Introduction

Given the sequential nature of events governing cancer, signal transduction pathways are the best defined ones. In this review, we will mainly focus on two signaling pathways related to proliferation, tumorigenesis, angiogenesis and malignancy, for these two pathways have been extensively exploited as drug targets for cancer therapy. We will start with the well-known Ras-Raf-MAPK signaling and proceed with the second one known as Akt/mTOR signaling. Eventually, we will conclude with drug therapies with a focus only on small molecule inhibitors that target the most important components of these pathways-protein kinases.

Signaling Mechanisms in Cancer

Receptor tyrosine kinase (RTK) signaling

Tyrosine kinase receptors are a large family of cell surface proteins that are related to diverse array of growth factors. These receptors are usually effective in epithelial tissues where the principal growth factor is the family of epidermal growth factors (EGF) and related signals. Since epithelial tissues are related to different carcinoma types that are the leading cancer types, understanding about RTK signaling will improve our knowledge, enabling us to develop therapeutic agents for different tumors.1

Ras-Raf-MAPK pathway / upstream events

Different growth factors, proto-oncogenes and cytokines activate cell cycle towards cell division via the small G protein called Ras that is a proto-oncogene whose activation drives the cell to undergo proliferation. Though Ras itself is not enough for transformation, a 20 fold increase in its activity may be sufficient for transforming the cells. Ras induces activation of serine/threonine kinase Raf,2,6 which is phosphorylated upon its recruitment to the plasma membrane.7,8 Activated Raf phosphorylates and activates Mitogen Activated Protein Kinase Kinase-1 (MAPKK1 / MKK1 / MEK1) which in turn do the same to Mitogen Activated Kinase-1 (MAPK1) type known as ERK (Extracellular signal Regulated Kinase). Numerous cell-adhesion receptors and growth factors take part in the control...
and regulation of anchorage-dependent cell-cycle entry and they trigger vast amount of signaling events only one of which is the MEK-ERK cascade[9]. Phosphatidylinositol 3-kinase (PI3K) signaling involving Akt/mTOR (mammalian target of rapamycin) pathway is another one linked to RTKs and will be discussed in the second part of the review in relevance with the specific mTOR inhibitor-rapamycin.

By the association with their respective ligands, growth-factor receptor tyrosine kinases such as the vascular endothelial growth factor receptor (VEGFR), the platelet-derived growth factor receptor (PDGFR), the fibroblast growth factor receptor (FGFR), and the receptor ERBB are activated. This event is followed by the binding of adaptor proteins such as growth-factor-receptor-bound protein 2 (GRB2). With the help of GRB2, the guanine-nucleotide exchange factor SOS induces RAS activation via GTP replacement in RAS. Activated RAS further catalyze RAF induction and downstream events. As we mentioned above, RAF phosphorylates MEK, which in turn activates ERK. This pathway is controled via some regulatory proteins that inhibit the RAS or RAF activity in positive and/or negative ways. Ras-Raf pathway also gets activated via adhesion of integrins to specific extracellular-matrix molecules. Such interactions activate focal adhesion kinase (FAK) and phosphatidylinositol 3-kinase (PI3K), which may also be activated by RAS and will also yield RAF activation via a different path (Figure 1).

For inhibition of Ras oncogene, farnesyl transferase inhibitors (FTI) are being widely used as potent drugs since farnesyl transferase activity is known to be crucial for Ras activation, delivering Ras a farnesyl group in a post-translational modification which leads to its recruitment to the site of plasma membrane. However, recently, some other Ras variants have been found to

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**Figure 1**

Simplified overview of RTK related MAPK and PI3K-Akt/mTOR pathways. Arrows indicate activation

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be responsible of the activity downstream Ras regardless of the presence of FTIs. This downstream activation has been shown to be due to three different constitutively active Ras mutants called KRAS, HRAS and NRAS along with a RAF mutant called BRAF. All these mutations promote the pathway towards ERK stimulation. Statistically, KRAS is involved in colon cancer by 45%; in pancreas cancer by 90%; in NSLC by 35%; papillary thyroid by 60% (along with HRAS and NRAS). NRAS is involved in melanoma by 15%; in ALL and AML by 30%. On the other hand, BRAF is involved in colon cancer by 12%; in ovarian cancer by 30%; in melanoma by 66%; in papillary thyroid cancer by 35-70%. Therefore, Ras activation is a common feature in melonomas, gliomas, adenocarcinomas, breast and ovarian cancer and other carcinomas where epithelial tissue is involved. Understanding more about this pathway will surely help us find the common features in them.

