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Signal transduction inhibition

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INTRODUCTION

The relatively narrow therapeutic window characteristic of the vast majority of traditional chemotherapeutics has led to pursuit of targeted therapies with the hope of providing both improved efficacy and reduced toxicity. These targeted therapies use agents that impact specific factors or cellular pathways that have been shown to have abnormal activity in neoplastic cells and that drive cancer progression. The diversity of potential targets is currently most impressive, with an astounding number of new possibilities emerging. However, translation of these targets into useful approaches for clinical drug development has provided both exciting successes and disappointing failures. Some of the most attractive targets for molecular-targeted strategies for the treatment of cancer include the family of receptor and non-receptor tyrosine kinases (TKs) and more specific areas of the growth factors and epidermal growth factor receptor (EGFR) inhibitors; the Ras/Raf/MAPK pathway; the phosphatidylinositol-3 kinase (PI3K)/Akt/*PTEN* pathway, gene expression modification with antisense oligonucleotides and RNAi; and endothelial cell and angiogenesis-associated factors such as the vascular endothelial cell growth factor (VEGF) (1).

Although some of these approaches have produced clinically useful agents, primarily due to the nature of the abnormality targeted, other approaches have not been as successful because of the redundancies and overlapping nature of complex signal transduction pathways. In many cases, although the aberration is present in cancer cells, it does not drive tumor behavior. Indeed, one of the most significant challenges is to distinguish molecular events that drive tumor behavior from epiphenomena that arise secondarily to an important event or due to the inherent genetic instability in tumors (4). Although single-agent therapy remains a goal, experience with conventional cytotoxic therapy and with experimental agents suggests that combining these new, targeted agents with either conventional therapies or one another is likely to provide the best efficacy. Indeed, given the complex crosstalk between signaling pathways, it is likely that it will be necessary to hit several critical signaling nodes to attain optimal outcomes. However, as combinations of targeted therapeutics may be more effective, they may also be more toxic, a process that can be quantitated only in patient studies (4).

Tyrosine Kinases: Kinases are enzymes that regulate the function of other proteins/enzymes and other target molecules. Tyrosine kinases accomplish signal transduction by transferring an activating gamma phosphate group from ATP to a tyrosine residue on the substrate molecule, usually another protein. Serine and threonine kinases are a separate group of transduction enzymes that target serine and threonine residues rather than tyrosine residues on substrate molecules. In addition to proteins, a number of other intracellular signaling molecules, such as membrane lipids or intracellular inositol rings are regulated by their phosphorylation status through the action of specific kinases. Phosphatases remove phosphates on the substrate molecules and thus oppose kinase activity and inhibit the signal transfer. These 2 enzyme classes act as switches; they activate and deactivate pathways according to the signals received by the cell. Although in general, phosphorylation events positively regulate signaling, there are circumstances in which phosphorylation inhibits function. So depending on the cell system and the target, kinases and phosphatases can be either positive or negative regulators. Cellular signaling frequently involves phosphorylation of all 3 amino acid residues (tyrosine, threonine, and serine), sometimes at multiple sites on one protein, or of other signaling molecules such as lipids and on multiple components in a pathway, suggesting that multiple kinases and kinase classes function collaboratively to orchestrate the final outcome of signal transduction. An extracellular signal activates a membrane-bound receptor, such as EGFR, which forms either homodimers with another EGFR or heterodimers with other family members to initiate the signal relay across the cytoplasm, culminating in changes of gene transcription in the nucleus and subsequent regulation of important cellular functions such as cell proliferation, migration, or survival (5).

