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Review Article

Emerging tactical strategies for fighting the war on cancer based on the genetic landscape

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Abstract: Although it is well-established that cancer is driven by genetic mutations resulting in the acquisition of oncogenes and the loss of tumor suppressors, until recently many of the genomic details remained obscure. As a result of recent high-throughput DNA sequencing, basic insights into the spectrum of protein coding mutations in many cancers are now known. These findings provide an unprecedented framework of understanding and present new avenues for diagnosis, treatment, and prevention of cancer. In this article we discuss several high impact areas of global sequencing projects including developing drugs that specifically target cancer cells, creating personalized tools for better treatment and monitoring, and developing pre-symptomatic diagnostic tests. Capitalizing on these and other advances represent a new turning point in the war on cancer.

Keywords: Genetic mutations, cancer, diagnosis, therapy, oncogenes, tumor suppressors, high through-put DNA sequencing, review

Introduction

When the "War on Cancer" began in 1971, the notion that cancer was a genetic disease was not well appreciated. During the last forty years, the molecular basis of cancer has been elucidated by the discovery of genetic mutations including within key oncogenes and tumor suppressors. Consequently, clinical cancer research is slowly transitioning from histopathological analysis to the molecular analysis of tumors. In the near future, most cancers will be classified based on their genetic abnormalities. At the forefront of the battle are new sequencing studies directed at partial and whole cancer genomes that provide an overall understanding of the molecular wiring of cancer cells. This new information, together with other advances, has enabled a paradigm shift from generalized to personalized treatment of cancer employing molecular signatures for cancer diagnosis and prognosis, new cancer-specific drugs and genome-guided therapy. In this short review, we summarize some of the recent findings and other high impact areas that should improve the diagnosis, treatment, and prevention of cancer.

Understanding the landscape of the battlefield

Spectacular advances in high-throughput DNA sequencing technology now enable researchers to comprehensively catalogue the gene mutations and other genetic changes in patient tumors. These large-scale DNA sequencing projects provide a new highly informative vantage point, termed the genetic landscape, for different cancers [1-8]. Understanding the genetic landscape through high-throughput sequencing reveals the type of protein coding mutations that are commonly found in different types of cancers as well as the spectrum of cancer proteins found in a given tumor type. This includes the identification of previously unrecognized tumor suppressors and oncogenes. For example, oncogenic mutations within the phosphatidylinositol 3-kinase p110 catalytic subunit (PIK3CA) were unknown until exon sequencing identified protein coding mutations in 32% of tumors from colon cancer patients [9]. Subse-

Table 1. Examples of high frequency protein coding mutations identified by high-throughput sequencing

Protein Coding Mutation	Frequency	Reference
PIK3CA	32% of colon cancer	[9]
IDH1	12% of glioblastoma multiforme	[3]
ARID1A	57% ovarian clear cell carcinoma	[1, 11]
FOXL2	97% granulosa-cell tumors	[14]
DAXX or ATRX	43% of pancreatic neuroendocrine tumors	[12]

quent follow-up studies demonstrated PIK3CA to be a commonly mutant cancer protein, and it now has the distinction of being the most frequently mutated gene in breast cancer [10]. As shown in **Table 1**, other previously unrecognized cancer proteins with a high mutation frequency have been identified by high-throughput DNA sequencing and include the chromatin remodeling ARID1A protein in ovarian clear cell carcinoma [1, 11], the DAXX and ATRX chromatin/transcriptional remodeling proteins in pancreatic neuroendocrine tumors [12] and the citric acid cycle enzyme isocitrate dehydrogenase-1 (IDH1) in glioblastomas [3]. Analysis of these large-scale sequencing projects of tumors reveals that mutations within cancer proteins fall into two general groups: driver mutations and passenger mutations. Driver mutations are genetic changes within the coding sequence of proteins that confer a selective advantage to the cancer cell. Passenger mutations also involve coding sequence mutations in all cancer cells of the tumor, but these mutations do not alter the basic characteristic of growth and survival of the cancer cells. At present the sheer number of new cancer protein targets identified by high-throughput sequencing, many whose normal biologic activities are still not completely known, provide high impact areas for further understanding the pathogenesis of cancer and new targets for oncological drug development.

