IMPACT OF GENE TECHNOLOGIES ON PERSONALISED CANCER THERAPY

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Abstract

Given that cancer is driven by inherited and acquired defects in our genetic code, the ability to profile genes and their activities has the potential to impact substantially on the prevention and treatment of cancer. The last decade has seen very rapid advances in the ability to measure both an individual's genetic predisposition to cancer and the mutational load within a cancer sample. Identification of germline mutations in genes such as BRCA1, BRCA2 and MSH1, that are associated with greatly increased risk of breast, ovarian, colorectal and other cancers, has led to the development of integrated management strategies for measurement and management of genetic risk in a clinical setting. Very recently, technical advances have made it possible to identify genes that confer a much lower, but still significant, risk of cancer. Low-risk genes will present major challenges in devising risk-management strategies, because complex gene-gene and gene-environment interactions are likely to have a significant impact on overall cancer risk. The ability to measure somatic DNA changes in the cancer genome has led to clinically available tests that can predict response to treatment and aggressiveness of disease. The availability of such tests will increase as their utility is validated and technical advances make them faster, cheaper and more comprehensive. The current revolution in DNA sequencing technology promises the availability of affordable whole genome sequence information within a few years.

Our genetic predisposition to cancer

Cancer can be thought of as a corruption of the DNA software code that controls normal cellular processes. Errors in the code may be present in the germline or acquired throughout life, as somatic mutations. Some germline changes can have a profound impact, increasing the risk of cancer greatly and these are referred to as being 'highly penetrant' and 'high-risk'. For example, germline mutations in BRCA1 that inactivate the protein can result in a 60-70% lifetime risk of breast cancer in women.[1] For high-risk cancer genes there is a close correspondence between presence of the mutation in an individual and appearance of the disease (cancer), making it possible to identify such mutations through the use of linkage studies involving families with strong cancer predisposition pedigrees. A number of high-risk cancer genes were identified in the 1990s and over the last decade a great deal has been learned about approaches to genetic testing for high-risk families. Integrated risk-management strategies for individuals carrying high-risk genes are now an established aspect of modern cancer care (overviewed *Cancer Forum*, November 2007 Vol 31 No.3).

High-risk genes account for a small proportion of all cancers and it appears that inherited cancer risk for most people is determined by the concerted impact of a number of genes in their genome, each of which may individually confer low risk, but which interact in an additive or even synergistic manner. As such, genetic cancer risk for most people is probably more akin to being dealt a good or bad hand of genes, rather than being the product of a single gene. A low risk gene implies that it is weakly penetrant, that is, only a minor proportion of individuals with the genetic change will manifest the disease. As a result, traditional linkage studies are ineffective at identifying such genes and other approaches must be used to find them. Whereas a high-risk mutation typically has a profound impact on a gene, a low risk change may have only a subtle impact on protein abundance or activity and therefore may not be readily obvious. Indeed, many low risk changes can be viewed as genetic polymorphisms that constitute part of normal human variation.

The HapMap is an international consortium that aims to identify the millions of single nucleotide polymorphisms (SNP) in the human population that confer difference between one person and another.[2] The identification of these SNP's and the development of technologies to type hundreds of thousands of SNP's in large numbers of individuals in an affordable manner made it possible to perform SNP-based genome-wide association studies to search for low risk genes.[3] In the last two years these studies have led to the identification of low-risk genes for cancers of the breast, prostate and colon.[4-10] As predicted, most of these genes increase risk slightly (less than two-fold), but appear to have a synergistic interaction. The impact of the SNP on gene function has not been obvious for some low risk genes, and in such cases it is not known whether the defect is associated with the specific SNP or whether the SNP simply marks a more significant nearby change. For example, a very robust association has been found between SNPs at chromosome position 8q24 and colorectal and prostate cancer risk, although the mechanism of action of the genetic change was not identified in these studies.[8-11] Genome-wide association studies require thousands of cases and controls to generate robust statistical associations. As a result, many studies are at the limit of what is possible and there is a substantial risk of finding chance associations between the presence of a given SNP and cancer risk. The most compelling findings are those that are replicated in completely independent studies as has been achieved for several new, low-risk breast and colon cancer genes. The finding that the presence of

some low risk genes can increase the risk of disease in individuals bearing high-risk mutations, such as in BRCA1 or BRCA2, adds weight to their importance.[12]

Although the field is at a very early stage, low risk cancer genes can clearly be found using advanced genetic technologies and very large patient cohorts, established through the formation of international research consortia. While they may confer relatively low risk individually, their effect appears to be compounded through gene-gene interactions.[4] Collectively, low risk genes are likely to account for a large proportion of cancers, if as expected the risk alleles are frequent in the population. Despite this progress, it is presently unclear how and when testing for these genes should be integrated into clinical practice. We don't yet fully understand how most low risk genes interact with the rest of the genome to confer overall risk – a key parameter in deciding risk-management options. Additionally, it is possible that the impact of many low risk genes will be strongly influenced by the environment (gene-environment interaction). While such interactions remain to be defined, it is likely that modification of diet and lifestyle factors may become the predominant approaches to the preventative management of individuals carrying low risk genes, rather than more drastic surgical interventions. Cost and the potentially negative psychological impact of testing will need to be weighed against the advantages of detection of low but significant genetic risk.

