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Full Length Research Paper

Cancer investigation: A genome perspective

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The completion of human genome project has evolved many techniques used to locate the human genes. The focus is mainly on the genome, transcriptome or proteome to recognise distinctive characteristics that may explain the basis of human disease and potentially envisage prospect outcomes. Cancer is one of the recent deadliest diseases. Various cancer types root problems in the generalised dealing. The objective of these investigative pursuits is to ultimately individualize treatment for each patient based on their exclusive gene expression prototypes.

Keywords: Human genome, cancer, functional genomics, transcripts, microarray, proteomics.

INTRODUCTION

Cancer is a series of biological consequences starting with growth and survival of neoplastic cells at the primary site, invasion, angiogenesis, intravasation, extravasation and finally growth at the secondary site. The disease is deadly and cure is thought to be impossible due to lack of recognition of specific biomarkers indicating detection and diagnosis of the disease at early stages (Riccardo, 2005). Newer scientific research methodologies have come into prime focus with the accomplishment of human genome project. It has opened the perception of big science in the field of biology, where huge data is generated and advanced computational technologies are required to analyze the data. For example, (a) the necessity to know a phenotype in order to define a new gene is not required, instead of starting with a phenotype and tracing it back to a sequence of DNA in order to discover the genes, it is now possible to start with the genome sequence and look for signals indicative of promoters, exons, splice junctions, and similarity to known sequences in order to discover new genes. In fact, most genes are now identified based on their DNA sequence. (b)Comparative genome analysis is used to search and identify homologous genes in an evolutionarily-related organism and the differences amongst closely related organisms also can be resolved using this technique. (c)Identification of virulence factors in various bacterial strains was done by comparing pathogenic and nonpathogenic strains (Dobrindt, 2005; Dobrindt et al., 2003; Schmidt et al., 2004; Wick et al., 2005). Similar concepts have been used for the study of cancer. For example, Hematological malignancies are diagnosed by karyotyping (Bayani and Squire, 2002; Jotterand and Parlier, 1996).

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THE GENOME

The genome is supposed to be the whole set of DNA sequence of the germ line cells of an organism. Knowledge of this DNA sequence will lead to valuable information about gene functioning. Some organisms have segmented genomes as in case of humans. Microscopic observations can visualize such units. Such units are studied previously to check their role in the cell. (Jackson, 1978; Martin and Hoehn, 1974; Pogosianz and Prigogina, 1972). Genome can be studied on nucleotide level. Whole gene analysis uniquely identifies the region of the genome that plays important role in the diseases. Cancer development is generally due to single point mutations in the gene. (Claus, 1995; Den Otter et al., 1990; Weinberg, 1983). Hence, whole genomic and focused approaches should be combined and used to find out the genes interfering cancer development. For years it is known that oncogenic mutations and suppression can manipulate the development of cancer. Specific mutations can be identified using large-scale sequencing of the genome in many cancers (Capella et al., 1991; Casey et al., 2005; Frank et al., 1999; Li et al., 1998). The type of mutation in a tumor can be a better source to have a choice of treatment. Large genomic

rearrangements are associated with cancer in many cases. These are observed in the microscope but functional genomic techniques and can be employed to detect smaller changes. (Beheshti et al., 2003; Hoque et al., 2003; Mundle and Sokolova, 2004; Squire et al., 2003; Weiss et al., 2003).

THE TRANSCRIPTOME

The huge number of mRNA transcripts that are formed by copying or splicing of genome segments can be termed as transcriptomes (Velculescu et al., 1997). This includes all the mass of RNA that encode the different proteins which determines the gene functionality. The term comprises all the transcripts that are formed in the specific biological and all other possible conditions. A gene can provide more than one transcript. Hence, the transcriptome study will provide a big scenario of the cellular functions. Variation in transcriptome is related to the variation in cell, tissue and even in the organism. Microarray and large scale nucleotide sequencing enables the complete study of transcriptome to get the clear view of inner cellular complexity. In case of cancers, transcriptomes are better source to categorise tumors into its subclasses, which can be helpful in treatment. (Golub et al., 1999; Perou et al., 2000; Ramaswamy et al., 2001). Diagnosis of the patient can be carried out using a single or smaller or even larger group of transcripts. For example, expression level of the estrogen receptor (ER) detects the subsequent consequence in some breast tumors (Leal et al., 1995; Perin et al., 1996). Such biological markers must be associated with other tumors. Transcriptome analysis thus has the advantage of identification of decisive transcriptional marker through the screening of transcripts. Introduction of novel designed drugs requires sophisticated transcriptome analysis (Rhodes and Chinnaiyan, 2005).

