

MOLECULAR APPROACHES TO CANCER MANAGEMENT IN THE POSTGENOMIC ERA

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Probably all disease states except trauma are influenced by genetic factors. In the last decade of the 20th century all avenues of biomedical research led to the gene. The human genome contains all the information necessary from conception until death. The completion of the draft sequence of the human genome in the Human Genome Project has resulted in sequencing of the entire human genome. This remarkable achievement will reveal the genetic instructions that specify the molecular components, the design and the operating software of the human body. This knowledge will transform medicine, giving us the means to see and to understand human anatomy, physiology and pathophysiology in molecular detail. This development will dramatically accelerate the development of new strategies for the diagnosis, presentation and treatment of diseases. Especially for common complex diseases such as cancer, genetic differences contribute to the risk of contracting the disease, clinical course of disease and responsiveness to different treatments.

Endeavors in the Human Genome Project and technical advances in molecular biology are expected to have a revolutionary effect on oncology practice, including how anticancer drugs are discovered and developed, how cancers are detected and classified and finally how patients are treated and monitored. Since the discovery of oncogenes, tumour suppressor genes and more recently genes of apoptosis cancer has become one of the most important diseases in the design of approaches based on genetics and genomic research. The explosion of information generated by large-scale genomic related technologies has resulted in exponential increase in the number of genes and proteins available for pharmaceutical and diagnostic research development. The increasing understanding of complex molecular pathways involved in cancer will shift clinical practice from empirical treatment to treatment based on molecular taxonomy of disease. The two important emerging fields in cancer in

the post genomic era include the development of new diagnostic tests based on micro array technology and the development of new drugs in the emerging field of pharmacogenomics.

MICROARRAY TECHNIQUE

In order to fully understand gene function, one must know when and where the gene is expressed, what affects its expression levels, and in a larger sense how does the gene's expression correspond to the expression of other genes that work together to carry out cellular processes. Facilitating this very task cDNA microarrays allow scientists to quantify the mRNA expression of thousands of genes simultaneously, whereas traditionally the elucidation of genes involved in carcinogenesis was accomplished one gene at a time. Furthermore, construction of genome wide expression patterns of all tissues and disease states will be possible in the near future. This will undoubtedly have an enormous impact on our understanding of cell biology and prove an important link between molecular genetics and clinical medicine.

BASICS OF DNA MICROARRAYS

In microarrays or the so-called gene chips, rows and rows of oligonucleotides are lined up in dots on a miniature silicon chip or glass slide or sheet of nitrocellulose. Each spot represents a unique sequence from a named gene or an expressed sequence tag (EST). An EST is an oligonucleotide sequence from a cDNA clone, which corresponds to a cDNA molecule. ESTs allow the identification of coding regions in genome wide sequences and are used to identify new genes. Currently over one million ESTs are deposited in large data banks. Considering estimates of the total number of human genes after the Human Genome Project, which is around only 40,000, there must be considerable overlap in the EST bank. Using a non-redundant set of ESTs, corresponding cDNAs are selected and spotted on a microscope slide using commercially available robots. One slide can hold up to 10,000 probes. The total RNA or mRNA extracted from tumour tissue and one control cell population is amplified and labeled with a fluorescent or radioactive pulse. It is applied to the slide surface and after appropriate hybridization, relative intensity of signal for each spot on the slide is measured which corresponds to abundance of

matching mRNA with appropriate pattern recognition software, it is possible to assemble a global score.

GENE EXPRESSION PROFILING IN HUMAN TUMOURS

Through the evaluation of large-scale expression profiles using microarrays, tumours can be classified and important molecular pathways discerned. One of the first studies that used gene expression data to supplement standard cancer histopathology studied the clinical heterogeneity of diffuse large B cell lymphoma, the most common subtype of non-Hodgkin's lymphoma. Correlation of specific gene expression patterns with diagnosis, classification and prognosis of neoplasia has been studied in progression of human breast cancer using microarrays. One study recently demonstrated the ability of these expressions profiles to distinguish acute myeloid leukemia and acute lymphoblastic leukemia. Microarrays are probably the technology of future in clinical diagnosis and classification of cancer.

DRUG DEVELOPMENT

Microarray based high throughput gene expression analysis will facilitate rapid identification of disease specific genes and pathways which has immediate implication for drug development. In the simplest scenario genes over expressed in diseased cells viz. Her-2/neu in breast cancer, could serve as potential drug target. A recent report shows how variation in transcript levels of particular genes identified using microarray, relates to mechanisms of drug sensitivity and resistance. This would allow clinicians to offer tailor made therapy in future. DNA microarrays mark a new age in the field of drug development i.e. pharmacogenomics.

PHARMACOGENOMICS

Pharmacogenomics can be used to predict how that individual will respond to certain drugs and personalized, safer and more efficient approach to medication. This requires a cost effective genotyping and progress in identifying markers of adverse drug response.

PHARMACOGENOMICS AND CANCER

Pharmacogenomics may be more crucial to the treatment of cancer than to any other illness. Current therapies of cancer have exhibited

limited success with efficacy only in 20-40% of cases. Moreover penalty of administering optimal therapy that employs high doses of extremely toxic drugs is severe due to the associated side effects. Pharmacogenomics can lead to optimized therapeutic regimens, resulting in improved quality of life and life expectancy in cancer patients.

MOLECULAR DIAGNOSTICS - THE ULTIMATE GOAL IN CANCER PHARMACOGENOMICS

Applying pharmacogenomics to cancer is to develop diagnostic tests predictive of therapeutic response and identifying patients who will respond well to specific treatments as well as those at high risk for severe drug induced toxicity. Diagnostics that guide the choice of therapy for an individual promise to significantly improve the long-term outcome of cancer treatment.

APPLYING PHARMACOGENOMICS TO DRUG DEVELOPMENT IN CANCER

The first step in developing molecular diagnostics is to identify genetic markers predictive of toxicity or efficacy. In general prediction of toxicity is associated with markers in normal cells e.g. SNPs (Single Nucleotide Polymorphisms) and Haplotypes. Predicting efficacy on the other hand requires markers in tumour cells; the following 3 analytical tools are used to identify these markers.

- (i) Genotype/Haplotype analysis: Most genes contain multiple DNA alterations including SNPs, which may affect therapeutic response. The first step is to define the SNPs in such putative genes. Haplotyping identifies the groups of these critical SNPs, which occur together in each gene. After the Human Genome Project, creation of a genetic map based on more than million SNPs has been of great help in identification of SNPs linked to alterations in therapeutic response.
- (ii) Loss of Heterozygosity (LOH): During early phases of tumour formation, substantial amounts of DNA are lost. This phenomena referred to loss of one of the two alleles at a locus is referred to as LOH, and is an important determinant of gene activity in the tumour since it affects both gene copy and its function.

(iii) mRNA expression analysis: Expression levels of specific genes have been shown to predict response to chemotherapy with great accuracy. mRNA expression profiling by microarrays provides a thorough screen for differences predictive of therapeutic efficacy.

Exquisitely pure tumour tissue samples for identification of above genetic markers are obtained by state of the art laser capture micro-dissection methods to isolate tumour cells from surrounding normal tissue.

After the completion of Human Genome Project, the annotation of full complement of human genes and identification of novel cancer causing genes is expected to provide newer gene targets for development of new and better anti cancer drugs. This is the ultimate aim of the pharmacogenomics related to cancer in this post genomic era and the goal may not be too far to attain predicted by the rapid rate of progress.