

Review

Novel Strategy of Anti-angiogenic Therapy for Uterine Cervical Carcinomas

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Abstract. *In uterine cervical carcinomas, lymph node metastasis, recognized as a common form of metastasis, and recurrence after curative resection are critical to patient prognosis. The growth of secondarily spreading and initial recurrent lesions must be suppressed to improve patient prognosis. Chemotherapy and radiation are often not very specific to cancer cells and produce severe effects on normal cells, especially bone marrow and renal cells. On the other hand, anti-angiogenic therapy is specific to the rapidly growing vascular endothelial cells in tumors, without any effect on slow growing vascular endothelial cells and other normal cells. Therefore, anti-angiogenic therapy should be an excellent strategy to suppress the growth of secondarily spreading and initial recurrent lesions. However, if a particular angiogenic factor is suppressed by anti-angiogenic therapy for a long period, another angiogenic pathway, a process recognized as tolerance. The angiogenic factor expression and the effects of angiogenic transcription factors in uterine cervical carcinomas are herein reviewed and novel therapeutic trends are introduced.*

Angiogenesis is essential for the development, growth and advancement of solid tumors (1). The manner of advancement, specific to each tumor, is fundamental to patient prognosis. In uterine cervical carcinomas, lymph node metastasis, recognized as a common form of metastasis, and recurrence after curative resection critically affect the patient prognosis.

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When a cluster of cancer cells is smaller than 2 mm in diameter, angiogenesis does not occur. However, when the cluster size reaches 2 mm, angiogenesis inevitably develops (1). Thereafter, a new capillary network is rapidly formed, nourishing the tumor and supporting growth. Therefore, attaining a diameter of 2 mm is critical for subsequent tumor advancement (1). Unfortunately, when a secondarily spread lesion is discovered, its size is usually already larger than 2 mm in diameter, meaning that angiogenesis in the lesion has already begun. Consequently, although the detachment and invasion of cancer cells can be suppressed, the growth of the secondarily lesion will not be suppressed. Such growth must be suppressed if patient prognosis is to improve and the inhibition of angiogenesis could be an excellent strategy to achieve this aim. Furthermore, chemotherapy is often not very specific to cancer cells, producing severe effects even on normal cells, especially bone marrow and renal cells. On the other hand, anti-angiogenic therapy is specific to the rapidly growing vascular endothelial cells in tumors, without any effect on slow growing vascular endothelial cells and other normal cells.

However, if a particular angiogenic factor is suppressed by anti-angiogenic therapy for a long period, another angiogenic factor might be induced by linked alternative angiogenic pathway, a process recognized as tolerance. Therefore, the manner of angiogenic factor expression and the influence of angiogenic transcription factor are herein reviewed and new strategies for anti-angiogenic therapy in uterine cervical carcinomas are introduced.

Tumor Angiogenesis

Neovascularization encompasses two concepts, vasculogenesis and angiogenesis. Vasculogenesis is the formation of a vascular plexus by differentiation from hemangioblasts to endothelial cells without any existing vascular system. For example, a primary vascular plexus is formed from hemangioblasts in a fetus by the process of vasculogenesis. Angiogenesis is the formation of new capillaries from existing capillaries and occurs in tumors supporting their growth and advancement.

Angiogenic factors originating from tumors induce and activate matrix metalloproteinase, plasminogen activator, collagenase and other enzymes in endothelial cells. The enzymes dissolve the basement membrane of the endothelial cells, after which the endothelial cells proliferate and migrate under the influence of the angiogenic factors. Additionally, angiogenic factors induce the production of integrins in the endothelial cells, which then form immature capillary tubes. In normal tissue, the capillary is matured upon being covered with pericytes. However, the pericyte-covering layer of capillaries in tumors consists of fewer cells than normal with inferior function. Therefore, cancer cells can easily invade through immature capillary. Microvessel density can be evaluated by counting the microvessels using immunohistochemical staining for CD31, CD34 or factor VIII-related antigen specific to the endothelial cells. If there is a positive correlation between increased microvessel density in tumor and the level of an investigated factor, it is plausible that such factor is an angiogenic factor.

