

New perspectives in cancer diagnosis and treatment by gene profiling

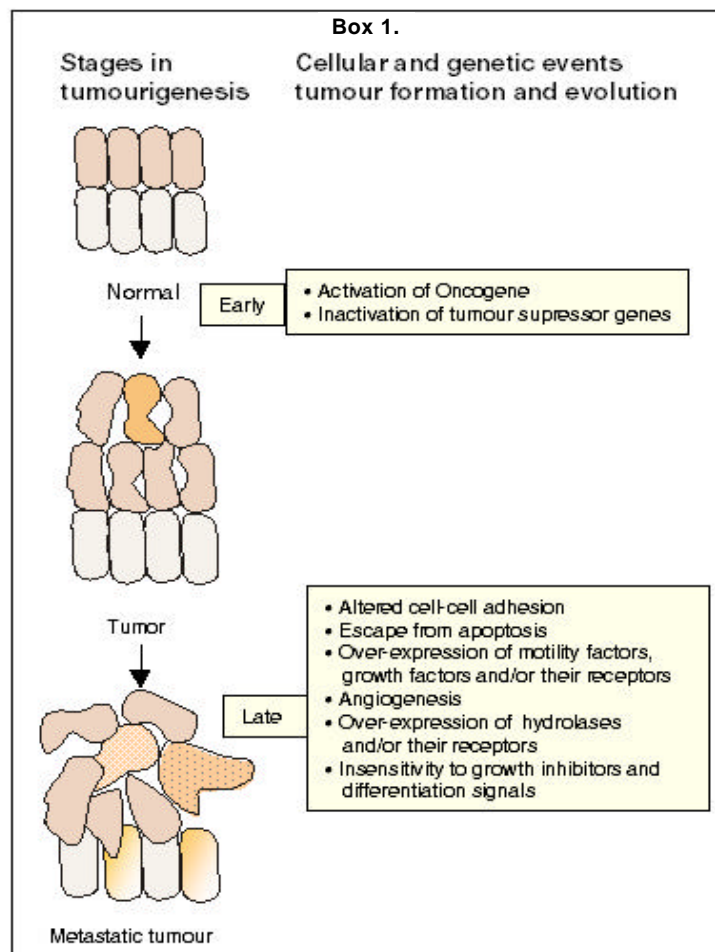
Cancers are caused by a variety of genetic alterations. Mutations in oncogenes or tumour suppressor genes represent the primary genetic lesions – their activation and inactivation, respectively, trigger carcinogenesis. A large number of mutational events and altered programme of gene expression, however, set in when primary tumours evolve to their final malignant state. In colorectal cancers, for instance, mutations leading to inactivation of adenomatous polyposis coli (APC) tumour-suppressor triggers cancerous transformations, resulting in constitutive expression of downstream genes which are involved in the control of cell proliferation and apoptosis¹. During these early stages of progression, colorectal cancers display chromosomal instability and aneuploidy² and sometimes overexpress Csk (COOH-terminal Src tyrosine kinase). Csk in turn down-regulates cSrc (cytoplasmic tyrosine kinase) which regulates cell proliferation. Csk and cSrc thus serve as markers for identification of early polyps³. Metastatic colorectal cancers often display mutation in the *p53* tumour-suppressor gene which controls programmed cell death or apoptosis⁴. Tumour evolution is thus marked by recruitment of a large number of genes that facilitate its malignant transformation⁵ (Box 1). Therapeutic strategies for different tumours can be designed if their abnormally expressed markers are first identified.

