Xenogeneic homologous genes, molecular evolution and cancer therapy*

TIAN Ling (田 聆) and WEI Yuquan (魏于全)**

Center for Biotherapy of Cancer and Cancer Research Center, First University Hospital, West China Medical Center, Sichuan University, Chengdu 610041, China

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Abstract Cancer is one of the main causes for death of human beings to date, and cancer biotherapy (mainly immunotherapy and gene therapy) has become the most promising approach after surgical therapy, radiotherapy and chemotherapy. However, there are still many limitations on cancer immunotherapy and gene therapy; therefore great effort is being made to develop new strategies. It has been known that, in the process of evolution, a number of genes, the so-called xenogeneic homologous genes, are well-conserved and show the structural and/or functional similarity between various species to some degree. The nucleotide changes between various xenogeneic homologous genes are derived from mutation, and most of them are neutral mutations. Considering that the subtle differences in xenogeneic homologous genes can break immune tolerance, enhance the immunogenicity and induce autologous immune response so as to eliminate tumor cells, we expect that a strategy of inducing autoimmune response using the property of xenogeneic homologous genes will become a new therapy for cancer. Moreover, this therapy can also be used in the treatment of other diseases, such as autoimmune diseases and AIDS. This article will discuss the xenogeneic homologous genes, molecular evolution and cancer therapy.

Keywords: xenogeneic, molecular evolution, cancer immunotherapy, gene therapy.

According to the reports of WHO, about 10 million patients suffer from cancer and about 6.6 million die each year on the globe. It is also estimated that there will be 300 million new cases and 100 million patients will die in the next 25 years. Cancer has become one of the main killers for human beings. Now therapeutic strategies for cancer are shifting from physical (radiotherapy) or chemical (chemotherapy) method to biological method (biotherapy); the latter includes mainly immunotherapy and gene therapy. Biotherapy is now the most promising approach to cancer therapy and may rank fourth cancer therapy following surgical therapy, radiotherapy and chemotherapy[1].

Cancer immunotherapy and gene therapy for inducing tumor-specific immune response to malignant cells by whole tumor cell vaccine, genetically modified tumor vaccine, dendritic cell vaccine, peptide and protein vaccine, etc. have been developing rapidly. However, except for melanoma tumor antigens, information on the characteristics of antigenic peptides and cytotoxic T lymphocyte (CTL) epitopes presented by most of human solid tumors (heptocarcinoma, lung cancer gastric cancer, and so on) is very limited. Furthermore, most of the identified antigens are self-molecules. As

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** Corresponding author, E-mail: yuquanwei@mail.sc.cninfo.net

expected, the reaction of the host to these self-molecules may show immune tolerance and it is difficult to induce effective immune response if the host is immunized by these self-molecules[1,2]. Therefore, great efforts are being made to develop new strategies for cancer immunotherapy and gene therapy.

With the great success in sequencing human genome, the genomic sequences of chicken, pig, horse, zebra fish, and other animals, have been clarified[3~5]. The problem confronting us now is how to effectively use this information in medicine and cancer therapy.

In recent years, our laboratory has been engaged in the study on the xenogeneic homologous genes of different species for cancer immunotherapy and gene therapy. Here we give a review of our recent achievements and make a brief discussion on xenogeneic homologous genes, molecular evolution and cancer immunotherapy.

1 Xenogeneic homologous genes

Homology, the foundation in the course of evolution, exists in different organisms. Before Darwin, homology belonged to morphology, which was explained as the ideal archetypes of philosopher's design. Darwin expounded homology from the scope of naturalistics rather than teleology, and construed it as a consequence of modification of the common ancestor. This kind of modifications completely complied with some naturalistic mechanisms, which are genetic programs and developmental pathways[6]. However, Gavin thought that homology might or might not be generated from the inheritance of the common ancestor, but it is absolutely generated from the similarity of genes or the similarity of developing process[7]. In the view of modern molecular biology, homology is a qualitative concept, which describes the relationships between genes. This relationship is based on a quantitative similarity and shows the match degree between two compared sequences. Homology suggests that the compared sequences have the common origin and go divergent during evolutionary process[6,8]. The counterparts of homologous sequences are homologs. If the homologs are whole genes precisely, they are then called homologous genes. Homologs have the same origin or similar biological functions.

