Published in final edited form as:

Med Hypotheses. 2007 ; 69(2): 414–421. doi:10.1016/j.mehy.2006.10.065.

POTENTIAL THERAPEUTIC IMPLICATIONS OF INTRACRINE ANGIOGENESIS

Richard N. Re, M.D. and Julia L. Cook, Ph.D.

Research Division, Ochsner Clinic Foundation, 1514 Jefferson Highway, New Orleans, Louisiana 70121, Tel: 504-842-3139, Fax: 504-842-3899 rre@ochsner.org

Abstract

Angiogenesis is a requirement for tumor growth beyond a diameter of a few millimeters and is, therefore, a major target for cancer therapy. The intracellular actions of certain extracellular signaling proteins (*intracrine*s) have been reported, and it is clear that intracrine such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), angiogenin, angiotensin, and endothelin, among others, are involved in angiogenesis. We have proposed that intracrine networks play an important role in angiogenesis, and have suggested that very similar intracrine networks exist in some tumor cells. These notions have implications for the development of anti-angiogenesis therapies because they suggest that the inhibition of intracellular intracrine trafficking pathways may be an effective therapeutic target. Here the participation and regulation of intracrine in angiogenesis is explored, as are the actions of various anti-angiogenic factors.

Keywords

Intracrine; Angiogenesis; Microtubules; Cytoskeleton

INTRODUCTION

As demonstrated by Folkman and colleagues, angiogenesis--the development of new vessels from pre-existing vessels--is a requirement for tumor growth beyond a diameter of a few millimeters.¹ Moreover, these investigators have shown that angiogenesis is regulated by the interplay of pro-angiogenic and anti-angiogenic factors, suggesting that understanding the signaling modalities used by these factors could prove beneficial in the search for effective therapies.

Many extracellular signaling proteins are known to act within the cellular interior, and we some time ago, designated these factors *intracrine*s. We have advanced an hypothesis regarding the actions of intracrine signaling molecules, be they hormones, growth factors, enzymes, or DNA binding proteins and have argued that these intracrine participate in cellular differentiation, hormonal responsiveness, and memory.²⁻⁹ We have argued that this occurs through the production of intracellular positive, yet finite gain, intracrine feedback loops which are long-lived. Intercellular trafficking of intracrine then contributes to tissue-wide differentiation. Intracrine frequently traffick to nucleus and nucleolus, and often appear to be involved in

Correspondence to: Richard N. Re.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ribosome regulation.²⁻⁹ A list of intracrines is found in Table I. Recently, we applied these principles to angiogenesis.⁹ Important for this discussion is the observation that intracrines are manifestly involved in angiogenesis; for example, vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), angiogenin, angiotensin, platelet derived growth factor (PDGF), and endothelin are intracrines. It is interesting in the context of the present discussion that virtually every intracrine that trafficks to nucleolus appears to regulate angiogenesis; moreover, the pleiotropic nucleolar protein, nucleolin, translocates to the surface of endothelial cells that are actively involved in angiogenesis and shuttles growth factors from the cell surface to nucleus/nucleolus. We have suggested that in the case of tumor angiogenesis, intracrine loops involved in vessel formation find parallels in intracrine loops involved in tumor proliferation (*intracrine reciprocity*) and in this respect tumor angiogenesis parallels intracrine-driven tissue differentiation. This view then suggests that strategies directed at inhibiting intracrine driven angiogenesis could find more global application in the therapy of human neoplasms.⁹ All these ideas are discussed elsewhere in detail.²⁻⁹ Against this background, recent observations have both supported and extended these suggestions regarding the role of intracrines in angiogenesis and the implications of that action for cancer therapy.

First, new information has been developed which bears on the notion of intracrine reciprocity. Angiogenin is a well-established angiogenic factor whose angiogenic activity, like that of bFGF, depends on its trafficking to nucleus.¹⁰ It has recently been shown that endogenous angiogenin also drives the proliferation of melanoma cells.¹¹ Although up-regulation of angiogenin in these cells down-regulates bFGF expression, it enhances bFGF growth effects. Conversely, down-regulation of angiogenin, up-regulates bFGF expression but blocks bFGF growth stimulation in these cells. Thus, angiogenin appears to be required for the proliferative activity of bFGF in this model. This circumstance is parallel to the finding that angiogenin appears to be essential for the angiogenic activity of both bFGF and VEGF; neamine, a small molecule inhibitor of angiogenin nuclear trafficking, inhibits endothelial cell proliferation to bFGF and VEGF.¹⁰ Whether angiogenin is strictly required or simply facilitative for bFGF-induced proliferation, these recent findings in melanoma cell serve as yet another example of intracrine reciprocity and underscores the possibility that small molecule inhibitors of angiogenin action (for example, neamine) could serve an important role in tumor therapy.⁹⁻¹¹

Second, recent findings have shed light on the mechanism of action of anti-angiogenic factors and these findings both appear to be consistent with a role for intracrine action in angiogenesis and also suggest additional therapeutic approaches.

