Opinion

Molecular tumor profiling: translating genomic insights into clinical advances

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Abstract

Molecular profiling of the transcripts or proteins within an individual tumor may in future provide important prognostic and therapeutic clinical information both for the affected individual and for their extended family, but for the time being traditional genetics and pathology retain their place in the clinic.

Extensive research over the last 50 years has revealed that cancer is a genetic condition in which a cell loses its genomic integrity. Tumorigenesis is associated with the accrual in cells of multiple genomic alterations. Base substitutions can inactivate tumor suppressor genes or cause constitutive activation of proto-oncogenes. Alternative mechanisms of tumorigenesis include large genomic deletions, large and small intragenic deletions, chromosomal translocations, aberrant promoter methylation and other epigenetic events. These alterations allow the cell to escape the normal tissue-dependent restraints on growth and differentiation that may be cell-autonomous or cell-dependent. It has been proposed that the accumulation of genetic abnormalities enables the cell to escape the physiological tissue framework by the acquisition of six essential cell-transformation traits: growth-signal autonomy; evasion of apoptosis; insensitivity to growth-inhibitory signals; sustained angiogenesis; limitless replicative potential; and the capacity to invade and grow metastatically [1]. The molecular aberrations and cellular mechanisms required to effect these cellular phenotypes no doubt vary greatly between different types of tumor, and even within tumor types classified by current histopathology as similar. Furthermore, the molecular route by which the tumor cell achieves certain biological endpoints may be as important as the endpoints themselves in terms of clinical prognosis and response to therapy.

The accurate diagnosis and classification of tumors is thus of fundamental clinical importance both as a prognostic indicator and as the determinant of the most effective treatment modality. Although the current basis of tumor taxonomy is tumor grade, stage and type in conjunction with other histopathological indices, it is becoming increasingly apparent that an individual's family history, as an indirect marker of inherent genetic susceptibility, also provides valuable prognostic and therapeutic indicators, both for the patient and for their extended family. The research attempting to reclassify tumors according to their molecular evolution has focused mainly on breast cancer, the commonest form of female cancer, which affects one in eight women at some-time in their lives, but the principles discussed here are equally applicable to all types of cancer, whether of childhood or adult onset.

Major breast-cancer susceptibility genes

Although the majority of cancers are 'sporadic', occurring in individuals with no family history of the condition, 20% of all colorectal and 30% of breast cancer patients have some family history of the condition, which in itself confers one of the strongest risk factors for developing the disease; for example, there is a two-fold increase in breast cancer in first degree relatives of the index case [2]. In approximately 5% of breast cancer cases the individual is found to be part of a large multi-case 'cancer family', in which the genetic predisposition to cancer is inherited as a single autosomal dominant trait, due in the majority of cases to a germ-line heterozygous mutation in either *BRCA1* or *BRCA2* [3,4].

Both these genes have products that appear to function in pathways involved in DNA repair, gene transcription and chromatin structure [5,6]. In each case the mutant susceptibility allele is inherited as an autosomal dominant germ-line trait, whilst transformation occurs as a recessive phenotype after loss of the wild-type allele in the transformed cell [7,8]. Importantly, the presence of a germ-line *BRCA* mutation does increase the possibility of the patient developing both ipsilateral and contralateral breast disease and other distinct tissue-specific tumors, such as male breast cancer, pancreatic and prostate cancer in the case of a *BRCA2* mutation [9], and ovarian cancer in the case of a *BRCA1* mutation [10]. The tissue-specific nature of the cancer predisposition seen in the inherited cancer syndromes has yet to be explained. The discovery of a germ-line *BRCA* mutation in a family means that predictive testing can be offered to unaffected individuals. On the basis of an estimation of their risk, afforded by predictive testing, such individuals can make better-informed decisions as to surveillance regimes, prophylactic surgery (mastectomy and/or salpingo-oophorectomy) or experimental chemoprevention strategies such as tamoxifen. The corollary of this is that mutation-negative patients within these families can be reassured that they are at normal population risk and can be withdrawn from high-risk screening programs.

