Implication of Angiogenesis in Thyroid Cancer

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Angiogenesis, the formation of new blood vessels from vascular endothelium, is essential for the growth and metastasis of tumor. Without angiogenesis, tumor cannot grow beyond 1 to 2 mm in size, because new blood vessels are needed to provide nutrient and oxygen to the growing tumor mass. Without angiogenesis, all solid tumors would be necrotic at the center and have only a few millimeter thin shell of viable tissue.

Tumor angiogenesis has certain interesting aspects that challenge our usual perspectives on tumor growth. Angiogenesis is regulated by paracrine action. For example, tumor cells secrete angiogenesis factors and the endothelial cells in the normal surrounding tissue then respond to these growth factors by proliferation and migration. Angiogenesis requires the normal endothelial cells to proliferate then “invade” into the abnormal tumor tissue. This is the opposite perspective of how we usually think of cancer, where the cancer cells proliferate and invade into the normal surrounding tissue.

Angiogenesis and Thyroid

Angiogenesis plays an important role in the growth of benign thyroid tissue, such as multinodular goiter and Graves’ disease, and thyroid cancer. Angiogenesis allows the primary tumor and the metastasis to grow in size. The size of tumor is a prognostic factor for patients with thyroid cancer, the larger the tumor the worse the prognosis. (1) In fact, papillary thyroid cancers smaller than 0.5 cm are clinically insignificant, and rarely invade or metastasize. This benign course has been postulated to be due to the lack of angiogenesis in these “occult cancers”. When sufficient cells within the tumor have switched to the angiogenic phenotype, neovascularization may begin for the growth of tumor.

1) Microvasculature and thyroid cancer

Tumor microvessel count has been found to correlate with tumor metastasis and patient prognosis for many cancers, including endocrine tumors. Microvessel count in thyroid cancers are increased compared to normal thyroid tissue. Microvessel count was found to correlate with the prognosis of patients with medullary thyroid cancer (MTC). (2) That is, tumors from patients who died from MTC had significantly more microvessels than those from patients who did not die from MTC. In these patients the microvessel count was the only clinically significant prognostic indicator besides TNM stage.

Although both benign (hyperplastic or neoplastic) and malignant thyroid tissues can have increased angiogenesis, malignant and invading tissues may stimulate more angiogenesis. In microinvasive follicular thyroid carcinomas, We found that follicular thyroid carcinomas have increased microvasculature and increased expression of angiogenic factor, such as VEGF, compared with follicular adenomas. (3)

2) Angiogenic factors in thyroid tissue

Various growth factors produced by the cancer cells stimulate tumor angiogenesis via paracrine action. Angiogenesis growth factors that are found in thyroid tissue or cancer are discussed below.

Basic fibroblast growth factor (bFGF, FGF-2) is a potent mitogenic factor for endothelial cells. Thyroid cells produce both bFGF and its receptor FGFR-1. Since bFGF has a mitogenic effect on both thyroid cells and endothelial cells, it has both an autocrine and paracrine effect. Rats treated with methimazole and on a low iodine diet develop goiters. Immunohistochemical staining of these experimental goiters show that the increased angiogenesis is accompanied by an increased immunohistochemical staining of angiogenesis stimulators, basic fibroblast growth factor (bFGF) and transforming growth factor-beta 1 (TGF β1), and a decreased immunohistochemical staining for an angiogenesis inhibitor, thrombospondin-1 (TSP-1). (4)

In human differentiated thyroid cancers, both bFGF and FGFR-1 are highly expressed. Immunohistochemical staining of bFGF in the thyroid cells is most intense for thyroid cancers, followed by adenomas, followed by normal thyroid tissue. (5) Expression of bFGF may correlate with the degree of malignancy in differentiated thyroid cancer, the more intense the cells stain for bFGF the more extensive the lymph node metastasis by the cancer.

Placental-derived growth factor (PIGF) mRNA is expressed in normal thyroid gland goiters. PIGF is a dimeric glycoprotein...
similar to VEGF. PIGF-1 induces angiogenesis and it stimulates the migration and proliferation of endothelial cells. PIGF-I induced angiogenesis in a dose-dependent manner, and it had a comparable effect to that of VEGF and bFGF under similar condition and concentration.

Other growth factors, such as epidermal growth factor (EGF), transforming growth factor alpha and beta (TGF-α and β), angiogenin and tumor necrosis factor-alpha (TNF-α) have also been implicated in tumor angiogenesis. These factors do not directly stimulate proliferation of endothelial cells, and probably induce angiogenesis indirectly.

Non-thyroid cells within the thyroid cancer tissue, such as the tumor infiltrating lymphocytes, also secrete angiogenesis factors and may contribute to tumor angiogenesis.