CYTOSKELETON TRAFFICKING

Because of our interest in intracrine action, we were intrigued by the evidence suggesting that the trafficking of some hormone and neurotransmitter receptors occurs along the cytoskeleton through the mediation of chaperone proteins.¹²⁻¹⁴ This led us to consider the implications of such cytoskeleton-related trafficking of receptors, and likely of intracrines as well. In particular, it led us to consider the relationship of angiogenesis, intracrine action, and the cytoskeleton.

The anti-angiogenic factor endostatin, the globular domain found at the C-terminus of Type XVIII collagen, has the capacity to block the growth of certain tumor cells but only after internalization and trafficking to tropomyosin microfilaments which stabilize the actin cytoskeleton. Similarly, the anti-angiogenic activity of the kringle domain of urokinase is associated with its internalization and trafficking to nucleus (nucleolus?).^{15, 16} These findings are consistent with the notion of intracrine reciprocity on the one hand and with intracrine participation in angiogenesis on the other. Moreover, it has been shown that bFGF, like

angiogenin, stimulates angiogenesis only after internalization and trafficking to cytoskeleton and nucleolus.^{2-9, 17} These observations immediately suggest that endostatin could bind to, or otherwise interact with, bFGF after internalization thereby inhibiting its translocation to nucleus and therefore inhibiting its angiogenic potential, just as it suppresses tumor proliferation by acting at the cytoskeleton. The urokinase kringle domain could similarly interfere with intracrine nuclear trafficking. Also, angiostatin, one of the the most potent anti-angiogenic factor yet identified, consists of the first four kringle domains of plasminogen. Recent evidence indicates that an angiostatin fragment consisting of the first three kringle domains can directly bind the intracrine angiogenic peptide angiogenin and this binding appears to be causative in the ability of the kringle peptide—and by extension of angiostatin—to block angiogenesis.¹⁸ It is entirely possible that, as suggested by the case of endostatin suppression of tumor growth, this binding occurs in the intracellular space as angiogenin trafficks to nucleus. Collectively, these findings support our earlier suggestion that the interruption of intracellular intracrine trafficking to nucleus/nucleolus is a potentially fruitful therapeutic target in both angiogenesis and tumor proliferation.⁹ These results, taken in the context of intracrine action in angiogenesis and tumor growth, also suggest that the cytoskeleton may be an important target for inhibiting both angiogenesis and tumor cell proliferation. This view suggests that cytoskeleton disruption, for example, could prove a beneficial strategy based, in large part, on the interruption of intracrine trafficking. Presumably, anti-angiogenic factors could either enter cells and bind angiogenic factors trafficking the cytoskeleton or could disrupt cytoskeleton structure or function to prevent the trafficking of angiogenic intracrine.

Against this background one can note that considerable interest, independent of any considerations of intracrine action, has developed in the application of tubulin binding agents in the suppression of both angiogenesis and cancer cell proliferation. Tubulin is a major component of the microtubule component of the cytoskeleton and vinca alkaloids such as vincristine are well-known cytotoxic therapeutic agents that bind to tubulin, disrupt the microtubular component of the cytoskeleton, and lead to cellular arrest at G(2)/M. Newer agents, such as taxol, bind to, and stabilize, microtubules and, again, cellular arrest at mitosis occurs and appears to be the proximate cause of cell death.¹⁹ However, the arguments discussed above suggest that, particularly at lower doses, these tubulin and microtubule binding agents may be effective in the inhibition of angiogenesis and tumor proliferation by virtue of their ability to disrupt cytoskeleton-dependent intracrine trafficking to nucleus.

