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Review

Key cancer cell signal transduction pathways as therapeutic targets

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ABSTRACT

Growth factor signals are propagated from the cell surface, through the action of transmembrane receptors, to intracellular effectors that control critical functions in human cancer cells, such as differentiation, growth, angiogenesis, and inhibition of cell death and apoptosis. Several kinases are involved in transduction pathways via sequential signalling activation. These kinases include transmembrane receptor kinases (e.g., epidermal growth factor receptor EGFR); or cytoplasmic kinases (e.g., PI3 kinase). In cancer cells, these signalling pathways are often altered and results in a phenotype characterized by uncontrolled growth and increased capability to invade surrounding tissue. Therefore, these crucial transduction molecules represent attractive targets for cancer therapy. This review will summarize current knowledge of key signal transduction pathways, that are altered in cancer cells, as therapeutic targets for novel selective inhibitors. The most advanced targeted agents currently under development interfere with function and expression of several signalling molecules, including the EGFR family; the vascular endothelial growth factor and its receptors; and cytoplasmic kinases such as Ras, PI3K and mTOR.

1. Introduction

The past decade has witnessed a major leap in the understanding of the molecular mechanisms involved in tumour pathogenesis and progression. Several signalling molecules that play a critical role in these processes have been identified and are now recognized as potential therapeutic targets. In parallel, a wide array of new agents of different classes and with diverse mechanisms of action has been synthesized and is now under clinical evaluation. The following short review highlights the most prominent signalling targets in the context of current drug development.

2. Epidermal growth factor receptor (EGFR) and HER2/ErbB-2

Growth factor peptides and their receptors are often overexpressed in human cancer cells and are involved in cell proliferation, differentiation and survival. One of the most studied growth factor receptor systems is the HER (also defined erbB) family. This family consists of four distinct, but structurally similar, transmembrane tyrosine kinase (TK) receptors, named HER1/erbB-1 (better known as endothelial growth factor receptor [EGFR]), HER2/erbB-2, HER3/erbB-3 and HER4/erbB-4) [1].

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The EGFR is a 170-kDa transmembrane protein able to bind several ligands, such as endothelial growth factor (EGF), transforming growth factor-α (TGF-α), heparin-binding EGF, amphiregulin, betacellulin, epiregulin and neuregulin G2b [2]. Ligand binding is followed by receptor dimerization and TK auto-activation which in turn, triggers a cascade of intracellular signalling pathways [1,3]. No ligand has been identified for HER2, therefore, this receptor usually heterodimerizes with either EGFR or HER3, and binds to their ligands for receptor activation [1,3]. Activation of EGFR or HER2 signalling triggers multiple and integrated biological responses, including mitogenesis, apoptosis, cellular motility, angiogenesis and regulation of differentiation [1]. The downstream propagation of activated EGFR/HER2 signalling involves at least two major pathways: the Ras/mitogen activated protein kinase (MAPK)-dependent pathway and the phosphatidylinositol 3-kinase (PI3-K)-dependent pathway [1,3].

The HER receptors and their ligands are frequently overexpressed in many tumour types, including those of epithelial and mesenchymal lineage, and have been implicated in cancer pathogenesis and are often associated with advanced disease and poor prognosis [4]. For instance, the degree of HER-2 overexpression in breast cancer is differentially associated to adverse prognosis and may influence the decision making for treatment [2,5]. A large body of preclinical studies and clinical trials conducted so far suggests that targeting the EGFR and HER-2 may provide a significant contribution to cancer therapy [5–7]. In general, the most promising strategies currently used to target EGFR include monoclonal antibodies that prevent ligand binding and favour the physiological process of EGFR receptor internalization and degradation; and 'small molecules' that inhibit the EGFR-TK autophosphorylation and downstream signalling. The most used and successful strategy to inhibit HER-2 is by monoclonal antibodies, particularly trastuzumab, although novel small molecules able to inhibit simultaneously the TK domain of EGFR and HER-2 have been synthesized and are now under clinical development [2,5].

