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Minireview paper

Immunotherapy of tumors with vaccines based on xenogeneic homologous molecules

Yu-quan Wei[1]
[1]Center for Biotherapy of Cancer and Cancer Research Center, West China Hospital, West China Medical School, Sichuan University, Chengdu, Sichuan 610041, PRC.

This review summarizes and discusses a new vaccine strategy based on xenogeneic homologous molecules by the breaking of immune tolerance against the growth factors or their receptors associated with tumor growth in a cross-reaction between the xenogeneic homologs and self-molecules. The xenogeneic vaccine 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. It may be of importance for the further exploration of the applications of xenogeneic homologous genes identified in human and other animal genome sequence projects in cancer therapy. [© 2002 Lippincott Williams & Wilkins.]

Key words: Angiogenesis, cancer vaccine, gene therapy, immune tolerance, xenogeneic homologous gene.

Introduction

Several lines of direct and indirect evidence indicate that the human immune system can mount cellular responses and humoral responses against autologous tumors.[1–7] It has been documented that lymphocytes in human tumor microenvironments are found to be directly cytotoxic to the tumor cells by *in situ* observations of lymphocyte–tumor cell interaction.[4–7] Furthermore, cytotoxicity assays with an autologous combination of fresh effectors and target cells have been performed.[7–12] Blood lymphocytes from 5–80% of human cancer patients, depending on the histological types of tumors and metastatic status, expressed autologous tumor killing activity.[7–12] This killing activity tested at the time of surgery was correlated strongly with a postoperative clinical course.[11,12]

Correspondence to Y-q Wei, Center for Biotherapy of Cancer and Cancer Research Center, West China Hospital, West China Medical School, Sichuan University, Guo Xue Xiang, Number 37, Chengdu, Sichuan 610041, PRC.
Tel/Fax: (+86) 28 5501675;
E-mail: yuquawei@mail.sc.cninfo.net

More than 80% of patients with positive results in the autologous tumor killing tests at the time of surgery remained tumor-free and alive more than 5 years after curative operation, whereas all autologous tumor killing activity-negative patients developed local and/or distant metastases by 2 years and died by 5 years.[11,12] The strong correlation of autologous tumor killing activity with disease-free interval and total survival indicates that this activity is a meaningful prognostic indicator, suggesting that lymphocytes against autologous tumor cells may be the main effector in the immunological defense system against growth and metastasis of tumors, and providing evidence for immunological control of tumor growth and metastasis.[11,12] In clinical trials, the induction of autologous tumor killing activity by biological response modifiers has improved the clinical outcome in cancer patients who naturally have no such potential.[11–13] Thus, considerable emphasis should be placed on a strategy that induces autologous tumor killing activity in cancer patients.[7,11–13] One of approaches for the induction of the autologous tumor killing activity is based on active immunotherapy with cancer vaccines.

Various strategies for cancer vaccines, including whole tumor cell vaccines, genetically modified tumor vaccines, dendritic cell vaccines, and peptides and protein vaccines, have been developed to induce tumor-specific immune responses against autologous malignant cells.[1–3] Thus, active specific immunotherapies with cancer vaccine-based tumor antigens represent very promising approaches for cancer therapy.[1–3] However, to date, with the few exceptions of melanoma antigen, there is limited information on the identity and density of antigenic peptides and cytotoxic T lymphocyte epitopes presented by human solid tumors.[1–3] In addition, most of the identified tumor antigens are self-molecules.[1–3] As expected, the reaction of the host toward these

self-molecules may show immune tolerance to them if the host is immunized by vaccines based on these self-molecules. Efforts are therefore continuing to develop new strategy for cancer vaccines. One of approaches designed to circumvent these facts is the induction of autoimmunity against constitutive molecules associated with angiogenesis or cancer cell growth with xenogeneic homologous molecules between mice and other species.[14–17]

Immunotherapy of tumors with vaccines based on xenogeneic homologous receptors associated with angiogenesis as antigen