Markers apart, the cancerous cells usually exhibit characteristic cyto-pathology. In fact, as a most general practice, microscopic analysis of the tumour sections dominates the world of cancer diagnosis. Cytological classifications of tumours are, however, not without pitfalls. Consider the example of basal cell carcinoma (BCC), which frequently displays mutation in the *p53* gene. In an experimental mouse model, carcinoma with remarkable cytological resemblance to BCC can be induced by over-expression of *GLI-1* oncogene⁶. These *GLI-1*-induced carcinoma in mice displayed markers like *K5* (*keratin5*), as in BCCs of man. However, unlike the latter this experimentally-induced BCC in mice did not display any *p53* mutation, a hallmark of BCC. This observation calls for a cautious approach

in cancer classification and appreciation of the caveat that cyto-pathology is a gross indicator of the underlying cause and that even similar types of cancers can display distinct set of molecular markers. A substantial improvement in the current level of cancer diagnosis and treatment would demand that cyto-pathology be supported by extensive molecular analysis of markers which have gone awry in the cancerous cell types. Indeed, understanding the latter aspect of a cancerous growth, namely, the multitude of genetic alterations involved in cancerous transformation, could hold the key to cancer diagnosis and treatment.

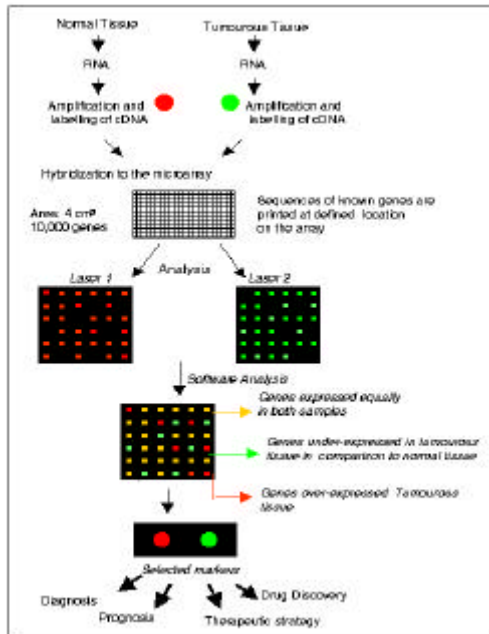
Take, for instance, the case of difused large B-cell lymphoma (DLBCL). Amongst patients displaying comparable clinical presentations and cytology, only

about 40% attain complete remission, the rest succumb⁷. Could this variable response to therapy be due to an underlying difference in the genetic alterations in the DLBCL of the two groups of patients? Addressing questions of this magnitude is a daunting task, when one considers the available compendium of a hundred genes implicated in cancer⁸. Answers to these questions thus cannot be readily discerned by compiling the differences in the pattern of expression of a few genes at a time. Instead, it calls for an entirely different approach where difference between normal and tumourous tissue, in terms of expression of the total complement of the genome, is investigated at once. One of these approaches in genomic sciences, which is in the process of revolutionizing



Box 2.

Microarrays are glass slides carrying individual signatures of as many as 10,000 genes or more which can be hybridized to their complementary sequences. cDNA microarrays have gene-specific poly-nucleotides (0.6–2.4 kb) robotically spotted on the matrix. For profiling gene expression of tumorous tissue, RNA from tumours as well as normal tissue are first extracted, which serves as a template for cDNA synthesis during which fluorescent tags are introduced. As seen from the figure, cDNA from tumours is



labelled red (e.g. Cy5) and that from the normal tissue is tagged with a green label (e.g. Cy3). The mixture of these labelled molecules is used for hybridization of the microarray. Laser scanning and software analysis quantitates this differential binding. RNA over-expressed in tumorous tissue appear as red spots on the matrix, while those expressed in normal tissue but under-expressed in tumours appear green. Genes that are expressed equally in both tumorous and normal tissues display an orange fluorescence.

Genes over- and under-expressed in tumours are potential markers of clinical value which may be used for tumour diagnosis, prognosis, therapy and drug discovery.

our understanding of genetic basis of carcinogenesis, is the technique of examining expression of hundreds of genes in tiny chips called microarray⁹ (Box 2).

