The use of molecular profiling for diagnosis and research in non-Hodgkin's lymphoma

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Abstract

Molecular profiling facilitates the understanding of the genetic processes underlying the development of cancer, and makes it possible to use specific signatures to prognosticate clinical outcome and to predict response to specific treatments. There has been a great increase in the availability of tools for exploring genetic abnormalities in cancer cells, which have allowed a more comprehensive characterization of the mutations, translocations, and copy-number variations that may affect the development of cancer or therapy response. An improved understanding of the molecular basis of cancer is helping also in the identification of new molecular targets for therapy.

Introduction

A key aim in the management of cancer is to associate the diagnosis to the treatment, so that treatments are more effective and the probability of curing patients is improved. For this, the concept of molecular cancer taxonomy is essential – a system that allows clinicians to assign treatments to patients, based on the morphology/immunophenotype/molecular features of the disease. Taxonomy requires the precise definition of clinicopathologic entities and molecular markers that are specific to each condition. This classification system leads to the identification of underlying molecular alterations, targets for therapy and predictive and prognostic markers for patient stratification.

The cancer sequence

Cancer molecular profiling has greatly facilitated a more comprehensive understanding of the processes underlying the development of cancer cells. An early stage is the presence of inherited plus acquired genetic mutations and epigenetic changes in the cells, which at this stage are not yet neoplastic cells, but acquire a selective survival advantage. Cancer cells then acquire the capacity for self-renewal, so-called *stemness*, al-

though it is not clear at this time whether all cancer cells have this capacity or only a minority of them, or even whether this is just transiently acquired. These cells develop into a tumor when they get the capacity for local infiltration, probably through development of a signature identified as epithelial-mesenchymal transition. Eventually, these cells gain the capacity for distant metastasis, requiring drug treatment. Finally, during therapy, these tumors can develop genomic instability, which allows them to escape the therapy. The steps for this sequence are still a matter of investigation, and new concepts and data may dramatically change our current understanding of cancer.

Genetic sequencing of cancer cells

A key advance in our study of cancer has come from the development of technology and reduction of the cost of gene sequencing, which has produced a great increase in the amount of sequencing data that are starting to be available.[1] This has led to the formation of the International Cancer Genome Consortium. The goal of this consortium is to coordinate the generation of comprehensive catalogs of genomic abnormalities (somatic mutations) in tumors from 50 different cancer types and/or subtypes that are of clinical, scientific, or social importance across the globe.[2] For each of these 50 cancer types/subtypes, the objective of the consortium is to sequence at least 500 cases. This project is ongoing, for example in breast cancer, lymphoma, and other common types of cancer – some of the tumor types have still to be defined. It is the most ambitious project that has ever been developed in the field of biomedicine or cancer research, as it encompasses the sequencing of 50,000 genomes.

Multiple mutations in cancer cells

An important finding discovered in recent years is that most cancer samples have multiple somatic mutations, rather than the small number that was originally expected. In that context, it is not surprising that targeted therapies that are directed at single mutations are not fully effective. A study of tumor cells in pancreatic cancer found that these tumors had an average of 63 genetic alterations, the majority of which were point mutations, and some cancers had more than 100 genetic alterations (Figure 1).[3] The frequency of mutational changes is nevertheless changing among different tumor types, with higher frequency in tumors associated with smoking or exposure to ultraviolet light, such as lung cancer and melanoma. The mutations characterized in pancreatic cancer are not distributed randomly across the genome, but affect particular signaling pathways and processes in the cells, forming a complex system of changes. The

challenge for researchers, therefore, is now to understand the data that are available on genetic changes, and move on from that to discover the implications for targeted therapy of the patients.

Therapy driven by molecular diagnosis

The concept we are now trying to sustain and develop is phrased as *therapy driven by molecular diagnosis*. Research strategies aim to match the individual genetic variability of tumor samples and patients with therapies adjusted to these variables. This will be achieved through the combination of: early diagnosis for screening of patients at risk; the definition of prognostic markers that would allow us to assign individual treatments and stratify patients by their individual risk; and the development of therapies for the newly identified therapeutic targets.

