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Review article

The anticancer potential of thrombospondin-1 by inhibiting angiogenesis and stroma reaction during cervical carcinogenesis

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ABSTRACT

Tumor growth is angiogenesis dependent. Angiogenic switch (the acquisition of an angiogenic phenotype) is essential for cervical carcinogenesis. Thrombospondin-1 (TSP-1) is an endogenous angiogenic inhibitor with multiple functional domains and interacting receptors. The disruption of TSP-1 fence (the expression in basal epithelia) occurred concordantly during the transition from low-grade squamous intraepithelial lesion into high-grade squamous intraepithelial lesion. This concordance suggests that TSP-1 plays a role in the regulation of angiogenic switch during cervical carcinogenesis. Tumor vasculature as a therapeutic target offers a paradigm shift for anticancer therapy. Endothelial cells do not appear to acquire resistance during antiangiogenic therapy. Low-and-frequent dose "metronic" chemotherapy is found to be antiangiogenic, which is more effective in targeting tumor endothelia than traditional large, single bolus doses. Meanwhile, the invasion process of cancers is associated with stroma reaction, which is characterized by fibroblasts' activation. In addition to the well-known angiogenesis inhibitor, TSP-1 also has a novel role of blocking activated fibroblasts (myofibroblasts) from invading cancer. Activated fibroblasts during stroma reaction could be used as an efficient drug delivery system to prevent or slow the local growth of cancer cells. Elucidation of the mechanism by which fibroblasts are recruited into cancer stroma could lead to new insights into not only the mechanisms of cancer progression but also strategies for cancer treatment. A better understanding of stromal contributions to cancer progression will likely result in the identification of new therapeutics targeting the stroma.

Angiogenesis and stroma reaction are important during carcinogenesis

Tumor growth is angiogenesis dependent

Angiogenesis is defined as the formation of new blood vessels by proliferation of new capillaries from preexisting microvessels. This process is distinct from vasculogenesis, which is defined as the formation of blood vessel *de novo* from angioblasts.[1,2] Angiogenesis involves degradation of the basement membrane surrounding an existing capillary or venule, migration of endothelial cells through the basement membrane to create a sprout, proliferation of endothelial cells, formation of a lumen within the new sprout and joining of two sprouts to from a functional capillary loop, and vessel maturation.[3,4] The idea that tumor growth is angiogenesis dependent was first proposed in 1971, allowing antiangiogenic therapy to be used to treat cancer.[5] The development of a solid tumor progresses from a prevascular phase to a vascular phase. The prevascular tumor does not induce angiogenesis, is limited in size, and rarely metastasizes. The vascularized tumor induces host microvessels to undergo angiogenesis. The best characterized example is the hypoxia-dependent

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angiogenic switch in which host cell-derived endothelial cells invade into the tumor stroma to form new blood vessels.[6] Thus, blocking angiogenesis can result in tumor dormancy, in which tumors could not expand beyond a microscopic size.[7] Within the dormant tumors, the proliferating tumor cells are balanced by apoptotic tumor cells and few, if any, microvessels.[8]

Angiogenic switch during cervical carcinogenesis

Cervical cancer usually develops by a sequence of gradual, stepwise events starting at low-grade squamous intraepithelial lesion (LSIL) and progressing through high-grade SIL (HSIL), until invasive cancer ensues.[9] Tumor development and metastasis is a complex process that includes transformation, proliferation, neovascularization, and metastatic spread. The angiogenic switch-acquisition of an angiogenic phenotype that is induced by a change in the balance of angiogenesis activators and inhibitors, is essential for tumor growth and metastasis.[2,10] During carcinogenesis, Toussaint-Smith et al[11] and Bequet-Romero and Lopez-Ocejo[12] have described an increase in vascular endothelial growth factor (VEGF) expression associated to the expression of human papillomavirus type 16 oncoproteins E6 and E7. HPV oncoproteins E6 and E7 disrupt the functions of the tumor suppressors p53. Loss of p53 results in upregulation of VEGF and downregulation of thrombospondin-1 (TSP-1). HPV 16 E6 and E7 oncoproteins may contribute to the development of cervical cancer not only by disrupting cell cycle regulation but also by creating a microenvironment that fosters the growth of tumors.[11,12] It has been reported that tumor microvasculature, accompanied by the overexpression of VEGF, was progressively upregulated during the process of cervical carcinogenesis.[13] However, the timing of angiogenic switch during cervical carcinogenesis remains controversial.[14] A debate exists regarding the ability of cervical intraepithelial neoplasia to induce angiogenesis.[14–16] Smith-McCune and Weidner[14] found a significant increase of microvessel density in the cervical intraepithelial neoplasia III lesions compared with those underlying low-grade lesions.[14] By contrast, reports from Abulafia et al[16] show that microinvasive squamous cell carcinoma is angiogenic, but not carcinoma *in situ*. Wu et al[17] examined different severities of cervical lesions in the same slide, to eliminate the heterogeneity. The data showed that the angiogenic switch in cervical carcinogenesis occurred during the transition from LSIL to HSIL, and the neovascularization was largely confined to a narrow zone immediately underneath the dysplastic epithelium. It further suggests that cervical carcinogenesis is angiogenesis-dependent.

