CANCER GENETICS

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From the time Boveri observed that chromosomal changes are a feature of cancer, it has been thought to be a disease caused primarily by alterations in the genome of the affected cells. Today, the notion of cancer being a consequence of genetic alterations, is almost intuitive and the advances in molecular biology and genomics have given us many tools to understand and possibly to combat cancer. Since science has always existed in a continuum, the genetic alterations in cancer have to be understood in the context of cellular organization , differentiation , tissue organization host response and susceptibility angiogenesis etc.

The properties that are taken to typify cancer cells are also present in normal cells. These include cell division, migration and even invasion (as exemplified by the trophoblast cells). However what marks out cancer cells is dysregulation and inappropriate expression of these attributes. Typically the genetic alterations in cancer can be said to include three major types of genes, oncogenes, tumour suppressor genes and genes that preserve the integrity of the genome. It must be kept in mind that cancer is a multi-step process and several genetic alterations are required for a full blown cancer phenotype.

Oncogenes

These are today known to be cellular genes that when mutated and/ or inappropriately expressed in a manner that increases their activity result in a malignant phenotype. Classical examples include src, ras and myc oncogenes. These genes are very much the key components of cellular regulatory processes eg. the src gene is codes for a tyrosine kinase, the ras gene for a G protein and the myc gene for a nuclear protein that is involved in DNA replication.

Oncogenes were first discovered in acutely transforming retroviruses (Rous Sarcoma Virus). When these viruses infect immortalized but untransformed cells in culture they generate a neoplastic phenotype. It was subsequently found that these viral

oncogenes were not naturally occurring viral genes but picked up from the cellular genome and subsequently mutated or over expressed to generate cellular transformation. A single mutated oncogene cannot transform primary cells and the requirement for oncogene co-operativity is in concordance with the multi-step theory of carcinogenesis derived from classical studies. Oncogene co-operavity usually requires cooperation between oncogenes belonging to different groupings eg. nuclear (eg myc with cytoplasmic eg ras).

Tumour Suppresor Genes (TSG)

These genes can be compared to the brakes of a car, and function in the cell to regulate cell division. Loss of genetic matter is also a key event in the generation of neoplasia, and the same can be demonstrated by cytogenetic techniques. Molecular tools have been able to further define the loss of genetic matter. Typically there is loss of one allele of a TSG while the other is inactivated by point mutation. The concepts of TSGs were demonstrated first with the Retinoblastoma gene (RB). Commonly affected TSGs include the p53 gene (affected in almost half the human malignancies) the Wilms tumour gene the p16gene etc.

Genes controlling genomic integrity

These have also been called caretaker genes. Inactivation of such genes leads to genomic instability and thus markedly increases the probability of alterations in the oncogenes and the TSGs. DNA mismatch repair genes have been extensively studied and include the hMSH2 and hMLH1 genes which are commonly affected in human malignancies. Again as the case of most oncogenes and TSGs, homologues of such genes can be traced back to the yeasts indicating the fundamental similarity of these biological processes. DNA mismatch repair defects manifest as unusually rapid expansion and contraction of microsatellite repeat sequences. Inherited defects in such genes are exemplified in Hereditary Non Polyposis Colon Cancer (HNPCC), where analysis of microsatellite repeats in leucocyte DNA forms a basis of diagnosing the affected siblings in a family. The affected individuals are subjected to regular investigations including colonoscopy. Increased genomic instability also includes several other aspects, the implications of which are under study. These include aneuploidy including genetic loss and translocations, increased

frequency of point mutations, other repeat mediated recombinations, increased tendency for gene amplification etc. The p53 gene which has been described as the guardian of the genome functions as both a caretaker gene and as a TSG. A similar role has been attributed to the Brca II gene.

The instability of the cancer genome could contribute extensively to therapeutic resistance, which is perhaps the most frustrating aspect of tumour therapy.

Viruses and Human Cancer

The viruses shown to be extensively involved in human cancer are the Hepatitis Viruses (B and C) and the Human Papilloma Virus (HPV) which are involved in liver and cervical cancers respectively. Several other viruses (like Herpes viruses) have also been implicated from time to time. However there is not much hard evidence as yet for the involvement of acutely transforming retroviruses. However Human T Cell Leukaemia Viruses I and II have been shown to be involved in outbreaks of T cell leukemia especially in Japan.

In terms of sheer numbers and morbidity and mortality, cervical and liver cancers are both very important. While there are a number of significant publications regarding the mechanistic basis of these cancers, it is important that these be treated as preventable cancers. The Hepatitis B vaccine is also an important vaccine for cancer prevention. Vaccines for HPV are at the experimental stages, however this knowledge could be useful for early diagnosis screening and behavioral intervention.

