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Genomic landscapes of cancers: prospects for targeted therapies

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Until now tumors have been classified based on two criteria: their localization (site of occurrence) and their appearance (histology). These criteria are also currently used as primary determinants of prognosis and to establish the best treatments. For many decades, it has been known that patients with histologically similar tumors have different clinical outcomes. Furthermore, tumors that cannot be distinguished based on histological analysis can respond very differently to identical therapies. As a result, patients are sometimes exposed to over-aggressive surgical and therapeutic regimens. Here, we will discuss how the knowledge that cancer has a genetic basis and the progress in the analysis of the cancer genome are revolutionizing the way that tumors are classified and treated.

What has been done

The availability of the human genome sequence and the progress in 'omics' technologies have led to a race to analyze the entire cancer genome. In this regard, one of the most successful approaches has been the systematic mutational profiling of gene families.[1,2] In light of their amenability as therapeutic targets, the sequences of kinase genes (collectively known as the 'kinome') were the first to be analyzed.[2,3] These approaches led to the discovery of somatic mutations in kinases such as *BRAF*, *EGFR*, *JAK2* and *PIK3CA* in many tumor types, including colorectal (CRC), breast and lung carcinomas, gliomas, melanomas and leukemias (**Table 1**). Functional studies have shown that the mutations generally activate the corresponding proteins and are oncogenic in cell and mouse models. These features render mutated kinases extremely attractive as therapeutic targets. Accordingly, intense drug-discovery programs led to the identification of inhibitory compounds, some of which are already in clinical use (e.g. those targeting the EGFR, BRAF and JAK2 kinases), while others are presently undergoing clinical trials (**Table 1**). A number of conclusions can be drawn from the experience gathered by the high-throughput genotyping of cancer genomes and the pharmacological targeting of the cancer alleles that have been recently discovered.

The first conclusion is that the genomic landscape of human cancers contains very few mountains (genes mutated at moderate to high frequency) and a lot of small hills (genes mutated at low frequency).[2,4] Interestingly, genes that are mutated at high frequency are often shared by different tumor types (e.g., *TP53*, *PTEN* and *PIK3CA*), indicating that they play a critical role in the process of tumor progression. Tumor-specific cancer genes (such as *APC*) in CRC also exist, suggesting that they play a critical but tissue-specific role in the transformation process. Furthermore, recent data show that the mutational pattern of tumors sharing identical sites of occurrence and histological features can be very different. For example, in CRC a recent analysis indicated that, with the exception of *APC* which is nearly always mutated, different subsets of genes are altered in individual tumors.[4]

The second conclusion is that because many genes are mutated at low frequency, it is hard to discriminate which mutations are 'drivers' of the tumorigenesis process and which are simply 'passengers' (not causally related to cancer progression).[2] For example, while the previously mentioned genes (*BRAF*, *EGFR*, *JAK2* and *PIK3CA*) are mutated at a prevalence that unequivocally confers them the status of cancer genes, many others (most of which

Gene	Tumor type	Mutation type	Drug
ABL	ALL, CML	Translocation	Dasatinib, Imatinib, Nilotinib
BRAF	CCC, CRC, Melanoma Ovarian, Thyroid	Missense Mutation	Sorafenib
EGFR	CRC, Glioma, NSCLC	Amplification, Deletion Missense Mutation	Erlotinib, Cetuximab, Gefitinib, Lapatinib, Panitumumab, Vandatenib
ERBB2	Breast, Lung, Ovarian	Amplification, Deletion Missense Mutation	Lapatinib, Trastuzumab
FGFR2	Endometrium	Missense Mutation	
FGFR3	Bladder, Head and Neck, MM	Missense Mutation Translocation	
FLT3	ALL, AML	Missense Mutation	Sorafenib
JAK2	ALL, AML	Missense Mutation Translocation	Erlotinib, Imatinib
KIT	AML, GIST	Missense Mutation	Dasatinib, Imatinib, Nilotinib
MET	HCC, Head and Neck NSCLC, RCC	Amplification Missense Mutation	
NTRK3	Breast (secretory)	Translocation	
PDGFRA	GIST	Missense Mutation	Dasatinib, Imatinib, Nilotinib
PIK3CA	Bladder, Brain, Breast CRC, Endometrium Lung, Oesophagus	Amplification Missense Mutation	
RET	Thyroid	Missense Mutation Translocation	Sunitinib, Vandetanib