Recent studies have demonstrated that, unlike HER-2 expression, EGFR expression is not a robust predictor of response to EGFR targeted therapy, since some patients with EGFR overexpressing tumours are refractory to EGFR inhibitors, while on the other hand, patients with a low degree of EGFR expression still respond to EGFR antagonists. Several factors affect the activation status of EGFR, including receptor mutations; receptor heterodimerization; increased expression of ligands; and activation of alternative pathways. The recent demonstration of mutations in the ATP binding pocket of the EGFR-TK domain, occurring in patients with non small cell lung cancers (NSCLC), provides new clues in the understanding of EGFR functions and the mechanistic bases of response to EGFR targeted therapies. In fact, mutations in exons 18–21, leading to increased EGFR activity through overactivation of PI3-K/Akt signalling have been reported. These mutations confer a genuine sensitivity to EGFR-TK inhibitors, such as gefitinib or erlotinib [8]. On the other side, there are mutations in exon 20 that confer resistance to TK inhibitors [9]. Although this matter is still under intensive investigation, these findings taken together could be very helpful in identifying the subgroups of patients potentially responsive to EGFR TK inhibitors. Recently, other factors responsible for inefficient therapeutic blockade of both EGFR and HER2 have been identified, including the activation of alternative pathways downstream to EGFR/HER-2 or the lack of cellular effectors, such as PTEN (discussed below) [6,10].

3. Ras

The signal transduction cascade activated by growth factors receptors, cytokines (IL2, IL3, GM-CSF), and hormones (insulin, Insulin-like growth factor-IGF), involves the 21-kDa guanine–nucleotide-binding proteins encoded by the *ras* proto-oncogene, of which H-, N-, and K-*ras* 4A/4B are prototypical [11]. The aberrant activation of Ras proteins is implicated in facilitating virtually all aspects of the malignant phenotype, including cellular proliferation, transformation, invasion and metastasis. Ras activity is regulated by cycling between inactive GDP-bound and active GTP-bound forms. Once in its GTP-bound form, RAS activates several downstream effector pathways that mediate cell proliferation and suppression of apoptosis. Hydrolysis of GTP by Ras is facilitated by GTPase-activating proteins (GAPs) such as p120GAP and NF1 [11].

Point mutations in the *ras* gene (at residues 12, 13 or 61) are oncogenic because they render Ras insensitive to GAP stimulation, resulting in a permanently active GTP-bound Ras form, which continuously activates the downstream pathways in the absence of any upstream stimulation [11]. Furthermore, continuous activation of Ras protein can still occur even in the absence of any mutation in the *ras* gene, as a result of continuous upstream signals, particularly by activation of HER family receptors. Thus, the therapeutic strategy of targeting Ras in cancer should not include only tumours with proven oncogenic mutation. Overexpression of Ras isoforms is reported in many solid and haematologic malignancies, while mutations in K-ras seem to play an important role in NSCLC, as they are described in approximately 30% of adenocarcinoma histotypes. Recent studies have demonstrated that K-ras and EGFR mutations are mutually exclusive in lung adenocarcinomas and are associated with a worse outcome [12].

Ras protein function depends on its physical association with the inner surface of the membrane and the consequent transmission of the signal to Raf-1, which involves complex post-translational modifications of the protein. The first and most critical step in this process is farnesylation, a reaction catalysed by the enzyme farnesyl-transferase [13]. Inhibition of farnesylation by farnesyl transferase inhibitors (FTIs) may be sufficient to abrogate Ras-dependent cell signalling and transforming functions. On the basis of this, several different FTIs have been now tested in a large number of clinical studies as single agents as well as in combination with standard chemotherapy.

The Raf kinase proteins include three isoforms, A-RAF, B-RAF and RAF-1, which differ in their expression profile, regulation and ability to function in the context of the Ras/Raf/MAPK pathway. Activating mutations in the B-RAF gene have been demonstrated in 70% of human malignant melanomas and 15% of human colon cancers [14]. To date, the most advanced Raf protein antagonist is the multitargeted kinase

inhibitor Sorafenib (BAY43-9006), a drug that is active in different tumours, particularly in advanced renal cancer.

