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Targeting Angiogenesis from Premalignancy to Metastases

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Strategies to target angiogenesis have been extensively studied for decades, and a strong body of data supports the potential of angiogenesis targeting for cancer prevention. Using transgenic mouse models, Folkman, Hanahan, and colleagues (1, 2) showed that an "angiogenic switch" occurs early (before progression to invasive cancer), reflecting a change in the net balance between angiogenic stimulators (e.g., vascular endothelial growth factor [VEGF]) and inhibitors. The hypothesis that angiogenesis begins early in tumor progression, typically during premalignancy in the lung and other sites, is further supported by studies in human clinical specimens (3, 4). A seminal early study, which was conducted in a transgenic mouse model of pancreatic islet cell carcinogenesis, showed that antiangiogenic agents have a spectrum of effects from preventing tumor formation (chemoprevention) to slowing the growth of small tumors (early intervention) to suppressing established tumors (5).

Several antiangiogenic agents are now standard for the treatment of cancer. Bevacizumab, a monoclonal antibody against VEGF, is being used in combination with chemotherapy for the treatment of metastatic colorectal cancer, metastatic breast cancer, and non–small cell lung cancer (NSCLC), and small molecule tyrosine kinase inhibitors (TKI) such as sunitinib and sorafenib that can inhibit a variety of proangiogenic factors are part of the armamentarium for the treatment of renal cell carcinoma and have attracted the interest of investigators for the treatment of lung cancer (Table 1). Despite recent improvements in therapeutics, lung cancer and most other solid tumors remain incurable unless diagnosed at early stages. Distant metastasis is the hallmark of lung cancer spread and remains the most common cause of failure after potentially curative therapy. Animal models with or without metastatic potential can be helpful in the study of new therapeutics including antiangiogenic agents, as shown by Gandhi et al. (6) in this issue of the journal. The authors describe a novel study in two genetically engineered mouse models of NSCLC. One model expresses a conditional

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activating mutation in *Kras* , the other expresses the conditional mutant *Kras* with concurrent conditional deletion of *Lkb1*. Combining mutant *Kras* expression with concurrent *Lkb1* loss produces a novel model with metastatic potential and a mixture of histologic changes including adenocarcinoma, squamous cell carcinoma, and precursors of both. The *Kras* alone model does not have metastatic potential and generally produces a spectrum of changes from atypical adenomatous hyperplasia to adenomas to adenocarcinoma.

Daily administration of sunitinib, a multitargeted angiogenesis inhibitor, decreased tumor development and size, increased tumor necrosis, slowed tumor progression, and prolonged survival in both the metastatic (*Lkb1/Kras*) and nonmetastatic (*Kras*) mouse models. The drug was active across the entire spectrum of tumorigenesis in the *Kras* model, resulting in a striking improvement in survival far exceeding that of any other cytotoxic or molecular-targeted agent studied in the model thus far. An interesting finding is that the incidence of local or distant metastases was not altered by sunitinib treatment in the *Lkb1/Kras* model. These findings suggest that sunitinib may be active in patients with early-stage NSCLC with *Kras* mutations. The failure of sunitinib to reduce metastasis but still prolong survival suggests that efforts that focus on interventions targeting the primary tumor could be successful in improving clinical outcome. Alternatively, this finding may reflect the limitations in extrapolating therapeutic approaches in animal models with discrete genetic alterations to human cancers, which are characterized by a diverse and heterogeneous spectrum of mutations. Validation of these findings in other metastatic versus nonmetastatic mouse models of NSCLC would suggest that they have broad biological relevance.

Sunitinib is an oral, small molecule, multitargeted receptor TKI that was approved by the U.S. Food and Drug Administration for the treatment of renal cell carcinoma and imatinib-resistant gastrointestinal stromal tumor in 2006. Receptor TK targets of sunitinib include platelet-derived growth factor receptors (PDGFR), VEGF receptors (VEGFR)-1, VEGFR-2, and VEGFR-3, KIT (CD117), RET, CSF-1R, and flt3. The plethora of molecules inhibited by sunitinib likely contributes both to its therapeutic benefit and known side effects. Furthermore, the key, discrete target, which must be inhibited to achieve antitumor effects, is difficult to elucidate in many cancers. Both VEGFRs (including VEGFR-1, VEGFR-2, and VEGFR-3) and PDGFR contribute to angiogenesis. Sunitinib has shown antiangiogenic effects *in vitro* and in a murine model of established/advanced lung cancer (7). There is a curious absence of reports on the effect of sunitinib on NSCLC xenografts. There are, however, clinical data on sunitinib in NSCLC. A phase II trial in patients with previously treated, advanced NSCLC showed modest single-agent activity with an objective response rate of 11% (8). Phase III clinical trials of sunitinib in combination with erlotinib as second-line therapy (NCT00457392) and as single-agent maintenance therapy after combination chemotherapy (NCT00693992) are ongoing in patients with advanced NSCLC. New anticancer agents are typically tested in advanced, incurable disease. If they fail to show sufficiently compelling effects on patients with typically poor outcomes, they may never be evaluated in early-stage disease or in the adjuvant setting of primary or secondary prevention. Models of carcinogenesis may assist clinical investigators in the selection of certain promising agents to be tested in a chemoprevention setting.