Consequently, any treatment addressed to RTK signaling, especially Ras-MAPK-ERK pathway, will be effective in different types of cancers though not a single drug will possibly be sufficient to completely cure a certain type due to other mechanisms involved in different levels during tumorigenesis.

MAPKs and TFs / downstream events

Protein kinases are of two main classes: serine/threonine kinases which phosphorylate their substrates on serine and threonine residues; and tyrosine kinases which phosphorylate substrates on tyrosine residues. An important group of kinases are in the form of serine-threonine kinase. Especially MAPKs, MAPKKs and MAPKKKs are essential in many regulatory pathways and are included in the "serine-threonine kinases" group. MAPKs are subdivided into three other substrate kinases; ERK, p38, JNK/SAPK. ERKs (or ERK1/2) are mitogen activated kinases whereas p38 and JNK are activated upon cellular stress or proinflammatory stimulants.

ERKs can induce phosphorylation and translocation of membrane-associated and cytoplasmic components, such as RSK (ribosomal S6 kinase). Once ERKs enter into nucleus, they phosphorylate related transcription factors (TFs) including fos, elk1, myc, and stat’s. All of these molecules are related to one or more survival mechanisms in the cell cycle that can be summarized as the following: myc is a well-known oncogene; ELK1 is Ets-related transcription factor that mediates growth factor stimulation of the c-fos promoter; FOS has been shown to function as a sensor for ERK signal duration. When ERK activation is transient, its activity fades away before FOS protein accumulates. On the other hand, normal ERK activation results in phosphorylation of FOS by ERK and its stabilization. This event further triggers progression into cell cycle entry by the activation of cyclin D1. STATS, as their names imply, are activators of DNA transcription that is obviously required for increase in gene expression for the subsequent series of events leading to cancer (progression to cell cycle and gaining cell motility).

Drug Discovery Targeting Signaling in Cancer

Until the early 1980s, drug discovery for cancer has traditionally focused on targeting DNA synthesis and cell division, resulting in drugs such as antimetabolites, alkylating/cross-linking agents and mitosis inhibitors. Although these drugs do show efficacy and are still widely in use, their lack of selectivity for tumor cells over normal cells lead to high toxicity with severe side effects. However from the 1980s onwards, discovery of intracellular signaling and its many components has opened up a new era for targeted therapies. The following part in this review will mainly focus on drugs as "small molecule inhibitors" targeting several protein kinases/phosphatases of two of these pathways: MAPK and Akt/mTOR signaling.

Mode of action of kinases includes recognition of the substrate molecule by its substrate-recognition site and transfer of a phosphate molecule to the bound substrate on its tyrosine/threonine residues from ATP that binds to a nearby site known as ATP-binding site or catalytic sites. Initially, research to develop potential inhibitors of kinases had mainly focused on this ATP-binding site with an attempt to come up with an effective ATP competitive compound.

The first protein kinase inhibitors

Historically, first ATP competitive kinase inhibitor developed in early 1980s was a modification of long known calcium-binding protein calmodulin antagonist. When the naphthalene ring was replaced by isoquinoline, Hiroyoshi Hidaka observed that resulting compound was only inhibiting several protein kinases. However, lack of specificity and the fact that compound worked only at high concentrations were two serious drawbacks of this first trial. Later came on a surprising discovery that staurosporine, an antifungal
agent that is produced by bacteria of the genus Streptomyces, was a nanomolar inhibitor of Protein Kinase C (PKC) that is capable of activating both Raf-MAPK and NF-kB signaling pathways (Figure 1). However, this was still a non-specific inhibition. Soon, chemically modified and more specific versions of this compound have been developed. 7-hydroxy staurosporine (UCN-01) blocks G2-to-M cell cycle progression possibly by inhibiting cell cycle checkpoint control kinase CHK1.