Invasion process of cancer cells is associated with stroma reaction

Our understanding of cancer has largely come from the analysis of aberrations within the tumor cell population. There is emerging evidence to highlight the important role of tumor microenvironment in tumorigenesis.[18] Stroma reaction, also known as stromagenesis, is a host response of mesenchymal alteration induced in cancer that produces a progressive and permissive mesenchymal microenvironment, thereby supporting tumor progression.[19] Paget[20] first proposed "seed and soil hypothesis" to highlight the influence of tumor growth by interactions between malignant cells and the tumor stroma in 1889. In the "seed and soil hypothesis" [20] of cancer biology, cancer cells are the "seeds," and the microenvironment is the "soil" in which the "seeds" must find a receptive environment.[21] The normal host microenvironment is nonpermissive for neoplastic progression, and tumor-reactive stroma promotes neoplastic growth and metastasis.[22] Activation of the local invasive environment seems to create a permissive field for the malignant cells.[23]

Stroma reaction is characterized by fibroblast activation

Activated fibroblasts, also called myofibroblasts, are defined by the expression of α-smooth muscle actin, desmin, vimentin, etc. in the fibroblasts.[24] Activated fibroblasts can produce noncellular scaffolds in response to extracellular stimuli, and create an environment promoting tumor progression.[25] In addition, activated fibroblasts within the tumor stroma have a propensity to migrate and invade like cancer cells.[26] The proliferative activity of activated fibroblasts in cancer-induced stroma is closely linked to tumor progression, lymph node, and distant organ metastasis of breast cancer.[27] Normal stromal cells may prevent epithelia from becoming tumorigenic.[28] Fibroblasts, as the major component of stroma, are recruited and can convert into smooth muscle actin-positive fibroblasts, i.e., myofibroblasts or activated fibroblasts, during stroma reaction.[29,30] Myofibroblasts appear at the invasion front during stromal changes in cells.[31]

TSP-1 and cervical carcinogenesis

TSP-1 is a matricellular protein with diverse functions

Thrombospondins (TSPs) consist of a family of five extracellular proteins that participate in cell-to-cell and cell-to-matrix communications.[32] Among them, TSP-1 is a 450-kDa homotrimeric matricellular glycoprotein with potent antiangiogenic effects. In many tumor types, TSP-1 can block *in vivo* neovascularization and decrease malignant tumor growth (e.g., skin, prostate, and bladder cancers),[33,34] whereas in others (e.g., breast cancer) it promotes cancer cell adhesion, migration, and invasion.[35] The differential effects of TSP-1 on tumorigenesis indicate that TSP-1 exerts different biological functions in different cell types. Also, TSP-1 interacts with multiple extracellular macromolecules and cell surface receptors, thus exerting a wide range of responses.[36,37] The hypothesis of this review is "Can TSP-1 could inhibit cancer progression via targeting endothelial cells (EC) in angiogenesis and fibroblasts (F) in stroma reaction?" (Figure 1).

TSP-1 acts as "an angiogenic fence" during cervical carcinogenesis

Wu et al[17] proposed a "TSP-1 fence" in which TSP-1 is mainly localized on basal cervical epithelial cells, and arrayed like a barrier in normal cervical epithelium or LSIL. TSP-1 decreases significantly during the transition from LSIL to HSIL, which is concomitant with the increase of microvessel density counts. The temporal and spatial concordance of TSP-1 downregulation and the emergence of angiogenic imply that the "TSP-1 fence" may act as an angiogenic barrier to inhibit angiogenesis that occurred in the early phase of cervical carcinogenesis. The disappearance of the angiogenic barrier may induce a vigorous angiogenic response for tumor growth and perhaps tumor metastasis.[38] TSP-1 does not appear to contribute directly to the structural integrity of connective tissue elements. Instead, TSP-1 acts by modulating the activity and bioavailability of protease and growth factors and by interaction with cell-surface receptors.[39,40] Matrix metallopreoteases (MMPs) have been shown to play an active role in the neovascularization of tumors through their ability to degrade the extracellular matrix.[41,42] Bergers et al[43] showed that the switch from vascular quiescence to angiogenesis involves MMP-9, which is upregulated in angiogenic islets and tumors. TSP-1 acts as a multifunctional modulator of angiogenesis by modulating through the activity and bioavailability of MMP-9.