The number of tubulin/microtubule binding agents which recently have been shown to be anti-angiogenic (by inhibiting endothelial cell proliferation, migration, or both) is relatively large. For example, colchicine, a classical tubulin binding agent, blocks angiogenesis but at concentrations not achievable clinically; 2-methoxyestradiol, on the other has recently been found to be a tubulin binding agent that inhibits angiogenesis at clinically achievable concentrations.²⁰ Also, a variety of anti-angiogenic tubulin/microtubule binding agents (taxotere, epothilone B, discodermolide, vincristine, 2-methoxyestradiol, and colchicines) have recently been shown to down-regulate hypoxia-inducible factor-1 alpha (HIF-1alpha) protein levels as well as HIF-1alpha transcriptional activity in human ovarian cancer 1A9 cells. Epothilone B treatment down-regulated HIF-1alpha protein in the parental 1A9 cells but had no effect in 1A9/A8 cells which are resistant to the microtubule effects of epothilone B. Confocal microscopy showed impaired nuclear accumulation of HIF-1alpha in parental 1A9 cells at epothilone B concentrations that induced extensive microtubule stabilization. HIF-1alpha in 1A9/A8 cells resistant to the microtubule action of epothilone, was unaffected.¹⁹ Thus a disordered tubulin network results in aberrant HIF-1alpha trafficking and function. Moreover, pseudolarix acid B (PAB), a product isolated from a traditional Chinese medicinal plant, has been shown to inhibit endothelial cell proliferation, migration, and tube formation.²¹ This compound arrest cells at the G(2)/M phase and disrupts the cytoskeleton. The biological

effects of PAB occur at non-toxic concentrations of the drug and can clearly be ascribed to tubulin depolymerization secondary to drug binding to a tubulin site distinct from the binding sites of colchicines and vinblastine.

Integrin binding also appears to play an important role in the function of some anti-angiogenic agents. Canstatin is the non-collagenous domain of collagen IV alpha-chains and is anti-angiogenic.²² In both endothelial cells and tumor cells it stimulates a mitochondrial apoptotic mechanism through procaspase-9 cleavage and this, in turn, is mediated by cross-talk between α 5 β 3- and α 5 β 1-integrin receptors. Several other domains of collagen type IV (arrestin, tumstatin) are anti-angiogenic and bind to integrins including α 5 β 3.^{23, 24} Also, angiostatin is known to bind to F1Fo ATP synthetase on the surface of endothelial cells, but also to α 5 β 3 integrins.^{25, 26} Similarly, endostatin appears to interact with integrins and actin filaments.²⁷ Collectively, these latter findings suggest that integrin binding is an important factor in the anti-angiogenic activity of many compounds.

One can also note that hypoxia-induced tumor invasiveness can depend on recycling of plasma membrane proteins that are involved in metastatic invasion. Rab 11 is a small GTPase protein involved in vesicular trafficking and recycling of, among other proteins, α 6 β 4 integrin, a protein involved in invasion.²⁸ Cytoskeletal disruption inhibits Rab 11 trafficking and therefore alters cell surface integrin expression and limits tumor invasiveness. Similarly, the fungal T(2)-RNase, Actibind, binds actin and, by interfering with the actin network, inhibits the elongation of pollen tubes in plants.²⁹ It inhibits tube formation in human umbilical vein endothelial cells and also inhibits colony formation by human colon and cervical cancer cells. In an *in vivo* rat model it was shown to inhibit tumor growth. It also inhibits cancer cell motility. All these effects are apparently independent of its RNase activity and rather dependent upon disrupting the cytoskeletal actin network.²⁹ Recent work clearly shows that Actibind binds to cell surface actin and disrupts the intracellular actin network. Of note in this regard is the fact that angiogenin also binds to cell surface actin.³⁰

Although many more examples could be provided, the recent evidence noted above indicates that agents which interact with various components of the cytoskeleton can reduce the proliferation and migration of endothelial cells and tumor cells alike, resulting in suppression of angiogenesis and tumor growth. Many anti-angiogenic factors bind to one or another cytoskeleton component, and in cases where microtubule binding occurs, check-point arrest often occurs at G(2)/M, likely because of aberrant microtubulin function.^{19-21, 31} However, it seems clear that other mechanisms must also be operative in anti-angiogenesis because endostatin, for example, affects the cytoskeleton via interactions with actin and integrins (as well as with other structures) but does not produce G(2)/M mitotic arrest or obvious microtubule disruption.³² Moreover, G(2)/M arrest resulting from microtubulin disruption often is associated with the induction of apoptosis through a poorly understood process that likely involves MAD2 ("mitotic arrest deficient 2") and phosphorylation of Raf, MEK1/2, and Bcl-2.^{26, 33} In order to explain the actions of factors that interact with the cytoskeleton, several mechanisms have been proposed. Among these are: (i) G(2)/M mitotic arrest associated with cell death secondary to apoptosis or polyploidy resulting from mitotic spindle dysfunction (ii) interruption of second messenger intracellular signaling because of cytoskeleton disarray (iii) inhibition of cellular migration/invasion because of cytoskeletal disruption. While both angiostatin and endostatin interact with components of the cytoskeleton, additional modes of action have been proposed for them. For example, in the case of angiostatin induction of p53-, Bax-, and tBid-mediated release of cytochrome c into the cytosol and activation of the Fas-mediated apoptotic pathway in part via up-regulation of FasL mRNA, down-regulation of c-Flip, and activation of caspase 3 has been described.³⁴ Moreover, it is direct binding of angiostatin, to F1Fo ATP synthase located on the endothelial cell surface that appears to trigger cellular apoptosis.^{26, 35} F1Fo ATP synthase is a mitochondrial enzyme responsible for ATP