Tools for molecular diagnosis

Analysis of the genomes of cancer patients now allows the characterization of several types of genetic changes. These include point mutations, insertion or deletion of sequences, inter- and intrachromosomal translocations, and copy-number changes.[4] These somatic mutations can be examined using DNA microarrays, to investigate single-nucleotide polymorphisms (SNPs), copy-number variations, and gene-expression signatures. Changes in gene expression and protein products can also be measured using tissue immunohistochemical microarrays. In addition, microRNA (miRNA) arrays are starting to be used, and techniques for analyzing miRNAs have been applied using formalin-fixed, paraffin-embedded tissue studied using quantitative polymerase chain reaction (QT-PCR)

on samples. Identification of SNPs allows the analysis of gene polymorphisms in patients and copy-number variation in tumors. However, interpretation of these results is not simple – millions of SNPs can be identified in an individual, but most of these cannot be linked to specific risk for a disease, or to response to therapy. In most cases, the impact of an SNP is not individually important, although in some cases it may produce a different gene product or alter the regulation of a gene with significant pathogenic results.

The use of tissue microarrays has led to the development of antibodies that can be used to identify specific cancer phenotypes. For example, germinal center B-cell-expressed transcript 1 (GCET1) has been used to characterize B-cell lymphomas,[5] and the expression of B-lymphocyte-induced maturation protein-1 (BLIMP-1) has been found to constitute the best marker of plasma cell differentiation among normal and neoplastic B-cells.[6] Immunohistochemical staining in tissue microarrays can also be used to quantify the expression of key regulatory proteins (e.g. Bcl-2, Lyn, Syk). These may be highly important in the future because, by using such microarrays, expression of multiple markers can be visualized at the same time in a tumor sample. Moreover, as tumor cells are unstable and their genetic composition changes with time, this technique should allow us to monitor differences both between tumors and within the same tumor over time.

Identifying molecular risk

Tumor specimens can be analyzed to identify specific molecular risk scores. For example, in samples from patients with advanced Hodgkin's lymphoma, quantitative reverse-transcription PCR was applied to 30 genes to develop a molecular risk score.[7] There are likely to be hundreds of markers that affect patients' molecular risk in this condition, but it is not feasible to work with large numbers of markers, so they were reduced to a reasonable number, to focus on the best predictors. These were integrated into an 11-gene model, incorporating genes from four functional pathways: the cell cycle, apoptosis, macrophage activation, and interferon regulatory factor 4. This produced a single molecular risk score for individual patients, which predicted response to treatment and 5-year failure-free survival (defined as the time interval between treatment initiation and treatment failure or last follow-up). Importantly, in this and other studies it has been found that the molecular risk was independent of clinical risk. Thus, molecular and clinical risk could be combined, and in this way it was possible to identify about 25% of patients who were at very high risk of not responding to therapy, carrying a very unfavorable prognosis (Figure 2).[7]

Identifying treatment targets

Lamb *et al*. have reported their development of a reference database of gene-expression profiles from cell lines. These can be mined for connections with *gene signatures* of specific tumors or experimental conditions, to form a *connectivity map* that relates tumor signatures with response to specific treatments.[8] Multiple practical applications of this approach have already been generated. For instance, an expression profile of genes thought to play a role in the pathogenesis of chronic lymphocytic leukemia revealed variability in the expression of two gene clusters associated with B-cell-receptor signaling and mitogen-activated protein kinase activation. Variations in the expression of these two clusters identified three groups of patients who had different risks of treatment-free survival.[9] Using such techniques, therefore, gene expression may be used to predict patients' treatment needs at early stages of the disease. These potential drug sensitivities can be tested *in vitro* using measurable pharmacodynamic markers. In this way, the introduction
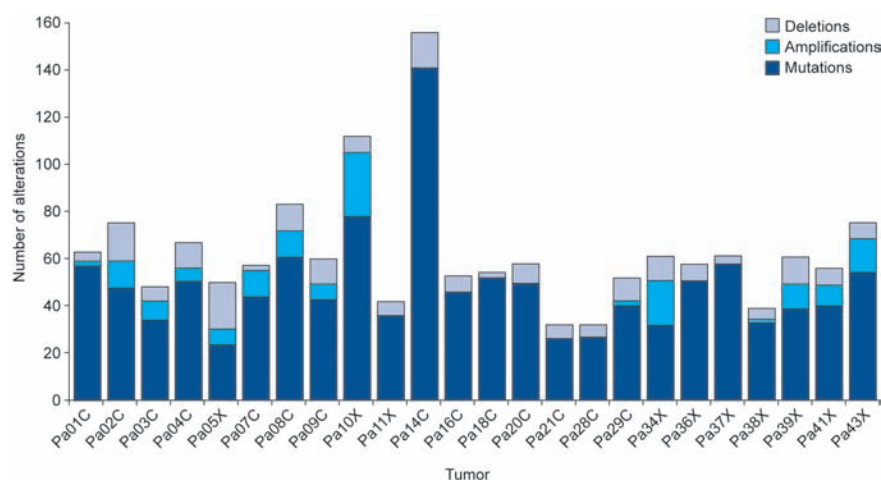