Although one must be careful when extrapolating findings from therapeutic models to models of prevention, agents that are active in patients with cancer may also be effective in preventing the initial tumor or a second primary malignancy. Second primary tumors occur frequently in long-term survivors of lung cancer and other aerodigestive malignancies (9, 10). Successful molecular-targeted prevention of solid tumors would certainly obviate the later need for relatively toxic combinations of chemotherapy and radiation and/or major surgical resections, which are usually required for curative cancer therapy. For example, the epidermal growth factor receptor (EGFR) TKI erlotinib is Food and Drug Administration approved for the treatment of NSCLC and is now being tested in a large phase III study to assess the ability of EGFR inhibition to prevent recurrence or a second malignancy in patients with resected NSCLC in the Randomized Double-blind Trial in Adjuvant NSCLC with Tarceva trial (NCT00373425). To date, there are no reports of the ability of antiangiogenic agents to prevent cancer in individuals at high risk for a primary cancer or for the development of a second malignancy after curative treatment for their initial cancer. Although cancer chemoprevention remains an enticing concept, it has been challenging to perform clinical prevention studies, and most agents tested to date have not shown efficacy (11–13). Screening potentially active compounds with an acceptable toxicity profile in the clinic has been challenged primarily by the need for large sample sizes and long-term follow-ups and the lack of reliable intermediate surrogate markers for cancer.

Mouse models of cancer are frequently used to test the potential efficacy of anticancer agents and thus prioritize drugs for clinical development. Subcutaneous xenografts consist of human tumor cell lines (or a small tumor mass) in a setting where tumor volumes can be readily measured. However, inoculation of tumor cells below the skin cannot mimic the tumor microenvironment. In orthotopic xenograft models, human tumor cell lines are injected into the organ under investigation, although rapid tumor outgrowth (generally 1-2 weeks after inoculation) and the requirement for an immunocompromised host significantly limit these models (14). Indeed, data generated in tumor xenografts have correlated poorly with the activity of the compound under investigation in phase II clinical trials (15). The engineering of mice to produce discrete genetic alterations that mimic mutations found in human tumors can provide an opportunity to assess *in situ* tumor development in an immunocompetent animal. The Wong laboratory has developed a variety of *de novo* mouse models of lung cancer progression including targeted models that can be used to assess response to molecular targeting agents (14, 16–20). In the study described by this group in this issue of the journal (6), conditional activation of oncogenic *Kras* and concurrent loss of the tumor suppressor *Lkb1* generated mice with metastatic lung cancer, whereas mice with *Kras* mutations alone develop lung cancers that do not metastasize.

Inactivation of the LKB1/STK11 kinase often accompanies activation of KRAS in human lung cancers (21). LKB1 is a serine/threonine suppressor kinase that plays a role in diverse cellular pathways and seems to function as a tumor suppressor. Inactivation of this gene in the germline by mutations leads to the development of the Peutz-Jeghers hereditary cancer syndrome. This gene is also somatically inactivated in about one third of NSCLC. Although inactivation of LKB1 has been shown to modulate metastatic potential in mouse models of lung cancer (18), its significance in human NSCLC metastasis is not fully understood. *KRAS* mutations, which are associated with tobacco-related carcinogenesis, are seen in

~15% to 20% of all NSCLC and in ~25% of adenocarcinomas, which account for ~50% of NSCLC tumors (22, 23). The observation that LKB1/KRAS mutant human NSCLC cell lines are more sensitive to inhibition of mitogen-activated protein kinase and mammalian target of rapamycin pathways suggests that tumors that harbor these mutations represent a genetically and functionally distinct subset that may be uniquely responsive to selected molecular targeting agents (24). A recent study reported sequencing LKB1 and *Kras* in 310 NSCLC tumors excised from Caucasian and Asian patients (25). LKB1 was mutated in 11% of these tumors, which were more likely to be adenocarcinomas in the Caucasian patients. In addition, mutations in LKB1 were associated with *Kras* mutations but independent of EGFR mutations, suggesting a potential link between genetic alteration of LKB1 and *Kras* in NSCLC.

In addition to KRAS and LKB1 mutations, EGFR mutations have been extensively studied in NSCLC particularly in the context of clinical response to EGFR TKIs. In general, *Kras* mutations are largely mutually exclusive of activating EGFR mutations, and NSCLC tumors with *Kras* mutations are not responsive to EGFR TKIs (26). Therefore, novel targeted therapies for *Kras*-driven NSCLC are needed. The observations of Gandhi et al. (6) suggest that sunitinib may have a role in the prevention and treatment of *Kras*-driven NSCLC. This is a novel finding because *Kras* mutations have not been reported in renal cell carcinoma, and there is no evidence to date that sunitinib is active in mutant *Kras* tumors. Tumors without *Kras* mutations, but harboring mutations of other oncogenes including RET, KIT, and PDGFRA, have been reported to respond to multikinase inhibitors such as sunitinib (27, 28). VEGF-targeted therapies have also been associated with improved clinical responses in renal cell carcinoma patients who harbor loss-of-function mutations of Von Hippel-Lindau (29). Further investigation is required to determine if mutations of KRAS confer sensitivity to antiangiogenesis strategies including sunitinib. This sensitivity would represent a major advance because these tumors seem to be refractory to EGFR-targeting agents and are generally associated with a poor prognosis.

