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Review

The molecular basis of targeting protein kinases in cancer therapeutics

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ABSTRACT

In this paper, we provide an overview of targeted anticancer therapies with small molecule kinase inhibitors. First, we discuss why a single constitutively active kinase emanating from a variety of aberrant genetic alterations is capable of transforming a normal cell, leading it to acquire the hallmarks of a cancer cell. To draw attention to the fact that kinase inhibition in targeted cancer therapeutics differs from conventional cytotoxic chemotherapy, we exploit a conceptual framework explaining why suppressed kinase activity will selectively kill only the so-called oncogene 'addicted' cancer cell, while sparing the healthy cell. Second, we introduce the protein kinase superfamily in light of its common active conformation with precisely positioned structural elements, and the diversified auto-inhibitory conformations among the kinase families. Understanding the detailed activation mechanism of individual kinases is essential to relate the observed oncogenic alterations to the elevated constitutively active state, to identify the mechanism of consequent drug resistance, and to guide the development of the next-generation inhibitors. To clarify the vital importance of structural guidelines in studies of oncogenesis, we explain how somatic mutations in EGFR result in kinase constitutive activation. Third, in addition to the common theme of secondary (acquired) mutations that prevent drug binding from blocking a signaling pathway which is hijacked by the aberrant activated kinase, we discuss scenarios of drug resistance and relapse by compensating lesions that bypass the inactivated pathway in a vertical or horizontal fashion. Collectively, these suggest that the future challenge of cancer therapy with small molecule kinase inhibitors will rely on the discovery of distinct combinations of optimized drugs to target individual subtypes of different cancers.

Tissue homeostasis is an intricate balance of cell proliferation and death. Tipping the balance toward cell growth causes cancer. Typically, to transform a normal cell into a tumor cell, changes in genes must occur, particularly in those playing a role in regulating cell growth and self-destruction (apoptosis). The affected genes are primarily comprised of either oncogenes with gain-of-function, or of tumor suppressor genes with loss-of-function. Oncogenes function to promote cell growth and survival. Analysis of a comprehensive survey of all tumors, the catalogue of human somatic mutations in cancer (COSMIC) [1], indicated a mutation rate of 33% in the Ras protein. Activating mutations at codons 12, 13, and 61 [2] promote oncogenesis. On the other hand, tumor suppressor genes either arrest cell division or induce cell death. The fact that the p53 tumor suppressor is functionally inactivated by mutations in approximately half of the sporadic human tumors [3], has earned its name the "cellular gatekeeper for growth and division" [4] or the "guardian of the genome" [5]. Usually cancer pathogenesis results from critical genetic alterations with a combination of out-of-control growth and failure of anti-proliferative responses. Analysis of genome-wide alterations in DNA copy number and somatic mutation data [6] indicated a 70% accuracy in subtypes discrimination in melanoma, suggesting that different cancer types developed along distinct genetic pathways. Intuitively, identification of genetic abnormalities that affect the crucial cell-signaling pathways in cancer cells is the very first step in targeted therapeutic development.

Cancer is a complex genetic disease. A decade ago, Hanahan and Weinberg conceived a very useful conceptual framework consisting of a common set of six characteristic capabilities acquired by cancer cells through mutagenic processes [7]. The six hallmarks of cancer include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis. Recently they extended the concept of cancer biology by including two enabling characteristics of genome instability and mutations for

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expediting the hallmark acquisition and tumor-promoting inflammation for fostering multiple hallmark functions, as well as two emerging hallmarks of reprogramming energy metabolism and evading immune destruction [8]. Cancer cells achieve these abilities mainly by rewiring existing cellular programs that normally take place during development. These programs coordinate delicate processes such as cell proliferation, migration, polarity, apoptosis, and differentiation during embryogenesis and tissue homeostasis. In line with Darwinian evolution, cancer cells confer the capability to proliferate and survive through random mutations and epigenetic changes followed by clonal selection of cells [9] under circumstances that would normally be deleterious.

Even though an experimental model of human cancer indicated that to drive healthy cells to cancer cells necessitates at least five or six genes [10], an emerging cancer theme named oncogene addiction [11–13], a term coined in 2000 by Weinstein [14,15], states that tumor cells can seemingly exhibit dependence on a single activated oncogenic pathway or protein to maintain their malignant proliferation and survival. The beautiful hypothesis of oncogene addiction implies that in response to constitutively deregulated pathways due to a single abnormally activated protein, the cellular circuit (network) of tumor cells is adaptively transformed into a unique rewired (addicted) state. It further implies that inactivation of the single oncogene will lead to devastating effects on the addicted state of cancer cells; but not on the normal state of healthy cells. The proof of concept has been reinforced by several reported findings in a variety of examples, including human cancer cell lines, mouse tumor models, and clinical cancer studies [16].