An inspiring discovery came in 1991 by Stuart Schreiber from the field of immunology. He and his colleagues identified the mode of action of a long-ago approved immunosuppressant drug named cyclosporine. Schreiber showed that cyclosporin is forming complex with its intracellular receptor protein named cyclophilin, that together inhibit a Ca+2-calmodulin dependent protein phosphatase named calcineurin. Additionally, he showed that FK506 of a bacterium origin associates with FK-binding protein (FKBP) in a similar manner and this also inhibits calcineurin (Table 1).

**Rapamycin and Akt/mTOR signaling pathway**

Rapamycin is an antifungal agent produced by Streptomyces hygroscopicus, with a history dating back over 30 years. Long after its dismissal due to undesirable side effects as an immunosuppressant, rapamycin managed to win back its pharmaceutical attention years later again for its immunosuppression properties—this time evaluated in a more precise way. Rapamycin was also associating with above mentioned FKBP, but unlike FK506-FKBP complex, Rapamycin-FKBP was not inhibiting calcineurin. In 1991, Michael Hall identified the molecular target of Rapamycin-FKBP complex in yeast to be a protein kinase he named "Target of Rapamycin" (Tor). This created a great scientific excitement because this was the the first time research reversed direction and went from the drug to its kinase. Very soon, the mammalian homologue [mTOR (or FKBP-rapamycin associated protein-FRAP-)] and other components of this mTOR signaling pathway were identified. Rapamycin got FDA approval in 1999, becoming the first approved drug for clinical use that inhibits solely one protein kinase specifically (Table 1).

mTOR is involved in Phosphatidylinositol-3 Kinase (PI3K)/Akt (Protein Kinase B)/ Phosphatase-and-TENsion homologue (PTEN) pathway. Growth-factor receptor protein tyrosine kinases activate PI3K, which in turn phosphorylates PIP2 to convert it into the essential second messenger PIP3 (phosphatidylinositol 3,4,5 triphosphate). PIP3 activates 3-phosphoinositide-dependent protein kinase-1 (PDK1) and brings Akt in contact with PDK1. Akt, having been activated by PDK1, exerts its effect by promoting cell survival and proliferation strongly through several diverse ways. mTOR/FRAP is the kinase substrate of Akt, activated upon phosphorylation by Akt. This explains why its specific inhibitor rapamycin has proven to be a very promising anti-tumor drug. (The last component of this pathway is the tumor suppressor phosphatase PTEN that dephosphorylates PIP3 back to PIP2, regulating Akt activity negatively) (Figure 1).

**ATP-competitive inhibitors**

Given the fact that small molecule inhibitors of kinases that have been developed are mainly ATP-competitive inhibitors, it was surprising to the scientific community how these compounds were actually working at all. One challenge was to compete sufficiently with very high intracellular ATP concentration level (2-10 mM), and the other was to ensure drug specificity since ATP-binding site is an evolutionarily well conserved region in nearly all kinases. Studies done with relatively specific p38 MAPK inhibitor SB203580 unraveled the mystery. Analysis of three-dimensional structure of p38 MAPK in complex with SB203580 displayed that specificity is determined largely by interaction of the compound with the residues that lie nearby -but out of- the ATP-binding pocket of the kinase.