THE PROTEOME

The proteome is the complete set of proteins expressed by the entire genome. As some genes code for multiple proteins; the size of proteome is greater than the total number of genes. Thus proteome is a potential target for the researchers to get clear insights into the biological phenomenon through their investigative approaches. (Kahn, 1995). Large scale and high throughput techniques are utilized to investigate proteome. Mass spectroscopy and antibody-based techniques are focused in localizing, quantitating, and structurally characterizing individual proteins or small groups of proteins. This can be achieved only if the proper structure and function of the proteins is elucidated. Proteomics is referred as the large scale analysis of the total protein content of the cell, or fluid. (Petricoin and Liotta, 2004; Stults and Arnott, 2005; Wulfkuhle et al., 2003). The proteome can be studied by evaluating many proteins from a cell through High-throughput mechanisms or microarray techniques to study a single protein in a multitude of tissue samples. The transcriptional and post transcriptional modifications cause variation in the proteome. This is an interesting feature to study the genome functioning and cell interactions. However, it is fascinating that some cellular responses occur in the proteome and does not necessarily involve genomic or transcriptome variations. This can only happen if protein undergoes modification or as a result of protein-protein interactions or with proteins and other macromolecules. The change in expression levels of a protein or its modifications are studied in proteomic experiments in response to a stimulus. One can study a single protein or group of proteins that determine a cellular response to the treatment. In case of cancer differences in the proteome due to the mutation of a single gene, following drug treatment, or between groups of patients separated by their clinical characteristics can be studied. (e.g., histology or survival outcome) (Dephoure et al., 2005; Soreghan et al., 2003) Early detection of diseases using selective markers or the possible mechanism of drug resistance at molecular level can be achieved by evaluating proteome. (Alexander et al., 2004; Bhattacharyya et al., 2004; Hondermarck et al., 2001; Petricoin et al., 2005; Zhang et al., 2004).

Techniques for cancer investigation

As the cancerous cell advances, cellular metabolism is altered dramatically as a consequence of genetic changes in the genome. Genes may lose their functionality or can promote cellular growth. Tumor formation varies in steps and hence genetic variation amongst similar tumors is important. These variations also change the way of response to eradication. To understand a tumor in a better way, it is necessary to know its genomics. Tools used for human genomics could be routinely used for diagnosis and suggest treatment of cancer (Mount and Pandey, 2005; Yeatman, 2003). Recently, microscopic observations are made to identify tumors based on their pathology. This also involves genome analysis in a crude manner. Chromosomal anomalies are associated with other forms of cancer (Gronwald et al., 2005; Kimura et al., 2004; Meijer et al., 1998; Micci et al., 2004). High resolution screening of genomes is done nowadays using various tools (Jones et al., 2005; Nakao et al., 2004).

Expressed sequence tags

As an outcome of the human genome project it is now possible to go for high throughput sequencing. Numbers

of sequence based techniques have evolved to extend biological understanding, especially diseases. Now, priority is given to transcriptome than genome. Complementary DNA libraries and sequencing is used to generate expressed sequence tags (ESTs) libraries (Kawamoto et al., 2000; Okazaki et al., 2002; Stapleton et al., 2002). These ESTs are single sequencing reads from cDNA. Variations in the human genome could be studied using these ESTs as demonstrated in 1991 (Adams et al., 1991). Not only the categorization of sequences from a cell is possible but the comparison can be made with other cells (Carulli et al., 1998; Kawamoto et al., 2000; Lindlof, 2003).

