and the terminating moiety.

Global advantages of second-generation or cyclic-array strategies, relative to Sanger sequencing, include the following: (i) *in vitro* construction of a sequencing library, followed by *in vitro* clonal amplification to generate sequencing features, circumvents several bottlenecks that restrict the parallelism of conventional sequencing. (ii) Array-based sequencing enables a much higher degree of parallelism than conventional capillary-based sequencing. As the effective size of sequencing features can be on the order of 1 μm, hundreds of millions of sequencing reads can potentially be obtained in parallel by rastered imaging of a reasonably sized surface area. (iii) Because array features are immobilized to a planar surface, they can be enzymatically manipulated by a single reagent volume. Collectively, these differences translate into dramatically lower costs for DNA sequence production.

The advantages of second-generation DNA sequencing are currently offset by several disadvantages. The most prominent of these include read-length (for all of the new platforms, read-lengths are currently much shorter than conventional sequencing) and raw accuracy (on average, base-calls generated by the new platforms are at least 10-fold less accurate than base-calls generated by Sanger sequencing). Although these limitations create important algorithmic challenges for the immediate future, we should bear in mind that these technologies will continue to improve with respect to these parameters, much as conventional sequencing progressed gradually over three decades to reach its current level of technical performance.

Cell Energy Medicine

Dr. Gautam Sarkar

INTRODUCTION

Every cell and tissue in our body requires energy to function optimally. Without energy none of our physiological functions would function optimally. Be it the nerves, muscles, bones, skin, gastrointestinal system, or the immune system, a low-energy status would compromise their function. Once compromised, this status of low energy can manifest in different forms, depending on the tissue, i.e., affected the most.

Cell energy can be affected by several factors. But perhaps the commonest reason in most individuals is inappropriate nutrition. Surprisingly enough, nutrition is a field of medicine, i.e., appreciated by neither the medical profession nor the general public. When appreciated partly, the understanding is often misplaced and misdirected. More often than not, this precious branch of medicine is not even considered a part of medicine, and is relegated to dieticians.

In this presentation, an attempt would be made to highlight a dimension of medicine that had hitherto laid unprojected. It would also stress upon the extremely important role that doctors trained in biochemistry would have, in the days to come, in expressing this important field of medicine.

Gas Chromatography Mass Spectrometry or Tandem Mass Spectrometry: Dilemma for Newborn Screening

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INTRODUCTION

Newborn screening identifies conditions that can affect a child's long-term health or survival. Early detection, diagnosis, and intervention can prevent death or disability and enable children to reach their full potential. Each year, millions of babies are routinely screened for certain genetic, endocrine, and metabolic disorders. Developed nations run their own newborn screening program. Most developed countries screen for a standard number of conditions, but some may screen for more.

Metabolic disorders represent major bulk of inborn errors of metabolism. At present, more than 300 different types of metabolic disorders are recognized, and the number is increasing every year. No single test can identify all these disorders. These include various aminoacidopathies, fatty acid oxidation disorders, carbohydrate disorders, mitochondrial disorders, etc. Both gas chromatography/mass spectrometry (GCMS) and tandem mass spectrometry (TMS) are widely used for simultaneous detection of multiple metabolites in urine and blood samples respectively. There are laboratories using only GCMS or TMS for sample analysis, which is causing a dilemma as to which technology is superior.

Since its first application by Tanaka in 1966, GCMS has been used worldwide in the diagnosis of inborn errors of metabolism because of its high accuracy, sensitivity, and power of analyzing multiple compounds simultaneously. More than 100 metabolic conditions can be simultaneously screened using GCMS for abnormal markers from the air-dried urinary filter paper. Analysis of organic acids is of utmost importance for diagnosis of a wide range of amino acids and organic acid disorders. The GCMS helps in identifying these conditions; GCMS is time consuming; it may not detect glyceroluria, volatile compounds, such as phenols, and short chain organic acids are not quantified. Also, small amounts of uric acid, xanthine, hypoxanthine, and sugars are not recovered.

