Why targeted therapy hasn’t worked in advanced cancer

Jack L. Arbiser

Department of Dermatology and Winship Cancer Institute, Emory University School of Medicine, and Atlanta Veterans Administration Medical Center, Atlanta, Georgia, USA.

In this issue of the JCI, Nissen et al. report that a reciprocal interaction exists between the growth factors FGF2 and PDGF-BB, causing tumors to exhibit increased angiogenesis and metastatic potential (see the related article beginning on page 2766). Both FGF2 and PDGF-BB signal through tyrosine kinase receptors, which have been the target of tyrosine kinase inhibitors for cancer therapies. These inhibitors are usually small molecules that inhibit the kinase activity of a receptor or nonreceptor tyrosine kinase, preventing downstream signaling. The results of this study shed light on why tyrosine kinase inhibitors have been useful for the treatment of only a small number of advanced cancers. Currently, a major focus of pharmaceutical companies is to develop ever more potent and specific tyrosine kinases. The results presented here suggest that this approach may not be successful.

Tyrosine kinases are a large family of enzymes that phosphorylate target proteins, resulting in either activation or inactivation of these proteins. This family includes many peptide receptors as well as nonreceptor proteins and is well represented in oncogenic fusion proteins, such as the BCR-ABL protein, which is generated from the Philadelphia chromosome. These proteins activate multiple signaling pathways, including those involving PI3K, phospholipase C{gamma}, MAPK, and STAT activation and the generation of reactive oxygen species. The study by Nissen et al. in this issue of the JCI demonstrates that activation of a combination of several tyrosine kinase receptors results in a high-
Demonstration of synergy between multiple receptor tyrosine kinases

Folkman was the first to hypothesize that tumors produce proangiogenic factors, which promote local tumor growth, invasion, and metastasis (6). This observation, in addition to the ability to culture microvascular endothelial cells, led to the purification of the first known angiogenic factors, FGF2 (also known as basic FGF) and VEGF, both of which signal through tyrosine kinase receptors (6). It has been assumed that tumors synthesize multiple angiogenic and growth factors, but this production has been assumed to be a redundancy that only becomes functional if a tumor is challenged with an inhibitor of a specific tyrosine kinase, in which case the tumor can display resistance by switching its dependence to an alternate growth/angiogenic factor. The findings reported by Nissen et al. (1) demonstrate that tyrosine kinases play a nonredundant role in the stimulation of angiogenesis and metastasis in normal tumor physiology, even when tumors are not challenged by tyrosine kinase inhibitors. Furthermore, the coexpression of two growth factors (FGF2 and PDGF-BB) in mice was shown to confer properties not seen with overexpression of either growth factor independently. While FGF2 and PDGF-BB signal through tyrosine kinases that show activation similar to PI3K and Ras, these growth factors may show differential activation of molecules in downstream signaling pathways, including reactive oxygen species, Akt, and phospholipase D (7, 8). In certain circumstances, FGF2 stimulates phospholipase D strongly and Akt poorly, while PDGF-BB is a potent activator of Akt (9–11).

In their study, Nissen et al. (1) have elegantly shown a synergy in vitro and in vivo between PDGF-BB and FGF-2 in the stimulation of angiogenesis, recruitment of an embryonic vascular phenotype, and an enhancement of metastasis. First, in murine corneal neangiogenesis studies, the coinplantation of FGF2 and PDGF-BB led to tumor-like neovascularization, as opposed to the effect of either FGF2 or PDGF-BB alone. Implantation of these growth factors individually resulted in attenuated vessels that rapidly regressed. Second, they demonstrated that FGF2 induces both the transcription of PDGF-BB in endothelial cells and the elevations of phosphorylated MAPK and phospholipase Cγ, both of which are required for optimal tumorigenesis. Third, murine fibrosarcoma cells coexpressing FGF2 and PDGF-BB exhibited rapid tumor growth in vivo and the development of primitive vascularplexes in the tumors, reminiscent of what is observed in human tumors. Surprisingly, the vessels in the tumors were not highly invested with pericytes (which normally envelop and stabilize the vessel exterior). Despite the known chemoattractant effects of PDGF on pericytes (12), pericyte recruitment was inhibited and tumor vessels appeared to be disorganized. Finally, the incidence of pulmonary metastases was increased in tumors coexpressing FGF2 and PDGF-BB in comparison with tumors expressing a single growth factor (1). While the minimum requirements for the transformation of human cells have been elucidated in several tissue types, the rationale for a solid tumor to express multiple growth factors in the absence of selective pressure remains poorly understood.