generation. Similar in its action to nucleolin, it is also found on cell surface where it plays a role in the internalization of such factors as lipoprotein A1 and appears to be one of several mitochondrial proteins which traffick between mitochondria and cell surface. Parenthetically, it is unknown if angiostatin is internalized. Angiostatin also inhibits endothelial cell migration, and inhibits plasminogen interaction with its receptor.^{35, 36} In the case of endostatin, down-regulation of a variety of genes including immediate-early response genes is also seen.³⁷ Thus, the mechanisms by which angiostatin and endostatin actually inhibit angiogenesis remain unclear.

While accepting the mechanisms which have been proposed to explain the actions of agents acting at the cytoskeleton, the intracrine view suggests that cytoskeleton disruption plays an additional role in the suppression of angiogenesis and of tumor proliferation: the inhibition of intracrine trafficking from the extracellular space to nucleus and other intracellular sites, as well as the inhibition of endogenous intracrine trafficking to intracellular targets. This, in turn, prevents the establishment of new self-sustaining intracrine loops, and interrupts established intracrine loops. Although microtubules are most often thought of in the context of vesicular trafficking to the cell membranes, there is evidence for microtubule involvement in the nuclear trafficking of the intracrine PTHrP.³⁸ Also, the tumor-suppressor protein p53 has been shown to utilize the cytoskeleton in its nuclear-cytoplasm trafficking, and an intact cytoskeleton is required for nuclear shuttling of the glucocorticoid receptor in NIH 3T3 cells.^{39, 40}

Collectively, this data and the studies related to the cytoskeleton interactions of the angiogenic and anti-angiogenic factors discussed above raise the possibility that key intracrines involved in angiogenesis utilize the microtubulin network in their intracellular trafficking to nucleus and other locations. Indeed, there is evidence that nuclear localization signal sequences themselves can direct trafficking of proteins along microtubules, but it is not yet clear how often this occurs.⁴¹ In addition, it has been suggested that it is the association of PTHrP with RNA that allows it to travel along the microtubule network to nucleus.³⁸ This is of interest because several angiogenic intracrines are known to bind RNA (e.g. bFGF, angiogenin, PTHrP, tryptophanyl-tRNA synthetases), consistent with the previously stated view that intracrines, in general, and angiogenic intracrines in particular, are closely associated with ribosomal biology and nucleolus.^{2-9, 42} In any case, this view of the role of the microtubulin network and, more generally, of the cytoskeleton in the action of angiogenic and anti-angiogenic intracrines has definite implications.

First, it suggests that the co-existence of inhibitory effects of cytoskeleton-disrupting drugs on endothelial cells and cancer cells is based on the notion of intracrine reciprocity—that is, on the use of the same nuclear trafficking intracrines by both tumor cells and endothelial cells—rather than on the fact that all growth requires a normal cytoskeleton. Major interruption of microtubule architecture may be incompatible with cellular survival once mitosis is initiated, but the utility of some of the microtubule-binding anti-angiogenic agents discussed above seems, at least in part, to be the result enhanced susceptibility of tumor cells and endothelial cells to these agents. The ability of PAB, discussed above, to inhibit angiogenesis while remaining non-toxic to somatic cells exemplifies this point, as does the observation that vinblastine is almost one hundred times more inhibitory against lymphoma cells than against proliferating normal granulocytic cells.^{21, 43} This kind of differential sensitivity arguably could be the result of differences in cellular mitotic rates or of the pharmacologic characteristics of the drugs used such as differential binding to endothelial cells (and to some tumor cells) as appears to be the case for angiostatin and urokinase.^{16, 26, 35, 36} However, it is not clear that these effects alone completely explain the differential sensitivity of tumor cells and activated endothelial cells to microtubule-binding agents.⁴⁴ The intracrine view suggests that the enhanced use of similar, cytoskeleton-facilitated, intracrine positive feedback loops by both tumor and endothelial cells at least partly explains their common sensitivity to these agents. Thus, endostatin could interrupt angiogenesis without producing G(2)/M arrest or major

cytoskeletal disruption by indirectly interrupting intracrine trafficking through its effects on integrins and the actin cytoskeleton. This possibility is supported by the observation that integrin $\alpha(v)\beta3$ is involved in the internalization of FGF receptor by endothelial cells and that interference with integrin function can block bFGF-induced angiogenesis.⁴⁵ Moreover, the intracrine perspective raises the possibility that even the apoptosis seen following mitotic arrest induced by vinca alkaloids could result from disrupted intracrine trafficking and subsequent phosphorylation events leading to programmed cell death.