On the contrary, TMS is able to pick up amino acid disorders like argininemia, argininosuccinic aciduria, citrullinemia, homocystinuria, maple syrup urine disease, nonketotic hyperglycinemia, phenylketonuria, tyrosinemia type I, various fatty acid and organic acid disorders like carnitine palmitoyltransferase I and II deficiency, carnitine transporter defect, various acyl coenzyme A dehydrogenase deficiencies, isovaleric acidemia, malonic aciduria, methylmalonic acidemia, etc. This is determined by identification of various amino acids and acylcarnitines in blood sample. Concentration of individual metabolites and certain ratios of metabolites help in making final diagnosis. The TMS has its limitation of not analyzing all possible organic acids. Many of the abnormal analyte elevation found in TMS screening are not pathognomonic of a single disorder and can be produced by several different genetic disorders.

A two-tier approach with blood sample analysis by TMS followed by urine analysis by GCMS is required for diagnosis and confirmation of specific organic acid disorder. If designed as a routine protocol in newborn screening laboratories, it will help early diagnosis of organic acidemias, thus helping early initiation of treatment.

Human Leukocyte Antigens: Importance in Organ Transplant

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INTRODUCTION

Major histocompatibility complex (MHC) is a set of cell surface proteins essential for acquired immune system to recognize foreign molecules in humans, which in turn determine histocompatibility. The major histocompatibility gene family is divided into three subgroups: Classs I, II, and III.

The transplant of organs is one of the greatest therapeutic achievements of the twentieth century, which is defined as moving of the organ from one body to another, or from a donor site to another location on the person's own body to replace the recipients damaged or absent organ.

In organ transplantation, the adaptive immunity is considered as the main response exerted to the transplanted tissue, as the principal target of the immune response is the MHC molecules expressed on the surface of donor cells. The innate and adaptive immunities are closely interrelated and should be viewed as complementary and cooperating. When a human transplant is performed, human leukocyte antigen (HLA) molecules from a donor are recognized by the recipient's immune system triggering an alloimmune response. Matching of donor and recipient for MHC antigens is known to have a significant positive effect on graft acceptance. Talk will be on MHC introduction, classification, different testing methodologies, and clinical HLA-associated diseases.

Role of Inflammation and Matrix Metalloproteinase-9 in Vascular Stability and Coronary Artery Disease

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INTRODUCTION

Cardiovascular disease is an epidemic in India, with ischemic heart disease having one of the highest burden worldwide. Furthermore, myocardial infarction continues to be an acute coronary event, i.e., unpredictable. In spite of success in lowering low-density lipoprotein (LDL), the epidemic of coronary artery disease continues to surge. The focus of research has therefore, justifiably shifted from dyslipidemia to inflammatory processes.

The focus of our study has been inflammatory processes, glycated LDL, and metalloproteinases. We studies levels of glycated LDL and matrix metalloproteinase (MMP)-9 in patients with acute myocardial infarction with and without diabetes mellitus. We also studied tumor necrosis factor (TNF)-α, interleukin (IL)-6, and adiponectin in patients with stable ischemic plaque (proven by coronary angiography). We further did polymerase chain reaction restriction fragment length polymorphism analysis of genetic polymorphism of MMP-9 (1562C/T) and TNF-α. The studies were case–control studies with patients from GB Pant Hospital. All special tests were by enzyme-linked immunosorbent assay or chemiluminescence and routine tests on Beckmann AU 680 autoanalyzer.

Serum levels (mean ± standard deviation) of TNF-α were 362.20 ± 236.45 pg/mL in cases and 186.94 ± 177.33 pg/mL in controls ($p = 0.000$), IL-6 in cases was 33.19 ± 5.77 pg/mL and in the control group was 13.73 ± 2.65 pg/mL ($p = 0.003$), whereas the adiponectin levels in cases were lower (5.97 ± 0.80 μg/mL) as compared with the control group (6.60 ± 0.4 μg/mL). The TNF-α emerged as the best biomarker associated with ischemic heart disease with maximum area (0.792) under the receiver operating curve. This shows that severity of atherosclerosis is an imbalance between inflammatory (TNF-α, IL-6, nuclear factor-κB) and anti-inflammatory (adiponectin) cytokines. Levels of glycated LDL and MMP-9 were found to be significantly elevated ($p = 0.004$) in acute myocardial infarction cases with diabetes mellitus (DM; 5.6 ± 1.1 ng/mL) as compared with controls with DM (4.8 ± 0.9 ng/mL).