Some kinases are membrane bound, and others reside in the cytoplasm. Thus, development of agents to modify kinase activities has included antibodies targeted to the extracellular portion of the membrane-bound kinase, e.g., trastuzumab, as well as small molecules that act in the cytoplasm, e.g., imatinib mesylate (1,2). Inhibition of intracellular TK activity has been achieved using small molecules whose structures are based on the structure of ATP. This characteristic allows these molecules to affect not only tumors that overexpress TK activity, but also those with normal TK expression or mutated, constitutively activated forms of TK. These competitive inhibitors of the ATP-binding site on the kinase can be specific for individual kinases or have a broader spectrum inhibiting multiple kinases, such as the effects of imatinib mesylate on Abl, Kit (CD 117), and platelet-derived growth factor (PDGFR), and have the clinical benefit of oral bioavailability (6).

Ras/Raf/MEK/MAPK PATHWAY

Ras proteins occupy a pivotal role in signal transduction, from RTKs and external growth stimuli to a variety of intracellular effector pathways, including the downstream targets of Raf and MEK (a mitogen-activated protein kinase kinase), leading to cell proliferation. As a response to growth-inducing signals, the inactive membrane-bound Ras-guanosine 5'-diphosphate (Ras-GDP) is switched to the activated membrane-localized Ras-GTP, which subsequently acts on downstream components (5,7). Normal control of Ras signaling involves GTP hydrolysis by GTPase-activating proteins, which rapidly convert Ras to the inactive Ras-GDP state. To perform signal transduction, the Ras protein also undergoes a series of post-translational modifications that facilitate its localization to the inner surface of the cell membrane and subsequent activity. The first of these modifications requires farnesylation (lipid prenylation) of the Ras protein at a C-terminal tetrapeptide sequence (the CAAX motif) in a step catalyzed by the enzyme farnesyltransferase (FTase). The introduction of this hydrophobic modification allows the Ras protein to associate with the membrane lipid bilayer more effectively. Other protein prenyltransferases, the geranylger-anyltransferases (GGTase) also catalyze prenylation of several proteins, such as Ras (5,7).

Mammalian cells have at least 3 ras proto-oncogenes: K-*ras*, H-*ras*, and N-*ras*, which, upon activating mutation, result in constitutive proliferation and anti-apoptotic signaling. Overall, *ras* mutations have been identified in about

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30% of all human cancers, such as the K-*ras* mutations in NSCLC, colorectal cancer, and pancreatic cancers; H-*ras* mutations in bladder and kidney cancers; and N-*ras* mutations in melanomas and hematologic cancers (8). Ras signaling is also influenced by aberrant upstream signaling, as Ras functions to transmit extracellular signals from growth factors and their receptors to intracellular pathways. With the established involvement of Ras in cancer and the necessity of prenylation for Ras activity, targeting of the FTases with inhibitors presented an attractive strategy to block this pathway.

Several classes of FTase inhibitors exist. Although many have been studied preclinically, few have progressed far in clinical development. Early clinical trials of both orally active and parenterally administered FTase inhibitors show similar toxicities, including schedule-dependent myelosuppression and gastrointestinal effects. Studies in solid tumors have also been disappointing. Monotherapy showed no objective responses in tumors, including NSCLC, SCLC, pancreatic, colorectal, and prostate (1,5). However, preliminary results suggest that R115777 might have some benefit in hematologic malignancies (9).

The lack of specificity of the FTase inhibitors suggests that these agents might also inhibit farnesylation of some of the 300 known proteins possessing a CAAX motif. This inhibition might account for some of the antiproliferative activity attributed to these agents (1,5). Additionally, redundant pathways might subvert the impact of the FTase inhibitors on Ras. Geranylgeranylation of K-Ras and N-Ras after blocking farnesylation may offer a means for continued signal transduction. Thus, although the exact mechanism of action of these agents is not as clear as once thought, preclinical data still suggest that FTase inhibitors might play a role in therapy if properly used (1,5).