VEGF in Thyroid Tissue and Thyroid Cancer

1) Vascular endothelial growth factor (VEGF)

VEGF is a unique angiogenic factor, because it is a true paracrine growth factor. VEGF is a 34–42 kDa, heat and acid stable, dimeric, heparin binding glycoprotein. The different splice variants of VEGF account for the different molecular weights. VEGF binds to membrane VEGF receptors 1 and 2 (flt-1 and flk-1/KDR) that are receptor tyrosine kinases, and activates the phospholipase C signaling system. Embryologically VEGF promotes vasculogenesis by stimulating the cells via VEGF receptors 1 and 2. In existing blood vessels such as those at the edge of a cancer, VEGF promotes angiogenesis in cooperation with angiopoietin 2, which inhibits the Tie 2 receptor. Tie 1 and Tie 2 receptors are also receptor tyrosine kinases that are structurally similar to VEGF receptors. Tie 2 binds to angiopoietins. These receptor tyrosine kinases are crucial for embryological development. Transgenic mice overexpressing angiopoietin 2 or knock out mice without functioning VEGF receptors or Tie receptors do not survive. VEGF not only stimulates proliferation of endothelial cell but also increases vascular permeability and was also named vascular permeability factor (VPF).

2) The role of VEGF in thyroid tissue

Sato et al were the first to show that normal and Graves thyroid tissue expressed VEGF mRNA. (6) Viglietto (7) showed that thyroid cancer cell lines expressed VEGF, and that VEGF expression paralleled the tumorigenic potential of the cell lines.

In our own studies, we found higher VEGF mRNA expression and VEGF production and secretion in the thyroid cancer cell lines (papillary, follicular, Hurthle and medullary cancer cell lines) than in the primary cultures of normal thyroid cell. (8) We found that thyroid cancer cell lines of follicular cell origin expressed more VEGF than those of parafollicular cell origin. Primary and metastatic thyroid cancers, however, appeared not to differ in their VEGF expression. Immunohistochemical staining of papillary, follicular, and Hurthle cell cancers for VEGF was also stronger than those of medullary thyroid cancers, benign, or hyperplastic (Graves’ disease) thyroid tissue. Number of microvessel density and expression of VEGF and receptors (flt-1/KDR, flt-1) were also higher in thyroid cancer, especially in anaplastic thyroid cancer, than in follicular adenomas. (9)

3) Regulation of VEGF expression in thyroid tissue

Growth factors (e.g., TSH, EGF), inhibitors (e.g., tamoxifen) and activate oncogenes (e.g., ras) that are known to modulate thyroid cancer cell growth may also have an indirect effect on thyroid tumor growth and metastasis by regulating the expression of VEGF and tumor angiogenesis.

TSH stimulates the production and secretion of VEGF by the thyroid cell via both the adenylate cyclase and the protein kinase C signal transduction pathways. (4) When rats were made hypothyroid by PTU, and have an elevated serum level of TSH, the thyroid cells expressed more VEGF mRNA, while the endothelial cells from the thyroid gland expressed more VEGF receptors.

Thyroid cancers, in general, secrete more VEGF than normal thyroid cells. In our studies of human thyroid cancer cell lines, we found some to have a constitutively elevated VEGF secretion and others to have an exaggerated increase in VEGF secretion in response to TSH stimulation. (10) XTC-1 Hurthle cell cancer cell line has a 3 fold higher basal VEGF secretion than normal thyroid cells. Whereas TPC-1 (papillary), FTC133 (follicular) and MTC 1.1 (medullary) thyroid cancer cell lines secreted significantly more (2~5 fold) VEGF than normal thyroid cells when stimulated with TSH. These increased VEGF by TSH stimulation promotes the vascular endothelial cell proliferation in vitro and angiogenesis in vivo. (11) TSH may, therefore, promote growth in some thyroid cancers by stimulating VEGF secretion and angiogenesis.

Ras oncogene, which is activated in some thyroid cancers and follicular adenomas may also have an effect on angiogenesis via VEGF. H-ras transformed rat intestinal epithelial cells have an increased expression of VEGF. When the mutant RAS protein was disrupted by treatment with L-739,749 (a protein farnesyltransfase inhibitor) the expression of VEGF was suppressed.

Angiogenesis Inhibitors

Since angiogenesis is essential for the growth of primary tumor
and metastasis, inhibiting angiogenesis may prevent cancer growth, and it potentially can be used to treat patients with cancer.

TNP-470, 6-O-(N-chloroacetyl-carbamoyl)-fumagillin, is a semisynthetic analogue of fumagillin that inhibits in vivo tumor growth. The exact mechanism of action for TPN-470 is unknown, although it inhibits the methionine aminopeptidase activity of type 2 methionine aminopeptidase (MetAP2). Hama et al, in an experimental model for thyroid cancer, treated xenotransplanted human anaplastic thyroid carcinoma in nude mice with injection of TNP-470. (12) Intratumoral injection of TNP-470 completely inhibited tumor growth. Peritumoral injection also significantly inhibited tumor growth. Whereas subcutaneous and intraperitoneal injections were less effective.

