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Targeting Oncogenic Drivers

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Abstract

Cancer is a genetic disease caused by a series of somatic and/or germline mutations. The roles of oncogenes and tumor suppressors in cancer molecular origin have been well established. Targeting oncogene products has become an attractive therapeutic strategy with great clinical success, whereas tumor suppressors are considered 'undruggable' because current technology is not able to restore tumor suppressor function in metastatic disease. Although systematic approaches to discover genetic alterations have become available to individual patients, differentiating driver from passenger mutations and identifying and validating drug targets remain challenging. Protein tyrosine kinases play crucial roles in virtually all cellular processes and possess structural features that render them 'druggable'. Monoclonal antibodies and small-molecule inhibitors represent two major classes of targeted therapeutic agents, each possessing its own strength and weakness. Although initial successes have been achieved, targeted therapy faces many challenges that need to be addressed and hurdles to overcome.

Cancer is the most common human genetic disease, developing after a series of gene alterations that lead to uncontrolled growth of cells. While a small number of hereditary cancer syndromes are directly caused by germline mutations, the vast majority of human cancers are driven by sequentially accumulated somatic genetic and epigenetic changes. These genetic alterations ultimately lead to acquisition of the hallmarks of malignancy comprising resistance to cell death, sustained proliferative signaling, activation of invasion and metastasis mechanisms, angiogenesis induction and replicative immortality [1]. Additionally, inheritable genetic variations also contribute to cancer development by influencing the susceptibility to malignancy transformation [2, 3].

Genetic alterations may promote cancer development through either constitutive activation of proto-oncogenes or loss of function of tumor suppressor genes [4, 5]. Proto-oncogenes are normal cellular genes that encode proteins involved in cell proliferation and cell-death protection. When deregulation occurs, either through changes in the protein-coding segment or by alteration of their expression levels, proto-oncogenes are converted to oncogenes, which promote cancer development. The first oncogene isolated was from a cancer-inducing retrovirus, the Rous sarcoma virus, which transmits sarcomas to infected chicks through incorporation of the v-src oncogene to host genomes [6]. Since the discovery of v-*src*, numerous other proto-oncogenes have been identified. They can be converted to oncogenes through various genetic alterations such as point mutations, deletions, duplication/amplification and chromosome translocations, as well as epigenetic alterations. Following the understanding of oncogenes, a second group of genes in tumor development, the tumor suppressor genes, also entered the center stage of research in the molecular origin of cancer. In contrast to oncogenes, tumor suppressor genes encode proteins that prevent tumor development or progression [4]. This is usually accomplished through maintaining genome integrity and constraining cell proliferation by regulating the cell cycle, promoting apoptosis and facilitating DNA repair. The first gene recognized as a tumor suppressor gene was a gene involved in cell cycle control, the retinoblastoma (*RB*) gene [7–9]. Loss of *RB* was found to be the cause of hereditary as well as sporadic retinoblastoma. Since in general one copy of a tumor suppressor gene is sufficient to control cell proliferation, both alleles of a tumor suppressor gene have to be lost or inactivated in order to promote tumor development. This may occur through alterations such as point mutations, deletions, chromosome deletions and translocations, and epigenetic silencing [10, 11]. In addition to oncogenes and tumor suppressors, genetic materials acquired from viruses, including human papilloma virus, Epstein-Barr virus, hepatitis B virus, human T lymphotropic virus 1 and human herpes virus 8, can also promote tumor development. This usually occurs through mechanisms such as inactivation of cellular tumor suppressors or alteration of the transcription of the neighboring genes after integration into the host genome, although viral genes may function as oncogenes themselves as well [12, 13]. The understanding of the molecular origin of cancer development has led to investigations of new therapeutic agents [14]. While strategies to restore tumor suppressor gene functions have been hindered by technical hurdles of ineffective gene delivery, numerous new therapeutics targeting oncoproteins have been evaluated in clinical studies with many approved for clinical use.