The use of the two genetically distinct mouse models in the study by Gandhi et al. (6) allowed the investigators to distinguish the effects of sunitinib on the formation of the primary lung tumor from the consequences in metastasis. Although angiogenesis may contribute to development of the initial tumor, it has been thought to be even more important in mediating the processes of growth, invasion, and metastasis. Therefore, the finding of an effect on primary tumor development and growth but not on metastasis is somewhat surprising. The lack of effect on metastatic disease may have been due to the activation of other escape mechanisms in metastasis involving, for example, Src and hypoxia-inducible factors 1 and 2. Given that sunitinib did not decrease *in vitro* growth of tumor-derived cell lines from either mouse model, it is likely that the decreased tumor volumes were mediated by the effects of sunitinib on other cells in the microenvironment at the primary tumor site. In the absence of a direct effect on the tumor cells, assessment of pharmacodynamic end points in the tumors may not show target inhibition. Perhaps even more surprising was the significant prolongation of survival conferred by sunitinib treatment in both mouse models, an effect that was independent of metastasis. Complicating the relevance of these findings is the observation that metastases in human lung cancer can occur early, even with very small primary tumors. Therefore, the relationship between primary tumor size,

metastases, and survival is nonlinear and biologically complex. Preclinical observations from mouse model studies are an important part of the multistep process of developing novel antiangiogenic and other anticancer agents, but their relevance to human complexity is subject to painstaking validation by clinical research. Also in this issue of the journal, Hasina et al. (30) report their study in a carcinogen-induced (4-nitroquinoline 1-oxide) model of oral carcinogenesis, finding that the angiogenesis inhibitor ABT-510 (a mimetic of Thrombospondin) significantly decreased the incidence of oral dysplasia and carcinoma. Sunitinib and other small-molecule TKIs of angiogenesis are currently candidate drugs with high priority for a place in the treatment and prevention of lung cancer and may have activity in other malignancies of the aerodigestive epithelium as well.

Disclosure of Potential Conflicts of Interest

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Table 1.

Multitargeted receptor TKIs that target angiogenesis

Agent	Molecular targets	Trial phase/setting	Manufacturer
ABT-869	VEGFR-1, VEGFR-2, VEGFR-3, PDGFR, KIT, Flt-3	Phase II/advanced NSCLC	Abbott/Genentech
Axitinib (AG-013736) BIBF 1120 (Vargatef)	VEGFR-1, -2, -3, PDGFR, KIT VEGFR, PDGFR, FGFR	Phase II/advanced NSCLC Phase III/advanced NSCLC	Pfizer Boehringer Ingelheim
Cediranib (AZD2171; Recentin)	VEGFR-1, VEGFR-2, VEGFR-3, PDGFR, KIT	Phase III/advanced NSCLC	AstraZeneca
Motesanib diphosphate (AMG 706)	VEGFR-1, VEGFR-2, VEGFR-3, PDGFR, KIT, RET	Phase III/advanced NSCLC	Amgen
Pazopanib (GW786034)	VEGFR-1, VEGFR-2, VEGFR-3, PDGFR, KIT	Phase III/stage I NSCLC; Phase II/advanced NSCLC	GlaxoSmithKline
PTK787/ZK 222584 (Vatalanib)	VEGFR-1, VEGFR-2, VEGFR-3, PDGFR, KIT	Phase I/II/advanced NSCLC	Novartis
Sorafenib * (BAY 43-9006; Nexavar)	VEGFR-1, VEGFR-2, VEGFR-3, Raf, PDGFR, Flt-3, KITt, RET	Phase III/advanced NSCLC	Bayer
Sunitinib † (SU011248; Sutent)	VEGFR-1, VEGFR-2, VEGFR-3, Flt-3, PDGFR, Flt-3, KIT, RET, CSF-1R	Phase III/advanced NSCLC	Pfizer
Vandetanib (ZD6474; Zactima)	VEGFR-2, EGFR, RET	Phase III/advanced NSCLC	AstraZeneca
XL647	VEGFR-2, EGFR, HER2, EphB4	Phase II/advanced NSCLC	Exelixis
XL999	VEGFR-2, FGFR, PDGFR, Flt-3, RET, KIT, SRC	Phase II/advanced NSCLC	Exelixis

Abbreviations: FGFR, fibroblast growth factor receptor; CSF-1R, colony stimulating factor 1 receptor.

*Food and Drug Administration approved for renal cell carcinoma and hepatocellular carcinoma.

†Food and Drug Administration approved for renal cell carcinoma and imatinib-resistant gastrointestinal stromal tumor.