Comparative genomics pointed out that there are 70 ~ 100 thousands of genes in all mammals[3], a certain number of which are rather conserved. These genes show certain degree of similarity in structures and/or functions between different species. They are xenogeneic homologous genes. For example, the epidermal growth factor receptor (EGFR) gene of human beings has the homology of 88% ~ 93% with mice, 72% ~ 83% with birds, and 40% ~ 56% with fruit flies.

The structural similarity between xenogeneic homologous genes determines their functional similarity, but the subtle differences between them again guarantee that they perform their functions in somewhat different ways. But how were these subtle differences generated and how did they evolve? Can we make use of these subtle differences to overcome the immune ignorance or immune tolerance to enhance immunity and induce autologous immune response against tumor cells? The questions remain to be answered.

2 Xenogeneic homologous genes in molecular evolution

Evolution is the mainstream of life. Homologous genes go divergent in the course of evolution and

result in some structural differences between species. We can then deduce the relationships of these organisms by comparing their differences. Cytochrome c is a kind of protein related to cell respiration, which is usually composed of 104 amino acids, and exists in the cytoplasm of prokaryote of aerobe and eukaryote cells. By comparing the amino acid sequences of cytochrome c among 30 species, it was found that about half of these amino acids are the same, and about 30 amino acids are located in fixed positions and the rest of them are replaced by other amino acids. For example, the amino acid sequence of cytochrome c of human beings is completely the same as that of chimpanzees, and only one amino acid changes from macaques, nine from rabbits, but 35 from wheat and 40 from yeast[8~10]. All this suggests that the cytochrome c of these organisms are homologous to each other, and the changed amino acids reflect the genetic relationships between them. Fig. 1 shows an evolutionary tree of animals based on nucleotide changes in cytochrome c gene.