Table 1. Kinases mutated in cancer and their clinically available inhibitors
Kinases altered in > 10% of the indicated tumors; common types of genetic alterations; FDA- and/or EMEA approved drugs are listed. Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CCC, cholangiocarcinoma; CML, chronic myeloid leukemia; CRC, colorectal carcinoma; EMEA, European agency for the evaluation of medicinal products; FDA; Food and drug administration; GIST, gastrointestinal stromal tumor; HCC, hepatocellular carcinoma; MM, multiple myeloma; NSCLC, non-small cell lung cancer; RCC, renal cell carcinoma.

were identified only recently) are currently not unambiguously defined.[4] The classification of the latter as bona fide cancer genes will likely depend either on their mutational profiling in very large tumor databases or (most likely) on the functional analysis of their mutated alleles. The emerging need for functional studies has become clear, with the identification of, amongst others, two frequently mutated genes, *OBSCN* and *TTN,*[2,4-6] that have never before been linked with any type of cancer. *OBSCN* and *TTN* code for giant proteins and thus far have been mainly known for their role in cardiac and skeletal muscle, where they are required for the assembly and organization of sarcomeres and the sarcoplasmic reticulum.[7] The identification of mutations in *OBSCN* and *TTN* in multiple cancer types[2,4-6,8] suggests that the cellular functions of these partner molecules could be related to a common tumor progression mechanism. However, considering the large size of the *OBSCN* and *TTN* genes, it is presently hard to determine whether mutations in these genes act as 'drivers' or are only 'passengers' in the development of cancer.[2] Functional studies will have to answer this question, thus establishing whether the cellular functions controlled by OBSCN and TTN may offer new therapeutic opportunities.

The third conclusion is that the frequency and distribution of the mutations affecting cancer genes can be used to redefine the histology-based taxonomy of a given tumor type. In this regard, *EGFR* mutations are paradigmatic, as they are present only in a specific subset of lung cancers. The occurrence of *EGFR* mutations therefore molecularly defines a subtype of lung adenocarcinomas that occur mainly in nonsmokers, tend to have a distinctly enhanced prognosis and typically respond to EGFR targeted therapies (see below and [9]). It is likely that the taxonomy of tumors will be rewritten using the presence of genetic lesions as major criteria. This will improve diagnosis and will be used to determine therapeutic regimens based on the genetic landscape of individual tumors.

A fourth conclusion is that the profile of mutations in individual cancer genes often displays striking patterns. One of the best examples of this feature is the occurrence of mutations in a 'mutually exclusive' fashion. This is the case in CRCs with *BRAF*, whose mutations are mutually exclusive with the ones affecting *KRAS*.[10] Likewise, *PIK3CA* and *PTEN* mutations are found in different sets of breast and brain cancers.[11,12] In both cases, the mutual exclusivity pattern indicates that the genes operate in the same signaling pathway. For instance, the serine–threonine kinase BRAF directly binds to and activates the small GTP-binding protein KRAS. Similarly, the lipid kinase PIK3CA converts PIP2 into PIP3, while the lipid phosphatase PTEN performs the opposite reaction. Dissecting the signaling pathways controlled by cancer genes can lead to opportunities for therapeutic intervention. For example, while constitutively active KRAS (in virtue of its peculiar biochemical properties) and inactive PTEN (considering the difficulties in reactivating an enzyme) are not easily 'druggable', their downstream effectors such as MAPK and AKT kinases clearly are.

Perhaps the most relevant conclusion stemming from the high-throughput profiling of cancer genomes is the realization that mutated kinase genes represent chinks in the tumor's armor that can be therapeutically exploited.[13] A number of evidences indicate that cancer cells are often physiologically dependent on the continued activity of activated oncogenes for maintenance of their malignant phenotype. This phenomenon is commonly referred to as 'oncogene addiction'.[14,15] This has been convincingly shown through genetic approaches in cancer cells. For instance, the metastatic potential of CRC cells carrying oncogenic *PIK3CA* alleles has been linked to the presence of the mutated *PIK3CA* allele.[16] Similarly, the knock down of mutated *BRAF* in melanoma cells causes growth arrest and promotes apoptosis.[17] The 'oncogene addiction' theory is further supported by experiments and clinical studies involving the pharmacological inhibition of genetically altered kinases. The concept of addiction to genetically altered kinases is well illustrated in chronic myeloid leukemia (CML), where the inhibitor imatinib can cause complete regression of advanced tumors by specifically inhibiting the tyrosine kinase activity of the *BCR-ABL* oncoprotein.[18] Similarly, lung cancers carrying mutated *EGFR* alleles are addicted to these genetic lesions as shown by the striking clinical effects of small molecule inhibitors of the EGFR kinase, such as gefitinib and erlotinib.[19]

