Molecular Pathways

Targeting Multiple Kinase Pathways: A Change In Paradigm

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Abstract

Anticancer drugs that target protein kinases include small molecule inhibitors and monoclonal antibodies. Feedback loops and cross talk between signaling pathways impact significantly on the efficacy of cancer therapeutics, and resistance to targeted agents is a major barrier to effective treatments. Increasingly, therapies are being designed to target multiple kinase pathways. This can be achieved using a single agent that inhibits multiple signaling pathways or a combination of highly selective agents. In this review we discuss the principles of specifically targeting multiple kinase pathways with particular reference to angiogenic signaling pathways. Clin Cancer Res; 16(7); 1973–8. ©2010 AACR.

Dysregulation of protein kinases in cancer cells is extremely common. Consequently protein kinases are attractive targets for anticancer drugs, including small molecule inhibitors that usually act to block binding of ATP or substrate to the catalytic domain of the tyrosine kinase and monoclonal antibodies that specifically target receptor tyrosine kinases (RTK) and their ligands. The most exquisite example of successful targeted therapy is perhaps that of imatinib, designed specifically to target an abnormal, constitutively active BCR-ABL tyrosine kinase found in >90% of cases of chronic myeloid leukemia (1). In solid malignancies, it is unusual for a single kinase abnormality to be the sole cause of disease and it is unlikely that tumors are dependent on only one abnormally activated signaling pathway. Instead multiple signaling pathways are dysregulated. Furthermore, even single molecular abnormalities may have multiple downstream effects. Thus, unless it is possible to target a single key underlying defect, it is likely that therapies will be more effective by inhibiting a number of downstream targets.

Advantages of such a multitargeted approach include the potential for increased efficacy and reduced resistance by simultaneous inhibition of multiple pathways and common escape pathways. Disadvantages include possible increased cost and toxicity. Another important question is whether simultaneous or sequential administration of targeted drugs produces superior efficacy. The theoretical background for simultaneously targeting multiple targets is not the same as simultaneously using multiple agents. Employing sequential use of non-cross-resistant therapies may in some cases result in improved outcomes. Interestingly, even agents with similar modes of actions, such as sunitinib and sorafenib, seem to show a rather low level of cross-resistance as shown by two clinical trials comparing the sequential use of sunitinib and sorafenib and vice versa (2, 3). Sequential therapy may also be associated with a more favorable toxicity profile, but ultimately, this is a question that will need to be resolved in clinical trials.

Multiple pathways can be targeted either by using a single agent that inhibits multiple signaling pathways or by using a combination of highly selective agents. Although use of a single multitargeted agent offers convenience, potential limitations include difficulties in obtaining sufficient potencies against multiple targets in tumor cells without excessive toxicity from cross-reactivity with normal tissues. Differing affinities for the receptors may result in relatively greater inhibition of one target to achieve adequate inhibition of another resulting in toxicity. In contrast, combining selective agents with the aim of achieving additive or synergistic effects may allow high target selectivity with reduced systemic effects, though this is at the risk of potential pharmacodynamic and pharmacokinetic interactions between the drugs. Ideally, combination therapies should use effective agents with differing mechanisms of action and adverse effect profiles.

In this review we discuss the principles of specifically targeting multiple kinase pathways.