Protease inhibitors may inhibit angiogenesis since proteolysis is an important aspect of invasion and angiogenesis. Degradation of the extracellular matrix facilitates cell migration. Rats injected with Dunning MAT-LyLu prostate cancer cells develop lung metastasis. Serine protease urokinase (u-PA) stimulates tumor angiogenesis. To determine the role of u-PA in cancer, gene coding for a mutant u-PA, which binds to the receptor but has no proteolytic activity, was used to transfected these prostate cancer cell. Rats injected with the transfected cells had smaller tumors and fewer metastasis. Matrix metalloproteinase (MMP) may also stimulate tumor angiogenesis. The activity of MMP is inhibited by tissue inhibitor of metalloproteinases-1 (TIMP-1). TIMP-1 has been found to have antiangiogenesis effect.

1) Tyrosine kinase inhibitors

Antiangiogenesis drug may also target down-stream in the signaling pathway of the angiogenesis factor. PD 166866, 6-aryl-pyridine [2,3-d]-pyrimidines, is a specific inhibitor of the human fibroblast growth factor-1 receptor (FGFR-1) tyrosine kinase [34]. Similarly, wortmannin is a fungal metabolite that selectively inhibits phosphatidylinositol 3-kinase 3-kinase, and it has been found to inhibit angiogenesis in bioassay of chick embryo chorioallantoic membranes.

2) Anti-VEGF antibody

Kim et al and Warren et al have shown that murine monoclonal anti-VEGF antibody inhibited the growth of xenotransplanted human cancer cells in nude mice and prevented liver metastasis. (13,14) In our own study, we injected nude mice subcutaneously with human thyroid cancer cells (papillary TPC-1, follicular FTC-133 and Hurthle XTC-1). (15) These mice developed large subcutaneous tumors and sometime developed systemic metastasis. For the treatment group, we began intraperitoneal injection with anti-VEGF antibody when the tumor became apparent (2 mm). Treatment with anti-VEGF antibody dramatically inhibited tumor angiogenesis and prevented growth of the subcutaneous tumors and development of metastasis. The humanized form of anti-VEGF antibody is currently in clinical trials for treatment of various cancers.

3) Angiostatin and endostatin

Angiostatin is produced by cancer-mediated proteolysis by urokinase (uPA) that converts plasminogen to angiostatin. In addition to systemic administration, gene therapy using angiostatin is another potential treatment. Gene transfer of a cDNA coding for mouse angiostatin into murine T241 fibrosarcoma cells suppressed primary and metastatic tumor growth in vivo. A potential disadvantage of antiangiogenesis therapy is that it may only be tumorstatic and not tumoricidal. Endostatin is a 20 kDa C-terminal fragment of collagen XVIII that specifically inhibits endothelial proliferation, tumor angiogenesis and tumor growth. When mice bearing Lewis lung carcinoma, T241 fibrosarcoma or B16F10 melanoma were treated with endostatin, the tumors regressed. When the tumors re-grew, the mice were re-treated with endostatin. After 2 to 6 treatment cycles the tumor no longer recurred.

4) Other angiogenic inhibitors

There are numerous other angiogenesis inhibitors. FR-118487 suppressed liver metastasis by inhibiting angiogenesis in a rabbit colon cancer model. Clarithromycin is a macrolide antibiotic. It suppressed tumor-induced angiogenesis by inhibiting endothelial cell tube formation. Eriochrome Black T (EBT) is an analog of suramin, a cancer chemotherapeutic agent. EBT inhibited angiogenesis in the chick chorioallantoic membrane assay with more potency and less toxicity than suramin. Irosogludine inhibited tumor growth and angiogenesis in xenotransplants of human glioma and hepatoma cells. 2-methoxyoestradiol is an endogenous estrogen metabolite. It inhibited endothelial cell proliferation and migration in vitro, and angiogenesis and growth of solid tumors in mice. Thrombospondins (TSPs) have antiangiogenic activity and inversely correlated with tumor growth and survival rate. SR 25989 is a member of the thienopyridine family. It inhibits angiogenesis by upregulating thrombospondin-1 (TSP-1) probably mediated by p53. Manumycin (farnesyltransferase inhibitor) and Paclitaxel have anticancer effect to anaplastic thyroid cancer by inhibition of angiogenesis.

Summary

Similar to most other solid tumors, growth and metastasis of
thyroid cancer depend on tumor angiogenesis. Various paracrine factors, such as VEGF and bFGF, are produced by the cancer cells and stimulate the normal endothelial cells to proliferate and migrate and to develop new vessels that provide nutrients to the growing tumor. Microvasculature count is a prognostic factor in many solid tumors, and may have prognostic importance in some thyroid cancer. The production and secretion of angiogenesis factors by thyroid cancer cells are likely to be regulated by TSH and its associated signaling pathways that also regulate thyroid cell function and growth. Antiangiogenesis therapy, such as TNP-470, anti-VEGF antibody, angiostatin and endostatin, may in the future be an option for treatment of advanced of aggressive thyroid cancers.

REFERENCES