Second, the idea that some of the efficacy of these agents results from inhibition of intracrine trafficking implies that additive or synergistic effects can be achieved if diverse intracrine trafficking pathways could be attacked at multiple points, either in series or parallel. For example, combining agents that bind to microtubules with nucleolin-binding agents could in principle further reduce the nuclear trafficking of key angiogenic intracrine proteins such as midkine and pleiotrophin which are shuttled by nucleolin from cell surface to nucleus. Recall that nucleolin is found on the surface of activated (that is, angiogenically active) endothelial cells and also on some cancer cells. Inhibition of nucleolin trafficking could well contribute to suppression of both angiogenesis and tumor cell proliferation. Similarly, combining small molecular blockers of angiogenin nuclear trafficking with tubulin-disrupting agents, and possibly with anti-integrin antibodies, would also be expected to be beneficial both in inhibiting angiogenesis and the proliferation of certain tumors such as melanoma (see above). Combining endostatin and angiostatin, which interact with the cytoskeleton in different ways, already has been shown to be effective.⁴⁶

Third, if the suggestion that RNA binding facilitates trafficking along the cytoskeleton is correct, intracrine angiogenic agents such as VEGF would be expected to bind RNA and to thereby utilize the microtubulin network in their nuclear trafficking.²⁻⁹ While no such VEGF/RNA interaction has thus far been reported, its existence would support the view of angiogenesis proposed here.

Some indirect support for the notion that interruption of intracrine trafficking can be efficacious comes from recent data suggesting that the ability of the anti-angiogenic factor, pigment epithelium derived factor (PEDF), to block VEGF-induced angiogenesis derives from PEDF-driven cleavage of the cytoplasmic tail of VEGF receptor1 (VEGFR1); this, in turn, prevents the nuclear translocation of the receptor and therefore prevents angiogenesis.⁴⁷ The authors of this study presented no data regarding possible trafficking of VEGF with the receptor to nucleus, but note that both bFGF and its receptor translocate to nucleus in target cells.^{47, 48} The proposition we have presented here suggests that VEGF does traffic to nucleus and that the interruption of the trafficking of VEGF and of other angiogenic agents could be a fruitful area for the development of new therapies.

CONCLUSION

The above findings, when viewed in the context of intracrine physiology, suggest that inhibiting the intracellular trafficking of intracrine proteins to nucleus and likely to other sites such as mitochondria, should be viewed as a major target for anti-angiogenesis therapy. That is, rather than focusing on microtubular disruption or apoptosis *per se*, we suggest targeting the multiple ways by which such trafficking can be interrupted. If this view is correct, it immediately suggests ways to develop more effective and durable therapies than have heretofore been possible. Thus, as noted above, instead of randomly combining agents or focusing on a single mechanism such as tubule disruption or inhibition of migration, this notion suggests combining drugs that act at various cytoskeleton components with those that act on intracrine shuttle proteins, such as nucleolin and possibly F1FoATP synthase. Tropomyosin, an intracellular protein that binds internalized endostatin and is integral to endostatin action, has been reported

on cell surfaces and may be a shuttle protein--and therefore it may be a therapeutic target amenable to antibody or small molecule inhibition; interestingly, endothelial cell surface tropomyosin is up-regulated by bFGF.^{15, 49} The intracrine view of angiogenesis further suggests that synergy will result from the combined use of inhibitors of intracrine trafficking such as neamine (inhibits angiogenin trafficking), thromboxane A2 (inhibits integrin-mediated bFGF internalization), and possibly herceptin (inhibits the activity of multiple angiogenic factors after cell surface binding and possible internalization).^{10, 50, 51} Anti-integrin antibodies, which are anti-angiogenic in their own right, and antibodies directed at the shuttle proteins discussed above could also prove effective, as could small molecule inhibitors of these proteins.^{4-9, 52} Cell surface actin could similarly be an effective target.^{29, 30} If intracrine-associated RNA is in fact an integral component of the cytoskeletal trafficking of angiogenic intracrines, this offers yet another therapeutic target.³⁸ Moreover, the intracrine view points the way to a more directed search for anti-angiogenesis drugs and drug combinations by focusing on agents which interrupt the trafficking of these factors. Finally, the notion of intracrine reciprocity suggests that any effective drug combinations which are developed for the suppression of angiogenesis may well also directly suppress tumor growth.