Identification of Cancer Therapy Targets

One of the most exciting developments in biomedical research over the past decade has been the technological advances in genome sequencing. The availability of next-generation sequencing (NGS) has revolutionized cancer genome sequencing. Com-

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pared with previous sequencing methods, NGS executes reactions in a massively parallel fashion, and thus millions of short sequence fragments are read simultaneously, and the reaction steps occur in parallel with the detection steps. This not only allows rapid sequence reading, but also provides improved coverage and increased accuracy since the same segment of a DNA sequence is read repeatedly [15]. The dramatically reduced cost and turnaround time of NGS makes sequencing of entire cancer genomes for each individual patient technologically and financially possible [16, 17]. With the availability of this new technology on the horizon for clinical care, the pressing question now is how to identify mutations that play causal roles in cancer.

Genetic alterations described in tumors include single-base substitutions, small insertions and deletions (indels), amplifications, homozygous deletions, and translocations. These alterations can be compiled into two categories, "drivers" and "passengers"; while the former confer growth advantage on cancer cells carrying them and are required to sustain proliferation of cancer cell, the latter are not. While more than one driver is likely required to sustain tumor growth, the number of driver mutations required in each tumor type has not been well established [13]. Since cancer is genetically unstable, a cancer cell often bears numerous genetic alterations. The ultimate assay to distinguish drivers from passengers is the transformation assay, which however is often not feasible due to the sheer volume of mutations existing in cancer cells. Alternatively, predictions can be made based on other factors. Whether a mutation can lead to potential functional impact due to protein coding sequence alterations may be predicated using computational tools. Mutations in genes that have been previously identified as cancer related or involved in cellular signal transduction pathways that play significant roles in malignancy transformation are more likely to be driver mutations [16, 18, 19]. Since passenger mutations are assumed to occur randomly throughout the genome, whereas driver mutations occur frequently in cancer-related genes, a gene that exhibits higher prevalence of somatic mutations than expected by chance is considered more likely to be a driver mutation, even though it has been recognized that passenger mutations may occur in clusters, and driver mutations can exist at low frequency in cancer cells [18, 19]. Since cancer is an evolutionary process with continuous acquisition of new genetic alterations, additional driver mutations can be acquired after cancer development, and clones harboring these mutations will expand under positive selection due to enhanced growth potential and/or treatment resistance [20–23]. Moreover, a preexisting passenger mutation may become a driver mutation by contributing to treatment resistance and clonal expansion [24].

In addition to identifying drivers, cancer genome profiling-based therapy selection faces other hurdles. The discordance between primary tumor and recurrent/metastatic lesions and intra-tumor heterogeneity raise the question of whether a single biopsy sample can be used to determine the genomic landscape of the entire disease [25]. Whether the driver mutations discovered are actionable, and how to design clinical studies to match patients with targeted agents are also among the challenges remaining to be addressed [26].

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Approaches to Inhibit Driver Genetic Alterations

Therapeutic targeting of driver genetic alterations has become a major focus of oncology. The development of new therapies is often based on inhibition of disease-associated molecular interactions. Modern biology has facilitated the search for new small molecules that potently and selectively modulate the functions of molecular targets via this approach. Additionally, monoclonal antibodies, which exert their antitumor activity through neutralizing and/or depleting targeted molecules or target positive cancer cells, have also become a vital drug class in targeting cancer-related molecular pathways [27, 28]. Other types of targeted therapeutic agents such as peptide mimetics and siRNAs have also emerged and are being evaluated in clinical studies [29, 30].

