Review

Cancer Immunotherapy of Targeting Angiogenesis

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Tumor growth and metastasis are angiogenesis-dependent. Anti-angiogenic therapy may be a useful approach to cancer therapy. This review discussed tumor angiogenesis and immunotherapy of targeting tumor angiogenesis from two main aspects: (1) active vaccination to induce effective anti-angiogenesis immunity; (2) passive immunotherapy with anti-pro-angiogenic molecules relevant antibody. Evidence from the recent years suggested that anti-angiogenic therapy should be one of the most promising approaches to cancer therapy. *Cellular & Molecular Immunology*. 2004;1(3):161-166.

Key Words: cancer, immunotherapy, angiogenesis

Introduction

The generation of new blood vessels, or angiogenesis, is important not only for some physiological processes, but also for the development of pathologic conditions such as cancer, rheumatoid arthritis, psoriasis, retinopathies, etc. Angiogenesis is a complex multistep process that includes endothelial proliferation, migration, differentiation and degradation of the extracellular matrix, etc (1, 2). Several lines of direct and indirect evidence indicated that the growth, persistence and metastases of solid tumors are angiogenesis-dependent (2). At the same time, recent studies indicated that angiogenesis also played an important role in the pathogenesis of hematopoietic malignancies (3, 4). As a novel strategy for cancer therapy, anti-angiogenic therapy attempts to stop new vessels formation around a tumor and break up the existing network of abnormal capillaries that feed the cancerous mass (5).

The complexity of the angiogenic process suggested that the angiogenic process could be temporarily turned on and off (1, 2). During the process of malignant tumor progression, it is very important that tumor cells could acquire an angiogenic phenotype, which was characterized by the expression of a large number of pro-angiogenic molecules. Once the pro-angiogenic molecules exceed the amount of endogenous anti-angiogenic molecules which were thought to maintain vascular homeostasis in normal tissue, the angiogenic switch was turned on and tumor angiogenesis was initiated (5, 6). The pro-angiogenic molecules include vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet-derived endothelial cell growth factor (PD-ECGF), transforming growth factor-α/β (TGF-α/β), tumor necrosis factor-α (TNF-α) and so on. And the characterized endogenous anti-angiogenic molecules are interferon α/β (IFN-α/β), angiostatin, endostatin, vasostatin, tumostatin, platelet factor-4, IL-10, IL-12, thrombospondin-1, etc. At the same time, endothelial cells in the angiogenic vessels within solid tumors express proteins on their surface that are absent or barely detectable in the normal quiescent vascular endothelium, such as $\alpha_v\beta_3$ integrin and receptors for certain angiogenic growth factors (7, 8). As a result, therapy targeting tumor angiogenesis attempts either to block sigal transduction between angiogenic growth factors and their corresponding receptors or to increase angiogenic inhibitors within tumors. This review does not intend to address the impact of anti-angiogenic molecules, e.g., endostatin, vasostatin on cancer therapy, but will focus on the development of the anti-angiogenic immunotherapies that target pro-angiogenic molecules, and we will divide anti-angiogenic immunotherapy of tumors into two major classes and overview its current development: (1) active vaccination to induce effective anti-angiogenesis immunity; (2) passive immunotherapy of tumors with anti-pro-angiogenic molecules relevant antibodies.

1. Active vaccination to induce effective anti-angiogenesis immunity

The breaking of immune tolerance against important molecules such as some growth factors and receptors associated with angiogenesis should be a useful approach to cancer therapy by active immunity. However, an

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Abbreviations: VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; PD-ECGF, platelet derived endothelial cell growth factor; TGF, transforming growth factor; TNF, tumor necrosis factor; MMP, matrix metalloproteinase.

immune response to growth factors or their corresponding receptors is difficult to induce because of the immune tolerance acquired during the development of the immune system. Yet, as we known, many genes were highly conserved during the evolutionary process, which was characterized by varying degrees of gene similarity among different species, and many counterparts of the genes of human and mouse could be identified from the genome sequence of the fruit fly *Drosophila melanogaster* and of other animals such as *Xenopus laevis* (9). Thus, one approach designed to circumvent these facts is to induce autoimmunity against constitutive molecules associated with angiogenesis with xenogeneic homologous molecules. In addition, being one of the most powerful antigen presenting cells, dendritic cells (DCs) represent a mostly used target to elicit strong anti-tumor immune response against tumor angiogenesis associated antigen.