The seminal insights and experimental findings of Folkman and colleagues have revolutionized thinking regarding the treatment of human neoplasia.¹ Novel therapies are in development, but, up until now, these have not lived up to their theoretical promise. We propose that viewing angiogenesis from the intracrine perspective can lead to the development of more effective drugs and improved therapeutic strategies.

Acknowledgments

This work was supported by Ochsner Clinic Foundation and NIH/NHLBI HL072795.

References

1. Folkman J. Angiogenesis. *Annu Rev Med* 2006;57:1–18. [PubMed: 16409133]
2. Re R. The nature of intracrine peptide hormone action. *Hypertension* 1999;34:534–8. [PubMed: 10523322]
3. Re RN. The origins of intracrine hormone action. *Am J Med Sci* 2002;323:43–8. [PubMed: 11814142]
4. Re RN. Toward a theory of intracrine hormone action. *Regul Pept* 2002;106:1–6. [PubMed: 12047903]
5. Re RN. The intracrine hypothesis and intracellular peptide hormone action. *Bioessays* 2003;25:401–9. [PubMed: 12655647]
6. Re RN. Implications of intracrine hormone action for physiology and medicine. *Am J Physiol Heart Circ Physiol* 2003;284:H751–7. [PubMed: 12578810]
7. Re RN. Intracellular renin and the nature of intracrine enzymes. *Hypertension* 2003;42:117–22. [PubMed: 12860832]
8. Re RN, Cook JL. The intracrine hypothesis: An update. *Regul Pept* 2005;133:1–9. [PubMed: 16226324]
9. Re RN, Cook JL. An intracrine view of angiogenesis. *BioEssays*. 2006In Press
10. Hirukawa S, Olson KA, Tsuji T, et al. Neamine inhibits xenografic human tumor growth and angiogenesis in athymic mice. *Clin Cancer Res* 2005;11:8745–52. [PubMed: 16361562]
11. Song J, Wang J, Yang J, et al. Influence of angiogenin on the growth of A375 human melanoma cells and the expression of basic fibroblast growth factor. *Melanoma Res* 2006;16:119–26. [PubMed: 16567967]
12. Chen ZW, Chang CS, Leil TA, et al. GABAA receptor-associated protein regulates GABAA receptor cell-surface number in *Xenopus laevis* oocytes. *Mol Pharmacol* 2005;68:152–9. [PubMed: 15814572]
13. Leil TA, Chen ZW, Chang CS, et al. GABAA receptor-associated protein traffics GABAA receptors to the plasma membrane in neurons. *J Neurosci* 2004;24:11429–38. [PubMed: 15601949]