One of the most significant implications of detecting low risk genes may be in targeted early detection testing. It is estimated that only about half the population account for ~90% those at risk of breast cancer.[13] In addition to significant cost savings through more targeted screening, the ability to identify those most at risk may make early detection testing feasible for low incidence cancers for which population-based screening is impractical. For example, population-based early detection testing for ovarian cancer is hampered by an unacceptably low positive predictive power of current testing regimes, however this might be improved through more focused screening of those most at risk.[14]

Genetic profiling of the cancer genome

The genomes of cancer cells carry a range of somatically-acquired changes which include alterations in gene copy number (amplifications/deletions), gene expression, methylation, novel gene fusions (translocations) and point mutations. Some of these changes are so called passenger mutations – inconsequential events acquired as a result of an unstable genome – which are distinct from important driver mutations that provide selective advantages to the cancer cell.[15] While it is believed that the constellation of driver mutations within a given cancer cell generally act in concert, some mutations can be of sufficient importance that reversing their effect can have a profound impact on the growth of the tumour and therefore represent excellent therapeutic targets. Amplification of HER2 in breast cancer, the BCR-ABL translocation in chronic myeloid leukaemia, epidermal growth factor receptor (EGFR) in lung cancer, and C-KIT mutations in gastrointestinal stromal tumours are good examples of mutations that result in a state of oncogene addiction by the cancer cell that when inhibited with agents such as trastuzumab, gefitinib and imatinib, lead to a significant therapeutic response.

These oncogenes and their corresponding diseases also exemplify the value of developing diagnostic molecular tests in conjunction with a targeted therapeutic, since detecting the presence of the driver oncogene provides a strong predictor of therapeutic response to the molecularly targeted agent. Recent studies showing that the presence of K-RAS or PTEN mutations can attenuate responses to molecular agents targeting HER2 or EGFR mutations, extends the concept of using molecular diagnostics to probe the network of other genetic events that may influence therapeutic response.[16-18] This concept is further exemplified by the development of gene expression profiling tests such as Oncotype Dx and Mammaprint, which monitor the expression of multiple genes to provide prognostic information that can guide clinical decision-making.[19-22] These tests, available commercially and in increasing clinical use, have been developed from a large number of DNA microarray-based studies performed over the last decade. While still in clinical development, it appears likely that such tests will also impact on the management of disease, such as diffuse large cell B lymphoma and carcinoma of unknown primary.[23,24] Key mutational events can also provide biomarkers of the presence of disease. The development of highly sensitive polymerase chain reaction based tests are widely used to monitor therapeutic response and recurrence in chronic myeloid leukaemia and other types of leukaemia.[25]

Given the importance of targets such as HER2 and BCR-ABL, systematic screens for mutations in thousands of genes in cancer genomes have commenced in order to provide new therapeutic approaches. Pioneering studies involving screens of all protein kinases in the genome led to the identification of B-RAF mutations in melanoma and other cancers, a potentially important new therapeutic target.[26] More recently, researchers have screened all known protein coding genes in a handful of breast and colorectal cancer samples, leading to the identification of several hundred new 'CAN' genes, putative driver mutations for these diseases.[27,28] Organised international consortia such as the Cancer Genome Atlas and the International Cancer Genomics Consortium are embarking on screens that aim to catalogue all significant mutation events in common cancers by screening hundreds of cancer samples for each disease. While this work is providing an unprecedented view of the cancer genome, a sobering finding so far has been the general absence of new, common, high-frequency mutations that could be ideal therapeutic targets.[27] These findings point to the importance of developing highly multiplexed tests that can search for a range of possible mutations in an individual's cancer to assist clinical decision-making. OncoMap provides such an example, where mutations in multiple therapeutically relevant genes are screened by mass spectrometry.[29]

Future of genetics in personalised medicine

There are now numerous examples of the value of genetic profiling in measuring genetic risk, monitoring disease response and recurrence, in prognostication and prediction of therapeutic response. The recent development of Poly ADP-ribose polymerase inhibitors targeting tumours with germline mutations in BRCA1 or BRCA2 is a potent example of how knowledge of germline status can be exploited therapeutically.[30] Although some cancers, particularly leukaemia and some sarcomas, appear to be predictably driven by dominant common oncogenic events, the pattern that appears to be emerging for most solid cancers is one of molecular heterogeneity both within and between individual cancer patients.[28] Very recent studies have identified new genes associated with small, but significant increases in cancer risk. These studies also suggest a complex pattern of events where overall genetic cancer risk will be dependent on the interplay of multiple genes within an individual's genome. All these findings point to the need for rapid, affordable and particularly, ultra-high throughput methods of probing the germline and the cancer genome.

The last few years have seen unprecedented innovation in DNA sequencing technologies.[31] So-called 'next generation' sequencers have already reduced the cost and increased the throughput of DNA sequencing several orders of magnitude, effectively replacing sequencing factories with desk-top boxes. Although not quite there yet, novel sequencing technologies are very likely to make available affordable whole genome sequencing, within the next few years. Such capability will have a profound impact on our ability to measure germline genetic risk and probe molecular change in cancer genomes. Integrating this welter of complex information into evidence-based medicine that works for the patient will be the great medical challenge of our time.

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