1.1 Active vaccination strategies based on xenogeneic homologous molecules

1.1.1 Vaccination with proliferating endothelial cells and pro-angiogenic molecules

Endothelial cells

Endothelial cells play a key role in the formation of new blood vessels in solid tumors, and express proteins on their surfaces such as $\alpha_v\beta_3$ integrin and receptors for certain angiogenic growth factors, which are absent or barely detectable in normal quiescent vascular endothelium (7, 8). So endothelial cells may be a potential target for anti-angiogenic therapy. As we shown above, we noted the proteins on the endothelium of new vessels in mice are homologous to those in humans and in other species to varying extents (9), so we tried to use the autologous angiogenic endothelial cells as vaccine to break immune tolerance. We prepared paraformaldehyde-fixed human and bovine endothelial cells as vaccines and tested their ability to induce anti-tumor immunity in several tumor models in mice. Immunotherapy of tumors using xenogeneic endothelial cells as vaccines was effective in affording protection from tumor growth, inducing regression of established tumors and prolonging survival of tumor-bearing mice. Autoantibodies against some receptors associated with angiogenesis in solid tumors might be provoked in a cross-reaction by the immunization of xenogeneic endothelial cells and the autoreactive immunity targeting receptors associated with angiogenesis on microvessels in solid tumor was probably responsible for the anti-tumor activity. Western blot analysis for detection possible antigens responsible for the cross-reaction showed that at least two bands with molecular sizes of 220 and 130 kDa had sizes similar to those of the known angiogenesis-associated molecules vascular endothelial growth factor receptor (VEGFR)-2 and α_v integrin (10). These observations may provide a new vaccine strategy for cancer therapy through the induction of an autoimmune response against the tumor endothelium in a cross-reaction.

VEGF/VEGFR-2

VEGF has been known to be a potent vasculogenic and angiogenic factor. VEGF induces endothelial cell proliferation, promotes cell migration, and inhibits endothelial cell apoptosis (11). In vast majority of cancers, VEGF is often at elevated levels and blocking its activity usually results in tumor angiogenesis inhibition and consequently tumor growth inhibition (12). Still we noted that the *Xenopus* homologue of VEGF is 75% and 73% identical in mouse VEGF164 and human VEGF165, respectively, at the amino acid level by searching the SwissProt database. Based upon the xenogeneic homologue of VEGF between mice and *Xenopus*, we designed a vaccine protocol based on *Xenopus* VEGF and it induced both protective and therapeutic anti-tumor immunity by overcoming the immune tolerance of mouse VEGF in a cross-reaction in mouse tumor models such as Meth A fibrosarcoma, MA782/5S mammary cancer and H22 hepatoma. VEGF-specific autoantibodies were identified by Western blotting analysis and ELISA assay. VEGF-mediated endothelial cell proliferation was inhibited *in vitro* by immunoglobulins from *Xenopus* VEGF-immunized mice. The elevation of VEGF in the sera of tumor-bearing mice was abrogated with *Xenopus* VEGF immunization. The anti-tumor activity and the inhibition of angiogenesis were acquired by the adoptive transfer of purified immunoglobulins. IgG1 and IgG2b were substantially increased in response to *Xenopus* VEGF. Anti-tumor activity and production of VEGF-specific autoantibodies could be abrogated by the depletion of $CD4^+$ T lymphocytes. Angiogenesis was apparently inhibited in tumors and corneal angiogenesis was inhibited. Based on the findings mentioned above, we may rule out the possibility that the anti-tumor activity with *Xenopus* VEGF may result from the non-specifically augmented immune response against tumor growth in host mice. Because our findings demonstrated that no increase in NK activity of spleen cells or in the level of cytokines such as IFN-α, IFN-β, TNF-α, or β-chemokine in sera was found in the immunized mice, we can also exclude the possibility that the anti-tumor activity may result from a nonspecifically augmented immune response (13).