SAGE

SAGE is another useful technique used to evaluate transcriptomes (Velculescu et al., 1995). It involves short sequence generation from cDNA using enzymes and then concentrating them in a large string for sequencing. These generated short sequences are the specific markers for transcripts. Abundance of the transcript within the transcriptome can be measured quantitatively by the frequency of short sequence tags. Variation in the expression of gene can be studied under experimental conditions using this technique (Sengoelge et al., 2005; Zucchi et al., 2004). SAGE is also employed to evaluate expression of undefined genes. Yet SAGE has its own limitations. It is mainly based on complexity of cloning and hence expensive. Secondly, it is also difficult to identify sequences with short tag size; below 21 base pairs significant cDNA databases or sequenced genomes are required by some SAGE applications (Liu, 2005). SAGE technique was used to recognise transcripts that are having enhanced metastatic potential like keratin K5, cystatin S, the human homologue of yeast ribosomal S28, and the p32 subunit of human pre-mRNA splicing factor SF2(Parle-McDermott et al., 2000.). Over expression of PGP9.5, a neurospecific peptide that functions to remove ubiquitin from ubiquitinated cellular proteins, in turn protects their degradation by the proteasome-dependent pathway was demonstrated using the SAGE in more than 50% of primary lung cancers (Bittencourt et al., 2001).

Proteomics

Proteomic analysis is widely applicable to investigate clinical biomarkers (Alaiya et al., 2005; Bhattacharyya et al., 2004; Chen et al., 2005; Hondermarck et al., 2001; Srinivas et al., 2001; Steel et al., 2003; Zhang et al., 2004) . High through put proteomics was utilized to detect pancreatic cancer using serum of the patients (Bhattacharyya et al., 2004). Proteomics was applied in a multi-institutional study of women with ovarian cancer, benign pelvic masses or pathology (Zhang et al., 2004). They exposed three distinct protein markers exclusive to ovarian cancer that could possible be used for early detection tumor markers. Proteomics has been employed for a histological diagnosis to subtype tumors whether directly from tumor samples or in attempts at early detection (Borczuk et al., 2004; Seike et al., 2005; Steel et al., 2003). So one can easily detect the tumor and the nearby tissue for changes associated to tumor growth or to gaze for microscopic tumors in an or else normal looking tissue section. These kinds of investigation will perhaps offer important information about the appearance of tumors from microscopic disease sites that cannot be obtained by any other method.

Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE)

An immobilized pH gel gradient is used to separate proteins by their isoelectric points, followed by SDS PAGE to promote separation of the proteins on the basis of their molecular mass. A number of spots are resolved in a single gel representing different proteins, different isoforms of the same protein, or its post-translational modifications (Sivakumar, 2002.) Identification of biomarkers in case of breast cancer in nipple aspiration fluid has been carried out recently by 2D-PAGE. Breast carcinomas can be easily evaluated by investigating breast ductal fluids (Kuerer et al., 2002).

Mass spectrometry

Gel based techniques like isoelectric focusing, SDS-PAGE polypeptide sequencing, and liquid chromate-graphy methods like affinity, ion exchange, or reverse phase separations can be utilized to separate protein components within a complex protein mixture like serum, tissue or cellular extract. Each separated fraction is then coupled to tandem mass spectrometry peptide sequencing (LC-MS/MS) and database search to resolve its protein constituents. With mass spectrometry profiling or differential fluorescence techniques or isotopic labeling, evaluation of fractions from multiple samples can be done. Whole plasma or tissue has been analyzed by mass spectrometry (Caprioli, 2005; Steel et al., 2003). Mass spectrometry is practicable for direct tissue analysis at the single cell level (Danna and Nolan, 2006) and implicated on microdissected samples for purer tumor analysis (Greengauz-Roberts et al., 2005; Jain, 2002). Multiple locations across microscopic tissue slices can even be identified using this technique (Chaurand et al., 2004). Matrix-assisted laser desorption ionization time of flight (MALDI-TOF) utilizes peptide mass fingerprinting (PMF). Gel separated proteins digested by trypsin are measured at high accuracy (100 ppm or better).