In a clever application of this technique of molecular profiling, Alizadeh *et al.*¹⁰ addressed the questions of unpredictable prognosis of patients suffering from DLBCL. Using microarray technique, they made a comparison of the pattern of expression of a staggering number of genes (around 17,856) from 42 patients suffering from DLBCL. This study led to a broad classification of the DLBCL's into two distinct categories: one displaying expression profiles comparable to those of the germinal centre B-cell and the other exhibiting the characteristics of activated B-cells. The former showed characteristic

up-regulation of a number of genes, notably, those coding for cell-surface proteins CD10 and CD38, nuclear factor A-myb, LMO2 an inhibitor of B-cell proliferation and BCL-6, which is implicated in development of the germinal center. In contrast, the activated B-like DLBCL displayed its own unique signature genes like IRF4, which is involved in B-cell proliferation and genes which block apoptosis including FLIP and BCL-2. In short, gene expression profiling uncovered several distinct categories of markers within what was previously classified as a single type of cancer. The most remarkable aspect of this classification of DLBCL, however, was revealed when the prognosis of patients of these two categories of DLBCLs was compared. Patients with the germinal centre-like

DLBCL had 76% survivors, compared to 16% in those with the activated B-cell DLBCL category. Improved precision in therapy apart, in the immediate future, this study should then provide a means for predicting reasonably accurate prognosis of patients suffering from DLBCL.

Could all types of cancers be classified using this technique? Extensive investigations undertaken by the National Cancer Institute (NCI), Bethesda over the past several years under its Developmental Therapeutic Programme, is poised to comprehensively answer these questions. In one of its investigations, 60 cancer cell lines derived from tumours from a variety of tissues and organs were typed for the expression of around 8000 genes¹¹. Molecular profiling of the transcriptomes of these cell lines and subsequent computational analysis led to the identification of molecular signatures for cancer categories like leukaemia, CNS, colon, renal and ovarian tissues were identified. These signatures could find a huge potential in cancer diagnosis¹¹.

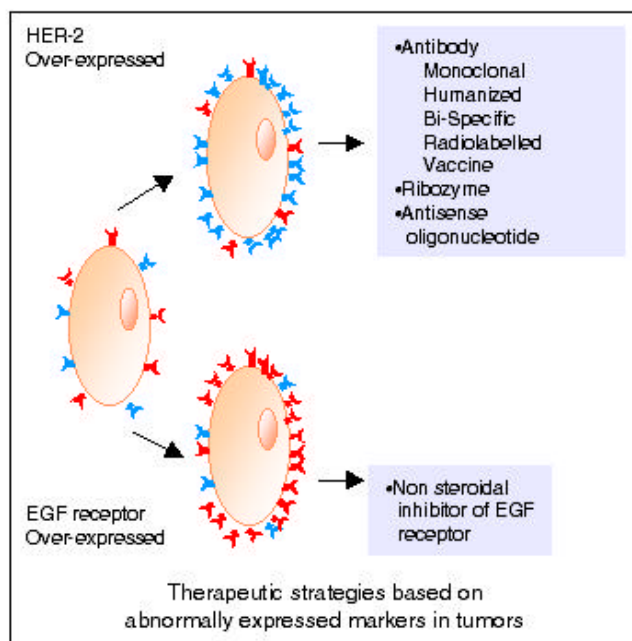
A nagging question, however, persists. Would it at all be practical to check for the large number of molecular signatures every time an oncologist is faced with the problem of tumour classification? The sheer cost and the magnitude of such analyses could thus hinder their widespread applications. The scenario, however, does not appear as bleak. Take for instance, the case of classification of acute lymphoblastic lymphoma (ALL), and acute myeloid lymphoma (AML)¹². Expression profile of 6817 genes when compared in 27 ALL and 11 AML lines, revealed about 1100 genes which expressed in a manner distinctive of one form or the other of lymphoma. While this appears an intimidating number of genes to be compared among various cancer types, the good news is that of these huge numbers, expression profile of only about 50 markers sufficed for the correct classification of 29 out of 34 randomly selected ALL and AML leukaemia samples! The huge efforts of the consortium, as undertaken by the NCI programme, in compiling the expression of profiles in the different cancer types will indeed be most useful, if these studies can narrow down the number of markers required for highly accurate diagnosis of different categories of tumours.

What promise do these tumour-specific markers hold in designing new therapy? This point has been well-illustrated in

Box 3.