VEGF receptor-2 (VEGFR-2, also called Flk-1 in mouse, KDR in human) is the main receptor responsible for the angiogenic activity of VEGF (14). Targeted inactivation of the gene for Flk-1 in mice resulted in the impairment of vasculogenesis and death of embryo at day 8.5 (15). Overexpression of KDR was found on activated endothelial cells of newly formed vessels (16). Moreover, VEGF/VEGFR-2 signaling pathway also played an important role in the development and progression of hematopoietic malignancies (17, 18). Sequence comparison analysis by searching the SwissProt database indicated that the primary sequence of quail VEGFR-2 (Quek-1) at the amino acid level was 67% and 70% identical with mouse homologue (Flk-1) and human (KDR), respectively. Immunotherapy with a vaccine based on quail homologous VEGFR-2 induced protective and therapeutic anti-tumor immunity in both solid and hematopoietic tumor models in mice. Autoantibodies against mouse VEGFR-2 (Flk-1) were identified by Western blot analysis and ELISA. Anti-VEGFR antibody-producing B cells were detected by ELISPOT. There was endothelial deposition of immunoglobulins within tumors. The inhibition of VEGF-mediated endothelial cell

proliferation and the anti-tumor activity were acquired by the adoptive transfer of the purified immunoglobulins from qVEGFR-immunized mice. Angiogenesis was apparently inhibited within the tumors, and the vascularization of alginate beads was also reduced. The anti-tumor activity and production of autoantibodies against Flk-1 could be abrogated by depletion of $CD4^+$ T lymphocytes (19).

FGFR-1

Being a specific and potent angiogenic factor, basic fibroblast growth factor (bFGF) exerts its biological activity through interaction with its high-affinity receptor, fibroblast growth factor receptor-1 (FGFR-1). FGFR-1 was expressed both on endothelial cells and on many types of tumors (20, 21). A comparison analysis made by searching the SwissProt database indicated that *Xenopus* homologue of FGFR-1 at the amino acid level was 80% and 74% identical with mouse FGFR-1 and human FGFR-1, respectively. So FGFR-1 may be used as another ideal target for anti-angiogenesis therapy. As the matter of fact, vaccination with the plasmid DNA vaccine based on the *Xenopus* FGFR-1 (pxFR1) was effective at anti-tumor immunity in several murine models and the autoimmunity response against FGFR-1 in mice was $CD4^+$ T lymphocytes dependent (22). The above observations showed that vaccination with xenogeneic proliferating endothelial cells or pro-angiogenic molecules could induce anti-tumor immunity and may be a feasible strategy for cancer therapy.

1.1.2 Vaccination with adhesion molecule receptors and matrix metalloproteinases

Integrins

The migration of endothelial cells is dependent on their adhesion to extra cellular matrix proteins, such as vitronectin, through a variety of cell adhesion receptors known as integrins. Integrins are heterodimeric transmembrane proteins consisting of α and β subunits with large ectodomains and short cytoplasmic tails. The selective expression of $\alpha_v\beta_3$ on activated endothelial cells suggested that this integrin may play an important role during angiogenesis and developmental neovascularization. The distribution pattern of integrin $\alpha_v\beta_3$ makes it an attractive target for tumor therapy (23, 24). To test this concept, a plasmid DNA encoding the ligand-binding domain of chicken integrin β_3 (P-BD-C) was constructed. The vaccine based on chicken homologous integrin β_3 as an antigen could induce both protective and therapeutic anti-tumor immunity in several tumor models in mice. An autoimmune response against integrin β_3 in mice may be provoked in a cross-reaction by the immunization of chicken homologous integrin β_3 vaccine and the autoantibody targeting to integrin β_3 is probably responsible for the anti-tumor activity. The anti-tumor activity and the production of integrin β_3-specific autoantibodies (manifested by significantly elevated IgG1 and IgG2b) could be abrogated by the depletion of $CD4^+$ T lymphocytes (25).