Angiogenesis Signaling Pathways

Angiogenesis is crucial for tumor progression and metastasis and is increasingly a target for cancer therapies. The vascular endothelial growth factor (VEGF) family of proteins consist of numerous subtypes, including VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placenta-growth factor-1 (reviewed in ref. 4), most of which bind to cell membrane-associated RTKs, the VEGF-receptors (VEGFR). The binding of VEGF ligand to its receptor initiates activation of downstream signaling pathways, including the RAF-mitogen activated protein kinase (MEK)-extracellular regulated kinase (ERK) and...
phosphoinositide 3-kinase (PI3K) pathways, which ultimately lead to endothelial cell activation, proliferation, migration, and survival (Fig. 1A). Increased VEGF expression is found in a variety of human tumors including colorectal cancer (CRC), non-small cell lung cancer (NSCLC), breast and ovarian cancers, and is correlated directly with increased neovascularization within the tumor (reviewed in ref. 5). Drugs targeting the VEGF pathways include the monoclonal antibody bevacizumab and the small molecule inhibitors sunitinib, sorafenib, and valatinib. Additional positive regulators of angiogenesis and their receptors include fibroblast growth factor (FGF, FGF-receptor), platelet derived growth factor (PDGF, PDGF-receptor), angiopoietin 1 and 2 (Tie2 receptor), and transforming growth factor-β (TGF-β, TGF-β-R). Moreover, increasing evidence suggests a link between the EGF receptor (EGFR) and HER2 pathways and VEGF-dependent angiogenesis and preclinical studies have shown both direct and indirect angiogenic effects of EGFR signaling (reviewed in ref. 6). Upregulation of tumor pro-angiogenic factors and EGFR-independent tumor-induced angiogenesis have been suggested as a potential mechanism by which tumor cells might overcome EGFR inhibition.

Targeting the PI3K and RAF-MEK-ERK Pathways

The PI3K-Akt signaling pathway is inappropriately activated in many cancers (Fig. 1A; reviewed in ref. 7). This activation may result from RTK-induced activation of PI3K; tumors in which PI3K is activated by multiple RTKs are invariably resistant to a single specific RTKI (reviewed in ref. 7). Alternatively, several genetic abnormalities are known to activate PI3K-Akt signaling. These include loss of the PTEN tumor suppressor (reviewed in ref. 8), somatic activating mutations in the class la PI3K catalytic subunit (p110α; ref. 9) or regulatory subunit (10), and genetic alterations of all three Akt isoforms (reviewed in ref. 7). PI3K also binds directly to ras (11), and there is increasing evidence that Ras activation modulates PI3K function, and vice versa, though the importance of this interaction is as yet unquantified. Several small molecules that inhibit the PI3K-Akt signaling pathway are in clinical development, including mammalian target of rapamycin (mTOR) inhibitors, PI3K inhibitors, dual PI3K-mTOR inhibitors, and Akt inhibitors (reviewed in refs. 7, 12). Interestingly, the p110 subunits of PI3K and mTOR share similar structures, and small molecule inhibitors of p110 often also inhibit mTOR.

Targeting Multiple Pathways Is Likely to Be the Optimum Therapeutic Strategy

Multitargeted therapies may target several points within a single pathway, or multiple distinct pathways. It is becoming increasingly clear that feedback loops and crosstalk between signaling pathways, including the PI3K/Akt and RAF-MEK-ERK pathways impact significantly on the efficacy of cancer therapeutics (Fig. 1A). For example, mTOR-complex 1 (mTORC-1) inhibition leads to activation of both PI3K signaling by abrogating feedback inhibition (13) and ERK signaling through a feedback loop, which depends on an S6 kinase-P110-Ras pathway (14). Such preclinical studies emphasize the potential of a combined therapeutic approach with mTORC1 and MEK inhibitors (Fig. 1B).

Likewise, in cancers with mutant RTKs or oncogenes such as Ras that activate both the RAF-MEK-ERK and PI3K pathway, blocking the PI3K pathway can actually up-regulate signaling of the RAF-MAPK pathway because the two pathways have cross-inhibitory effects (15). It is therefore likely that the most useful role for drugs targeting molecules downstream to the RTKs may be as combination therapies rather than as single agents. Administration of mTORC1 inhibitors concurrently with PI3K and MEK inhibitors could theoretically circumvent resistance because of the feedback loops described above, and combining PI3K and MEK inhibitors may alleviate problems associated with cross-inhibitory effects between the two pathways (Fig. 1B; ref. 16).