A variety of therapeutic approaches can be developed once a distinct target in a given cancerous cell is identified. The HER-2 marker illustrates this strategy. About 20% breast cancers display an over-expression of HER-2, named so by its resemblance to the human epidermal growth factor receptor. This receptor is a protein tyrosine kinase which plays a key role in controlling cell growth. Up-regulated HER-2 expression in breast and ovarian cancer patients made it one of the prime suspects in the etiology of these cancers and led to the development of various therapeutic strategies.

Antibody: The humanized anti HER-2 antibody, trastuzumab, in combination with a drug called paclitaxel was effective in the treatment of 60–70% cancer patients displaying over expression of HER-2 (ref. 16). When bound to the receptor, this antibody inhibits the growth-signalling molecules. In another approach, bi-specific antibody binding both to the HER-2 expressing cancer cells and also the CD3 cell surface marker bearing immune cells facilitates the recruitment of cytotoxic cells in the proximity of the tumorous cells, resulting in a four-fold enhancement in the cytotoxic activity against the cancerous cells¹⁷. Antibodies tagged with radioisotopes have been used to selectively destroy the target cancerous cells in the treatment of Hodgkin's disease¹⁸.



Vaccine: HER-2 derived MHC class II helper peptides could provide an immune protection for a period of more than one year in cancer patients¹⁹.

Ribozyme and anti-sense oligonucleotides: These are the emerging weapons against cancer. Ribozymes are RNA molecules that can act as enzymes by the virtue of their 3D structure. HER2-ECD mRNA ribozymes could bring about a dose-dependent down-regulation of the HER2-ECD expression in ovarian carcinoma cell line, by the cleavage of the HER-2/neu mRNA at the tyrosine kinase domain²⁰. Anti-sense oligonucleotides (ODNs), on the other hand, act by binding to the RNA molecule directly and thus generating duplex structures that are not accessible to the translation machinery of the cell or result in premature transcriptional termination or degradation of RNA molecules. In tissue culture experiments in combination with chemotherapeutic agents like doxorubicin, anti-HER-2 ODNs were found to synergistically inhibit cancer cell growth and activate apoptotic cell death mechanisms²¹.

Non-steroidal inhibitors: While cancers with an over-expression of the HER2 protein responded well to a combination of antibody and drug therapy, for breast cancers in which the estrogen receptor was over-expressed, non-steroidal antagonist tamoxifen was found to be effective²².

tumour experiments conducted in laboratory mice. In one recent study using mice, comparison of expression profile of experimentally-induced benign versus metastatic tumours revealed a set of 32 markers

specific to the latter. Further, RhoC was identified as one of the key molecules associated with the transition from benign to metastatic stages¹³. Inhibition of RhoC effectively inhibited this metastatic trans-

formation. Thus RhoC emerged as a definite indicator of tumour transition, which could be useful for the diagnosis as well as therapy. Although this example may appear experimentally contrived, applications of this strategy in cancer treatment are actually in the offing. The identification of *her-2* gene, albeit by classical methods of gene identification, opened up a number of interesting possibilities for cancer therapy, some of which are now in clinical practice (Box 3). With promising results from the already identified cancer-specific molecules, we can actually begin to look forward to a giant leap in cancer therapy, especially with the availability of modern genomic tools like microarrays.

Gene profiling of the patient's tumour could also direct the choice of the most appropriate drugs, as one of the results in a study by NCI illustrates¹⁴. 5-fluoro uracil (5-FU, a drug used for the treatment of colorectal and breast cancers) was found to inhibit the growth of 18 of the 60 cell lines whose molecular profiles were available. Analysis of the gene expression profiles revealed that 14 from among these cell lines, had a very low expression of the enzyme dihydropyrimidine dehydrogenase (DPYD is involved in thymidine catabolism). Since this enzyme is also involved in the catabolism of 5-FU, the above result readily suggests the effective use of 5-FU in the treatment of tumours with low levels of DPYD. This illustrates a case of how molecular profiling can further harness accurate use of even the prevailing drugs for their optimal therapeutic benefits.