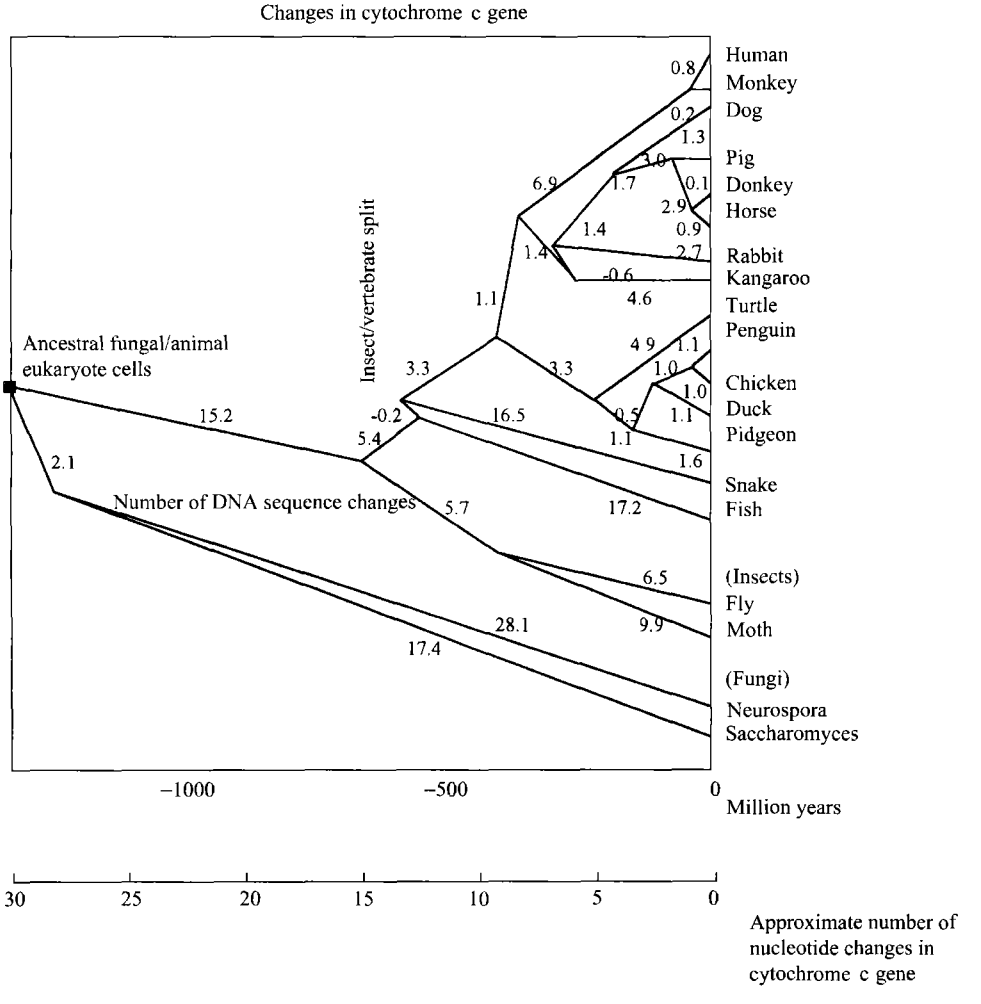

Fig. 1 An evolutionary tree (phylogeny) of animals based on nucleotide changes in the cytochrome c gene. Numbers show the average of nucleotide changes along each branch.

The biological evolution of gene is through the action of the multi-copy genes by duplication and

non-equal exchange at first, followed by the formation of the multi-member gene family or a new gene through accumulated mutations, gene rearrangement and natural selection[8~10]. Fig. 2 illustrates the possible evolutionary pathway of ancestral globin's gene structure of different species.

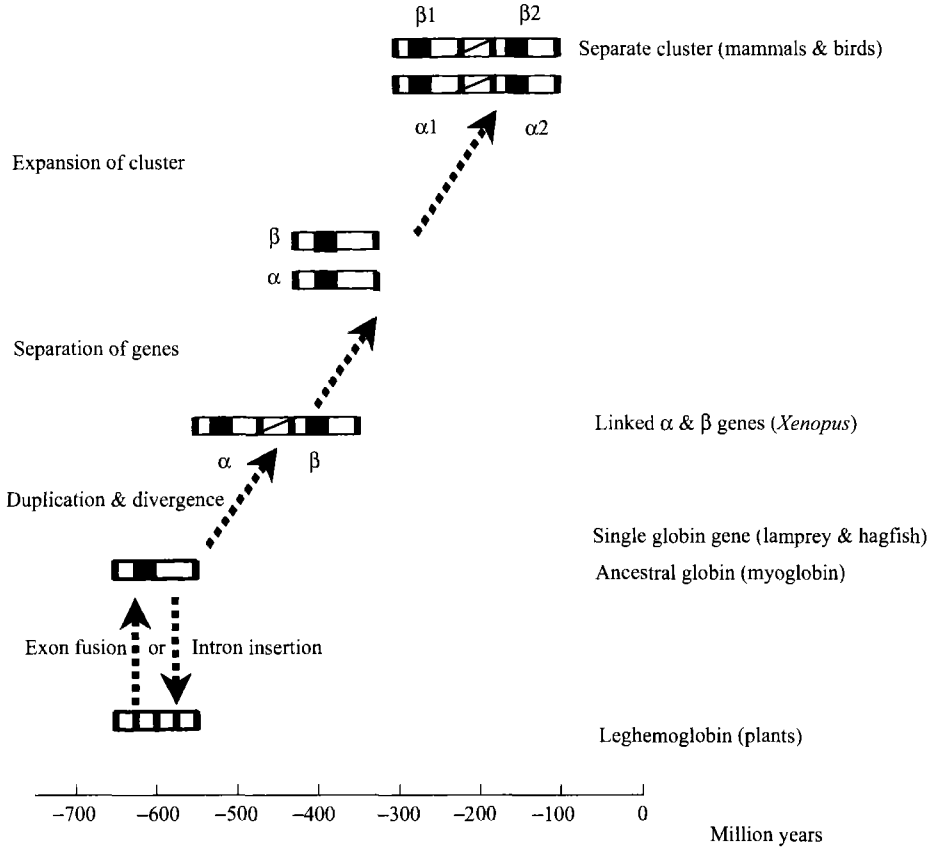

Fig. 2 The globin genes evolved by a series of duplications, transpositions and mutations from a single ancestral gene.