Angiogenic Factors

The main angiogenic factors in carcinomas of the reproductive organs are shown in Figure 1 and include basic fibroblast growth factor (bFGF), cyclooxygenase (COX)-2, vascular endothelial growth factor (VEGF), angiopoietins (Angs), interleukin (IL)-8, and thymidine phosphorylase (TP). Basic FGF is expressed in cancer and stromal cells, and might be associated with the underlying angiogenesis driving tumor growth and secondary spread (2). COX-2 is expressed in cancer cells and functions in conjugation with VEGF. The metabolites of COX-2, prostaglandin (PG)E1 and PGE2, have weak angiogenic activity (3), and induce VEGF production in osteoblasts, synovial fibroblasts and macrophages (4-6). The VEGF expressed in cancer cells is translocated to the vascular endothelial cells, where the Angs are expressed. Although Ang-1 induces the phosphorylation of the tyrosine kinase domain of tie-2, a receptor of Ang-1 and Ang-2, Ang-1 does not directly influence the proliferation of vascular endothelial cells. In Ang-1 knockout mice, normal vasculogenesis develops, but incomplete angiogenesis is induced, linked to lethal heart dysplasia (7). On the other hand, although Ang-2 also exhibits high affinity to tie-2, it does not induce phosphorylation of the tie-2 tyrosine kinase domain, while the overexpression of Ang-2 inhibits the signal transduction of Ang-1 linked to tie-2. Ang-1 is constitutively produced in pericytes, and stabilizes the interaction of the vascular endothelial cells with the pericytes. Stimulation of Ang-2 production in vascular endothelial and nearby stromal cells, for example by hypoxia, results in the initiation of angiogenesis with the dissociation of pericytes and the dissolution of the vascular endothelial basement membrane. Furthermore, VEGF acting

with Ang-1 enhances the formation of a capillary network, and VEGF with Ang-2 enhances its expansion (8). IL-8 levels correlate with microvessel and infiltrated macrophage counts, and the localization of IL-8 is similar to that of CD68 specific to macrophages. IL-8 secreted from macrophages affects the angiogenesis associated with cancer cell invasion (9). TP is expressed in stromal cells, and the metabolites of thymidine converted by TP as an enzyme play a role angiogenesis enabling the processes of tumor advancement (10). Additionally, angiogenesis is induced by hypoxia by means of the angiogenic transcription factor hypoxia inducible factor (HIF). HIF-1 belongs to the Per-Ahr/Arnt-Sim (PAS) family of basic helix-loop-helix proteins. HIF-1 consists of HIF-1 α and HIF-1 β (aryl hydrocarbon receptor nuclear translocator), or HIF-2 α and HIF-1 β as a heterodimer using the helix-loop-helix domain (11). HIF-1 α is localized in cancer cells and the levels correlate with microvessel counts. Although HIF-1 α is reduced by the proteasome after ubiquitination, hypoxia stabilizes and activates HIF-1 α (12, 13). On the other hand, HIF-2 α is expressed abundantly in various organs under normoxia, although the transcriptional activating properties of HIF-2 α are similar to those of HIF-1 α (14). HIF-1 α may be responsible for the VEGF expression mediated by hypoxia, and may be closely related to tumor angiogenesis (15-17). During angiogenesis, the angiogenic transcription factor E26 transformation specific (ETS)-1 is strongly expressed in vascular endothelial and adjacent interstitial cells (18) and ETS-1 levels correlate with microvessel counts (19). Once angiogenesis has ended, ETS-1 expression is distinctly down-regulated (20, 21). VEGF and bFGF induce ETS-1 expression in the early stage of angiogenesis, while the inhibition of ETS-1 expression leads to the suppression of angiogenesis (22, 23). The proteases urokinase type-plasminogen activator (u-PA), matrix metalloprotease (MMP)-1, MMP-3 and MMP-9 contain an ETS-binding motif, and ETS-1 converts vascular endothelial cells to an angiogenic phenotype by inducing the gene expression of these proteases and integrin β 3 (24, 25).

Possible Novel Strategies for Anti-angiogenic Therapy

Possible therapeutic strategies in uterine cervical carcinomas, corresponding to the main processes of angiogenesis are shown in Figure 1. Basic FGF levels increase with advancement regardless of histological type. However, the correlation between bFGF levels and poor patient prognosis is not so clear (2).