14. Li XC, Carretero OA, Navar LG, et al. AT1 receptor-mediated accumulation of extracellular angiotensin II in proximal tubule cells: role of cytoskeleton microtubules and tyrosine phosphatases. *Am J Physiol Renal Physiol* 2006;291:F375–83. [PubMed: 16478976]
15. Mallery SR, Morse MA, Wilson RF, et al. AIDS-related Kaposi's sarcoma cells rapidly internalize endostatin, which co-localizes to tropomyosin microfilaments and inhibits cytokine-mediated migration and invasion. *J Cell Biochem* 2003;89:133–43. [PubMed: 12682914]
16. Kim KS, Hong YK, Joe YA, et al. Anti-angiogenic activity of the recombinant kringle domain of urokinase and its specific entry into endothelial cells. *J Biol Chem* 2003;278:11449–56. [PubMed: 12529357]
17. Sorensen V, Nilsen T, Wiedlocha A. Functional diversity of FGF-2 isoforms by intracellular sorting. *Bioessays* 2006;28:504–14. [PubMed: 16615083]
18. Youn MR, Park MH, Choi CK, et al. Direct binding of recombinant plasminogen kringle 1-3 to angiogenin inhibits angiogenin-induced angiogenesis in the chick embryo CAM. *Biochem Biophys Res Commun* 2006;343:917–23. [PubMed: 16564503]
19. Escuin D, Kline ER, Giannakakou P. Both microtubule-stabilizing and microtubule-destabilizing drugs inhibit hypoxia-inducible factor-1 α accumulation and activity by disrupting microtubule function. *Cancer Res* 2005;65:9021–8. [PubMed: 16204076]
20. Stafford SJ, Schwimer J, Anthony CT, et al. Colchicine and 2-methoxyestradiol Inhibit Human Angiogenesis. *J Surg Res* 2005;125:104–8. [PubMed: 15836858]
21. Tong YG, Zhang XW, Geng MY, et al. Pseudolarix acid B, a new tubulin-binding agent, inhibits angiogenesis by interacting with a novel binding site on tubulin. *Mol Pharmacol* 2006;69:1226–33. [PubMed: 16424078]
22. Magnon C, Galaup A, Mullan B, et al. Canstatin acts on endothelial and tumor cells via mitochondrial damage initiated through interaction with α 5 β 1 and α 5 β 3 integrins. *Cancer Res* 2005;65:4353–61. [PubMed: 15899827]
23. Colorado PC, Torre A, Kamphaus G, et al. Anti-angiogenic cues from vascular basement membrane collagen. *Cancer Res* 2000;60:2520–6. [PubMed: 10811134]
24. Serini G, Valdembri D, Bussolino F. Integrins and angiogenesis: a sticky business. *Exp Cell Res* 2006;31:651–8. [PubMed: 16325811]
25. Tarui T, Miles LA, Takada Y. Specific interaction of angiostatin with integrin α (v) β (3) in endothelial cells. *J Biol Chem* 2001;276:39562–8. [PubMed: 11514539]
26. Champagne E, Martinez LO, Collet X, et al. Ecto-F1Fo ATP synthase/F1 ATPase: metabolic and immunological functions. *Curr Opin Lipidol* 2006;17:279–84. [PubMed: 16680033]
27. Wickstrom SA, Alitalo K, Keski-Oja J. Endostatin associates with lipid rafts and induces reorganization of the actin cytoskeleton via down-regulation of RhoA activity. *J Biol Chem* 2003;278:37895–901. [PubMed: 12851410]
28. Yoon SO, Shin S, Mercurio AM. Hypoxia stimulates carcinoma invasion by stabilizing microtubules and promoting the Rab11 trafficking of the α 6 β 4 integrin. *Cancer Res* 2005;65:2761–9. [PubMed: 15805276]
29. Roiz L, Smirnov P, Bar-Eli M, et al. ACTIBIND, an actin-binding fungal T(2)-RNase with antiangiogenic and anticarcinogenic characteristics. *Cancer* 2006;106:2295–308. [PubMed: 16586499]
30. Dudani AK, Ben-Tchavtchavadze M, Porter S, et al. Angiostatin and plasminogen share binding to endothelial cell surface actin. *Biochem Cell Biol* 2005;83:28–35. [PubMed: 15746964]
31. Keezer SM, Ivie SE, Krutzsch HC, et al. Angiogenesis inhibitors target the endothelial cell cytoskeleton through altered regulation of heat shock protein 27 and cofilin. *Cancer Res* 2003;63:6405–12. [PubMed: 14559830]
32. Hanai J, Dhanabal M, Karumanchi SA, et al. Endostatin causes G1 arrest of endothelial cells through inhibition of cyclin D1. *J Biol Chem* 2002;277:16464–9. [PubMed: 11815623]
33. Wang X, Jin DY, Wong HL, et al. MAD2-induced sensitization to vincristine is associated with mitotic arrest and Raf/Bcl-2 phosphorylation in nasopharyngeal carcinoma cells. *Oncogene* 2003;22:109–16. [PubMed: 12527913]