Advanced tumors activate signaling through several pathways

Many human tumors overexpress tyrosine kinase receptors, including common epithelial tumors such as those in lung and breast tissue (13, 14). While overexpression of growth factors that signal through
tyrosine kinase receptors is sufficient to transform even human cells (7), breast and lung cancers often demonstrate additional mutations, including mutations in K-ras in lung cancer and loss of PTEN in Her2-positive breast cancer, even in the absence of selective pressure (15, 16). Indeed, the presence of K-ras mutations in lung cancer predicts a poor response to EGFR antagonists despite the biochemical activity of the antagonist on the relevant receptor (17, 18). Several forms of signaling redundancy have been characterized at the biochemical level. First, we have previously observed the common activation of reactive oxygen species (e.g., superoxide, hydrogen peroxide) with Akt and MAPK activation in cell types that have lost the p16 tumor suppressor gene (19–21). These tumors arise in the presence of carcinogenic stimuli that induce reactive oxygen species such as chronic inflammation or viral infection. Reactive oxygen species drive NF-kB activation and trigger downstream effectors such as the angiogenic growth factor angiopoietin 2 (Ang-2) (Figure 1) (22). Ang-2 may play a critical role in the development of the primitive vascular plexuses observed by Nissen et al. (1) as well as in human tumors. Akt levels but activate another survival signal, the generation of phospholipase D (23, 24). While tumors with mutant p53 express tyrosine kinase receptors, they survive serum deprivation, a potential form of vascular plexuses observed by Nissen et al. (1) as well as in human tumors. Akt levels but activate another survival signal, the generation of phospholipase D (23, 24). While tumors with mutant p53 express tyrosine kinase receptors, they survive serum deprivation, a potential form of tyrosine kinase inhibition, through activation of phospholipase D and Ras (23).

The resistance of tumor cells to targeted therapy with tyrosine kinase inhibitors, due to the presence of multiple tyrosine kinase receptors or coexpression of tyrosine kinase receptors with oncogenic Ras or mutant PTEN, may appear to be a bleak outcome, but there is room for optimism because the response to targeted therapy is predictable and thus vulnerable. In a previous study of Epstein-Barr virus–induced Burkitt lymphoma, we demonstrated that treatment of these cells with N-acetyl cysteine resulted in an inhibition of NF-kB as expected, but also in a compensatory activation of p42/44 MAPK (20). While blockade of NF-kB may not have an immediate effect on tumor growth in vitro or in vivo, it may sensitize cells to MAPK inhibition. Similarly, sorafenib has been found to be ineffective against advanced melanoma (3), and we have recently demonstrated that aggressive melanoma involves both MAPK signaling as well as Akt/reactive oxygen species/NF-kB signaling (21). Blockade of reactive oxygen species may make cells more sensitive to MAPK blockade and sensitize cells to sorafenib. Finally, tyrosine kinase blockade in advanced solid tumors with mutant p53 may lead to activation of phospholipase D and sensitize tumor cells to phospholipase D blockade.

Screening by immunohistochemistry for a small panel of markers may allow the oncologist and pathologist to determine optimal therapy. The presence or absence of mutant p53 may help determine whether a tumor uses a reactive oxygen species–dependent or –independent signaling pathway. The presence of Ang-2 may also suggest that a tumor is using a reactive oxygen species–dependent pathway (22). Along with the use of stains already utilized for the detection of tyrosine kinase receptor signaling, both the presence of a mutation and the response to targeted therapy can be determined. Finally, the efficacy of targeted therapy can be improved against advanced malignancies. The current study by Nissen et al. (1) is the first to show a synergistic interaction of 2 growth factors that play a nonredundant role in tumor angiogenesis and metastasis. First, FGFR2 and PDGF-BB in combination play a role in organizing tumor neovascularization that is distinct from the effect of the presence of either growth factor alone. A possible mediator of this combined effect may be Ang-2, a growth factor known to antagonize pericyte investment of blood vessels (25, 26). Of interest, Ang-2 is known to be a poor stimulator of phospholipase A2, phosphoinositide 3-kinase and cytoskeletal reorganization in porcine aortic endothelial cells. (27, 28). Li, L., Sampat, K., Hu, N., Zakari, J., and Yuzhap, S.H. 2006. Protein kinase C negatively regulates Akt activity and modifies UVC-induced apoptosis in mouse keratinocytes. J. Biol. Chem. 281:3237–3243.