The advantages apart, some constraints still remain. The technology of microarrays or DNA chips is still in its infancy and a cautious approach in interpreting these results is necessary¹⁵. Since a large number of cDNA clones are handled simultaneously while manufacturing the arrays, the dangers of multiplying the errors loom large. Between 1 and 5% of the clones used to prepare the DNA to be coated on the matrix do not contain the appropriate sequence. Further, some stocks when verified for sequence actually contained more than one cDNA¹⁵. Moreover, the transcriptional profile generated by microarrays need not be the actual proteomic profile in all the cases. Thus any data generated by the microarray have to be counter-verified by other methods of analysis. In addition, a major barrier to the widespread use of the DNA chip technology

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is its prohibitory cost. As competition between the manufacturers increases and as technology advances, hopefully there will be solutions that would help overcome this barrier. Meanwhile, with the booming entry into the era of genomics, gene profiling microarrays will continue to provide us novel clues to cancer diagnosis and treatment.

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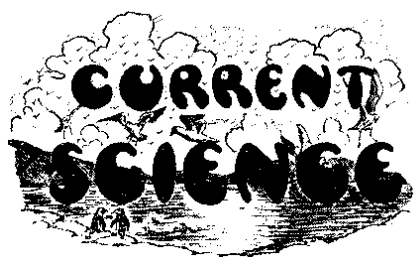
Hina Patel and Pradip Sinha*[†] are in the *Drosophila* Stock Center, School of Life Sciences, Devi Ahilya Vishwavidyalaya, Khandwa Road Campus, Indore 452 001, India

*Present address: Department of Biological Sciences and Bio-engineering, Indian Institute of Technology, Kanpur 208 016, India

[†]For correspondence.

(e-mail: pradips@iitk.ac.in)

FROM THE ARCHIVES



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Educational reform

According to the Press report published recently the Government of India is contemplating the appointment of a special committee to suggest reforms in the present educational syllabuses and methods, as a sequel to the report of the Sapru Unemployment Committee. The special committee is proposed to be assisted in its deliberations by two or more British experts on technological education. In the meantime the Central Government is considering the report of the Sapru Committee with a view to discover the extent and direction of the application of its proposals for relieving unemployment, in the light of the opinions received from the provinces. The main conclusion of the Sapru Commit-

tee is that the problem of unemployment will ultimately be solved by the institution of more technical schools, and by the improvement and adaptation of the existing system of education to the needs of a growing community. The Committee has also suggested measures of reform in the conditions of service with reference to wastage and recruitment. The general aspects of unemployment have been discussed by a number of special committees, the legislative bodies and the leaders of public opinion, who have expounded the causes and remedies of such unemployment. The announcement in the Press that a new committee is speedily to be appointed might lead to the impression that Government is not already surfeited with documents, and it is almost certain that this new committee, however much it may be strengthened by the British experts, is not going to be the last. The problem of unemployment is far too complicated in India to invoke the aid of educational reform alone to provide remedies.

The present system of education is not, as is commonly and frequently criticised, rooted in the life of the people. Its purpose is not relevant to their needs. It is the creation of Government

to suit the special needs of administration. It supports their interests and embodies their prejudices. It brings a foreign culture imposed upon the genius of the people from above by the ruling people who think that they know what is good for the country. The country suspects the motive behind it all. These criticisms are as familiar to us as the things by which we are surrounded from childhood. The people are undoubtedly keen for education, if it is good for something, but they are naturally indifferent to what is offered to them in the name of education leading their children nowhere. Education is mixed up with a multitude of other extraneous things, which have nothing to do with it, and of which people do not approve,—institutions and interests which in a very subtle but powerful way it bolsters up and perpetuates. They agree with everything we might tell them about the need and urgency of education, but the actual system and the purpose of education now in practice they distrust. This feeling which is undoubtedly widespread explains the prevailing indifference of the working classes to education. The reform of education must attack this indifference; it can do so only by making the schools so effi-