Therapeutic Monoclonal Antibody

The modern era of monoclonal antibody therapy began after the development of hybridoma technology in 1975, which enabled the continuous supply of specific monoclonal antibodies [31]. Among all antibody isotypes, immunoglobulin G (IgG) is the most frequently used monoclonal antibody in cancer therapy. Structurally, it comprises an antigen binding (Fab) and a constant fragment (Fc) region. While Fab binds specific antigen, Fc interacts with IgG receptors (FcγRs) and complement, mediating antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and complement-dependent cytotoxicity (CDC). Fc also interacts with neonatal FcR, which is responsible for the long serum half-life of IgG. Since murine antibodies are immunogenic, lack effector functions, and have a short half-life when used in humans, chimerization and humanization are required in order to achieve adequate efficacy [32]. Additionally, fully human antibodies obtained by screening phage display libraries expressing human antibody fragments or expressed by mice genetically engineered with human immunoglobulin gene sequences have also become available for clinical use [27, 33]. The antitumor activities of monoclonal antibodies are exerted through both immune and nonimmune mechanisms, including binding and depletion of growth factors, blockade of ligand and receptor interactions and signaling, and cellular depletion through ADCC, ADCP and CDC [27]. In addition to the antitumor effect medicated by naked antibodies, antibody-drug conjugates carrying drugs, toxins or radionucleotides have also been developed and gained approval for clinical use [34].

Since the approval of the first therapeutic monoclonal antibody rituximab in November 1997, thirteen monoclonal antibodies have been introduced to clinic for treatment of solid tumors and hematological malignancies (table 1). Currently, there are about 300 monoclonal antibodies at various stages of clinical development. Among them, 10 are undergoing evaluation in phase 2/3 or phase 3 clinical studies [35]. In general, treatments with currently approved monoclonal antibodies are better toler-

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Table 1. Monoclonal antibodies approved for clinical use

	Molecular target(s)	Mechanism of action	Treatment indications
Cetuximab	EGFR/ErbB1	mouse/human chimeric IgG1	squamous cell carcinoma of the head and neck, *KRAS* wild-type colorectal cancer
Panitumumab	EGFR/ErbB1	human IgG2	*KRAS* wild-type colorectal cancer
Trastuzumab	ErbB2/HER2/Neu	humanized IgG1	HER2-overexpressing breast cancer, gastric or gastroesophageal junction adenocarcinoma
Pertuzumab	ErbB2/HER2/Neu	humanized IgG1	HER2-overexpressing breast cancer
Bevacizumab	Vascular endothelial growth factor A	humanized IgG1	glioblastoma, colorectal cancer, non-small cell lung, renal cell cancer
Rituximab	CD20	mouse/human chimeric IgG1	follicular, CD-20-positive, B-cell non-Hodgkin lymphoma
Ofatumumab	CD20	human IgG1	chronic lymphocytic leukemia
Alemtuzumab	CD52	humanized IgG4	chronic lymphocytic leukemia
Ipilimumab	cytotoxic T lymphocyte-associated antigen 4 (CTLA-4)	human IgG1	melanoma
Tremelimumab	anti-CTLA-4	human IgG2	melanoma
Tositumomab and iodine I^{131} tositumomab	CD-20	murine IgG2 iodine-131 conjugate	non-Hodgkin's lymphoma
^{90}Yttrium-ibritumomab tiuxetan	CD20	murine IgG1 chelator tiuxetan conjugate	non-Hodgkin's lymphoma
Brentuximab vedotin	CD30	chimeric IgG1-monomethyl auristatin E conjugate	Hodgkin lymphoma, systemic anaplastic large-cell lymphoma

ated compared with conventional chemotherapeutic agents, although toxicity profiles may vary depending on the therapeutic targets, with the exception of infusion reactions, which have been reported across all agents. Skin rash and cardiac dysfunction were observed with epidermal growth factor receptor (EGFR)- and HER2-targeted agents, respectively; hypertension, thrombosis, hemorrhage and delayed wound healing were observed with vascular endothelial growth factor-targeted agents; infection and cytopenia are more commonly associated with monoclonal antibodies targeting hematological malignancies, while immune-related adverse events have been reported with immune-modulating agents. Most of the monoclonal antibodies approved for

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