Interaction of genes and environment

As most cancers are thought to have an environmental basis, there has been a lot of work on looking at the genetic footprints of environmental carcinogens. This approach has been successful for animal models for cancers induced by specific carcinogens. However the same has not been generally true for human cancers. There has been an extensive attempt to build up a data base of p53 mutations and establishing the mutational spectrum of different cancers. While it is known that mutations in some p53 codons are more prevalent in cancer of a particular organ (eg oesophagus vs brain), the fingerprint of a particular carcinogen has ben hard to define. The only exception has been liver cancer in regions with a high exposure to aflatoxin

which are shown to have a mutation in codon 249. However this is not always true. This observation could be a manifestation of different levels of aflatoxin exposure and or of other modifying factors like DNA repair.

The Human Genome Project(HGP) and Microarray Technology in Cancer

No discussion of modern cancer genetics is complete without a mention of the Human Genome Project and the role of microarray technology in the study of cancer genetics. While a detailed discussion of the Human genome Project is beyond the scope of this report, a detailed and precise knowledge of each individual gene and its regulatory elements has contributed (along with other knowledge) to the identification of a number of possible anti-cancer targets. This major quantitative and qualitative leap in our knowledge and understanding of tumours promises to change our way of thinking about cancer. The sheer power of microarray technology where one can study tens to hundreds of thousands genetic segments in one experiment gives a picture of the global nature of alterations in the cancer genome and gene expression, where earlier we had to be content with studying a much smaller number of events. It is also expected that the technological advances brought about by the HGP will translate themselves into techniques that give the clinical laboratory much more investigative power than it previously had.

Points of intervention

Genetic aspects of cancer potentially lend themselves to intervention in a variety of ways. These include predictive, diagnostic, prognostic and therapeutic aspects. However it is also a fact that right now most of the cancer interventions do not rely on the knowledge derived from genetic studies on cancer. Neverhteless, there are strong indications that this outlook is likely to change in the near future.

Drugs exploiting selective genetic and functional differences in cancer cells

Cancer therapy today is mostly based on anti-mitotics and this lack of selectivity results in increased toxicity. However increasing knowledge of definite genetic alterations in different classes of tumours can help in the design of drugs that will selectively attack tumour cells. The drug gleevec has provided proof of the principle that specific tyrosine kinase inhibitors can be used for cancer therapy, mainly

related to CML, but also valid for some other cancers as well. Similarly Epidermal Growth Factor Receptor based protein kinase has also been targeted by small molecular weight inhibitors with promising results. Another class of drugs are inhibitors of the farnesyl pathway. These target mutant ras proteins as ras is anchored o the plasma membrane by a farnesyl tail.

Gleevec has been shown to be clinically effective. Looking at the number of drugs in trials at various stages and the explosion in knowledge based drug design, the number of such molecules is bound to increase. Another example that has been around for some time is tamoxifen. If one goes by definition of oncogenes Estrogen Receptor also functions as one in the context of breast cancer, and tamoxifen is a specific anti cancer compound.

Small molecules no doubt have an advantage when it comes to therapy, however derivatised oligonucleotides have been shown to have useful properties in terms of specificity, stability and bio-availability and many eg anti c-myc oligos are in clinical trials, often in conjunction with conventional chemotherapy. Inhibitor RNA technology and ribozymes may be technologies of the future.

The examples so far are about inhibiting the actions of mutant or hyperactive oncogenes. What about restoring the functions of tumour suppressor genes? Gene therapy has been tried for restoring p53 function in a variety of tumours and shown to be effective in animal experiments and clinical trials. However the results of restoring p53 are expected to vary from tumour to tumour. Small molecules have also been designed to restore the function of mutant p53 and they hold out al lot of promise.

Antibody Based therapy in Cancer

Recombinant antibodies, including chimaeric and humanized antibodies have revolutionized antibody based therapy, and currently form the class of recombinant proteins in various stages of approval. Recombinant antibodies approved by the FDA include Rituximab (for Non-Hodgkin Lyphoma), Trastuzumab (Breast cancer) and Gemtuzumab (Acute myeloid leukaemia). Such antibodies target cell surface antigens on the surface of malignant cells, and apart from their direct effects have been shown to sensitize the tumour to other therapeutic modalities.

Diagnostic and Prognostic Markers

The immediate applicability of genetic studies to diagnostic and prognostic applications is growing. As genes ultimately have to function through the increased expression of proteins, IHC can play a role in addition to direct detection of genetic alterations. In breast cancer HER IHC already has place in the management of estrogen receptor (ER) negative cancer and herceptin has emerged as an emerging therapy for such tumours. Using classical therapeutic modalities HER over expression is a negative prognostic factor in breast cancer. Another oncogene that have been used for prognosis is Nmyc and its amplification is associated with poor prognosis in neuroblastoma. There have been a lot of studies on the mutational status of p53 in a variety of cancers but it is yet to become an accepted part of therapeutic strategies.

Loss of hetrozygosity (LOH) of 1p, 19q in oligoastrocytomas has turned out to be a valuable genetic marker of response to chemotherapy using procarbazine, carbamazipene and vincristine (PCV). Since chemotherapy is costly and toxic and the genetic markers are clearly able to distinguish responder from non responder genetic typing will soon become an accepted part of management of such tumours.