By comparing the amino acid sequences of some homologous proteins in different species, it was found that the extent of difference between the proteins is closely related to the time they evolved in different ways from a common ancestor. That is to say, the occurrence and accumulation of a gene mutation during evolution are constant generally. This is called evolutionary clock. It was also found that the evolutionary tempo of globins was about 1% base substitution per 10.4 million years by comparing the changes of globin in different species, and the timetable of globin divergence could therefore be deduced[8~10].

Genes sustain different negative selective pressure during evolution. Generally, the more important the gene product is, the more pressure its structure and function bear, and the slower the rate of mutation accumulation is. Even more, the negative selective pressure on different segments of the same gene also varies with its significance to gene function and/or structure.

The differences in bases between xenogeneic homologous genes derive from mutations. Most of them are neutral mutations, including synonymous variation that does not change its coded amino acids and silent mutation that does not affect its spatial structure of proteins. However, the biological significance of these mutations remains unclear yet. In recent years, we probed into the significance of the differences between xenogeneic homologous genes in the induction immune response, especially induction of autoimmunity against tumors.

3 Xenogeneic homologous genes and their significance in anti-cancer function

It has been known that tumor cells would produce one or more kinds of tumor antigens during its course of malignant transformation and proliferation. However, in most cases, tumor antigen is differentiation antigen with very weak immunogenicity that is not enough to induce active immune response. In addition, in the viewpoint of immunology, tumor cells themselves are the cells of host, capable of continuously expressing "normal" antigen (gene overexpression) and/or abnormal antigen (resulting from gene modification, mutation or deletion). We can therefore consider tumor antigen as self-antigen in this sense. In normal physiological state, a body cannot evoke immune response to self-antigen, which is immune tolerance[11~13]. In fact, self-antigen is the most compatible and the richest antigen the host immune system must tolerate. The induction and maintenance of self-antigen is mediated by several kinds of mechanisms by which the normal tissues can be protected from improper injury. However, when the host cryptic antigen is released or changes by some kinds of biological, physical or chemical reasons, it can induce autologuos immune response, which acts on the tissues or cells where the target antigen is located, resulting in pathological changes and blocking the function of the corresponding organelle. So if we can release the cryptic self-antigen of tumor cells or modify it to some extent, we could induce specific autologous immune response to autologous tumor cells, consequently regressing or suppressing tumors[14,15]. In other words, we can do cancer immunotherapy by inducing autologous immune response of a body.

In general, cancer vaccines are based on the weak immunogenicity of target tumor antigen. They are mixed with adjuvant in order to produce, recover or enhance anti-cancer immune response and kill the residual or invasive tumor cells. The potential target of anti-self-antigen or anti-tumor includes overexpressed protein, tissue-specific differentiation antigen, development proteins tumor cells abnormally expressed, and so on. How can we enhance the immunogenicity of target antigen then?

As we mentioned above, there exist a certain number of xenogeneic homologous genes between various species. We think that xenogeneic homologous genes between various species should have immunological significance in the course of evolution. We hypothesize that we could make use of the subtle differences in xenogeneic homologous genes deriving from evolution to break immune tolerance, enhance immunogenicity and induce autologous immune response of tumor cells and finally kill tumor cells[2,14,15,]. The mechanism is probably as follows: although the neutral mutation of xenogeneic homologous gene from evolution does not lose or change its function, it probably affects or changes its mode of immune response. When xenogeneic homologous genes are introduced into a host and express corresponding xenogeneic homologous protein, the host will recognize it as alien antigen and eliminate it by producing the specific antibodies or CTL. On the other hand, it will lead non-specific cross im-

mune reaction because of the similarity between xenogeneic homologous proteins and the related protein in the host, thereby inducing autologous immune response and breaking the immune tolerance of the body to this protein[15]. Maybe the immunological rejection during heterogenous transplantation is also due to the existence of xenogeneic homologs (such as genes, peptides and proteins).