4. PKC

PKC belongs to a class of serine–threonine kinases encompassing at least 12 closely related isozymes that have distinct and, in some cases, opposing roles in cell growth and differentiation. Activation of cell surface receptors, such as EGFR and platelet-derived growth factor receptor (PDGF-R), triggers the phospholipase to produce diacyl-glycerol which, in turn binds and activates PKC. Increased levels of PKCα, the most studied isoform, have been associated with malignant transformation in breast, lung and gastric carcinomas [15]. The presence of PKC isoforms with differential activation and tissue distribution raises the theoretical possibility to develop PKC isozyme-specific inhibitors. However, the complex nature of the many secondary messenger systems triggered by PKC activation makes selective drug action difficult. Few of the currently available pharmacological agents exhibit a high degree of selectivity for a specific PKC isoform. Among them, Affinitak, an oligonucleotide antisense PKCα [16]; and enzastaurin, a small molecule targeting the PKCβ that transduces the signals of the Vascular endothelial growth factor Receptor (VEGFR), are currently under clinical development.

5. AKT/PKB and mTOR

Akt, also known as protein kinase B (PKB) is a serine/threonine protein kinase that acts as a critical regulator of cell survival and proliferation. Akt interacts via the pleckstrin homology (PH) domain with the membrane lipid phosphatidyl–inositol–trisphosphate (PIP3) produced by PI3-K following activation by growth factor receptors. On the other hand, activation of Akt is physiologically prevented by PTEN, a phosphatase that removes from PIP3 the phosphate attached by PI3-K [17]. Akt is an indirect positive regulator of the mammalian target of rapamycin (mTOR), a central controller of eukaryotic cell growth and proliferation, through the phosphorylation and inactivation of mTOR inhibitors, such as tuberin (TSC2). Moreover, Akt seems also able to phosphorylate mTOR directly, although the functional significance of this remains to be determined [18].

The activation of Akt provides cells with a survival signal that allows them to withstand apoptotic stimuli, through phosphorylation/inactivation of proapoptotic proteins, such as BAD and Caspase 9, and transcriptional factors. Moreover, Akt is involved in the regulation of cell metabolism through inhibition of glycogen synthase kinase 3 (GSK3) [17]. A large body of experimental evidences confers to Akt and its regulators an important role in cancer pathogenesis: (a) Akt is overexpressed in a variety of human cancer types [19]; (b) Akt is constitutively activated in several cancers, including NSCLC, conferring chemoresistance [20]; (c) PTEN, one of the major antagonists of Akt activity, is frequently lost or inactivated by mutation in human cancer; and (d) conversely, the Akt positive regulator, PI3-K, or one of its downstream effectors, mTOR, are commonly up-regulated [17]. Furthermore, it has been demonstrated that increased PI3-K and Akt activation induced by different growth factor receptors, such as the IGF-1 Receptor (IGF-1R), is involved in the failure of EGFR- and HER2-targeted drugs in several types of cancer [21,22]. Therefore, Akt is an obvious attractive target for antitumour therapy and it has been postulated that inhibition of Akt, alone or in combination with standard cancer chemotherapeutics could reduce apoptotic threshold and preferentially kill cancer cells. The majority of inhibitors are phosphatidyl–inositol analogs and small molecules ATP-competitive antagonists, but they are low in specificity and have poor bioavailability. A new class of allosteric inhibitors, that are PH domain-dependent and exhibit Akt isozyme selectivity, could have sufficient potency and specificity to be tested as antitumour agents in animal models [19]. For the same reasons, mTOR and IGF-1R are also appealing novel therapeutic targets. Oral anti-mTOR rapamycin analogues, everolimus (RAD001) and temsirolimus (CCI-779), are now in phase II/III clinical evaluation and encouraging antitumour activity has been observed. Different classes of IGF-1R kinase inhibitors have also been recently synthesized and are being evaluated in preclinical studies.

6. The proteasome

Selective protein degradation is fundamental to regulation of vital cellular processes, including cell proliferation and survival. The ubiquitin-proteasome pathway plays a central role in protein degradation. Most cellular proteins are degraded by the 26S proteasome complex, leading to increased apoptosis in malignant cells by preventing release of the anti-apoptotic factor NF-κB. Proteasome inhibition is regarded as an attractive anticancer strategy. A selective proteasome inhibitor, bortezomib, is under clinical development and has shown efficacy in patients with relapsed and refractory multiple myeloma [23].