While high-throughput DNA sequencing of tumors greatly enhances our ability to identify novel protein coding mutations, it has also yielded other significant information including a much more extensive understanding of the commonalities and differences between different cancer types. For example, high-throughput sequencing has revealed that certain driver mutations such as p53, Ras, PIK3CA and PTEN are shared by many different types of cancer, while other coding mutations such as the recently identified IDH1 gene found in glioma and astrocytomas [3, 13] and FoxL2 in granulosa-cell tumors [14], are relatively specific to a certain tumor subtypes. DNA sequencing studies revealed differences in the mutational complexity of different cancer types. For example, sequencing of the coding exons of 518 diverse kinases in different cancers revealed that some cancer types, such as lung carcinoma had a high prevalence of mutations compared to testicular cancer or acute lymphoblastic leukemia (ALL) [6]. These differences in the frequency of mutations in many cases may explain the general characteristics and overall prognosis of diverse cancer types. Global sequencing projects also revealed selectivity among the types of pathways that are disrupted or activated in certain tumors. For example, both K-Ras and B-Raf activating mutations are found in colon tumors but rarely do both mutations occur together. Similarly, either the loss of PTEN or activation of PIK3CA are found in many tumors, but rarely do they occur together [15]. These results highlight key pathways within cancer cells. Lastly, analysis of the genetic landscape has revealed the heterogeneity of genetic changes within a single cancer subtype whereby a vast majority of protein coding mutations are not shared by other tumors [4]. One limitation to existing studies is the realization that cells within the primary tumor are heterogeneous and have their own mutational spectrum. One likely consequence of this heterogeneity is the finding of novel mutations in the metastatic tumor that were not reflected by the majority of cells in the primary tumor [16]. Thus, understanding low frequency mutations present within only a few cells of the primary tumor cells are critical areas of further study needed to block tumor metastasis and drug resistance. Nevertheless, the current state of the genomic cancer landscape provides a framework for mapping the major pathways that should be targeted for drug therapy. In many cases it offers the possibility of intervening at multiple

Table 2. Current and emerging targeted drug therapies

Type of tumor	Associated genetic alteration	Targeted drug action
Breast cancer	HER2 mutation	Anti-HER2 monoclonal antibody
Chromic myeloid leukemia	BCR-ABL translocation	BCR-ABL kinase inhibitor
Non-small cell lung cancer	Activating mutation in EGFR	EGFR kinase inhibitor
Melanoma	B-RAF oncogenic mutation	RAF kinase inhibitor
Breast cancer	BRCA1/BRCA2 deficiency	PARP inhibitor
Gastrointestinal stromal tumor	Mutations in KIT or PDGFR tyrosine kinases	KIT/PDGFR kinase inhibitor

pathways to arrest tumor growth or kill the tumor cells.

Targeted therapy and bunker busters

Better treatment of cancer patients will require a wide spectrum of highly effective targeted therapies. Many currently available chemotherapeutic drugs, simply target rapidly dividing cells and thus show relatively poor selectivity for killing cancer versus normal cells. More selective drugs based on tumor-specific mutations and gene amplifications are being developed to more efficiently match tumor cell vulnerability and provide greater long-term benefit with potentially fewer side-effects. Currently, some of the most effective cancer drugs are based on therapies that directly exploit specific genetic changes. For example, amplification of the HER2 gene in breast cancer is the basis of the highly effective Herceptin monoclonal antibody therapy [17]. Similarly, the anticancer drug imatinib represents a selective kinase inhibitor for the BCR-ABL kinase translocation found in chronic myeloid leukemia (CML).

Despite the identification of a large assortment of mutant oncogenes and tumor suppressors, there are still relatively few selective drugs for these pathways, in part because it is very difficult to develop drugs that specifically interact with transcription factors, proteins in cellular signaling pathways and other molecules. It is particularly difficult or impossible to identify drugs that can reactivate deactivated tumor suppressors carrying coding mutations or premature stop codon. One recent paradigm shift for cancer drug discovery that addresses these difficulties is the use of synthetic lethality which employs cell death as a metric for the efficacy of a specific drug against a protein mutation [18]. In synthetic lethality screens, the altered cellular activity based on oncogene activation or loss of tumor suppressor function provides the "Achilles heel" needed to kill the tumor cells. In an optimum synthetic lethality strategy, only the cancer cells with the protein coding mutation are susceptible to killing by the drug. For such cell-based screens, isogenic cell lines harboring the defined loss of tumor suppressors or harboring activation mutants of oncogenes are generally tested [18]. However, for speed and simplification, siRNA knock-down can be used as a rapid substitute when performing a loss of tumor suppressor screen. An example of the success of synthetic lethality is the finding that cell lines with BRCA1 and BRCA2 mutations show DNA repair defects and are effectively killed by an inhibitor of poly-ADP ribosylase (PARP), an enzyme involved in DNA repair [19, 20]. A subsequent human clinical trial showed objective anti-tumor killing activity for breast, ovarian, and prostate cancers by a PARP inhibitor in patients harboring BRCA1/2 mutations [21]. Beside mutations with BRCA1/BRCA2, PARP inhibition is also toxic against tumor cells harboring a loss of function of the commonly mutated phosphatase, PTEN [22]. PTEN deficient cancer cells were 200 times more susceptible to killing by the PARP inhibitor than wild type cells. Interestingly, the well-known anti-cancer drug methotrexate was found to selectively kill tumors cells containing a mutation in the DNA repair enzyme MSH2 [23]. These few studies suggest that synthetic lethality screens could identify other clinically useful targets. Of note, the exact biological activity of the mutant cancer protein need not been known to identify drugs by synthetic lethality screens. A list of several known or experimental targeted cancer therapies is shown in **Table 2**.