The prognostic significance of a *BRCA* mutation is still unclear for breast cancer, when it is separated from indirect effects such as earlier age of onset, or higher mitotic index and the higher histopathological grade associated with *BRCA1* tumors [11-14], although it appears to be an indicator of good prognosis in ovarian cancer [15,16]. Studies prospectively addressing this question are still ongoing. Interestingly, however, recent insights into BRCA2 [17] and BRCA1 [18] protein function, respectively, suggest that this group of breast tumors may not respond as well to taxanes, a current common chemotherapeutic agent, or indeed may be more sensitive to cisplatin than is sporadic breast cancer. These mechanistically driven hypotheses have yet to be shown to have clinical significance.

Knowing the *BRCA* status of a breast tumor has important clinical implications not only for the affected individuals themselves but also for their extended family. Unfortunately, the current means of ascertaining the *BRCA* status of tumors is dependent on the prospective identification of genetically predisposed families on the basis of their large multi-case affected kinships. Recently, however, with completion of the Human Genome Project comes the potential for 'reverse genetic diagnosis', based on the genetic phenotype of the tumors themselves, independent of family structure.

Redefining tumor taxonomy

Since completion of the draft human genome sequence, there has been great interest in identifying novel diagnostic, prognostic and potential therapeutic markers by establishing the genome-wide expression profiles (transcriptomes) of various tumor groups; this has been achieved through the rapid adoption of high-throughput microarray technologies. This type of approach is in contrast to the previous, labor-intensive candidate-gene-based approaches and has the major advantage of potentially measuring simultaneously the expression of all human genes and not merely a pre-selected, and thus biased, subgroup. The cancer phenotype is only partially described by its transcriptome, however, as functional protein levels are also modulated by post-translational modifications.

Somatic mutations in the *BRCA* genes are not found in sporadic breast cancers. This is in contrast to the molecular pathology of mutations in other cancer-predisposition genes, such as the familial adenomatous polyposis (*APC),* retinoblastoma (*RB1*) and *P53* tumor suppressors, and suggests that breast tumors arising in cells with a heterozygous *BRCA* mutation may form a distinctive pathological group. Down-regulation of *BRCA1* transcription has been noted in a few cases of sporadic breast cancer and found to correlate with epigenetic methylation of the *BRCA1* promoter [19]. This epigenetically mediated diminution in gene expression in sporadic tumors has not been shown with *BRCA2* [20]. It is significant, then, that gene-expression profiles [21,22] of *BRCA1*- and *BRCA2*-linked tumors allow them to be distinguished and classified separately from a group of sporadic breast tumors with similar hormone-receptor expression patterns, emphasizing once more the unique nature of these tumors. Unfortunately, the *BRCA*-associated tumor cohort size is small in the recent studies of gene expression in breast tumors [21,22].

In the case of ovarian cancer, Jazaeri *et al.* [23] showed that *BRCA1*- and *BRCA2*-associated cancers show distinct gene-expression profiles, but it is striking that, in comparison with the results from sporadic breast cancer, sporadic ovarian cancers displayed an expression motif mimicking the profile of either a *BRCA1*- or a *BRCA2*-linked germ-line ovarian tumor and do not form a separate third group. These findings could be explained by postulating that sporadic ovarian cancer can occur by two major, mutually exclusive pathways in which the *BRCA* genes are major players, evidenced by the loss of heterozygosity occurring at their genomic loci despite the absence of somatic mutations in *BRCA1* or *BRCA2*. More intriguingly, these distinct expression profiles could represent differing cellular origins for the various ovarian tumors. Germ-line mutations in either the *BRCA1* or *BRCA2* gene could predispose different ovarian cell types to cellular transformation. Germane to this suggestion in ovarian cancer is the expression profiling of breast cancer samples [24], which has allowed their stratification into two subtypes - luminal (similar expression profiles to cells that line the duct and are implicated in the majority of breast cancer) and basal (similar expression profiles to cells in the basal epithelium) - hinting

at two distinct cellular origins for breast cancer cells. It is notable that all the expression profiles from a group of *BRCA1*-linked breast tumors fell into the latter, basal subclassification, suggesting a possible common, homogenous cellular origin for *BRCA1* breast tumors [25]. These profile classifications were also found to have prognostic significance, as the tumors with a basal signature had a poorer prognosis than the luminal group. These results would suggest that a unique gene-expression signature may be one possible mechanism for the identification of *BRCA1*- and *BRCA2*-type breast tumors in families for which a detailed family history is not known or is small.