The generation of new blood vessels, or angiogenesis, is a complex multistep process that includes endothelial proliferation, migration and differentiation, degradation of the extracellular matrix, etc.[18–20] The complexity of the angiogenic process suggests that the existence of multiple controls of the system that can be temporarily turned on and off.[18,19] Angiogenesis is important for normal embryonic development and for the development of pathologic conditions such as cancer, rheumatoid arthritis, retinopathies, etc.[18–20] Several lines of direct and indirect evidence indicate that the growth and persistence of solid tumors and their metastases are angiogenesis dependent.[18–20] As a strategy for cancer therapy, antiangiogenic therapy attempts to stop new vessels from forming around a tumor and break up the existing network of abnormal capillaries that feeds the cancerous mass.[14,15,18–20]

Endothelial cells in the angiogenic vessels within solid tumors express proteins on their surface that are absent or barely detectable in normal quiescent vascular endothelium, including $\alpha_v\beta_3$ integrin, receptors for certain angiogenic growth factors, etc.[18–20] The proteins on the endothelium of new vessels in mouse have been known to be homologous with those in human and other species to varying extents.[21–23] For example, sequence comparison analysis by searching the Swissprot database in National Center for Biotechnology Information (NCBI) in our study indicates that the primary sequence of many angiogenesis-associated molecules of mouse such as $\alpha_v\beta_3$ integrin, vascular endothelial growth factor receptor (VEGFR), endoglin, etc., at the amino acid level is 75–90% identical with human homologs.[21–23] The breaking of immune tolerance against important molecules such as some receptors associated with angiogenesis on autologous angiogenic endothelial cells should be a useful approach for cancer therapy by active immunity.[14] However, the immunity to the self-molecules on angiogenic vessels is presumably difficult to elicit by autologous or syngeneic protein molecules as vaccine, due to immune tolerance acquired during development of the immune system. We have explored the feasibility of immunotherapy of tumors with xenogeneic homologous molecules as vaccine by the breaking of immune tolerance against those on autologous angiogenic cells in a cross-reaction between the xenogeneic homologous and self-molecules.[14,17]

To test this concept, we prepared vaccines by the use of proliferative endothelial cells cultured *in vitro*, like new vessels with proliferative activity within solid tumors. Human and bovine endothelial cells as vaccines were tested for the ability to induce antitumor immunity in several tumor models in mice.[14] Immunotherapy of tumors using xenogeneic endothelial cells as vaccine 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 may be provoked in a cross-reaction by the immunization of xenogeneic endothelial cells and the autoreactive immunity targeting to receptors associated with angiogenesis on microvessels in solid tumor was probably responsible for the antitumor activity.[14] These data also suggest that the identification of single xenogeneic homologous peptides and genes responsible for cross-reaction may provide new strategies for the development of vaccines based on single peptides or genes.

It has been known that $\alpha_v\beta_3$ integrin and VEGFR II play an important function during angiogenesis.[24–30] Blockade of the ligand binding domain of these molecules has resulted in the inhibition of angiogenesis *in vivo* or of endothelial cell proliferation *in vitro* and antitumor activity.[24–30] Sequence comparison analysis using the SwissProt database in NCBI indicated that the primary sequences of VEGFR II and α_v integrin of mice and humans were homologs that were 82 and 89% identical, respectively, at the amino acid level. We selected pairs of peptides for synthesis from the regions that shared the most identical amino acid sequences between humans and mice. Each peptide synthesized was 35 amino acids long. The possible peptides responsible for the cross-reaction were screened in two steps.[14] These peptides were probed with immunoglobulins from mice immunized with human endothelial cells, using ELISA. Mice were immunized with pairs of homologous peptides that showed immunoglobulin-positive binding and we determined their antitumor