Genetic markers from serum DNA: Tumours shed a considerable amount of DNA into the serum, which could be utilized for determining the presence or recurrence of the tumour and also give an indication of tumour load. This is technically difficult because of the presence of a normal background. How ever some determinations like serum mutant p53 levels have lot of potential.

Molecular typing of hematological malignancies has an important role because differences in behavior of different sub sets of leukemias and lymphomas. Molecular probes include studies of T cell receptor re arrangement, BCR-ABL fusions etc. These can be used for studies of tying, response to chemotherapy, follow up and the detection of minimum residual disease.

While molecular markers have an important role in tumour staging, with increasing knowledge of markers that may affect the transition from pre-neoplasia to neoplasia may help identify potentially threatening

lesions at sites accessible for biopsy or FNAC. These include tumours of the skin, breast, cervix, lymph nodes, liver etc.

Genetic Susceptibility to Cancer

While familial cancers do form a high percentage of the total cancer burden, they .remain a source of misery to the affected families. However the identification of specific germ-line mutations provides an opportunity to identify susceptible individuals and prevent or markedly improve the outcome by timely interventions. Similarly, those individuals in the affected families who do not carry the mutations can be spared the mental agony of uncertainty and the physical agony and costs of repeated screening. This screening for mutations in Brcal I And Brca II genes in breast cancer families is an established management strategy in the West. However before it is brought to Indian situation the frequency of such mutations in familial breast cancer in India remains to be determined. Li-Fraumeni's syndrome is rare and characterized by the inheritance of a mutated p53 gene. While these markers are not the stage to be incorporate into public health programs it is expected that with continuing research more genetic indicators for familial cancers will be discovered. Microsatellite instability is an indicator of HNPCC as discussed earlier. This is offered as an investigation to the affected families in advanced centers in the West.

The human genome project has brought an unprecedented amount of information, but most of the data is of general nature and not suitable for direct extrapolation to the diverse ethnic groups that constitute the Indian population. The Department of Biotechnology has initiated a ' People of India ' project to characterize the genetic make up of these groups with reference to their ethnicity. It is expected that this research will lead to the identification of suitable genetic markers (eg. single nucleotide polymorphisms and microsatellites) that indicate cancer susceptibilities of different populations.

Genetics and toxicity

Genetics also has something to offer to individuals receiving conventional chemotherapy. There is a considerable amount of literature linking various genetic polymorphisms to drug metabolism and toxicity. It is expected that such genetic signatures will constitute

a major expect of planning drug regimens in neoplasia. This also has the potential of reducing cost of management by tailoring chemotherapy to those patients who benefit the most.

Policy Aspects Related to Genetics

As most of the information and interventions related to the genetics of cancer cells is new and still evolving, it is difficult to assess the feasibility of each individual component, However it is clear that the impact of new information and technology will be felt on various aspects of cancer management.

There is no doubt that the cost of cancer chemotherapy is prohibitive. The fact that the results are often equivocal often make the costs unjustified. However, if because of the identification of genetic targets in cancer cells , there is a increased efficacy and reduced toxicity, resulting in increased cure rates or at least marked increase of quality of life, policy decisions have to be taken to supply these 'essential' drugs of the future at a reasonable cost. In an increasingly older population, with a correspondingly higher demographic predisposition to cancer this issue will increasingly confront policy makers. The issues of intellectual property, and the definition of what constitutes a life saving drug that needs to be made available cheaply to the population will need to be addressed.

Generic Production of Recombinant Proteins: a case for reducing costs

HBS Ag can be called a cancer vaccine, also many recombinant proteins like interferons are important in cancer therapy. The technology for producing such recombinant proteins is comparatively simple. For the moment product patents are not involved, and anyway for these and several other recombinant products, the patents are expected to expire soon. One aspect of policy intervention could be to reduce prices to better reflect the low costs of production of several recombinant products like HBS Ag and interferons. This will have a multiplier effect on healthcare.

Genetic predisposition to cancer: occupational and environmental exposure

It is expected that it will be possible (though not perhaps to a large extent in the near future), an increasing number of individuals and

groups with a significantly increased susceptibility to cancer. They can be counseled about employment and other modes of risk avoidance. However, like other similar offshoots of the Human Genome Project an ethical frame work needs to be made where the right to privacy, various aspects of discrimination at workplace, nature of health insurance etc can be incorporated into fair and balanced guidelines.

Genetic aspects of drug trials and the incidence of toxicity

The incidence of drug toxicity in an individual is often unpredictable and because of the occurrence of major toxic side effects in even a small percentage of individuals, many drugs are permitted for use. It is expected that in the field of anti-cancer drugs, as for other classes of drugs, genetic predictors of toxicity will be more precise. This will reduce risks and individualize therapy to the ' most effective and least toxic' combination for any individual. However this requires significantly more research into the genetic backgrounds of individuals, the various ethnic groups of India and clinical work ups of individual patients.