Molecular ions from the peptide samples are produced using a laser source and then introduced in an analyzer that resolves ionized fragments on the basis of their mass-to charge (m/z) ratio(Rowley A, et al., 2000.). The Surface-enhanced laser desorption and ionization time-of-flight (SELDI-TOF) is not only able to find single protein biomarkers but is also able to identify biomarker expression patterns (Seibert et al., 2004). Mass spectroscopy has the potential to be used for systematic identification and characterization of proteins that are helpful in diagnosis as prognostic markers. (Volker et al., 2005)

Single nucleotide polymorphisms

Approximately more than 1 million common variations in the human genome have been documented in the public database generated by sequencing diverse human genomes. A search for genetic difference in the form of single nucleotide polymorphism, which indicates human diversity, is continued in this HapMap project (Thorisson and Stein, 2003). Now more than 10 million genomes have been sequenced to find out more than 2 million differences counting rare SNPs (Altshuler et al., 2005; Botstein and Risch, 2003; Gu et al., 1998; McVean et al., 2005; Sherry et al., 2001). Many techniques have evolved to utilize this information with respect to cancer. Linking of specific genotype databases with diseases is now possible with individual SNPs as markers in the genome. Cancer involves large genomic insertions or deletions that can be recognized by SNP data (Hoque et al., 2003). Polymorphism found within the regulatory genes and variation in the protein function due to difference in the coding regions of some genes can affect the expression level of the gene (Marsh, 2005). Many cases have been found where expression level or specific polymorphism affected chemotherapeutic response in the patients (Landi et al., 2003). The correlation between an individual's reaction to chemicals and the polymorphisms in his genome is studied in pharmacogenomics (Bomgaars and McLeod, 2005; Marsh, 2005; Turesky, 2004). Microarray techniques are better than traditional sequencing to locate individual single point mutations and SNPs, if specific variants are known.

Microarray techniques

Microarray is the most promising technique for functional genomics. Screening with high density DNA microarrays allows the pattern of gene expression to be compared between tumor cells and normal cells. It involves complementary joining of two nucleic acid strands to generate duplexes. With the aid of this specific complementary binding, it is easily possible to locate the specific sequences in billions of different sequences. Entire transcriptomes or single nucleotides within the genome can be screened using microarray. Microarray analysis provides valuable information on disease pathology, progression, resistance to treatment, and response to cellular microenvironments and ultimately may lead to improved early diagnosis and innovative therapeutic approaches for cancer (Pascale and Jeremy, 2002). Alternative splicing provides variation in the transcripts by changing the exon joining patterns and difference in start and stop points. Almost 40% of human genome is supposed to show alternative splicing. (Brett et al., 2000; Mironov et al., 1999; Modrek and Lee, 2003). The alterations in the type of transcripts through alternative splicing can influence the host susceptibility towards the cancer (Mercatante and Kole, 2000; Milani et al., 2006). Such type of splicing also affect on the individual response to the therapy for same type of tumor (Mercatante and Kole, 2000). These variations in tumor types and also in the response towards tumor can be correlated using microarray technologies (Bracco and Kearsey, 2003; Veuger et al., 2002).