Vascular inflammation superimposed on the deposited modified LDL is the proposed pathogenesis of ischemic heart disease. The rupture of the plaque causes acute coronary events like myocardial infarction, and this vulnerability is mediated by inflammation and increased activity of metalloproteinases. A linking element between diabetes and cardiovascular complications could be the excess production of reactive oxygen species and hyperglycemia, resulting in increased oxidant products via multiple processes forming oxidized and glycated products. The molecular alarm signals sent by dysfunctional endothelium and modified LDL deposition are decoded by specific blood immune cells (monocytes, T lymphocytes, neutrophils, mast cells) and by the resident vascular cells that respond by initiating a robust inflammatory process, in which the cells and the factors they secrete hasten the atheroma development and thrombogenesis.

Vitamin D Immunity and Immunological Disorders

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INTRODUCTION

Vitamin D, the prohormone on activation in the body, leads to its metabolic effects. Presently, vitamin D is so strongly associated with its effects on bone formation including the calcium and phosphate metabolism that bone and vitamin D are like synonyms. Liver and kidneys play an essential role in this physiology.

However, studies in the last few decades have revealed the very important extraskeletal effects of vitamin D. The finding of extrarenal expression of enzyme CYP 27B (1-alpha hydroxylase) as well as of vitamin D receptor in many tissues and cell types establishes these extraskeletal effects. Presently, almost all tissues are known as the target tissues for vitamin D, e.g., heart, brain, skin, bowel, gonads, prostate, breast, parathyroid gland, etc.

Latest emerging view is that calcitriol is an immune modulator. All types of immune cells, monocytes, macrophages, dendritic cells, T and B lymphocytes, are influenced by vitamin D. Their basic activities are modified, leading to several effects e.g., vitamin D is known to decrease the inflammatory response, enhances the antimicrobial effects, suppresses autoimmunity, etc. Alteration of these functions is seen in many immunological disorders.

Vitamin D has long been known to be beneficial in many diseases. During the days when there was no definitive treatment of tuberculosis, sunshine was an important part of the overall regimen of recuperation in sanatoriums. The antimicrobial effect of macrophages in enhancing the expression of antimicrobial peptides like cathelicidin and beta-defensins is well known. The association of vitamin D with several immune disorders, particularly the autoimmune diseases like rheumatoid arthritis, systemic lupus erythematosus, type I diabetes mellitus, inflammatory bowel disease, autoimmune thyroiditis, etc., is well known. The role of vitamin D in immunological disorders including their pathogenesis and therapy is highlighted in the lecture.

Standardization of Serum/Urine Argininosuccinate Levels by Liquid Chromatography Technique: Establishing Urea Cycle Disorders

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INTRODUCTION

Among the inborn errors of all essential urea cycle, argininosuccinic aciduria is the second in incidence. Argininosuccinate (ASA) is one of the six intermediates in the urea cycle. Qualitative detection of ASA in urine of these patients is not difficult, but quantification of ASA is tricky. It is acid labile and degrades with moderately high temperature. It cannot be estimated like other 22 amino acids on reversed-phase high-performance liquid chromatography (HPLC). It needs stringent temperature (22° Peltier control) and pressure control and has to be eluted separately. Earlier assays relied on conversion of ASA to cyclic anhydrides prior to analysis. But caution should be put forth against this procedure since recovery may not be complete, making procedures not requiring such conversions preferable. We report here a simple and rapid assay in which ASA is determined directly in untreated urine and plasma by an accelerated reversed-phase HPLC procedure, which is based on separation of O phthaldialdehyde (OPA) – amino acids on reverse phase Zorbax Eclipse (C18) column.