MMP

Angiogenesis is prerequisite for tumor growth and metastasis. Being an invasive process, angiogenesis requires proteolysis of the extracellular matrix (ECM), proliferation and migration of endothelial cells. Inappropriate breaking-down of extracellular matrix components is thought to play an important role in pathological conditions, including arteriosclerosis, rheumatoid arthritis, and tumor invasive growth and metastasis. The matrix metalloproteinases (MMPs) are a family of extracellular endopeptidases that selectively degrade components of the extracellular matrix (26, 27). Numerous pathological and clinical studies showed that the MMPs were frequently overexpressed in various solid tumor cells and peritumoral stromal cells. Increased activity of MMPs appeared to allow tumor to remodel its surrounding microenvironment, to grow in a permissive space, and to develop supporting stroma, including angiogenesis (28, 29). It was reported that the abrogation of MMP-2 alone inhibited the transition from the prevascular to the vascular stage during tumor development and subsequently inhibited tumor growth. Moreover, tumor-induced angiogenesis and the invasion and metastasis of tumor cells were suppressed in MMP-2-deficient mice (30, 31). These findings indicated that MMP-2 alone played an important role in angiogenesis and tumor growth. Sequence comparison analysis by searching the SwissProt database indicated that the primary sequence of mouse MMP-2 at the amino acid level was 82% and 91% identical with chicken and human homologues, respectively. We found that the plasmid DNA vaccine based on chicken homologous MMP-2 (c-MMP-2) as a model antigen could induce both protective and therapeutic anti-tumor immunity. Autoimmune response against MMP-2 may be provoked in a cross-reaction by the immunization of c-MMP-2, and the autoantibody targeting to MMP-2 was probably responsible for the anti-tumor activity. The elevation of MMP-2 in the sera of tumor-bearing mice was abrogated with the vaccination of c-MMP-2. Transmigration of human endothelial cells and tumor cells through gelatin-coated filters *in vitro* was inhibited. The inhibition of the gelatinolytic activities in tumors of mice immunized with c-MMP-2 was found by using gelatin zymography. Angiogenesis was apparently inhibited within tumors, and chick choriallantoic membrane angiogenesis was also inhibited. The anti-tumor activity and production of autoantibodies against MMP-2 were $CD4^+$ T lymphocytes dependent (32).

The above observations indicated that the induction of the autoantibodies response to the vaccines based on xenogeneic molecules, which were responsible for anti-tumor activity, may involve $CD4^+$ T lymphocytes (10, 13, 19, 22, 25). $CD4^+$ T lymphocytes could "steer" and amplify immune responses through the secretion of cytokines and expression of the surface molecules (33, 34). Moreover, $CD4^+$ T lymphocytes were prominent in classic mouse models of autoimmunity, such as experimental allergic encephalitis, systemic lupus erythematosus and autoimmune gastritis (35-37). These findings may help explain the requirement for $CD4^+$ T lymphocytes in the induction of the autoimmune response against the self-molecules in a cross-reaction.

1.2 DC-based vaccines targeting tumor angiogenesis associated molecules

Dendritic cells loaded with tumor associated antigens have been successfully used as cellular vaccines to elicit effective anti-tumor immune response in different mouse models (38). Immunization with DCs pulsed with soluble Flk-1 induced neutralizing antibody and $CD8^+$cytotoxic T cell response, suppressed tumor angiogenesis, and strongly inhibited the development of metastasis in two mouse models. *In vivo* T cell depletion experiment showed that the anti-tumor effect was $CD8^+$ T lymphocytes dependent, and $CD4^+$ T cell only had a partial effect (39). The importance of $CD4^+$ versus $CD8^+$ T cell immunity in these studies may have been determined by the use of different target antigens, adjuvants and routes of immunization.

2. Passive immunotherapy of tumors with anti-pro-angiogenic molecule relevant antibody

With the technological advances made during the past decades, monoclonal antibodies (mAbs) represent an important class of therapeutic agents because of their ability to bind to specific antigens. The first murine mAbs were reported in 1975, and by 1980 mAbs had entered studies in humans. But the human immune system normally recognized murine mAbs as foreign materials, and thus produced human anti-mouse antibodies (HAMA) which restricted the clinical application of mAbs. The development of chimeric, humanized and human mAbs circumvented the problems associated with murine mAbs (40).

