Angiogenesis and Lung Cancer: Potential for Therapy

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The major cause of death from the most common type of lung cancer, NSCLC, is attributable to metastasis and is responsible for more than 155,000 deaths annually in the United States alone. The prognosis and choice of therapy for patients with lung cancer is currently based on clinical stage and tumor type (1). However, this staging system does not accurately determine an individual patient’s prognosis. For example, even with stage I resectable disease (the earliest possible stage), >40% of patients will not survive for five years (1). Because routine histopathological examination of primary lesions cannot always predict disease outcome, there is an urgent need to identify and validate new molecular markers to better identify patients at risk for recurrent and metastatic disease.

The publication in this issue by Marrogi et al. (2) tests the hypothesis that the level of NOS2, COX2, and VEGF proteins directly correlates with MVD as measured in surgical specimens of NSCLC and, subsequently, with the clinical outcome of the disease. The authors analyzed 106 cases of surgically resected NSCLC by immunochemistry for NOS2, COX2, and VEGF expression, and by CD31 staining for MVD. They report that the molecules are differentially expressed in approximately fifty percent of the cases and that the expression of NOS2 and COX2 correlates with the VEGF expression level as well as MVD in the tumors. Interestingly, overexpression of NOS2 was more common in surgical specimens of adenocarcinoma and large cell carcinoma as compared with squamous cell carcinoma, which suggests a heterogeneous expression for different cell types. Whereas the expression level of NOS2, COX2, and MVD correlated with disease stage, it did not predict nor correlate with survival. The authors conclude that angiogenesis is a complex process and that a better understanding of the biology of angiogenesis in lung cancer is critical for designing improved therapies. We agree!

The process of tumor metastasis is highly selective and has been shown to consist of a series of sequential, interrelated steps. To produce clinically relevant lesions, metastatic cells must complete all of the steps of this process that include angiogenesis (3). After the initial transformation and growth of these cells, vascularization must occur if a tumor mass is to exceed 1 mm³ in diameter. The synthesis and secretion of several angiogenic factors by tumor and host cells, therefore, play a key role in establishing a capillary network from the surrounding host tissues. Local invasion of the host stroma next occurs as a consequence of the enhanced expression of a series of enzymes (i.e., collagenases). Because thin-walled lymphatic channels and venules offer little resistance to penetration by tumor cells, they provide the tumor cell with easy entry into the circulation. Detachment and embolization of small tumor cell aggregates occur next, yet most of these circulating tumor emboli are rapidly destroyed. The tumor cells that survive in the circulation must then arrest in the capillary beds of organs and adhere to the vessel walls where extravasation into the organ parenchyma must occur, probably by the same mechanisms that influenced the initial extravasation process. Proliferation within the organ parenchyma completes the metastatic process, at which point the lesion must again develop a vascular network (i.e., angiogenesis) while evading the host immune system. It has been suggested that the metastatic potential of any individual tumor is determined by the balance between positive and negative regulatory factors, regulated by diverse genes. To understand this evolution for any given tumor would, hence, require a multivariate genetic analysis of multiple potential causative factors.

The growth and metastasis of a neoplasm is also dependent on the formation of adequate vascular support. Angiogenesis begins with an angiogenic stimulus followed by local degradation of the basement membrane surrounding the capillaries. Endothelial cell migration is then accompanied by the proliferation of cells at the leading edge of the migrating column. As they move, the endothelial cells begin to organize into three-dimensional structures to form new capillary tubes. All of these, then, require endothelial cell survival mandating the interplay of numerous factors, which can act in a positive or negative fashion (3). The five-step process resulting in neo-angiogenesis is shown in Fig. 1. For angiogenesis to occur, every one of these different steps must take place, hence, the multiple levels of regulation of this process. Furthermore, negative regulatory factors exist along the way, and their interaction must also be considered.

The extent of angiogenesis also appears to be an important prognostic factor for many solid tumors, and MVD has been shown to correlate with stage and survival in lung cancer in both prospective and retrospective case series (4–8). The lack of correlation of MVD with survival in the current study is somewhat puzzling but attests to the variable nature of this test. In fact, at least one study that evaluated 500 stage I NSCLC patients showed no significant differences in disease-free interval or survival between patients with high and low MVD (9). This technique is certainly prone to variability, and several studies now show considerable heterogeneity of the vasculature in NSCLC specimens even among different zones (blocks) of the same recurring tumor. Differences between the periphery versus the center (10), in terms of both vessels and growth...
factors/peptides, are common. For example, EGF, MMP, and bFGF are all overexpressed at the periphery of a prostate tumor, whereas VEGF is overexpressed in the center of the lesion (11). This regional heterogeneity within neoplasms is a concern as newer techniques (e.g., reverse transcription-PCR, microarrays) for analyzing tumors are explored which evaluate only a small fraction of the specimen. Furthermore, the staining technique chosen can have a major effect on the result (antibody chosen, conditions, etc.).