Cancers can be identified using transcriptomes. Microarrays are one of the important tools to provide evidences for cancer. Gene expression profiles of various histologically similar tumor types allow altering the treatment of choice. Different types of tumors based on gene expression profiles have been identified. (Bucca et al., 2004; Cao et al., 2004; Elek et al., 2000; Halvorsen et al., 2005; Hu et al., 2005; Khan et al., 1999; Lee et al., 2004; Smith,2002; Sorlie et al., 2001; Wrobel et al., 2005; Zhang and Ji, 2005) . With microarray, 78% accuracy in prediction of unknown tumor type identification has been achieved (Ramaswamy et al. 2001). Using microarray technique and cDNA, 84% accuracy was found to identify unknown tumor type. Significant gene expression differences between patients suggesting that several subtypes might exist were noted that can explain the response in therapeutic variations (Perou et al., 2000). A positive or negative therapeutic response prediction through identified gene markers has been done (Cheok et al., 2003; Kakiuchi et al., 2004; McLean et al., 2004; Staunton et al., 2001). A study of advanced non-small cell lung cancer revealed 51 genes that predicted a response to Gefitinib (Kakiuchi et al., 2004). Almost 9000 genes expressed in AML have been evaluated to check the response before and after therapy with methotrexate and mercaptopurine, given alone or in combination (Cheok et al., 2003). In a study of 8000 genes and 60 cell lines from central nervous system, renal, ovarian, leukemia, colon, and melanoma neoplasms established the strength of the genomic approach to differentiate among tumor subtypes, using cDNA microarray (Ross et al., 2000). Microarray techniques are being utilized to locate genes that may result in tumor progression and metastatic potential (Agrawal et al., 2003; Henshall et al., 2003; Ramaswamy et al., 2003; Sanchez-Carbayo et al.,

2003; van 'T Veer et al., 2002; Vasselli et al., 2003). 295 patients have been evaluated using 70 gene classifier with stage I or stage II breast Cancer (van 'T Veer et al., 2002). Hence, prediction of clinical responses based on gene expression patterns in tumors is achievable. A reverse-phase microarray approach (RPA) was utilized to compare expression of several pro-survival proteins in micro-dissected normal and prostate cancer samples. Protein expression was studied using antibodies. It was revealed that early step in the development of cancer is phosphorylation and activation of AKT/PKB (Paweletz et al., 2001). National Cancer Institute studied 60 human cancer cell lines (NCI-60) to screen compounds for anticancer activity, using RP Microarray and recognition of two promising pathological markers to distinguish colon from ovarian adenocarcinomas in the abdomen was achieved (Nishizuka et al., 2003).

Chip-on-chip technology

Identification of DNA-binding sites of transcription factors is necessary to study regulation of transcriptomes. ChIP-on-Chip technology also known as Location Analysis (LA) is used for the same, it comprises microarray chips with chromatin immunoprecipitation. *In situ* cross linking of specific transcription factor to its DNA-binding site is carried out. The DNA is then fragmented and immunoprecipitated using a transcription factor specific antibody. These fragments of DNA are amplified by PCR, labeled and then hybridized to array. The DNA-binding sites for specific transcription factors within the genome can be evaluated using this technique (Horak and Snyder, 2002). Verification of insilico predictions of target genes regulated by ER alpha was also done (Jin et al., 2004). Identification of new targets of the p53 gene responding to ionizing radiations was recognized using the same technique. (Jen and Cheung, 2005) Within a whole genome, the patterns of DNA methylation related to disease status can be investigated (Wilson et al., 2006). This technology provides insight into key mechanisms of methylation, histone modification, as well as DNA replication, modification, and repair. It has been used to understand not only cancer but also diseases such as diabetes and leukemia. It has also provided important insight to vital processes like cell proliferation, cell fate determination, oncogenesis, cell cycle, apoptosis and neurogenesis.

Conclusion

Completion of human genome project and advancement in whole genome analysis is providing ample opportunities for comprehensive analysis and interpretation of cancer genomes, exomes, transcriptomes, and proteomes as well as epigenomic components. The integration of these data sets with well-annotated phenotypic and clinical data will expedite improved interventions based on the individual genomics of the patient and the specific disease. Human diseases are no more a threat to scientists. This has been possible because of the advancement in technology and the fine approach. The recognition of DNA as origin of phenotypic expression, its role in gene expression and its manipulations has lead to many discoveries one of them is to know details about some diseases like cancer. With the human genome project completion and the detailing aspects of proteomics and genomics reinforced scientists to think in the vicinity of genes responsible for the development and viability of cancer. The recent era in approach would be significant in suggesting the treatment for cancer and also be able to predict the outcomes. It would be possible to opt for an individualized treatment in near future, with the help of microarray technology and proteomics. This requires generation, storage and processing of huge data from patients. This is feasible only with the help of computer scientists. Bioinformatics will have to play a major role in analyzing the data in order to provide a correct treatment plan for an individual.

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