In recent years, numerous mAbs targeting tumor angiogenesis showed clinical potential. Avastin (Bevacizumab), a humanized anti-VEGF mAb, has been approved by FDA as a first-line treatment for patients with metastatic colorectal cancer. By blocking VEGF, Avastin decreased tumor perfusion, vascular volume, microvascular density, interstitial fluid pressure and the number of viable, circulating endothelial and progenitor cells, and increased the fraction of vessels with pericyte coverage in rectal carcinoma patients (41). A humanized mAb to integrin $\alpha_v\beta_3$, Vitaxin, can functionally block the $\alpha_v\beta_3$ integrin and consequently suppress tumor growth by angiogenesis inhibiton in various animal models. Based on the selectivity of integrin $\alpha_v\beta_3$ on tumor endothelial cells and the effect of anti-$\alpha_v\beta_3$ in animal models, Vitaxin has been studied in phase I/II clinical trials (42). CD105 (endoglin) is highly expressed in the vascular endothelium of angiogenic tissues compared with that in normal endothelial cells. It is a receptor for transforming growth factor (TGF) β-1 and β-3 and modulates TGF-β signaling by interacting with TGF-β receptors I and/or II. Several studies revealed the upregulation of CD105 in a variety of tumor endothelial cells. Such properties suggested that CD105 would be a suitable target for anti-angiogenic therapy (43). Anti-CD105 antibody showed anti-tumor efficacy in a human skin/SCID mouse chimera model (44). And when conjugated to immunotoxin or radiolabeled, anti-CD105 resulted in long-lasting complete inhibition of human solid tumors in SCID mice (45, 46).

In the case of anti-angiogenic therapy of tumors, the anti-tumor effect may be somewhat weak and relatively transient with only mAbs, thus mAbs combined with either active immunotherapy or chemo/radiotherapy should be a better way forward.

Conclusions and perspectives

Angiogenesis plays an important role in both tumor growth and metastasis. Anti-angiogenic therapy has become another important treatment of cancer therapy. Compared with the traditional cancer therapies, anti-angiogenic therapy has the following advantages: (1) Ease of accessibility. Anti-angiogenic therapy is directed against microvascular endothelial cells that have been recruited into the tumor bed. (2) Broad applicability to many tumor types. Anti-angiogenic therapy can be used in variety types of primary tumors, as well as metastatic tumors. (3) Avoidance of tumor resistence mechanism. Targeted cells of anti-angiogenic therapy, such as endothelial cells, are genetically stable, and thus less likely to accumulate mutations that allow them to develop drug resistance in a rapid manner. (4) Thousands of tumor cells will starve and perish when only small segment of a microvessel fails. (5) Specific anti-angiogenic therapy has less toxicity (2, 47-49).

The observations mentioned above indicate that anti-angiogenic immunotherapy of tumors should be an exciting mode of cancer therapy. The induction of anti-tumor immunity by overcoming immune tolerance to self-molecules with xenogeneic counterparts may circumvent the fact that few tumor-specific antigens have been identified in human solid tumors and that the host usually shows immune tolerance to self-molecules as antigens. Also, a variety of mAbs had shown an exciting anti-tumor effect. But we should bear in mind that carcinogenesis is a complex progression, and no single anti-angiogenic therapy will be totally effective against all cancers. Thus, the challenge to us is to establish rational and effective combination therapies to do a better job.

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References

1. Folkman J. What is the evidence that tumors are angiogenesis dependent? J Natl Cancer Inst. 1990;82:4-6.
2. Zetter BR. Angiogenesis and tumor metastasis. Annu Rev Med. 1998;49:407-424.
3. Jerry WH, George MR, Paul JS. Evidence of increased angiogenesis in patients with acute myeloid leukemia. Blood. 2000;95:309-313.
4. Xu JL, Lai R, Kinoshita T, Nakashima N, Nagasaka T. Proliferation, apoptosis, and intratumoral vascularity in multiple myeloma: correlation with the clinical stage and cytological grade. J Clin Pathol. 2002;55:530-534.
5. Fan TP, Jaggar R, Bicknell R. Controlling the vasculature: angiogenesis, anti-angiogenesis and vascular targeting of gene therapy. Trends pharmacol Sci. 1995;16:57-66.

6. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. Nature. 2000;6801:249-257.
7. Ferrara N, Alitalo K. Clinical application of angiogenic growth factors and their inhibitors. Nat Med. 1999;5:1359-1364.
8. Fidler IJ, Ellis LM. The implications of angiogenesis for the biology and therapy of cancer metastasis. Cell. 1994;79:185-188.
9. Kornberg TB, Krasnow MA. The Drosophila genome sequence: implications for biology and medicine. Science. 2000;287:2218-2220.
10. Wei YQ, Wang QR, Zhao X, et al. Immunotherapy of tumors with xenogeneic endothelial cells as a vaccine. Nat Med. 2000;6:1160-1166.
11. Neufeld G, Cohen T, Gengrinovttch S, Poltork Z. Vascular endothelial growth factor (VEGF) and its receptors. FASEB J. 1999;13:9-22.
12. Kerbel RS. Tumor angiogenesis: past, present and the near future. Carcinogenesis. 2000;21:505-515.
13. Wei YQ, Huang MJ, Yang L, et al. Immunogene therapy of tumors with vaccine based on xenopus homologous vascular endothelial growth factor as a model antigen. Proc Natl Acad Sci U S A. 2001;98:11545-11550.
14. Gille H, Kowalski J, Li B, et al. Analysis of biological effects and signaling properties of Flt-1 (VEGFR-1) and KDR (VEGFR-2). J Biol Chem. 2001;276:3222-3230.
15. Shalaby F, Rossant J, Yamaguchi TP, et al. Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. Nature. 1995;376:62-66.
16. Vajkoczy P, Farhadi M, Gaumann A, et al. Microtumor growth initiates angiogenic sprouting with simultaneous expression of VEGF, VEGF receptor-2, and angiopoietin-2. J Clin Invest. 2002;109:777-785.
17. Dias S, Hattori K, Zhu Z, et al. Autocrine stimulation of VEGFR-2 activates human leukemic cell growth and migration. J Clin Invest. 2000;106:511-521.
18. Ferrajoli A, Manshouri T, Estrov Z, et al. High levels of vascular endothelial growth factor receptor-2 correlate with shortened survival in chronic lymphocytic leukemia. Clin Cancer Res. 2001;7:795-799.
19. Liu JY, Wei YQ, Yang L, et al. Immunotherapy of tumors with vaccine based on quail homologous vascular endothelial growth factor receptor-2. Blood. 2003;102:1815-1823.
20. Valesky M, Spang AJ, Fisher GW, et al. Noninvasive dynamic fluorescence imaging of human melanomas reveals that targeted inhibition of bFGF or FGFR-1 in melanoma cells blocks tumor growth by apoptosis. Mol Med. 2002;8:103-112.
21. Plum SM, Holaday JW, Ruiz A, et al. Administration of a liposomal FGF-2 peptide vaccine leads to abrogation of FGF-2-mediated angiogenesis and tumor development. Vaccine. 2000;19:1294-1303.
22. He QM, Wei YQ, Tian L, et al. Inhibition of tumor growth with a vaccine based on xenogeneic homologous fibroblast growth factor receptor-1 in mice. J Biol Chem. 2003;278: 21831-21836.
23. Gutheil JC, Campbell TN, Pierce PR, et al. Targeted antiangiogenic therapy for cancer using Vitaxin: a humanized monoclonal antibody to the Integrin $\alpha_v\beta_3$. Clini Cancer Res. 2000;6:3056-3061.
24. Hagedorn M, Bikfalvi A. Target molecules for anti-angiogenic therapy: from basic research to clinical trials. Crit Rev Oncol Hematol. 2000;34:89-110.
25. Lou YY, Wei YQ, Yang L, et al. Immunogene therapy of tumor with vaccine based on the ligand binding domain of chick homologous integrin β_3. Immunol Invest. 2002;31:51-69.
26. Gomis-Ruth FX, Maskos K, Betz M, et al. Mechanism of inhibition of the human matrix metalloproteinase stromelysin-1 by TIMP-1. Nature. 1997;389:77-81.
27. William G, Stetler S. Matrix metalloproteinases in angiogenesis: a moving target for therapeutic intervention. J Clin Invest. 1999;103:1237-1241.