The downstream MAPK effector pathway, which includes Raf and MEK, also offers sites for drug targeting. B-Raf mutations have been documented in a number of human cancers, including early ovarian cancers and melanomas, and the MAPK pathway is activated in approximately 30% of human malignancies (10-12). Raf is a family of serine-threonine kinases. Because they can also be activated by protein kinase C alpha and are known to contribute to multidrug resistance expression, they play a large role in oncogenesis (2). The only known substrates for MEK are the MAPK kinases Erk1 and Erk2 (extracellular signal-regulated kinases), whose substrates are cytosolic and nuclear proteins (probable transcription factors) (5). These downstream kinases have only recently garnered the interest that had previously been reserved for Ras; therefore, inhibitors of these kinases are not as far along in clinical investigations. BAY 43-90006 is an orally available potent inhibitor of Raf-1 that targets the ATP-binding site and is in clinical trials. CI-1040, an inhibitor of MAPK at a non-ATP site is also in early phase II clinical trials in pancreatic, breast, renal, and colorectal cancers (1,5). The response rates in renal cell cancers to BAY 43-9006 may, however, represent a rather promiscuous activity of this inhibitor, blocking for example the Kdr VEGF receptor.

PI3K/Akt/PTEN PATHWAY AS AN EMERGING TARGET

The PI3K pathway plays a role in cell growth; cell cycle progression (both during the G1 phase of the cell cycle and at the G2/M transition); protein translation; sensing the environment, particularly available nutrients and growth factors; motility; neovascularization; metastasis; drug resistance; and cell survival, particularly apoptosis and anoikis (matrix deprivation-induced apoptosis), as well as in transformation induced by many different oncogenes and tumor suppressor genes (13). Given the importance of these processes in transformation and tumor progression, the PI3K pathway is a potential driver of tumor behavior. Various elements of the PI3K/Akt/*PTEN* pathway are activated in at least 70% of all cancers, resulting in constitutive activation of the pathway. Indeed, the PI3K pathway is targeted for mutagenesis in human tumors more frequently than any other pathway, with the potential exception of the p53 pathway (14-16). However, the p53 and the PI3K pathways interact at multiple levels, suggesting that these 2 pathways are really a network and any attempt to distinguish between the pathways is moot. The PI3K pathway is targeted by amplification of the catalytic subunit of PI3K, Akt and S6K1, activating mutations in the catalytic subunit of PI3K and the regulatory subunit of PI3K, inactivating mutations in *TSC1/2*, *LKB1*, and *PTEN* and rearrangements of forkhead and *TCL1*. In addition, activation of Akt can bypass the effects of radiation and chemotherapy and also of targeted therapeutics such as gefitinib and erlotinib. This has not escaped the attention of the pharmaceutical and biotech industries, where there are multiple programs targeting many of the components of the pathway. CCI-779, RAD001, and AP23573, which target mTOR, a downstream component of the pathway, are in clinical trials and have demonstrated remarkable responses in a subset of patients with glioblastoma multiform, metastatic melanoma, malignant glioma, mantle cell non-Hodgkin's lymphoma, small cell lung cancer (SCLC), and renal cell carcinoma. Objective response rates have been as high as 10% to 20% in several of the tumors, with stable disease in a significant fraction of patients. Mantle cell lymphoma is particularly intriguing, as response rates may be as high as 44%. Why mantle cell lymphoma is highly sensitive is under extensive investigation. Ongoing clinical trials are evaluating mTOR inhibitors in combination with other targeted therapeutics and also in combination with chemotherapy.