In order to confirm the hypothesis mentioned above, we selected some cancer cell proliferation-associated molecules (EGFR, insulin-like growth factor receptor (IGFR), etc.) and tumor angiogenesis-associated molecules (vascular endothelial growth factor (VEGF), $\alpha v\beta 3$ integrin, endoglin, vascular endothelial growth factor receptor (KDR), fibroblastic growth factor receptor (FGFR), Tie2, etc.) from *Xenopus laevis*, bird, mouse, pig, bovine, even from fruit fly *Drosophila melanogaster* as target molecules. We isolated the counterparts of these homologous genes, immunized mouse models with the plasmid DNA vector or adenoviral vector inserted with these xenogeneic genes or their recombinant proteins or synthetic peptides, observed their activity of anti-tumors (including mammary cancer, lung cancer, melanoma, hepatocarcinoma and fibrosarcoma) and explored the possible molecular and immunological mechanisms. Some of the work is in progress.

We found that immunotherapy of tumors using fixed xenogeneic whole endothelial cells as vaccine was effective in affording protection from tumor growth, inducing regression of established tumor, and prolonging survival of tumor-bearing mice[2,14,15]. We used 3% paraformaldehyde-fixed human (HUVE, HDMVE and HUV-EC-C) and bovine (GEN-T) endothelial cells as vaccines to immunize mice intraperitoneally once a week for four weeks with different doses ($1 \times 10^2 \sim 1 \times 10^7$ cells/mouse). At the same time, a mouse endothelial cell line (SVEC4-10), a human vascular smooth muscle cell line (T/G HA-VSMC), an EBV-transformed human B-lymphoblastoid cell line (RPMI 7666) and PBS were used as controls. All the immunized mice were then challenged with $1 \times 10^5 \sim 1 \times 10^7$ live tumor cells after the fourth immunization. Tumor grew progressively in all unimmunized mice (PBS alone) or SVEC4-10 or T/G HA-VSMC or RPMI 7666 cells-immunized mice, but there was complete protection from tumor growth in HUVE, HDMVE, HUV-EC-C, or GEN-T-immunized mice. The protective effect was dose-dependent and long lasting[2].

The therapeutic efficiency of xenogeneic endothelial cells as vaccine was tested in mice with established tumors. The mice were treated with HUVE or GEN-T twice a week for four weeks starting at day 7 after the injection of Meth A fibrosarcoma, hepatoma or breast cancer cells when the tumor was visible and palpable. The vaccine injection produced retarded progression and final regression of the established tumors. The chance of survival of the tumor-bearing mice treated with GEN-T or HUVE was also significantly greater than that of the untreated mice or SVEC4-10-immunized mice. The mice immunized with xenogeneic endothelial cells were investigated for gross measures such as weight loss, ruffling of fur and life-span, and no adverse consequences were demonstrated. In addition, no pathological changes in liver, lung, kidney, spleen and brain were found by the microscopic examination[2].

In an attempt to explore the possible mechanism by which anti-tumor activity was induced with xenogeneic endothelial cells, we treated HUVE, HUV-EC-C, GEN-T, HDMVE, SVEC4-10 and tumor cells with various doses of immunoglobulins (Ig) isolated from HUVE- or SVEC4-10- immunized,

or unimmunized mice. Treatment with Ig from the HUVE-immunized mice resulted in apparent inhibition of proliferation of human, mouse and bovine endothelial cells, compared with those from SVEC4-10-immunized or unimmunized mice. By contrast, the treatment had no effect on proliferation of tumor cells. Adoptive transfer of Ig isolated from HUVE-, GEN-T- or HDMVE-immunized mice was effective in protection from the tumor growth. Adsorption of Ig with fixed-endothelial cells before adoptive transferring could abrogate its anti-tumor activity, but not affect with T/G HA-VSMC[2].