28. Albini A, Melchiori A, Santi L, et al. Tumor cell invasion inhibited by TIMP-2. J Natl Cancer Inst. 1991;83:775-779.
29. Kleiner DE, Stetler-Stevenson WG. Matrix metalloproteinases and metastasis. Cancer Chemother Pharmacol. 1999; 43 Suppl: S42-S51.
30. Schnaper HW, Grant DS, Stetler-Stevenson WG, et al. Type IV collagenase(s) and TIMPs modulate endothelial cell morphogenesis *in vitro*. J Cell Physiol. 1993;156:235-246.
31. Itoh T, Tanioka M, Yoshida H, et al. Reduced angiogenesis and tumor progression in gelatinase A-deficient mice. Cancer Res. 1998;58:1048-1051.
32. Su JM, Wei YQ, Tian L, et al. Active immunogene therapy of cancer with vaccine on the basis of chicken homologous matrix metalloproteinase-2. Cancer Res. 2003;63:600-607.
33. Schwartz RH. T cell clonal anergy. Curr Opin Immunol. 1997;9:351-357.
34. Romagnani S. The Th1/Th2 paradigm. Immunol Today. 1997;18:263-266.
35. Ohashi PS. T cell selection and autoimmunity: flexibility and tuning. Curr Opin Immunol. 1996;8:808-814.
36. De Silva HD, Van Driel IR, La Gruta N, et al. $CD4^+$ T cells, but not $CD8^+$ T cells, are required for the development of experimental autoimmune gastritis. Immunology. 1998;93: 405-408.
37. Kumar V, Stellrecht K, Sercarz E. Inactivation of T cell receptor peptide-specific CD4 regulatory T cells induces chronic experimental autoimmune encephalomyelitis (EAE). J Exp Med. 1996;184:1609-1617.
38. Avigan D. Dendritic cells: development, function and potential use for cancer immunotherapy. Blood Rev. 1999; 13:51-64.
39. Li YW, Wang MN, Li HL, et al. Active Immunization Against the Vascular Endothelial Growth Factor Receptor flk1 Inhibits Tumor Angiogenesis and Metastasis. J Exp Med. 2002;195:1575-1584.
40. Reichert JM. Therapeutic monoclonal antibodies: Trends in development and approval in the US. Curr Opin Mol Ther. 2002;4:110-118.
41. Willett CG, Boucher Y, di Tomaso E, et al. Direct evidence that the VEGF-specific antibody bevacizumab has anti-vascular effects in human rectal cancer. Nat Med. 2004;2: 145-147.
42. Gutheil JC, Campbell TN, Pierce PR, et al. Targeted anti-angiogenic therapy for cancer using Vitaxin: a humanized monoclonal antibody to the integrin $\alpha_v\beta_3$. Clin Cancer Res. 2000;8:3056-3061.
43. Duff SE, Li C, Garland JM, Kumar S. CD105 is important for angiogenesis: evidence and potential applications. FASEB J. 2003;9:984-992.
44. Takahashi N, Haba A, Matsuno F, Seon BK. Antiangiogenic therapy of established tumors in human skin/severe combined immunodeficiency mouse chimeras by anti-endoglin (CD105) monoclonal antibodies, and synergy between anti-endoglin antibody and cyclophosphamide. Cancer Res. 2001;21:7846-7854.
45. Seon BK, Matsuno F, Haruta Y, Kondo M, Barcos M. Long-lasting complete inhibition of human solid tumors in SCID mice by targeting endothelial cells of tumor vasculature with antihuman endoglin immunotoxin. Clin Cancer Res. 1997;3: 1031-1044.
46. Tabata M, Kondo M, Haruta Y, Seon BK. Antiangiogenic radioimmunotherapy of human solid tumors in SCID mice using ^{125}I-labeled anti-endoglin monoclonal antibodies. Int J Cancer. 1999;82:737-742.
47. Boehm T, Folkman J, Browder T, O'Reilly MS. Anti-angiogenic therapy of experimental cancer does not induce acquired drug resistence. Nature. 1997;390:404-407.

48. Shant K, Li CG. Targeting of vasculature in cancer and other angiogenic diseases. Trends Immunol. 2001;22:129.

49. Kerbel RS, Folkman J. Clinical translation of angiogenesis inhibitors. Nat Rev Cancer. 2002;2:727-739.