Another powerful approach for gaining insight into the drug susceptibility of tumors is to couple cell-based screening with integrated genomic profiling. Using this approach with a

panel of human non-small cell lung cancer cell lines, K-Ras mutant cells were identified as susceptible to an HSP90 inhibitor and cells harboring increased copy number of ABL2 and Src kinases were identified as sensitive to a Src/Abl inhibitor [24]. These results highlight the finding that specific subsets of tumors are susceptible to certain drugs, but the challenge remains in identifying these sensitivities. Once a clinically effective drug has been identified for a given target, additional challenges remain related to drug resistance. In one study, resistance to a Raf inhibitor was deciphered by a functional screen testing a large array of kinases that could overcome drug resistance [25]. From this study, COT kinase was identified to confer resistance to the Raf kinase inhibitor, which functions downstream of Raf in the MAP kinase pathway. Coupling functional approaches with genomic information is likely to be highly useful in the development of new anticancer drugs targeting both cellular misfunction and corrective adaptations in response to drug treatment.

Understanding downstream and connected signaling networks related to drug resistance and drug uptake are critical for improving treatment of cancer, but are only part of the fight against cancer. Human tumors *in vivo* show multiple mechanisms of resisting the effect of cancer drugs, which are often not well recapitulated in experimental model systems such as cell culture or mouse models. For example, one tactic used by solid tumors to resist cancer drugs is to produce a "bunker" consisting of the desmoplastic stroma containing connective tissue, cancer-associated fibroblasts and inflammatory cells that surround cancer cells. The bunker prevents efficient chemotherapeutic drug uptake. Olive et al. used an inhibitor of the hedgehog pathway which blocked desmoplastic stroma formation, increased blood vessel formation and increased exposure of the tumor cells to the chemotherapeutic agent [26]. However, the effect of the inhibitors was short-lived and was countered by additional tumor changes, suggesting that other potential combination strategies including targeting growth factor pathways, blocking adaptation to hypoxia and even inhibiting collagen biosynthesis might be needed to assure better long-term clinical response. It is possible that gene expression profiling and DNA sequencing might identify additional druggable targets responsible for these adaptive changes allowing one or more supplementary treatments.

Getting personal

With the increased realization that tumors are highly heterogeneous, the delineation of the exact molecular make-up of tumors before and even during therapy will begin to play a critical role in clinical decision making. Utilizing the genetic makeup of individual tumors to guide therapy was first shown with Her2 amplified tumors with Herceptin monoclonal antibody therapy [17]. Another example of how genetic makeup can guide therapy is the finding that a subset of non-small cell lung cancer patients with functionally active point mutations within the epidermal growth factor receptor dramatically respond to tyrosine kinase inhibitors such as gefitinib [27-29]. These and other studies suggest the development of rapid and inexpensive technologies to determine the spectrum of mutations and amplifications within tumors will become powerful new tools to determine the most effective therapeutic drug regime because it will identify the individuals most likely to respond to therapy. It is highly likely that this personalized information will be even more useful as more targeted therapies are developed. Clearly, new cheaper and faster sequencing technologies may become a standard method for monitoring the genetic makeup of tumors from cancer patients. The advent of the so called $1000 genome may make this a practicality, after all if a cancer can be cured by applying what is learned from sequencing a tumor biopsy, then a thousand dollar diagnostic test will be appreciated by all concerned.