34. Chen YH, Wu HL, Chen CK, et al. Angiostatin antagonizes the action of VEGF-A in human endothelial cells via two distinct pathways. *Biochem Biophys Res Commun* 2003;310:804–10. [PubMed: 14550275]
35. Wahl ML, Kenan DJ, Gonzalez-Gronow M, et al. Angiostatin's molecular mechanism: aspects of specificity and regulation elucidated. *J Cell Biochem* 2005;96:242–61. [PubMed: 16094651]
36. Dudani AK, Ben-Tchavtchavadze M, Porter S, et al. Angiostatin and plasminogen share binding to endothelial cell surface actin. *Biochem Cell Biol* 2005;83:28–35. [PubMed: 15746964]
37. Shichiri M, Hirata Y. Antiangiogenesis signals by endostatin. *FASEB J* 2001;15:1044–53. [PubMed: 11292666]
38. Lam MH, Thomas RJ, Loveland KL, et al. Nuclear transport of parathyroid hormone (PTH)-related protein is dependent on microtubules. *Mol Endocrinol* 2002;16:390–401. [PubMed: 11818509]
39. Giannakakou P, Nakano M, Nicolaou KC, et al. Enhanced microtubule-dependent trafficking and p53 nuclear accumulation by suppression of microtubule dynamics. *Proc Natl Acad Sci USA* 2002;99:10855–60. [PubMed: 12145320]
40. Harrell JM, Murphy PJ, Morishima Y, et al. Evidence for glucocorticoid receptor transport on microtubules by dynein. *J Biol Chem* 2004;279:54647–54. [PubMed: 15485845]
41. Salman H, Abu-Arish A, Oriel S, et al. Nuclear localization signal peptides induce molecular delivery along microtubules. *Biophys J* 2005;89:2134–45. [PubMed: 16040740]
42. Yu Y, Liu Y, Shen N, et al. Crystal structure of human tryptophanyl-tRNA synthetase catalytic fragment: insights into substrate recognition, tRNA binding, and angiogenesis activity. *J Biol Chem* 2004;279:8378–88. [PubMed: 14660560]
43. Smith SD, Trueworthy RC, Kisker SE, et al. In vitro sensitivity of normal granulocytic and lymphoma colonies to vinca alkaloids. *Cancer* 1983;51:417–22. [PubMed: 6600412]
44. Nygren P, Larsson R. Differential in vitro sensitivity of human tumor and normal cells to chemotherapeutic agents and resistance modulators. *Int J Cancer* 1991;48:598–604. [PubMed: 2045203]
45. Ashton AW, Cheng Y, Helisch A, et al. Thromboxane A2 receptor agonists antagonize the proangiogenic effects of fibroblast growth factor-2: role of receptor internalization, thrombospondin-1, and alpha(v)beta3. *Circ Res* 2004;94:735–42. [PubMed: 14963009]
46. Raikwar SP, Temm CJ, Raikwar NS, et al. Adenoviral vectors expressing human endostatin-angiostatin and soluble Tie2: enhanced suppression of tumor growth and antiangiogenic effects in a prostate tumor model. *Mol Ther* 2005;12:1091–100. [PubMed: 16169279]
47. Cai J, Jiang WG, Grant MB, et al. Pigment epithelium-derived factor inhibits angiogenesis via regulated intracellular proteolysis of vascular endothelial growth factor receptor 1. *J Biol Chem* 2006;281:3604–13. [PubMed: 16339148]
48. Myers JM, Martins GG, Ostrowski J, et al. Nuclear trafficking of FGFR1: a role for the transmembrane domain. *J Cell Biochem* 2003;88:1273–91. [PubMed: 12647309]
49. Donate F, McCrae K, Shaw DE, et al. Extracellular tropomyosin: a novel common pathway target for anti-angiogenic therapy. *Curr Cancer Drug Targets* 2004;4:543–53. [PubMed: 15578912]
50. Ashton AW, Cheng Y, Helisch A, et al. Thromboxane A2 receptor agonists antagonize the proangiogenic effects of fibroblast growth factor-2: role of receptor internalization, thrombospondin-1, and alpha(v)beta3. *Circ Res* 2004;94:735–42. [PubMed: 14963009]
51. Izumi Y, Xu L, di Tomaso E, et al. Tumour biology: herceptin acts as an anti-angiogenic cocktail. *Nature* 2002;416:279–80. [PubMed: 11907566]
52. Alghisi GC, Ruegg C. Vascular integrins in tumor angiogenesis: mediators and therapeutic targets. *Endothelium* 2006;13:113–35. [PubMed: 16728329]

INTRACRINES

Insulin	Pigmented Epithelium-derived Factor (a serpin)
Prolactin	Maspin (a serpin)
FGF (1,2,3,10)	Schwannoma-derived growth factor
Midkine	Leukemia Inhibiting Factor
Angiotensin	Endogenous Opioids (Dynorphin)
Angiotensin (RNase)	Phosphoglucose Isomerase/Neuroleukin
VEGF	Oxytocin
INF beta, gamma	Macrophage Colony-Stimulating Factor (CSF-1)
Interleukins	Hepatopoietin
NGF	Renin/Prorenin (aspartyl-protease)
PDGF	Leptin
PTHrP	Amphoterin (HMGB1)
Pleiotrophin	PD-ECGF/thymidine phosphorylase
Proenkephalin	TGF-alpha
Homeoproteins	IGFBP 3, 5
IGF-1	Granzyme A, B
Lactoferrin	Hepatopoietin
Heregulin	ESkine/CCL 27
Growth Hormone	Thioredoxin
Somatostatin	PAI-2 (a serpin)
Galectins	Angiotensin (1-7)
EGF	Reelin
Tat*	SHBG
TRH	Pancreatic Bile Salt-Dependent Lipase
LHRH	Macrophage Migration Inhibitory Factor
VIP	Urokinase
Defensins	Endothelin
Factor J	Ribosomal Protein S 19
PLA2-I	Trp-tRNA synthetase
ANP	Pituitary Adenylate Cyclase Activating Polypeptide
Hepatoma-Derived Growth Factor	Neuropeptide Y
Brain-derived Neurotrophic Factor	Erythropoietin
Gonadotropin	ACE
Chorionic Gonadotropin	Angiotensinogen
Lysyl-tRNA synthetase	ACheR