7. Angiogenesis

Angiogenesis is a complex process and represents a critical step for tumour formation and progression [24,25]. It is now evident that angiogenesis is not only essential for tumour growth, but it is also implicated in the initial progression from a pre-malignant lesion to a fully invasive cancer, and in the growth of dormant micrometastases into clinically detectable metastatic lesions [24,25]. Therefore, the targeting of angiogenesis has become a major therapeutic strategy for cancer treatment, and a wide variety of drugs interfering with this process are under development.

To grow beyond a critical size or metastasise to another organ, a tumour must recruit a network of new blood vessels. This switch to an angiogenic phenotype is regulated by a balance between pro- and anti-angiogenetic molecules [24,25]. Although this is a complex and coordinated process, requiring the sequential activation of a series of receptors by numerous ligands, there is consensus that signalling by the major angiogenic factor, VEGF, often represents a crucial rate-limiting step in pathological angiogenesis. There are five recognized isoforms of VEGF, named from A to D, of which VEGF-A is considered the most important for angiogenesis,

while others like VEGF-C and D seem to play a role for lymphangiogenesis. They are highly conserved, disulfide-bonded homodimeric glycoproteins of 34–45 Kd that belong to the PDGF superfamily of growth factors [25].

Although constitutively expressed by many tumour cells and transformed cell lines, VEGF expression is subject to several mechanisms of control. A key pathway is the regulation by oxygen concentration. Decreased intratumour oxygen concentration potently upregulates VEGF expression [25,26]. In addition, alterations in oncogenes and tumour suppressor gene function promote tumour growth by modulating the angiogenic response induced by VEGF [25].

The VEGFs mediate angiogenic signals to the vascular endothelium via high-affinity TK receptors. To date, three receptors have been identified: VEGFR-1 or Flt-1 (fms-like tyrosine kinase), VEGFR-2 or KDR (kinase insert domain-containing receptor)/Flk-1 (fetal liver kinase-1), and VEGFR-3 or Flt-4 [25]. Following its binding to cognate receptors, VEGF initiates a cascade of signalling events that begins with dimerization and trans-autophosphorylation of TK residues in the VEGFRs which in turn, activates phospholipase C-γ (PLC-γ), PI3-K, GAP, MAPK, and others.

Overexpression of VEGF in cancer cells may be an indicator of poor prognosis in many types of human tumours, including carcinomas of the breast, kidney, colon and prostate [27]. VEGF levels may have a potential value for predicting the effectiveness of conventional treatments, including radiotherapy, chemotherapy, and hormonal therapy in different diseases [27,28].

Recent experimental evidence has demonstrated that VEGF overexpression and secretion represent a major escape pathway when tumour cells develop resistance to selective EGFR inhibitors [29,30]. On this basis, different types of drugs directed against VEGF or its receptors have been developed. They include monoclonal antibodies, small molecules TKI, and antisense oligonucleotides. For some of them, including the monoclonal antibody bevacizumab, relevant results have already been obtained in the treatment of patients affected by colorectal, lung, renal and breast cancer. Among the small molecules antagonizing VEGF-R TK, a wide array of agents is under development. Some of them antagonize only one VEGF-R, often the KDR; some target all the VEGF-Rs; and still others are simultaneously active against VEGF-Rs and other receptor kinases, such as PDGF-receptor [27–29].

8. Conclusions

The association of selective inhibitors of key signalling proteins with conventional chemo-, hormone- and radiotherapy has become a common therapeutic option in several types of cancer. On the other hand, increased understanding of signal transduction propagation has also brought awareness that a large degree of cross-talk and redundancy exists among the different signalling pathways. This information is now being used to realize novel therapeutic strategies, based on the combination of different signalling inhibitors or the development of multitargeted inhibitors. The aim of this type of therapy is to block signalling pathways to avoid the occurrence of resistance due to the activation of compensatory mitogenic pathways. The wide use of genomics and proteomics will contribute in elucidating the functional role of genes and proteins, highlighting their role in different stages and contexts of tumour growth and, consequently, their potential value as therapeutic targets. The progress in the fight against cancer will dependent more and more on understanding the critical steps in tumour progression at the molecular level, and linking that to the development of drugs that can interfere selectively with these events.

Conflict of interest statement

None declared.

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