Lung cancers that are sensitive to EGFR inhibitors (EGFR-I) have PI3K and ERK activation that is under sole control of the EGFR (EGFR oncogene addiction). Such tumors can be rendered resistant to EGFR-Is simply by maintaining PI3K signaling, and reactivation of PI3K signaling is almost invariably seen in cancers that naturally develop resistance to EGFR-Is (reviewed in ref. 7). Combining PI3K inhibitors with EGFR-Is is a sensible strategy to increase the proportion of cancers that benefit from EGFR-Is, and delay the development of resistance in those tumors that are initially responsive (Fig. 1C). Similarly, there is evolving evidence that inhibition of both the PI3K-Akt and RAF-MEK-ERK pathways might be substantially more effective than inhibition of either pathway alone. For example in a mouse model of lung cancer that expressed mutant k-ras, the combination of PI3K inhibitors and MEK inhibitors was highly effective, although the use of either agent alone was not (16).

Clinical-Translational Advances

Combining anti-angiogenic agents in the clinic

Combination therapies with mTOR-Is. Although mTOR was only recently defined as a member of the PI3K pathway, it is the first node of the pathway to be targeted in the clinic. mTOR exists in two multiprotein complexes: mTORC1 and mTORC2. The rapamycin analogs temsirolimus (17) and everolimus (18) both inhibit mTORC1 and are approved as treatments for advanced clear cell renal carcinoma (ccRCC). Rapalogs have also shown single-agent activity in lymphoma but have failed to show any appreciable single agent activity in many other tumor types (reviewed in ref. 19). Selective ATP-competitive inhibitors of mTOR that are currently in clinical trials inhibit both mTORC1 and mTORC2 and impair cell growth and
Fig. 1. A, VEGF signaling and potential therapeutic targets. The binding of VEGF to its receptor initiates activation of both the PI3K-Akt and RAF-MEK-ERK pathways (1). Each pathway has its own distinct downstream effects. However, they also converge on at least two important downstream targets, mTORC1 (2) and Bcl2-associated agonist of cell death (BAD; ref. 3), which play a key role in apoptosis. Furthermore, Ras binds directly to PI3K, and each influences activation of the other pathway (4). mTORC1 inhibition leads to activation of both PI3K and ERK signaling by abrogating feedback inhibition (5). Potential points of therapeutic inhibition are highlighted. Components in the signaling pathway that are mutated in cancers are shown in pink.

B, combination therapies including mTOR Inhibitors. Combining mTOR-Is with VEGF antagonists such as bevacizumab may result in additive or synergistic efficacy. Alternatively, preclinical evidence suggests that combining mTOR-Is with MEK-Is and/or PI3K-Is may improve clinical efficacy by abrogating downstream effects induced by mTORC-I-induced activation of the RAF-MEK-ERK and PI3K pathways. C, combinations of EGFR-Is and PI3K-Is may improve clinical efficacy. Reactivation of PI3K signaling is a common mechanism of resistance to therapies targeting the EGFR. Combining these agents with PI3K-Is may be one way to overcome this resistance mechanism.
Increasingly "multitargeted" agents are being developed with the goal of inhibiting more than one pathway simultaneously. Multitargeted agents currently licensed for use include sorafenib, sunitinib, lapatinib, and pazopanib. Additional agents under investigation include vandetanib, an oral inhibitor of VEGFR and EGFR signaling that also has activity versus Ret TK. A phase II study in advanced NSCLC showed a significant prolongation of progression-free survival versus gefitinib, and vandetanib is currently being evaluated as a monotherapy in two randomized phase III studies in advanced NSCLC (37). Further studies are evaluating its use in colorectal cancer. Regorafenib is an orally active, potent multikinase inhibitor with a kinase inhibition profile targeting VEGFR, PDGFR, TIE2, KIT, RET, and FGFR. Preliminary data from an open label phase II study of previously untreated patients with metastatic ccRCC showed promising antitumor activity with disease control in 81% of patients (38). Vatalanib is another agent that inhibits all known VEGFRs, as well as PDGF-ß-R and c-kit, but is most selective for VEGFR-2. Addition of vatalanib to chemotherapy in mCRC did not improve overall survival in two large randomized controlled phase III studies (39, 40), though a meta-analysis of these trials suggested that vatalanib did significantly improve progression-free survival in patients with high levels of lactate dehydrogenase (41).