Microarray profiling has also been successfully used to delineate gene-expression signatures associated with poor clinical outcome [22,26], response to neoadjuvant therapy [27] and *a priori* potential to metastasis [28]. This latter finding runs contrary to current belief that primary tumors grow locally and evolve with time into aggressive tumors capable of metastasis. The common expression signature indicative of metastatic potential was delineated over a range of different tumor tissue types and thus would suggest that metastatic capacity is already inherent in the primary tumor at first diagnosis; this finding, if clinically substantiated, could form the basis of tailoring post-operative adjuvant treatment to individual tumor genotypes.

The clinical implications of this large body of research, both in identifying familial breast cancers from the larger cohort of sporadic tumors and in finding prognostic molecular markers for tailoring future therapy, are potentially huge. Even with conservative estimates of their influence on the identification of future novel therapeutic markers, gene-expression signatures would allow a more focused, targeted application of the treatment regimes currently available. But such a radical change in clinical emphasis from empiric treatment regimes to those based on individual tumor-specific molecular markers is highly dependent on our ability to take transcription profiles and translate them into a simple, fast, cost-effective and highly robust clinically applicable form. Alternatively, it may well be that, once the dust has settled from the microarray explosion, gene-expression motifs will emerge that directly correlate with protein levels and would therefore be amenable to immunohistochemical approaches, which are already in widespread use in pathology departments. One example of this would be to identify the basal breast cancer subgroup associated with poor prognosis [24] by means of keratin 5/6 and keratin 17 immunostaining.

Validation of protein expression on multiple paraffin-embedded tumor samples can be achieved easily and cheaply through the use of tissue microarrays. These comprise core samples of multiple individual tumors embedded and sectioned onto an individual slide; this has the effect of reducing the cost of the test and of intra-sample variation. Numerous studies [29,30] have validated the use of between one and four core 0.6 mm biopsies, rather than immunostaining of the whole tissue section as is current practice in pathology departments. Tissue arrays can contain multiple different tumor types, collections of histologically similar tumors with different clinical prognosis (based on clinical follow-up data), or samples representing different stages in tumor progression. This approach is highly dependent on the capacity to reduce a highly complex gene-expression profile to a feasibly small protein profile, the availability of antibodies and the ability to assay expression of the relevant proteins in a quantifiable manner.

Identifying novel tumor-susceptibility genes

To date, classical linkage analysis and candidate-gene approaches have yet to identify the genetic cause of the 80% of familial moderate- to high-risk breast cancer families not associated with *BRCA* mutations or another familial breast cancer syndrome (Table 1). This is due wholly or in part to complications from genetic heterogeneity, low penetrance or polygenic mechanisms. Arguably, the clustering of breast cancer seen in these families could be due to environmental factors, but twin studies [31] and the pattern of inheritance [32] would point towards a genetic susceptibility (although the two models are not mutually exclusive). A recent model [33] based on population and multi-case non-*BRCA* breast cancer families suggested a polygenic mode of inheritance in which genetic susceptibility is the product of multiple low- to moderate-penetrance alleles; this model predicts that half of all breast cancers will arise in the most susceptible 12% of

Table 1

Breast cancer susceptibility syndromes

Syndrome	Gene(s) responsible	Other main tumor types
Familial breast and ovarian cancer syndrome	*BRCA1* and *BRCA2*	Ovary, prostate and pancreas
Li-Fraumeni syndrome	*P53*	Sarcoma and brain
Cowdens syndrome	*PTEN*	Thyroid and endometrial
Mismatch repair syndrome	*MLH1* and *MSH2*	Gastrointestinal
Peutz-Jegher syndrome	*STK11*	Gastrointestinal

the population. If this susceptible population could be identified, it would have major clinical implications for the most effective targeting of population surveillance regimes.