Finally, genotyping of tumors has also proven to be valuable in identifying genes whose alterations are associated with primary and acquired resistance to targeted therapies. For example, secondary mutations in the *EGFR* genes or alterations in other tyrosine kinases have been associated with acquired resistance to erlotinib and gefitinib treatment of lung cancers.[19–21] Similarly, the development of resistance to imatinib in CML patients is associated with the acquisition of additional mutations in the kinase domain of the *BCR-ABL* oncogene.[18] Another example of the role of genetic alterations in defining tumor resistance to targeted therapies comes from the clinical use of monoclonal antibodies (cetuximab and panitumumab) directed against the extracellular domain of the EGFR receptor in metastatic CRC (mCRC). In this regard, recent data indicate that patients carrying tumors with *KRAS* mutations do not respond to cetuximab and panitumumab treatment.[22,23] The lack of response is associated with constitutive activation of the KRAS oncogenic pathway, which acts downstream of the EGFR receptor, thus bypassing the inhibitory activity of anti-EGFR therapies. In the future, the presence of *KRAS* mutations in CRC will likely be routinely used to select patients eligible for panitumumab and cetuximab treatment, thus showing the potential of tumor genotyping in the field of predictive medicine. Clinical trials designed to test multitherapies targeting both the EGFR and the KRAS pathways (such as MAP kinase inhibitors) could also be considered for mCRC patients carrying *KRAS* mutations. All data taken together, the future foresees personalized tumor treatment consisting of a cocktail of different inhibitors, based on the genetic profile of individual tumors.

What needs to be done

Unprecedented efforts in the high-throughput mutational profiling of common tumors, including lung, skin, breast and colorectal cancers, have led to the identification of genetic alterations that are likely to be the major drivers of these diseases.[2,4] However, the genetic landscape of cancers is by no means complete, and what we have learnt so far has raised new and exciting questions that must be addressed. One of the next imperatives is the definition of the oncogenomic profile of all tumor types. Especially, the less common although not less lethal ones are still largely mysterious to scientists and untreatable to clinicians. For some of these diseases hardly any new therapeutically amenable molecular targets have been discovered in the past years. For example, identification of 'druggable' genetic lesions associated with brain and pancreatic cancers could help in defining new therapeutic strategies for these aggressive diseases. To achieve this, more detailed oncogenomic maps of the corresponding tumors must be drafted. The latter will hopefully be completed in the coming years, thanks to the systematic cancer genome projects that are presently being performed.

Even in the case of common cancers, a lot of genomic profiling efforts still lay ahead. For example, in a significant fraction of breast and lung tumors, the mutations that are likely to be drivers have yet to be found.[2,4] This is not surprising considering that even in these tumor types, only a limited number of samples have been systematically analyzed so far. Therefore, low-incidence mutations that could represent potentially key therapeutic targets in a subset of tumors might have escaped detection. Consequently, the scaling-up of the mutational profiling to a large number of specimens for each tumor type is warranted.

In addition, the sequence of the entire cancer genome, and not only of the coding regions, should be examined. For example, the mutational profiling of the regulatory elements, such as promoters and enhancers, noncoding RNA such as miRNAs, and ultraconserved (but apparently gene-free) genomic territories should be attempted.[24,25] The availability of new high-throughput sequencing techniques will make these daunting tasks fairly realistic. The analyses of the cancer epigenome and gene copy number alterations are beyond the scope of this editorial, but will also be useful in identifying additional cancer genes. Last but not least, understanding of the cellular properties imparted by the hundreds of recently discovered cancer alleles is another area that must be developed. As a matter of fact, compared with the genomic discovery stage, the functional validation of putative novel cancer alleles – despite their potential clinical relevance – is substantially lagging behind. To achieve this, high-throughput functional studies in model systems that accurately recapitulate the genetic alterations found in human cancer must be developed. For example recent exciting progress in the introduction of cancer mutations in the genome of mammalian cells will likely be instrumental in the development of novel genotypespecific drugs.[26,27]

Conclusion

The high-throughput analysis of cancer genomes has identified genes whose mutations act as 'drivers' with respect to the tumor progression process. This information is being translated into the clinical arena at multiple levels. The genomic map of common cancers is redesigning the tumor taxonomy by moving it from a histological to a genetic based level. Tumor genotyping is helping clinicians to individualize treatments by matching patients with the best treatment for their tumors. Finally, the ongoing completion of a number of cancer genome projects will identify additional cancer alleles whose pharmacological exploitation will undoubtedly result in new therapeutic approaches.

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