MATERIALS AND METHODS

O phthaldialdehyde and barium salt of argininoscuccinic acid were from Sigma. A fresh OPA solution was used in methanol followed by addition of 50 µL of beta-mercaptoethanol and 11.2 mL of 0.4 mol/L sodium borate, maintaining pH of 9.4. It was left in the dark for 24 hours for derivatization and digestion to take place. Fluorescence detector (338 excitation and 425 emission filters) was used. Solvent A was methanol:tetrahydrofuran: Sodium acetate and sodium hydrogen phosphate in 2:2:96 (pH 7.5 with N/10 acetic acid). Solvent B was methanol:water in 65:35. A 10 µL of sample being injected, a peak at 6.86 minutes was found. The identity of this peak with ASA was established by the addition of commercial ASA to the control urine sample and one to patient's sample. The area of the peak at 6.86 minutes was proportional to the amount of ASA.

CONCLUSION

The important advantages of the present over previous assays for ASA are speed and simplicity. It takes only 8 minutes for the chromatographic procedure to take the next sample and does not depart from the routine HPLC procedure for amino acids. Further ASA/creatinine ratio can be done for estimation of daily excretion. Since heterozygotes have been reported to excrete significantly more ASA than normal humans (though much less than gross patients), this assay method appears suitable for detection of the carrier state.

Multitargeted Agents in Cancer Cell Chemosensitization: What we learned thus far?

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INTRODUCTION

Extended research over the past several years in unveiling the molecular causes of cancer have identified many molecular targets for cancer. This helped the scientists to develop many monotargeted therapies (so called the Magic bullets) which are highly specific to the key molecules actively involved in various signaling pathways for the treatment of this disease. However, cancer still remains one of the most dreadful diseases in the world, killing 8.2 million people annually. It has been well-established that development of chemoresistance in cancer cells against monotargeted chemotherapeutic agents is the major cause of failure of these agents. This chemoresistance is mediated by modulation of multiple survival pathways in cancer cells. Therefore, inhibition of these multiple survival pathways by nontoxic multitargeted agents may have high potential in inhibiting drug resistance and sensitizing cancer cells to these monotargeted agents. Numerous lines of evidence showed that curcumin, a natural polyphenol obtained from the rhizomes of Curcuma longa, is a highly potent, nontoxic chemosensitizer for cancer cells that target diverse molecular and cellular pathways. The safety and tolerability of this compound even at high doses have already been well established by several human clinical trials. This talk will summarize the high potential of this compound as a chemosensitizer with special emphasis to pancreatic cancer.

ABOUT THE SPEAKER

Dr Ajaikumar B Kunnumakkara is currently working as a faculty member in the Department of Biosciences and Bioengineering, Indian Institute of Technology, Guwahati, Assam, India. He earned his doctorate in 2006 from the Amala Cancer Research Center,

Thrissur, affiliated with the University of Calicut, Kerala, India. Dr Kunnumakkara did his first postdoctoral work at the University of Texas MD Anderson Cancer Center, Houston, Texas, USA (2005–2008), and his second postdoctoral work at the National Cancer Institute of National Institutes of Health (NCI/NIH), Bethesda, Maryland, USA (2008–2010), where he was subsequently employed as a NIH scientist from 2010 to 2012. Dr Kunnumakkara's research interests include identification of novel biomarkers for cancer diagnosis and prognosis; role of inflammatory pathways in cancer development; role of chromosomal translocations in the initiation, promotion, and progression of cancer; and drug development from natural products. He is credited with the publication of more than 120 research articles. He has more than 12,800 citations with an h-index of >39. Currently, his work is cited over 1,700 times in the literature annually. Dr Kunnumakkara has also edited three monographs entitled "Molecular targets and therapeutic uses of spices: Modern uses for ancient medicine," "Anticancer properties of fruits and vegetables: A scientific review," and Fusion Genes and Cancer.