Besides genomic biomarkers other molecular tests based on the mRNA profile of the tumor show important clinical promise. For example, several mRNA profiling technologies directed at breast cancer are already available and yield important information for prognosis based on gene expression within the primary tumor. For example, OncoTypeDX® uses RNA extracted from archival breast tumor blocks for analysis in a multigene assay [30]. From analysis of gene expression of 21 genes, prognostic information including the chance of recurrence are obtained. Similarly, the MammaPrint® uses an array format to assess an mRNA prognostic profile in determining long term outcome in breast cancer patients, but requires that the tissue

sample be snap frozen at the time of collection [31-33]. Additional technologies utilizing gene expression profiles are currently being developed to predict response to therapy thereby allowing a better chemotherapeutic choice for a particular tumor. Since cancer cells have the capacity to escape the effect of the chemotherapeutic agent by genetic mutation of target genes, future gene expression profiling technologies directed at drug resistance may have an impact on determining alternative drug therapies.

Genetic profiling after identification and treatment of the tumor can help to evaluate tumor burden and recurrence and thereby optimize treatment. In one study, detection of tumor DNA in serum was evaluated using a highly sensitive amplification approach [34]. Somatic mutations in APC, P53, K-Ras and PIK3CA within the tumor were first identified and then used to determine the presence of circulating tumor DNA in patient plasma. Interestingly, the lack of detectable tumor DNA in the plasma from some patients following surgery strongly correlated with disease-free survival [34]. A related approach exploited the unappreciated, tumor-specific gene translocations that occur in solid tumors [35]. In this study, high-throughput sequencing and bioinformatics analysis identified 4-10 unique translocations per colon cancer tumor. Based on this information, relatively simple and robust PCR was used to readily detect very low levels of tumor DNA in plasma showing these translocations. Similar to the findings of Diehl et al [34], there was a rapid drop in detectable tumor-specific DNA following surgery [35]. These and other tumor monitoring technologies could be used to determine if a patient is cancer free or requires more aggressive therapy, but will initially require more global approaches to understand and map the "baseline" cancer genome and mutational profile of a patient.

Risk assessment

Despite the large amount of information gleaned from the genetic landscape of many cancers, the challenge now is to use this information to develop diagnostic tests for *presymptomatic* cancer diagnosis. Pre-symptom or early stage diagnosis is critical for intervention because overall cancer burden is low, patients are often free of metastases and early intervention is known to be lifesaving. One recent study with human pancreatic cancer suggests that tumors remain relatively dormant for 10 years prior to metastasizing, offering a large window of opportunity for the detection and subsequent early stage surgery and/or treatment [36]. For a cancer surveillance technology to be practical in a clinical setting requires it to be non-invasive, relatively inexpensive and have high sensitivity and specificity. It is already possible to detect small amounts of tumor DNA in non-invasively collected samples. For example, a number of studies have shown that cancer-specific mutations can be identified in DNA isolated from stool samples obtained from colon cancer patients [37-39]. The next step is to implement DNA-based technologies for sensitive and practical screening of early stage tumors. Alternatively, proteomic, serologic, metabolomic and other technologies are currently being explored to detect cancer biomarkers in bodily fluids. Using mass spectrometry, mutant Ras proteins containing a single amino acid substitution were identified in pancreatic cyst fluid [40]. Further technical advances are needed in order to detect mutant proteins in more dilute biological samples, which can be collected less non-invasively from serum, urine or stool. Finally, it is possible that profiling the host immune system for cancer-specific signatures including the detection of humoral responses against tumor antigens such as p53 and other targets might be beneficial. Profiling antibody responses is particularly appealing because humoral responses represent an endogenously amplified response to a given tumor, making early detection a realistic possibility. In conclusion, new technologies directed at detecting pre-symptom and early stage disease are ongoing and would have a great impact on the prevention and treatment of cancer.

Summary

Our overall understanding of cancer is rapidly changing due to comprehensive DNA sequencing of many different types of human tumors. The challenge remains to identify which of the many protein mutations identified by high-throughput sequencing studies are clinically relevant. For example, the identification of driver mutations which contribute to the malignant phenotype, represent new targets for treatment of cancer. As the list of relevant driver mutations continues to grow, another goal will

be to develop low cost DNA sequencing or other technologies that can be used clinically to rapidly profile the spectrum of these mutations in a patient's tumor. This information along with gene expression profiles, real time monitoring of tumor burden and future biomarkers of tumor resistance will play an important role in implementing more precise and personalized treatment of cancer. The burgeoning field of drug development targeting cancer-specific mutations and other genomic alterations represents an untapped, future pharmacological arsenal. The discovery of new potent targeted therapies coupled with personalized tumor profiles should markedly enhance treatment. Additionally, increased early diagnosis based on cancer-specific alterations should translate into fewer late-stage disease cases. In the near future, it is likely that these many advances will improve quality of life and promote the long-term survival for many more cancer patients than currently available.

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