Among the subtypes of VEGF, the populations of VEGF₁₆₅ and VEGF₁₂₁ are dominant in uterine cervical carcinomas, and the levels are remarkably higher in some advanced stage adenocarcinomas of the cervix than in squamous cell

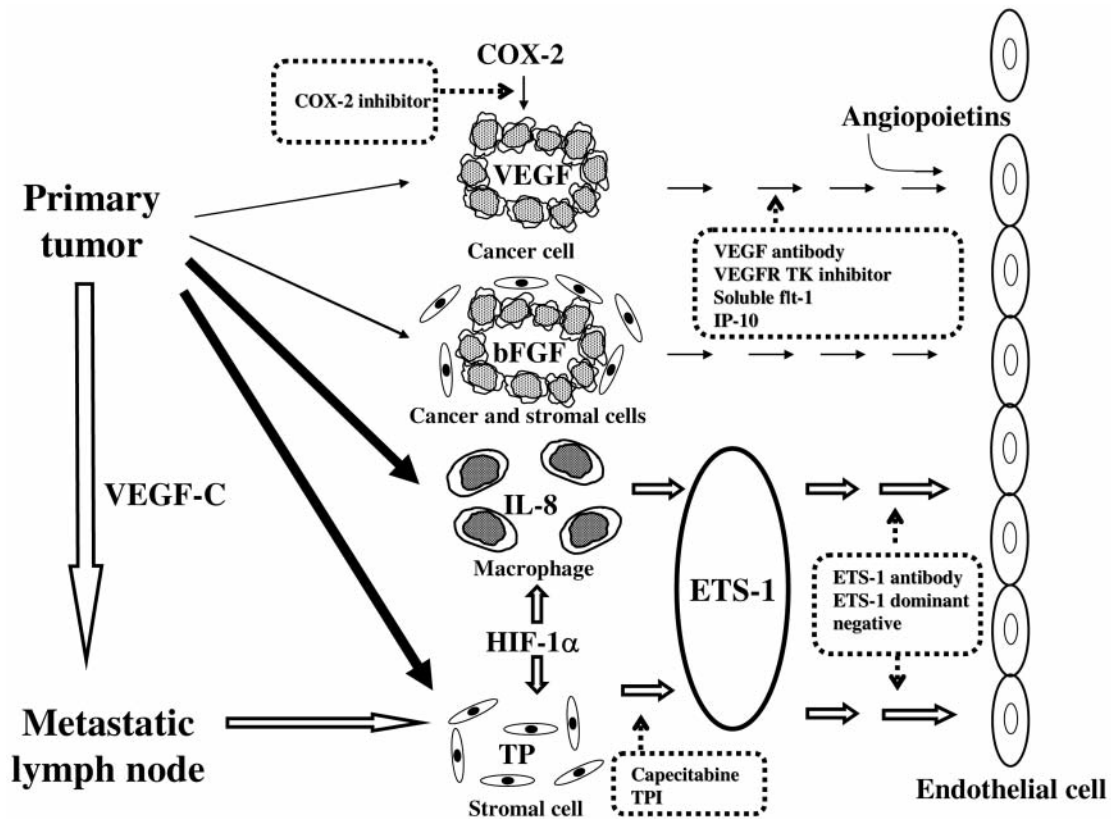


Figure 1. Angiogenesis and its inhibition in uterine cervical carcinomas. COX-2: cyclooxygenase-2; VEGF: vascular endothelial growth factor; bFGF: basic fibroblast growth factor; IL-8: interleukin-8; HIF-1 α : hypoxia inducible factor-1 α ; TP: thymidine phosphorylase; ETS-1: E26 transformation specific; VEGFR TK: VEGF receptor tyrosine kinase; flt-1: fms-like tyrosine kinase-1; IP-10: interferon-gamma-inducible protein-10; TPI: TP inhibitor.

carcinoma and other adenocarcinoma cases (26). The elevation of VEGF₁₆₅ and VEGF₁₂₁ might contribute to the relatively late progression via angiogenic activity in advanced adenocarcinomas of the cervix (26). Since VEGF is associated with COX-2 in promoting the angiogenesis of progressing tumors, the long-term administration of COX-2 inhibitors might be effective for the suppression of regrowth or recurrence after intensive treatment for advanced uterine cervical carcinomas. The ratio of Ang-2/Ang-1 increases up to late stage II and correlates with VEGF levels (not yet published). Therefore, Angs interacting with VEGF might promote angiogenesis at a comparatively early stage. It is interesting that the administration of a soluble extracellular domain of tie-2 inhibitors tumor growth yet conserves the binding domain of Ang-1 and Ang-2 (unpublished data).