In flow cytometric analysis, both human and mouse endothelial cell lines showed positive staining detected by the sera isolated from the HUVE-immunized mice, but negative staining by the sera from SVEC4-10-immunized or unimmunized mice (PBS alone). In addition, no positive staining was observed in tumor cells that reacted with the sera from immunized or unimmunized mice. Furthermore, the positive staining for the endothelial cells could be found by use of FITC-goat-anti-mouse IgG (Fab specific) as secondary antibody, but not by use of FITC-goat-anti-mouse IgM or IgA. These suggested IgG may be responsible for positive staining for the endothelial cells. Ig subclass response to the endothelial cells was determined by ELISA, and it was found that IgG1, IgG2a and IgG2b rose significantly, but IgM or IgA level increased little in sera obtained from the mice at day 7 after the fourth immunization, as compared with controls[2].

To identify the possible deposition of autoantibodies in endothelial cells, we stained microvessels of tumor tissues by immunohistochemical method. There was endothelial deposition of Ig within the tumor tissues from HUVE-immunized mice, but not within those of the unimmunized or SVEC4-10- or T/G HA-VSMC-immunized mice. In addition, no endothelial deposition of Ig was found in normal quiescent endothelium within the major organs such as kidney, liver, spleen, brain in the immunized or unimmunized mice. Angiogenesis was apparently suppressed within the tumors of the mice treated with adoptive transfer of Ig and within the tumors of GEN-T- or HUVE-treated mice. Sequential analysis of microvessel density was performed as the tumor was regressing in response to the vaccine. The microvessel density gradually decreased as a result of the prolongation of the vaccine treatment. Also, blood-vessel length and area of neovascularization in micropocket assay were inhibited by $68 \pm 6\%$, $72 \pm 5\%$ and $81 \pm 7\%$ respectively in the mice treated by the adoptive transfer of Ig isolated from mice immunized by HUVE cells, as compared with controls. Also, similar results were found by direct immunization with HUVE cells[2].

It was reported that the anti-tumor immunity depended on CD8 + T lymphocytes in some mouse models, whereas CD4 + T lymphocytes often had little, if any, influence[16,17]. Some molecular targets of tumor-specific CD8 + T lymphocytes have been identified in human and mouse. CD8 + T lymphocytes have been the focus of recent efforts toward development of therapeutic anti-tumor vaccine[16,17]. However, we found that mice depleted of CD4 + T lymphocytes by the injection of anti-CD4 monoclonal antibody (mAb) and vaccinated with xenogeneic endothelial cells were not protected from tumor challenge. At the same time, the mice depleted of CD4 + T lymphocytes did not develop detectable antibodies against the endothelial cells. In contrast, treatment with anti-CD8 or anti-NK mAb or control IgG failed to abrogate the anti-tumor activity. In addition, T lymphocytes isolated from the spleens of the mice immunized by xenogeneic endothelial cells showed no increase in cytotoxicity against syngeneic endothelial cells or tumor cells in ^{51}Cr release assay[2]. These data suggest that the

induction of the antibody response to the endothelial cells, which is responsible for xenogeneic endothelial cells-induced anti-tumor activity, may involve CD4 + T lymphocytes. It has been known that CD4 + T lymphocytes can steer and amplify immunal responses through secretion of cytokines and expression of surface molecules[18,19]. Also, it has been reported that CD4 + T lymphocytes play a prominent role in the classic mouse models of autoimmunity, such as experimental allergic encephalitis, systemic lupus erythematosus and autoimmune gastritis[19]. These findings may help explain the requirement for CD4 + T lymphocytes in the induction of autoimmune response against the tumor endothelium in a cross-reaction[2].