activity. Three pairs of the homologous peptides showed immunoglobulin-positive binding and their human homologs showed antitumor activity. These immunoglobulin-binding regions were represented by amino acid residues 330–364 and 545–579 within the extracellular fragment of α_v integrin in both human and mouse, and by residues 243–277 in human and the corresponding residues 243–277 in mouse within VEGFR II. Furthermore, two of three pairs of immunoglobulin-binding sites were located within the regions encompassing ligand binding domain (residues 247–261) of VEGFR II[33] and partially encompassing ligand binding domain (residues 139–349) of α_v integrin.[30] The other pair of immunoglobulin-binding sites (residues 545–579) was located outside the ligand-binding domain of α_v integrin. However, it has been reported that some antibodies against the non-binding domain can block the function of the integrin allosterically as well.[31] Next, we found that immunoglobulins isolated from mice immunized with xenogeneic peptides of α_v integrin and VEGFR II could identify the α_v integrin and VEGFR II, respectively, on the endothelial cells, and showed the inhibition of endothelial cell proliferation *in vitro*. Also, the adoptive transfer of immunoglobulins isolated from these xenogeneic peptide-immunized mice showed inhibition of the tumor growth.[14] These identified xenogeneic homologous peptides may provide us with another strategy for the development of peptide vaccines for cancer immunotherapy.[14]

A plasmid DNA encoding the ligand-binding domain of chicken homologous integrin β_3 (P-BD-C vaccine) was constructed and tested for antitumor efficacy by immunogene therapy in mice tumor models such as Meth A fibrosarcoma, MA782/5S mammary cancer and H22 hepatoma.[17] The vaccine based on chicken homologous integrin β_3 as an antigen could induce both protective and therapeutic antitumor immunity. 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 antitumor activity.[17] This vaccine strategy may be used to target other important molecules such as growth factor receptors on angiogenic endothelial cells associated with tumor growth. This suggestion is also supported by our unpublished data that the vaccine based on VEGFR isolated from quail or integrin α_v from chicken can induce antitumor effects through autoimmunity against the tumor endothelium in mouse. Thus, the observations may provide a vaccine strategy for immunogene tumor therapy through the induction of autoimmunity against the molecules on angiogenic endothelial cells associated with tumor growth in a cross-reaction with single xenogeneic homologous gene.[17]

Immunotherapy of tumors with vaccines based on xenogeneic homologous growth factors associated with angiogenesis as antigen

The generation of new blood vessels, or angiogenesis, is regulated by some growth factors, including VEGF and fibroblast growth factors.[18–20] VEGF has been known to be a potent vasculogenic and angiogenic factor. It has been reported that the abrogation of VEGF-induced angiogenesis, including the passive immunization of a neutralizing antibody against VEGF, can suppress tumor growth *in vivo*, suggesting that VEGF plays an important role in angiogenesis in tumor growth.[18–20] In addition, a comparison analysis made by searching the Swissprot database at the NCBI indicates that the *Xenopus* homolog of VEGF is 75 and 73% identical in mouse VEGF164 and human VEGF 165, respectively, at the amino acid level.[32–34] Thus, VEGF may be used as another ideal model molecule to explore the feasibility of immunogene tumor therapy with a vaccine based on a single xenogeneic gene by overcoming the immune tolerance of growth factors associated with tumor growth in a cross-reaction between xenogeneic homologous and self-molecules. A plasmid DNA encoding *Xenopus* VEGF was constructed. At the same time, the plasmid DNA encoding the corresponding mouse VEGF and empty vector were also constructed, and used as controls. The vaccines were tested for the ability to induce antitumor immunity in several tumor models in mice.[15]

Immunogene tumor therapy with a vaccine based on *Xenopus* VEGF could induce both protective and therapeutic antitumor immunity in mouse tumor models such as Meth A fibrosarcoma, MA782/5S mammary cancer and H22 hepatoma (Figure 1). 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 antitumor activity and the inhibition of angiogenesis were acquired by the adoptive transfer of purified immunoglobulins.