Besides the well-known fact that protein phosphorylation regulates most aspects of cell life [17], a compilation of cancer genes from the published literatures up to 2004 revealed that the most commonly encoded Pfam [18] domain is the protein kinase [19]. Together with the realization that deregulation of kinase activity has emerged as a major mechanism by which cancer cells evade normal physiological constraints on growth and survival [20], protein kinase has become the most intensively pursued anticancer drug target. Therefore, it is not surprising that more than 14 small molecule inhibitors have become available on the market for cancer treatments [20–22] since the first kinase inhibitor (imatinib) was approved by FDA in 2001 for chronic myeloid leukaemia (CML). In addition, the clinically validated examples of oncogene addiction have been associated with mutationally activated kinases [12]. In the first part of this Review, we seek to understand the cellular pathways linking between a single aberrant kinase oncogene and the acquired hallmarks of cancer cells.

1. Cellular pathways lead to hallmarks of cancer cells by single kinase activation

Even though all cells in a multicellular organism have the same genetic material from the germline, differentiated cells operate under distinct cellular circuits for specific functionalities. Distinct cellular networks coded by different sets of master transcription factors spell unique genetic programs which specify particular chromatin organization and epigenetic states. In each circuit, cell homeostasis is then dynamically maintained via interactions between nodes, whose concentrations are positively regulated by gene expression through transcription and/or translation control, and negatively regulated by a particular degradation system. Furthermore, the inherent interactions between nodes can change dynamically via post-translational modifications (PTM) by the associated enzymes. For example, phosphorylation (or its counteract dephosphorylation) of a node plays a significant role in rewiring the cellular circuit, especially of an enzyme (such as Akt) that executes the PTM on its (more than 100) connectors.

The outcome of the grand cellular phenotypes (cell fate), such as proliferation, cell death, and differentiation, normally relies on multiple inputs from many intracellular and extracellular cues (the bow–tie model [23,24]). The extracellular cues come mainly from cellular communications when a ligand secreted from one cell binds to a receptor residing on the surface of another. Extracellular ligand binding usually results in either an allosteric conformational changes at the intracellular part of the receptor or receptor dimerization (or oligomerization) that activates the initiation of a signaling pathway. In the case of receptor tyrosin kinases (RTKs), receptor dimerization due to a growth factor binding event activates the intracellular tyrosine kinase domains [25,26].

It is well established that activation of intracellular tyrosine kinase domains triggers a multitude of signaling pathways that act in concert to sustain cell proliferation and survival and to foster the malignant properties of cancer cells [27]. Triggered by the receptor tyrosine kinases, the two major downstream signals which lead to the hallmarks of the cancer cell are summarized in Fig. 1. The signaling cascades and components include the Ras–Raf–Mek–Erk and PI3K–Akt axes, STATs, NF-κB, and MAPKs of the JNK and p38 families. A complete map including every detail of the nodes in this cellular network with accompanying structural data is critical to fully understand cancer cell biology with the abnormality originating from deregulation of the growth factor signaling pathways. This paradigm holds for all signaling pathways in the cell. How to model protein–protein interactions and construct structural pathways is detailed and exemplified elsewhere [28–36].

2. Principles (the molecular basis) of targeted cancer therapy

The ideal anticancer strategy would be the one that selectively kills tumor cells while sparing normal cells. This is the goal of targeted cancer therapy. Even though the two traditional cancer treatments, chemotherapy and radiation, were not specifically designed to target tumor cells [37], the enhanced sensitivity to either DNA damage or cell cycle arrest due to the inherent replication stress in cancer cells has been exploited [38], seeking an optimal dose and schedule to kill tumor cells while minimizing the damage to normal cells.

The establishment of protein kinase inhibition as a targeted cancer therapy is mainly supported by the phenomenon of oncogene addiction [16]. Oncogene addiction describes the dependency of certain tumor cells on a single activated oncogenic protein or pathway to maintain their malignant properties, despite the likely accumulation of multiple gain- and loss-of-function mutations that contribute to tumorigenicity. The oncogene addiction hypothesis has been supported by many studies; whether generated as xenografts or via genetically engineered models, following acute inhibition of the oncoprotein, single oncogene-driven tumors undergo regression through apoptosis or proliferative arrest. Several models have been proposed to answer the key question of how exactly oncoprotein inhibition induces tumor cell death while sparing normal cells, including the genetic streamlining [39], oncogenic shock [16], and synthetic lethality [40]. The hypothesis of synthetic lethality has also been applied to the analogous scenario in non-oncogene addiction [41]. The three models share two common features: first, they address the differences between the cancer cell following the addicted oncoprotein activation, and a normal cell; second, they emphasize that after the removal of the addicted source, the differences either keep the normal cell alive or lead the addicted tumor cell to death. According to the synthetic lethality hypothesis, two genes are in a synthetic lethal relationship when loss of one or the other is still compatible with survival; but loss of both is fatal [40]. In a tumor cell, the addicted oncogene not only

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