The prognosis of patients with high IL-8 is extremely poor. Consequently, IL-8 can be regarded as a prognostic indicator (9). Although IL-8 mainly affects angiogenesis in the primary tumor, the suppression of IL-8 might not lead to the efficient inhibition of angiogenesis, because IL-8 is involved in cytokine networks with alternative angiogenic potential.

TP has a wide range of expression and is highly expressed in uterine cervical carcinomas regardless of clinical stage. The prognosis of patients with a high TP level in the primary tumor is worse than in those with low TP (27). Furthermore, TP was shown to increase remarkably in 8 of 40 metastatic lymph node lesions of uterine cervical carcinomas, and the prognosis of the patients with high TP in the metastatic lymph node lesions was extremely poor. Therefore, TP appears to contribute to progression in metastatic lymph nodes after the establishment of metastasis and is recognized as a prognostic indicator (28). TP together with VEGF-C expression in metastatic lymph nodes is key to evaluating patient prognosis and to building new strategies of anti-angiogenic therapy (28, 29). In addition, serum TP is positively correlated with clinical stage and tumor size and with the advancement of lymph node metastasis, parametrial involvement, and vessel permeation in both squamous cell carcinomas and adenocarcinomas (30). Serum TP level is thus recognized as a tumor marker regardless of the histopathological tumor type (30). VEGF-C expressed in cancer cells also directly contributes to lymph node metastasis as a non-angiogenic factor (29) and VEGF-C

antibody or inhibitor could suppress the process of lymph node metastasis and lead to better patient prognosis. Since capecitabine is converted to 5-fluorouracil (FU) by TP, resulting in remarkably increased 5-FU levels in metastatic lymph nodes containing high TP levels with capecitabine as the metabolite, 5-FU might be highly effective for the suppression of metastatic lymph nodes. Additionally, TP inhibitor (TPI) could directly suppress TP angiogenic activity in metastatic lymph nodes (28).

HIF-1 α levels correlate with IL-8 and TP levels in uterine cervical carcinomas, HIF-1 α appears to induce IL-8 and TP as an angiogenic mediator and is recognized as a candidate prognostic indicator. ETS-1 levels also correlate with IL-8 and TP levels and ETS-1 might be a prognostic indicator in uterine cervical carcinomas (19). Furthermore, ETS-1 antibody and/or dominant-negative ETS-1 status might be useful for suppressing the linkage of angiogenic factors to ETS-1 (19). Therefore, to avoid inducing alternative angiogenic pathways, a possible tolerance mechanism to an angiogenic inhibitor, the simultaneous suppression of the main target angiogenic factors IL-8 and TP, and the transcription factor ETS-1 might be highly effective (19).

Additionally, interferon-gamma-inducible protein 10 (IP-10) regulates lymphocyte chemotaxis, mediates vascular pericyte proliferation and acts as an endogenous angiostatic agent, thus inhibiting tumor growth (31, 32). IP-10 levels were shown to decrease with tumor advancement, and the prognosis of the 30 patients with low IP-10 expression in uterine cervical carcinomas was poor (66%), whereas the 24-month survival rate of the 30 patients with high IP-10 expression was 90% (33). Furthermore, IP-10 levels are significantly inversely-correlated with VEGF levels (33). IP-10 might function by suppressing the angiogenesis associated with VEGF and can be regarded as a prognostic indicator. Therefore, IP-10 activation might be effective for the suppression of regrowth or recurrence after the intensive treatment of advanced cervical carcinomas.

Conclusion

In uterine cervical carcinomas, the angiogenic factors bFGF, VEGF, COX-2, Ang, IL-8 and TP, and the angiogenic transcription factors HIF-1 α and ETS-1 are involved in tumor advancement *via* angiogenesis with the promotion of lymph node metastasis developed by VEGF-C. The keys to creating new strategies of anti-angiogenic therapy are: the overexpression of TP induced by HIF-1 α and linked to ETS-1 in metastatic lymph nodes; COX-2 inducible VEGF interacting with Angs at a comparatively early stage and an endogenous angiostatic agent, IP-10 which decreases with the tumor advancement associated with VEGF. Clinical trials of anti-angiogenic agents in advanced stage patients and after curative resection for uterine cervical carcinomas are soon to be underway.

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