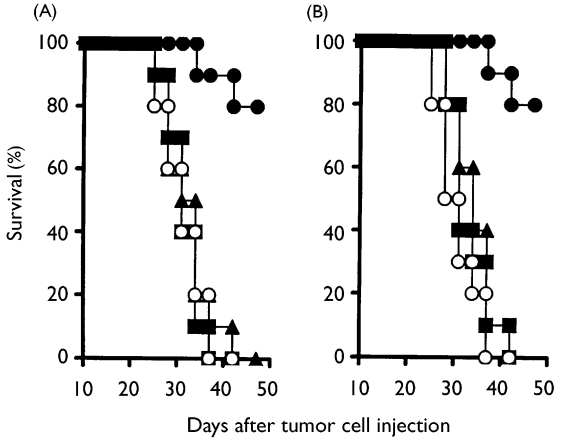

Figure 1. Induction of the protective antitumor immunity. Mice (10 mice in each group) were immunized intramuscularly with 100 μg of plasmid DNA encoding *Xenopus* homologous VEGF (solid circles), plasmid DNA encoding mouse VEGF (open circles), empty vector (solid squares) or non-immunized (saline alone) (solid triangle) once a week for 4 weeks. Mice were then challenged with 1×10^6 Meth. A fibrosarcoma (A) or H22 hepatoma cells (B) s.c. 1 week after the fourth immunization. A significant increase in survival in *Xenopus* homologous VEGF vaccine-treated mice, compared with the control groups, was found.

IgG1 and IgG2b were substantially increased in response to *Xenopus* VEGF. There were antitumor activity and production of VEGF-specific autoantibodies that could be abrogated by the depletion of $CD4^+$ T lymphocytes. Angiogenesis was apparently inhibited in tumors and corneal angiogenesis was inhibited. Thus, the autoimmune response against VEGF may be provoked in a cross-reaction by the immunization of *Xenopus* VEGF and the autoantibody targeting of VEGF is probably responsible for the antitumor activity.[15] Based on the findings mentioned above, we may rule out the possibility that the antitumor 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 the natural killer activity of spleen cells or in the level of cytokines such as interferon (IFN)-α, IFN-γ, tumor necrosis factor-α or chemokines in sera was found in the immunized mice, we can also exclude the possibility that the antitumor activity may result from a nonspecifically augmented immune response.

The mice immunized with these vaccines have been in particular investigated for the potential long-term toxicity in the present study, but no marked side effects was found. It has been reported that VEGF is often over-expressed and is required for angiogenesis within tumor, but it does not seem to have a continuous maintenance function for much of the adult vasculature.[35,36] Thus, these findings may help to explain the fact that no marked side effects were observed in the present study.

Direct injection of naked plasmid DNA intramuscularly can induce strong, long-lived immune response to the antigen encoded by the gene vaccine.[37,38] Gene transfer into muscle is simple, inexpensive and safe.[37,38] Cancer gene therapy using plasmid DNA is undergoing evaluation in clinical trials.[38] A DNA vaccine based on *Xenopus* VEGF as antigen could induce not only protective, but also therapeutic antitumor activity in several tumor models in mice without adverse effects as mentioned above. In addition, it is known that the *Xenopus* homolog of VEGF is 75 and 73% identical in mouse VEGF 164 and human VEGF 165, respectively, at the amino acid level. Furthermore, antibodies induced with XVEGF-p recognized not only mouse VEGF, but also human VEGF. The findings mentioned above suggest that XVEGF-p vaccine may have a potential application to the treatment of cancer patients.[15]

The proliferation of tumor cells themselves is also regulated by some growth factors, including transforming growth factor-α, amphiregulin and interleukin-6 under certain circumstances.[39,40] It is also conceivable that overcoming immune tolerance of these growth factors associated with cancer cell growth may be a useful approach to cancer therapy by active immunity with vaccines based on xenogeneic molecules

Role of $CD4^+$ T lymphocytes in xenogeneic homologous molecule-induced antitumor activity