A landmark event occurred in May 2001 when the first small molecule kinase inhibitor, Gleevec (STI-571/Imatinib) to be rationally developed by targeting a kinase specifically (the Abelson tyrosine kinase-ABL) was approved for clinical use in therapy for chronic myelogenous leukaemia (CML). Fused BCR-ABL oncogenic protein causes uncontrolled activation of MAPK cascade, leading to excessive proliferation of leukocytes of the myeloid lineage, hence the disease. Despite the relatively low incidence of CML, BCR-ABL was chosen to be the target of the first drug to be rationally developed, for this was also the first time that a protein kinase was showing a clear difference in its activity between normal and leukaemic cells. Exploiting knowledge gained from PKC (mentioned above) as a starting point, Gleevec was developed eventually...
through series of time-taking screening and modification steps.23,24 Having initially been developed as an ATP-competitive inhibitor, Gleevec has soon proved to be more than this: Structural studies showed that Gleevec inhibits the ABL kinase by extending much further into the ATP-binding site, promoting a transition that drives the kinase to adopt the inactive conformation.25 Even though this enhanced interaction with nearby residues around ATP-binding site has increased drug specificity remarkably, it did not take long for screening profiles to come up with two extra kinases getting inhibited with close efficacy: c-KIT and PDGF-receptor (Table 1).26

Emerging new drugs; non-ATP competitive inhibitors

A similar surprise was waiting for Alan Saltiel and his colleagues when they were trying to develop a small molecule inhibitor targeting MKK1 (MEK1) of Ras/Raf/MEK/ERK cascade (why MEK was chosen to be targeted although it is not an oncogene27). The first compound they developed, PD08059, inhibited a mutant form of MEK that can no longer be phosphorylated by ATP, but surprisingly did not inhibit MEK that had been activated by Raf.28 Structural studies showed that the more potent compound PD184352 (also called CI-1040)29 is actually binding more strongly to inactive form of the kinase, interacting with a hydrophobic pocket adjacent to, but distinct from, the ATP-binding site. This binding, due to a conformational change in the protein, ends in the ERK activation loop having no longer access to the catalytic ATP-binding site. Thus, even though ATP binds to its site, nearby-bound inhibitor does not allow the catalytic activity, preventing the subsequent phosphorylation of the ERK substrate by trapping MEK in a catalytically inactive state.2 More potent versions of CI-1040-like inhibitors- recently named as “non-ATP competitive inhibitors” by Ohren et al in a more convenient way.30 The finding of such inhibitors binding to a site apart from ATP-binding pocket accounts for their unique non-competitive mechanism of inhibition and explain the remarkable specificity of these drugs, as this binding site is in a region without sequence homology to other kinases.2

The future perspectives

Apparently, in the future drug discovery will seem to focus on these non-ATP competitive inhibitors for their remarkable superiority over ATP-competitive inhibitors. More advanced ways to screen for the inhibitors that will bind to the inactive forms of kinases will be essential in agreement with the development of the knowledge on catalytic and regulatory properties of kinases.3 Hopefully, this way will lead us finally to develop small molecule inhibitors that will compete for binding with the protein substrates, interfering in interphases where protein-protein interactions occur. Time will show us whether this will remain as a dream or not (for review of research on this32).

Table 1

Therapeutic drugs and their targets in cancer (adapted from14,31).

<table>
<thead>
<tr>
<th>Targets</th>
<th>Type</th>
<th>Inhibitor</th>
<th>Structure / comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>PKC/CHK 1</td>
<td>Ser/Thr kinases</td>
<td>UCN-01</td>
<td>Staurosporine analogue</td>
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<td>Calcineurin</td>
<td>Phosphatase</td>
<td>Cyclosporin</td>
<td>Imunosuppressant, FDA approved</td>
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<td>mTOR only</td>
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<td>Rapamycin</td>
<td>Inhibits by binding to FKBP, FDA app.</td>
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<td>SB203580</td>
<td>Small molecule-ATP competitive</td>
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<tr>
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<td>Tyr kinase</td>
<td>STI-571 (Gleevec)</td>
<td>Small molecule-ATP competitive with</td>
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<td></td>
<td></td>
<td></td>
<td>Enhanced interaction, FDA approved</td>
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<tr>
<td>MEK1/MKK1 only</td>
<td>Ser/Thr kinase</td>
<td>PD184352 (CI-1040)</td>
<td>Small molecule/non-ATP competitive</td>
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<td>RAS</td>
<td>GTP-binding kinase</td>
<td>R115777</td>
<td>Farnesy transferase inhibitor</td>
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<td>Some others NOT</td>
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<td>CDKs</td>
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<td>IMC-225 (Cetuximab)</td>
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References