To understand angiogenesis and the relevance of MVD within a tissue specimen requires understanding the multiple factors that drive the process. The metastatic potential of human neoplasms has been shown to correlate directly with the expression level of several independent genes that promote angiogenesis: VEGF, bFGF, and IL-8 and invasion: type IV collagenase (MMP-2, MMP-9 genes; Ref. 3). There have also been several reports that the expression of E-cadherin, which is involved in cell-to-cell cohesion, is inversely correlated with tumor progression and metastasis. Most of these correlative studies reached the inevitable conclusion that the expression of a given gene is necessary but insufficient to complete the multistep process of metastasis. We have demonstrated this in our own work, studying the molecular mechanism of experimental brain metastasis using a panel of human cell lines (12). The expression of VEGF mRNA and protein by the tumor cells was in direct correlation with angiogenesis and growth of brain metastasis in this study. Blocking VEGF expression using antisense transfection, furthermore, inhibited the metastatic potential of these different cell types. However, the presence of VEGF alone did not by itself induce the metastasis of these cell lines. Because each of the discrete links in the pathogenesis of a metastasis is regulated by one, or several, independent gene(s), the identification of cells with metastatic potential in heterogeneous neoplasms requires a multiparametric, multivariate analysis of gene expression.

Several studies have demonstrated that VEGF expression is strongly associated with angiogenesis and has the most consistent prognostic significance in solid tumors, including breast, gastric, and pancreatic cancer (3). Several case series (13, 14) have shown the importance of VEGF expression in the angiogenic process of, and as a prognostic factor for, NSCLC also, and the report by Marrogi et al. (2) indicates that VEGF may be a suitable target for novel therapies in the management of NSCLC. The inhibition of VEGF activity with monoclonal antibodies against soluble VEGF ligand, and VEGF receptor monoclonal antibodies have shown initial promise in clinical studies (15, 16). VEGF is an important angiogenic growth factor in NSCLC to the point that detection of highly vascular tumors not dependent on VEGF often indicates that there may be other factors that have a role in lung cancer tumorigenesis. For example, in one study, the levels of platelet-derived endothelial cell growth factor, bFGF, and VEGF as single factors are not prognostic in NSCLC (17). However, when the factors were combined in a multivariate analysis, only 43% of patients had metastatic involvement when the three factors were negative, whereas 77% showed metastasis when all of the factors were positive (17). Similarly, we have recently used an in situ hy-

Fig. 1 Angiogenesis is a complex process requiring multiple independent steps as shown. To complete the process, all of the steps must occur.
bridization technique to assess levels of bFGF, VEGF, interleukin 8, MMP-2, MMP-9, and E-cadherin in 60 paraffin-fixed samples of stage I NSCLC. Using a Cox univariate analysis, no one factor was significantly prognostic of recurrence. However, the ratio between type IV collagenase expression (mean of the expression of MMP-2 and MMP-9) and E-cadherin expression termed the MMP:E-cadherin ratio (measured at the periphery of each tumor), was significantly higher in patients with recurrent disease than in patients who remained disease-free ($P = 0.00003$; Ref. 18). Overall survival and disease recurrence from lung cancer was significantly associated with a higher MMP:E-cadherin ratio ($\geq 2$) by Kaplan-Meier survival analysis ($P = 0.0002$ and $P = 0.0001$, respectively). Multiple covariate analysis of overall and disease-free survival demonstrated that the MMP:E-cadherin ratio was a significant prognostic factor when corrected for age ($P = 0.0001$) (18).

In summary, by the time of diagnosis, most tumors are biologically heterogeneous and contain multiple subpopulations of cells with different properties. Because not all cells in a neoplasm are likely to give rise to metastasis, it is important to identify the more malignant populations of cells that portend an adverse prognosis. Therefore, to improve the survival rate of patients with early stage NSCLC and to better stratify patients with advanced disease, novel methods must be developed that identify individuals at increased risk who might benefit from more aggressive treatment. Along these lines, the recent study demonstrating significant angiogenic squamous dysplasia exclusively in bronchial biopsies from former smokers at high risk for lung cancer suggests that the switch to the angiogenic phenotype might occur at a preinvasive stage, and potential for early intervention in high-risk patients through this mechanism now exists (19). Because chemotherapy of lung cancer (although improved in the last decade) is still only marginally effective (and often toxic), routine adjuvant or neoadjuvant therapy for patients is not performed. The development of new therapeutic agents with novel targets of action (i.e., antiangiogenic agents and MMP inhibitors) must be based on a better understanding of the processes of metastasis and angiogenesis in human lung tumors. The report by Marrogi et al. (2) is a step in the right direction.

REFERENCES

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