It has been reported that a cellular rather than a humoral immune response is responsible for the rejection of the transplanted tumors.[3,42] In addition, antitumor immunity depends on $CD8^+$ T lymphocytes in some mouse models, whereas $CD4^+$ T lymphocytes often have little, if any, function.[2,3,42] Some molecule targets of tumor-specific $CD8^+$ T lymphocytes have been identified in human and mouse systems. $CD8^+$ T lymphocytes have been the focus of recent efforts in the development of a therapeutic antitumor vaccine.[2,3] However, mice depleted of $CD4^+$ T lymphocytes by the injection of monoclonal antibody against CD4 and immunized with vaccines based on xenogeneic molecules were not protected from tumor challenge.[14,15,17] These

mice did not develop detectable autoantibodies against the target molecules associated with angiogenesis.[14,15,17] In contrast, treatment with monoclonal antibody against CD8 or natural killer cells or control IgG failed to abrogate the antitumor activity. These data indicate that the induction of the autoantibodies response to the vaccine based on xenogeneic molecules, which is responsible for antitumor activity, may involve $CD4^+$ T lymphocytes.[14,15,17] $CD4^+$ T lymphocytes can 'steer' and amplify immune responses through the secretion of cytokines and expression of the surface molecules.[43,44] Moreover, $CD4^+$ T lymphocytes are prominent in classic mouse models of autoimmunity, such as experimental allergic encephalitis, systemic lupus erythematosus and autoimmune gastritis.[45–47] 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.

Conclusions and perspectives

Taken together, the observations mentioned above indicate that a vaccine strategy for cancer therapy based on xenogeneic homologous molecules could induce the autoimmune response against the self-receptors or cytokines associated with angiogenesis for tumor growth in a cross-reaction. Based on similar strategy targeting important molecules associated with angiogenesis by the induction of the autoimmunity discussed above, we also tested the ability to induce autoimmunity against constitutive molecules such as epidermal growth factor receptor (EGFR) on tumor cells themselves associated with tumor growth with a single xenogeneic homologous EGFR gene vaccine. The vaccine based on EGFR from the fruit fly *Drosophila melanogaster* or from birds can induce autoimmunity against EGFR-positive tumors in mouse models (unpublished data). These findings suggest that this vaccine strategy can be used to target other growth factors receptors or their growth factors associated with the proliferation of cancer cells themselves. Thus, the induction of antitumor 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.

The many genes were highly conserved during evolutionary process, which was characterized by the gene similarity to varying degrees among different species.[48] Many counterparts of the genes of human and mouse can be identified from the genome sequence of the fruit fly *D. melanogaster* and of the other animals such as *Xenopus laevis*.[48] Thus, the breaking of immune tolerance to the self-molecules involving angiogenesis or tumor cell proliferation with xenogeneic counterparts may be of importance to the further exploration of the applications of xenogeneic homologous genes identified in human and other animal genome sequence projects in cancer therapy.

To explore this potential application of immunotherapy with vaccines based on xenogeneic homologous molecules to the treatment of cancer patients, we are carrying out a variety of preclinical studies on xenogeneic vaccines to apply for clinical trials for the treatment of patients with advanced cancer.

References

1. Marincola FM. Cancer vaccines: basic principles. In: Rosenberg SA, eds. *Principles and practice of the biologic therapy of cancer*. Philadelphia, PA: Lippincott Williams & Wilkins 2000: 601–15.
2. Boon T, Coulie PG, Van den Eynde B. Tumor antigens recognized by T cells. *Immunol Today* 1997; **18**: 267–8.
3. Rosenberg SA. Progress in human tumor immunology and immunotherapy. *Nature* 2001; **411**: 380–4.
4. Wei Y-Q, Hang Z-B. *In situ* observation of inflammatory cell–tumor cell interaction in human seminoma (germinoma). *Hum Pathol* 1992; **23**: 421–9.
5. Hang ZB, Wei Y-Q. Direct ultrastructural evidence of lymphocyte-mediated cancer cell lysis in the microenvironments of Chinese nasopharygeal carcinomas. *Hum Pathol* 1991; **22**: 320–5.
6. Wei Y-Q, Hang Z-B. *In situ* observation of inflammatory cell–tumor cell interaction in human lung carcinoma. *Immunol Invest* 1989; **18**: 1095–104.
7. Wei Y-Q, Zhao X, Kariya Y, Fukada Y, Teshigawara K, Uchida A. Induction of autologus tumor killing by heat treatment of fresh human tumor cells: involvement of γ/δ T cells and Heat shock protein(HSP)70. *Cancer Res* 1996; **56**: 1104–8.
8. Zhao X, Wei Y-Q, Kariya Y, Fukada Y, Teshigawara K, Uchida A. Accumulation of γ/δ T cell in human dysgerminoma and seminoma: roles in autologous tumor cell killing activity and the formation of granuloma. *Immunol Invest* 1995; **24**: 607–10.
9. Wei Y-Q, Zhao X, Kariya Y. Impairment of host immunity by surgical stress and its prevention by urinastin. *Proc Jpn Soc Immunol* 1992; **22**: 297.
10. Wei Y-Q, Zhao X. The investigation of autologous tumor-killing activity in human tumors. *Biother Today* 1994; **2**: 33–5.

