Comment

Hold that Line: Angiomotin Regulates Endothelial Cell Motility

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The modern era of angiogenesis research is generally considered to have begun with the proposal by Folkman (1971) that tumors release diffusible factors that promote the formation of new blood vessels. Although there has been interest in this topic for 30 yr, the last 6–7 yr have seen an explosion of interest in this field. This explosion is evidenced in the number of meetings on the topic, the number of press reports, and especially the number of publications. A search of the Medline database reveals that in 1990 approximately 200 papers were published on the topic of angiogenesis, whereas in the year 2000 more than 1,700 papers appeared on the same subject. To what can we attribute this rise? My own feeling is that while the previous two decades were devoted to proving that angiogenesis was a real biological phenomenon and to the isolation of angiogenesis-stimulating factors, the 1990s were devoted to the identification, purification, and cloning of potentially effective angiogenesis inhibitors. This allowed the establishment of clinical trials to test the therapeutic efficacy of these agents and has stimulated a torrent of activity among biologists in both academic and industrial settings to find out more about the mechanisms by which these inhibitors work.

A large number of angiogenesis inhibitors have entered or will soon enter clinical trials. These have been identified and isolated using a variety of strategies. Some were chosen on the basis of their ability to interfere with key angiogenesis stimulators such as the vascular endothelial growth factor and its receptors (Presta et al., 1997; Fong et al., 1999) or key endothelial receptors for extracellular matrix molecules such as the αvβ3 integrin (Brooks et al., 1994). Others were isolated from tissues such as cartilage that are naturally devoid of blood vessels (Moses et al., 1999). Some were known cytokines such as interferon alpha (White et al., 1989) or interleukin 12 (Voest et al., 1995) that were subsequently found to have antiangiogenic properties. Still others were identified on the basis of their structural characteristics (D'Amato et al., 1994; Klauber et al., 1997) or on the basis of their ability to inhibit endothelial cell migration or proliferation (Ingber et al., 1990) or to function as a tumor suppressor (Good et al., 1990). Information regarding angiogenesis inhibitors in clinical trials can be found on the Cancer Trials web site of the National Cancer Institute (http://cancertrials.nci.nih.gov/news/angio/table.html).

One of the first angiogenesis inhibitors described in the 1990s was angiostatin, a molecule originally collected from the urine of tumor-bearing mice (O'Reilly et al., 1994). Angiostatin is a proteolytically generated fragment of plasminogen consisting of the first four kringle domains of the parent molecule. Angiostatin is thought not to be produced inside the tumor cells, but rather to be generated in the circulation by tumor-derived proteolytic enzymes (Gately et al., 1996). The observation that angiostatin as well as other angiogenic inhibitors are derived from molecules involved in hemostasis has encouraged speculation that these molecules may have a role in normal wound healing, as well as in tumors and other pathological states (Browder et al., 2000). Angiostatin is currently in clinical trials to determine its utility as an antitumor drug.

Despite the intense effort and research output on the topic of angiogenesis inhibitors, much remains to be learned. For many of these newly described inhibitors, we know little of their mechanism of action and less about the nature of the receptors that mediate their effects on endothelial cells. For this reason, the paper by Troyanovsky et al. (2001) is a welcome addition to our understanding of angiogenesis. This team has described angiomotin, a new, functional angiostatin-binding protein that mediates the inhibition of endothelial cell motility, a critical component of the angiogenic process (Zetter, 1980). Because of the importance of cell migration in the angiogenesis process, the discovery of a cell-surface protein that mediates the effects of angiostatin on endothelial cell motility represents a significant step forward for this field.

The data are convincing. Angiomotin was discovered using a yeast two-hybrid screen for proteins that interact with the first four kringle domains of plasminogen. It is described as a 72-kD cell surface–associated protein that is expressed in capillary endothelial cells as well as in actively angiogenic tissue such as placenta and solid tumors. Like other surface-associated proteins that can bind plasminogen and its derivatives, angiomotin does not appear to have a signal sequence, thus its association with the cell surface may be via protein–protein interaction. It is localized to the lamellipodia at the leading edge of migrating endothelial cells. Most importantly, transfection of control HeLa cells with angiomotin results in an increased re-

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sponse to migration-stimulating proteins. Furthermore, angiostatin treatment blocked cell migration and three-dimensional tube morphogenesis in cells transfected with angiomotin but not in vector-control cells. These results solidly implicate angiomotin in the angiostatin-mediated regulation of cell motility and capillary differentiation.

Angiomotin is not the first angiostatin-binding protein discovered. ATP synthase was previously described as a high affinity angiostatin-binding protein (Moser et al., 1999) that bound preferentially to angiostatin relative to the parent molecule plasminogen. Antibodies to ATP synthase were reported to inhibit the antiproliferative activity of angiostatin on endothelial cells. Plasminogen itself binds to other cell-surface proteins such as annexin II and alpha enolase (Miles et al., 1991; Hajjar et al., 1994). Should we be concerned, then, that a potent antiangiogenic molecule should have more than one potentially important cell surface binding molecule? Probably not. The positive and negative regulation of blood vessel growth is a critical determinant of tissue repair and of normal tissue mass. Angiogenesis has essential roles in embryonic development, tissue hyperplasia, and a variety of diseases. Vascular endothelial growth factor (VEGF), a positive regulator of angiogenesis, has at least three receptors, and fibroblast growth factor, another positive regulator, has more receptors than VEGF. It would be no surprise if a potent angiogenesis inhibitor such as angiostatin were to have multiple receptors. These multiple receptors could act independently or in concert, as is the case for the high- and low-affinity receptors for fibroblast growth factor (Yayon et al., 1991).

As always, a discovery of this type generates many new questions. Among those undoubtedly being asked in the Holmgren laboratory today are: (a) what is the binding affinity of angiostatin's interaction with angiomotin? (b) what is the nature of the binding interactions between angiomotin and plasminogen? (c) what signaling molecules are downstream of angiomotin? (d) does binding of angiostatin to angiomotin cause activation of the focal adhesion kinase previously shown to be modulated by angiostatin (Claesson-Welsh et al., 1998)? and (e) does angiomotin mediate other functions of angiostatin action such as endothelial proliferation in vitro or the inhibition of angiogenesis and tumor growth in vivo? There is considerable work left to be done.

Who are the beneficiaries of this new discovery? Those who will try to add more flesh to the bones of this new finding, those who will attempt to identify or construct novel small molecules that regulate angiogenesis via the angiomotin pathway, those curious about angiogenesis, and, with any luck, those who may have or will later acquire a disease characterized by untoward angiogenesis.

The author thanks Drs. S. Pirie-Shephard and K. Javaherian for their helpful comments and discussions.

Submitted: 22 February 2001
Accepted: 22 February 2001

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