**Development of resistance.** Resistance to targeted agents is eventually inevitable. The types of resistance may be classified as intrinsic resistance, which is present prior to starting treatment, and acquired resistance, which evolves during the course of therapy. Mechanisms of resistance include:

(a) upregulation of alternative RTKs that activate the same downstream targets. For example, amplification of the Met oncogene may lead to resistance to EGFR-Is by activating erbB-3 signaling (42);

(b) constitutive activation of downstream mediators. For instance activating mutations in k-ras have been significantly associated with lack of response to EGFR-Is (reviewed in ref. 42), and four separate studies of mCRC showed that adding an EGFR-I (cetuximab or panitumumab) to standard chemotherapy only benefits patients with wild-type k-ras (43–46);

(c) upregulation of the ligand for the targeted receptor. For example hypoxia-triggered upregulation of proangiogenic factors such as FGF and PDGF-ß in the presence of anti-VEGFR agents can restimulate tumor angiogenesis in a VEGF-independent manner (42);

(d) the existence of specific mutations within the targeted RTK. For example a T790M mutation in the EGFR confers resistance to ATP-competitive kinase inhibitors such as gefitinib and erlotinib by increasing the ATP affinity of the EGFR by more than an order of magnitude (47).

As we learn more about mechanisms of resistance it will become easier to devise intelligent strategies to overcome them. Targeting multiple pathways at multiple levels may be one strategy to surmount resistance.

**Conclusions**

To develop effective therapeutic strategies that overcome or prevent drug resistance we must continuously anticipate the potential mechanisms underlying resistance and devise methods to circumvent them. To achieve this, it is critical to design genotype-directed clinical trials, matching the
therapy to the patient, rather than testing random combinations in a broad selection of cancers. Assimilation of the most convincing preclinical data will assist in patient selection, and such a strategy is more likely to prioritize the most active therapies. In turn, preclinical models must be reevaluated and adjusted in view of the results from clinical trials.

Our ways of assessing tumor response need reevaluating. Kinase-inhibition often has antiproliferative rather than anti-apoptotic effects, and antitumor activity may not, therefore, necessarily lead to tumor regression. Instead, long-lasting disease stabilization may be seen, implying a need to modify the traditionally used Response Evaluation Criteria In Solid Tumors (RECIST) criteria to account for this phenomenon. It might be expected that combining therapies will increase toxicity. However this risk may be overcome with intelligent dosing strategies. In chronic myeloid leukemia, cytotoxicity with transient potent target inhibition using dasatinib is equivalent to prolonged target inhibition (48), and in metastatic ccRCC sunitinib shows activity either when administered within an intermittent 4-weeks-on, 2-weeks-off dosing schedule (50 mg/d) or with a continuous once daily administration of 37.5 mg, suggesting the potential for flexible dosing (49). Intermittent potent kinase inhibitor therapy may be a tool to minimize the toxicity of targeted therapy.

It is important to understand why therapies fail when they do. Is it because they do not potently inhibit their target in vivo, or because effective target inhibition does not produce the desired results? Pharmacodynamic assessments of target inhibition are necessary to answer this; at present this requires evaluation of cancer specimens after drug treatment, and developing less invasive ways of measuring target inhibition, potentially, for example, by assessing circulating tumor cells (50), would be invaluable. Equally, identification of robust biomarkers that can be used with confidence to select patients most likely to benefit from treatment is a crucially important part of the development of new agents. The holy grail for targeted therapies is unlikely to be a highly specific agent targeting a single molecule. Instead, approaches targeting more than one molecule in more than one pathway are likely to be the most successful. Informed combinations, directed at bypassing feedback loops and interrupting cross talk between signaling pathways may improve therapeutic outcomes.

Disclosure of Potential Conflicts of Interest

T Eisen, commercial research grant, Pfizer, Bayer, Astra Zeneca; honoraria from speakers bureau and consultant, Pfizer/Wyeth, Bayer, Astra Zeneca, GSK, Amgen, Bristol-Myers, Aveo, Immatics. The other author disclosed no potential conflicts of interest.

Acknowledgments

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Received 12/08/2009; revised 12/21/2009; accepted 12/22/2009; published OnlineFirst 03/09/2010.

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