11. Wei Y-Q, Uchida A. Biological and clinical significance of autologous tumor-killing activity in human cancer and its induction therapy. In: Tsuchiya M, ed. *Current topics in mucosal immunology*. New York: Elsevier 1994; 447–52.
12. Wei Y-Q, Zhao X. Autologous tumor killing activity and adoptive immunotherapy. *Chin J Cancer Biother* 1997; **4**: 173–5.
13. Guo Y, Wu MC. Effective tumor vaccine gene rated by fusion of hepatoma cells with activated B cells. *Science* 1994; **263**: 518–20.
14. Wei Y-Q, Wang Q-R, Zhao X, *et al*. Immunotherapy of tumors with xenogeneic endothelial cells as a vaccine. *Nat Med* 2000; **6**: 1160–6.
15. Wei Y-Q, Huang M-J, 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 USA* 2001; **98**: 11545–50.
16. Luo F, Wei Y-Q, Kang B. Immunotherapy of melanoma with xenogeneic melanocytes. *Chin J Oncol* 2000; **22**: 167–79.
17. Lou Y-Y, Wei Y-Q, Yang L. Immunogene therapy of tumor with vaccine based on the ligand binding domain of chick homologous integrin β_3. *Immunol Invest* 2002; **31**: in press.
18. Folkman J. What is the evidence that tumors are angiogenesis dependent? *J Natl Cancer Inst* 1990; **82**: 4–6.
19. Risau W. Mechanism of angiogenesis. *Nature* 1997; **386**: 671–4.
20. 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.
21. Quinn TP, Peters KG, De Vries C, Ferrara N, Williams LT. Fetal liver kinase 1 is a receptor for vascular endothelial growth factor and is selectively expressed in vascular endothelium. *Proc Natl Acad Sci USA* 1993; **90**: 7533–7.
22. Djaffar I, *et al*. A new alternative transcript encodes a 60 kDa truncated form of integrin beta 3. *Biochem J* 1994; **15**: 69–74.
23. Eichmann A, Marcelle C, Breant C, Le Douarin NM. Molecular cloning of Quek 1 and 2 two quail vascular endothelial growth factor (VEGF) receptor-like molecules. *Gene* 1996; **174**: 3–8.
24. Mandriota SJ, Menoud P, Pepper MS, *et al*. Transforming growth factor β1 down-regulates vascular endothelial growth factor receptor 2/fik-1 expression in vascular endothelial cells. *J Biol Chem* 1996; **271**: 11500–5.
25. Yeh CH, Peng HC, Huang TF. Cytokines modulate integrin $\alpha_v\beta_3$-mediated human endothelial cell adhesion and calcium signaling. *Exp Cell Res* 1999; **251**: 57–66.
26. Soker S, Fidder H, Neufeld G, Klagsbrun M. Characterization of novel vascular endothelial growth factor (VEGF) receptors on tumor cells that bind VEGF165 via its exon 7-endoded domain. *J Biol Chem* 1996; **271**: 5761–7.
27. Piossek C, Schneider-Mergener J, Schirner M, Vakalopoulou E, Germeroth L, Thierauch K. Vascular endothelial growth factor (VEGF) receptor II-derived peptides inhibit VEGF. *J Biol Chem* 1999; **274**: 5612–9.
28. Cheifetz S, *et al*. Endoglin is a component of the transforming growth factor-β receptor system in human endothelial cells. *J Biol Chem* 1992; **267**: 19027–30.
29. Eliceiri BP, Cheresh DA. The role α_v integrins during angiogenesis: insights into potential mechanisms of action and clinical development. *J Clin Invest* 1999; **103**: 1227–30.