APOPTOSIS MODULATION AND ANTISENSE OLIGODEOXYNUCLEOTIDES

The use of antisense oligodeoxynucleotides (AS-ODNs) involves synthetically producing a short sequence (usually an oligomer of about 20 nucleotides) of chemically modified DNA that will hybridize with a specific mRNA sequence to prevent the translation of the targeted mRNA into protein. It is thought that the mRNA in the AS-ODN complex is subjected to degradation by ribonuclease H, thus removing the mRNA from the pool of translatable RNAs (17). The AS-ODN enters the cell through endocytosis and, possibly, a receptor-mediated process. The AS-ODN approach has been validated with the approval of the antisense drug fomivirsen for the treatment of cytomegalovirus retinitis in patients with AIDS. A number of AS-ODNs are in clinical development and target proteins, such as H-Ras, c-Raf, and Bcl-2.1 The oncogene Bcl-2 is overexpressed in more than 50% of human cancers (18). It is known to code for an anti-apoptotic protein that can block cancer cells from undergoing programmed cell death (apoptosis) on exposure to anticancer therapies, such as traditional cytotoxic chemotherapy, radiotherapy, and monoclonal antibody therapy. Oblimersen sodium is an 18-mer oligodeoxynucleotide antisense sequence that hybridizes with a key region of Bcl-2 mRNA to block expression (18,19). Clinical trials of oblimersen in combination with cytotoxic therapies and biologic/immunotherapies are underway in metastatic breast cancer, SCLC, and hematologic malignancies (20). Initial reports from a phase I study in advanced SCLC showed that oblimersen use in combination with carboplatin and etoposide was well tolerated and produced encouraging results (21). However, follow-up studies have not been as encouraging (2). Whether this represents a problem with the approach, failure to deliver stable antisense oligodeoxynucleotides, or selection of a tumor type not dependent on Bcl-2 will require additional investigation.

Small interfering RNAs (siRNA) have demonstrated ability to downregulate gene expression by targeting RNAs to the RISC complex with subsequent degradation (22). Although in an earlier stage of evolution, siRNA appears to be a natural gene expression regulator and may have more generalizability than antisense. Small interfering RNAs has shown the ability to regulate gene expression and tumor growth in animal models.

MAMMALIAN TARGET OF RAPAMYCIN (mTOR)

mTOR as a central modulator of proliferative signal transduction is an ideal therapeutic target against cancer. Through extensive clarification of many signal transduction pathways, it has become clear that the mTOR kinase participates in critical events that integrate external signals with internal signals, coordinating cellular growth, and proliferation. mTOR receives signals that indicate whether transcription and translational machinery should be upregu-

lated, then efficiently transmits these signals to the appropriate pathways. Multiple components of pathways that signal through mTOR are deregulated in numerous cancer types. The development of inhibitors of mTOR is a rational therapeutic strategy for malignancies that are characterized by deregulated pathways that signal through mTOR. With the availability of a wide spectrum of mTOR inhibitors, clinical application in oncology of a select few of the rapamycin-related agents is under intense investigation.

Preclinical studies of rapamycin and the rapamycin analogs CCI-779, RAD001, and AP23573 have shown that mTOR may be an important target for the treatment of human cancers. Because inhibitors of mTOR are primarily cytostatic in cancer cells, combination therapy with cytotoxic chemotherapeutics may prove to be the most advantageous use of these drugs. Additionally, use of molecular profiling and surrogate biomarkers might eventually speed appropriate regimen selection and earlier assessments of treatment response.

The initial clinical data for mTOR inhibitors as potential treatment in a number of advanced human cancers are promising. Although SD was the predominant clinical benefit, noteworthy tumor regression has also been observed. Efforts are underway to validate biomarkers of drug activity in the ongoing clinical trials, and molecular profiling for appropriate patient selection may become a priority for investigation. The tolerability of the mTOR inhibitors and the lack of overt mechanistic overlap encourage the study of combination regimens of mTOR inhibitors with other modalities and agents.

CONCLUSION

The explosion of new therapeutic targets associated with signal transduction pathways has led to the development of rationally designed drugs and the emergence of biologic-based therapies that have provided both encouraging advances in outcomes and dramatic disappointments. However, interest has not waned, and this area of investigation remains in the early stages of discovery in terms of both the basic science of cellular control and appropriate regimen and patient selection for clinical study. Efforts continue to explore opportunities in this area for medical value. The optimal implementation of these drugs will require the concurrent development of targeted therapeutics with molecular markers able to identify tumors driven by particular targets and molecular imaging approaches able to identify patients who are responding early in therapy.

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