Figure 1. The number of genetic alterations detected through sequencing and copy-number analyses in each of 24 pancreatic cancers.[3] From Jones S *et al*. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science 2008;321:1801-6. Reprinted with permission from AAAS.
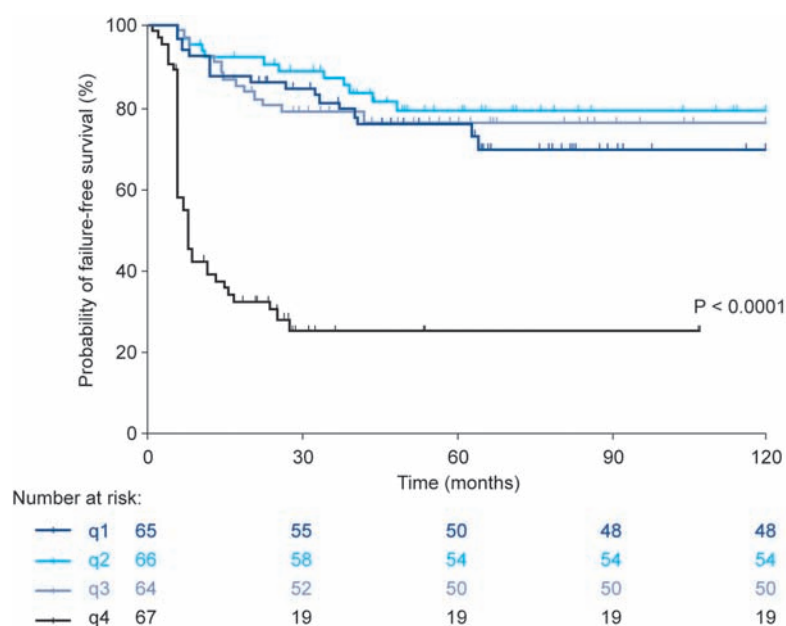

Figure 2. Integration of the molecular risk score and clinical risk (stage IV disease) identified a quartile of patients with particularly poor prognosis.[7] Refer to main text for definition of failure-free survival. This research was originally published in Blood. Sanchez-Espiridion B *et al*. A molecular risk score based on 4 functional pathways for advanced classical Hodgkin lymphoma. Blood 2010;116:e12-17.© The American Society of Hematology.

of new therapies for hard-to-treat cancers may be accelerated.

A second tool for identifying molecular treatment targets is gene set enrichment analysis (GSEA) – a computational method that determines whether an *a priori*-defined set of genes shows statistically significant differences between two phenotypes (www.broad.mit.edu/gsea). This approach has been used to screen a database of drug-associated gene-expression profiles for molecules whose profile overlapped with the gene-expression signature of glucocorticoid sensitivity in acute lymphoblastic leukemia. This indicated that the gene-expression profile of the mTOR inhibitor rapamycin matched the signature of glucocorticoid sensitivity. Further testing demonstrated that rapamycin could induce glucocorticoid sensitivity in neoplastic lymphoid cells.[10] This demonstrates how GSEA can be used to identify promising new or combination therapies. Likewise, in cutaneous T-cell lymphoma, this approach demonstrated that the histone deacetylase inhibitor vorinostat interfered with the signal-transduction pathway of the T-cell receptor, and synergized with phospho-inositide-3 kinase inhibitors.[11] Thus, the development of new drugs and combination therapies is being accelerated through the use of molecular diagnostic techniques.

Conclusions

It may be possible to stratify patients for targeted therapy using molecular diagnosis. This approach requires clear, specific markers that can be applied at diagnosis, and pharmacodynamic markers to assess responses to treatment. New drugs and combinations may also be assayed *in vitro* using these molecular techniques. Rapid and flexible clinical trials are needed to move ahead with the introduction of new drugs developed in this way.

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