30. Smith JW, Cheresh DA, *et al*. Integrin $\alpha_v\beta_3$-ligand interaction. Identification of a heterodimeric RGD binding Site on the vitronectin receptor. *J Biol Chem* 1990; **265**: 2168–72.
31. Lin EC, Carron CP, Meyer DM, Smith JW. A series of function blocking antibodies against the $\alpha_v\beta_3$ integrin bind allosteric to the ligand binding site and induce ligand dissociation. *Cell Adhes Commun* 1998; **6**: 451–64.
32. Cleaver O, Tonissen KF, Saha MS, Krieg PA. Neovascularization of the *Xenopus* embryo. *Dev Dyn* 1997; **210**: 66–77.
33. Breier D, AlbrechtU, Sterrer S, Risau W. Expression of vascular endothelial growth factor during embryonic angiogenesis and endothelial cell differentiation. *Development* 1992; **114**: 521–32.
34. Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science* 1989; **246**: 1306–9.
35. Yancopoulos GD, Davis S, Gale NW, Rudge JS, Wiegand SJ, Holash J. Vascular-specific growth factors and blood vessel formation. *Nature* 2000; **407**: 242–8.
36. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. *Nature* 2000; **407**: 249–57.
37. Rinaldi M, Ria F, Parrella P, *et al*. Antibodies elicited by naked DNA vaccination against the complementary-determining region 3 hypervariable region of immunoglobulin heavy chain idiotypic determinants of B-lymphoproliferative disorders specifically react with patients' tumor cells. *Cancer Res* 2001; **61**: 1555–62.
38. Roth JA, Cristiano RJ. Gene therapy for cancer: what have we done and where are we going? *J Natl Cancer Inst* 1997; **89**: 21–39.
39. De Luca A. Simultaneous blockage of different EGF-like growth factors results in efficient growth inhibition of human colon carcinoma xenografts. *Oncogene* 2000; **19**: 5863–71.
40. O-Charoenrat P, Rhys-Evans P, Eccles S. Expression and regulation of c-erbB ligands in human and neck squamous carcinoma cells. *Int J Cancer* 2000; **88**: 759–65.
41. Treon SP, Anderson KC. Interleukin-6 in multiple myeloma and related plasma cell dyscrasias. *Curr Opin Hematol* 1998; **5**: 42–8.
42. Barth RJJ, Bock SN, Mule JJ, Rosenberg SA. Unique murine tumor-associated antigens identified by tumor infiltrating lymphocytes. *J Immunol* 1990; **144**: 1531–7.
43. Murray JS. How the MHC selects T_h1/T_h2 immunity. *Immunol Today* 1998; **19**: 157–63.
44. Romagnani S. The T_h1/T_h2 paradigm. *Immunol Today* 1997; **18**: 263–6.
45. Ohashi PS. T cell selection and autoimmunity: flexibility and tuning. *Curr Opin Immunol* 1996; **8**: 808–14.

46. 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**: 1609–17.
47. De Silva HD, Van Driel IR, La Gruta N, Toh BH, Gleeson PA. $CD4^+$ T cells but not $CD8^+$ T cells are required for the development of experimental autoimmune gastritis. *Immunology* 1998; **93**: 405–8.
48. Kornberg TB, Krasnow MA. The *Drosophila* genome sequence: implications for biology and medicine. *Science* 2000; **287**: 2218–20.

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