Attempts to identify low-penetrance genes have used candidate-gene-based linkage-disequilibrium approaches, focusing on polymorphisms that may either be causally related to the cancer risk or are in strong linkage disequilibrium with the disease-causing variants in breast cancer patients compared with unaffected controls. Common polymorphisms in candidate breast cancer genes have been studied by looking for an association between a common polymorphism in a candidate gene and breast cancer in a large series of affected individuals compared to an age- and ethnicity-matched unaffected control group. Unfortunately, many studies appear contradictory in their conclusions, whilst the size of many studies may also preclude the identification of low-penetrance genes. Possible low-penetrance alleles have been identified in the estrogen-metabolism gene *CYP19*, the carcinogen-metabolism gene *GSTP1*, and the general tumor-suppressor *P53*, each of which confers 1.2- to 2-fold increase in the relative risk of developing breast cancer [34]. Other studies have included common variants of *BRCA1* [35] and other genes implicated in DNA repair [36]. Indeed the 1100delG polymorphism in the DNA-repair gene *CHK2*, first described in a family with Li-Fraumeni syndrome (see Table 1), has been found to cause a 1.7-fold relative risk of familial breast cancer. Interestingly, however, this increased risk is not found in families with a known *BRCA* mutation, suggesting an epistatic overlap in function between these proteins [37,38]. The relative lack of success of the candidate-gene approach underlines our lack of knowledge of normal breast tissue physiology, cell biology and the mechanism of cellular transformation. The choice of candidate genes for screening is at best speculative. There is a need to identify novel candidate tumor-susceptibility genes without the bias of our current knowledge of the pathways involved in tumorigenesis, or to attempt non-hypothesis-driven genome-wide linkage-disequilibrium studies [39,40].

An alternative approach to identifying novel tumor suppressor genes has involved interrogation of the transformed genome of established tumors. The Cancer Genome Project [41], based at the Wellcome Trust Sanger Institute (Hinxton, UK), is using the information and high-throughput technologies established by the Human Genome Project to characterize large genomic homozygous deletions and somatic mutations in an initial panel of 48 common adult epithelial tumors, as a means of identifying novel genetic events important in carcinogenesis. This approach will produce an extremely detailed unbiased description of the transformed genome. Alterations found in the initial panel of tumors will be sought in a larger tumor cohort. In addition, further cell-biological investigation will be required to separate those aberrations that are causative in nature from those that merely correlate with tumorigenesis. Array comparative genomic hybridization (array CGH) can also be used to identify chromosomal gains and losses within tumor cells, with a high resolution even in the presence of 60% normal cell contamination [42]. This makes it an excellent technique for the identification of chromosomal events early in tumor formation and, used in conjunction with human genome sequence, can generate a list of candidate genes very quickly.

What are the rate-limiting steps?

The completion of the Human Genome Project marked a paradigm shift in human genetic research. The knowledge of the human genome sequence allows in theory the characterization of all genomic diversity in both disease-associated and non-disease-associated genomes. This information, in association with high-throughput technologies, has allowed non-hypothesis-driven interrogation of tumor phenotypes at both the genomic and gene-expression levels. Proteomic research has not been considered in this article, but it constitutes the next descriptive level being pursued in 'translational' research that aims to bring the insights of the lab bench to bear on clinical practice. Unlike previous approaches, genome-wide scans have the advantage of painting a picture based on unbiased, non-pre-selected data points but - like pointillistic images - if viewed too closely the abundance of primary brushstrokes will obscure the actual image. The interpretation and reproducibility of the data thus become key rate-limiting steps. These problems will be solved by establishing functional, biological assays through which observational data can be filtered. When considering tumors, functional biological assays include establishing biologically relevant, mechanistically defined end-points in patient treatment and response. From a clinical perspective, substratification of tumors is only relevant if it can be achieved within a time scale relevant to the patient's care and, more importantly, can have a significant clinical impact on either treatment or prognosis. Similarly, the identification of low-penetrance genetic susceptibility alleles will only become clinically important if intervention, either at the environmental/behavioral level or by population surveillance, becomes a feasible reality both from the practical and the financial points of view. At present, for example, the clinical utility of the *CHK2* 1100delG allele, conferring a two-fold increase in breast cancer but present in 1.1% of the normal population, is extremely limited.

It is becoming evident that the current models used in cancer genetics studies for defining and using information about familial cancer risk will see a radical change as our knowledge increases. It will be interesting to see whether, in the next decade, current genetic practice will be turned on its head and 'reverse genetic diagnostics' will mean that tumor phenotype will alert us to increased family risk. The ethical implications of this approach in terms of informed consent and pre-test counseling for clinical diagnostic tests that may have wider genetic implications for unaffected relatives will

need to be addressed. Genome-wide unbiased approaches are already beginning to shed light both on the genetic changes that give rise to tumors and on the expression profiles that characterize established tumors with different origins and different prognoses. As these findings move ever closer to having specific implications for individual patients and their families, we will face new challenges in interpreting and applying them in the clinic.

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