We prepared vaccines using proliferous endothelial cells cultured *in vitro*, like new vessels with proliferous activity within solid tumors. In this context, the endothelial cells in culture may be heterogenous and may not express genes in the original tissue, while they may lose the expression of a number of antigens. However, we found that Ig or serum isolated from mice immunized with cultured xenogeneic endothelial cells showed positive staining not only for cultured endothelial cells but also for microvessels within tumor tissues or those within wound healing. These findings suggested that there might be still some common antigens or cross-reactive epitopes present between cultured and intratumoral endothelial cells, which is responsible for autoimmune response against the tumor endothelium in a cross-reaction. In addition, this suggestion is also supported by some findings that the cultured endothelial cells can still express some genes that are expressed in the microvessels within tumor tissues. For example, some molecules such as VEGFR II, $\alpha v\beta 3$ integrin and endoglin, which are associated with angiogenesis within tumor, could be found in primary culture of HUVE, HDMVE and bovine endothelial cells, as well as in HUV-EC-C and some bovine endothelial cell lines[20,21]. Some of these molecules were also present on the endothelial cells used in our study, as confirmed by us.

It has been known that αv integrin and VEGFR II play an important role during angiogenesis[22~24]. 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 the anti-tumor activity[22~24]. In our study, at least two out of six bands in Western blot analysis showed similar sizes of the known angiogenesis-associated molecules, VEGFR II and αv integrin[2]. We also found that these two molecules on the endothelial cells used in our study could be identified by the commercially available antibodies against VEGFR II and αv integrin in flow cytometry and in Western blot analysis, and Ig isolated from HUVE cell-immunized mice showed positive reaction against the recombinant extracellular parts of VEGFR II and αv integrin in ELISA assay. Three pairs of possible peptides responsible for cross-reaction could be identified within the extracellular parts of these molecules. Furthermore, two of three pairs of Ig-binding sites were located within the regions encompassing ligand-binding domain (residues 247 ~ 261) of VEGFR II and partially encompassing ligand-binding domain (residues 139 ~ 349) of αv integrin. The other pair of Ig-binding site was located outside the ligand-binding domain of αv integrin. However, it has been reported that some antibodies against the non-binding domain can block the function of integrin allosterically as well[25]. We found that Ig isolated from the mice immunized with xenogeneic peptides of αv integrin and VEGFR II could identify the αv integrin and VEGFR II respectively on the endothelial cells, and showed the inhibition of the endothelial cell proliferation *in vitro*. Also, the adoptive transfer of Ig isolated from these xenogeneic peptides-immu-

nized mice showed the inhibition of the tumor growth. Thus, the findings mentioned above suggest that the cross-reaction observed may be in part involved in the epitopes within VEGFR II and αv integrin on the endothelial cells. The other four bands in Western blot analysis could handly match the sizes of the known angiogenesis-associated molecules. Whether or not they belong to new angiogenesis-associated molecules for cross-reaction is an intriguing question to be further explored. The findings mentioned above also suggest that the high potency of the polyclonal serum isolated from xenogeneic whole endothelial cells in our study may result from the blockage of some important angiogenesis-associated molecules such as αv integrin and VEGFR II and from the targeting of these molecules to multiple different sites[2]. In addition, these identified xenogeneic homologous peptides may provide us another strategy for the development of peptide vaccines for cancer therapy.

Based on a similar strategy, we tested the ability of xenogeneic melanocyte cells to induce the autoimmunity against the tumor cells. The anti-tumor immunity was found in B16 melanoma in C57/BL mouse model with xenogeneic melanocytes isolated from the retinal pigment epithelium of pig or chicken as a vaccine[26].

The vaccine was prepared using 3% paraformaldehyde-fixed pigment cells isolated from the retinal pigment epithelium of pig or chicken. Mice were intraperitoneally or subcutaneously immunized once a week for four weeks in succession with different doses ($1 \times 10^6 \sim 1 \times 10^7$ cells/mouse). Tumor grew progressively in all controls, but there was protective effect on tumor growth in immunized mice. The therapeutic efficiency of xenogeneic pigment cells was also found in the established tumors[26].

In an attempt to explore the possible mechanism by which anti-tumor activity was induced with xenogeneic pigment cells, we found that there was no anti-tumor activity induced by xenogeneic pigment cells in the nude mice, suggesting that T cells may be required for the anti-tumor response. Besides, the tumor killing activity of CTLs was examined. T cells isolated from spleen of immunized mice exhibited higher cytotoxicity against syngeneic melanoma cells than those from control groups. This cytotoxicity could be blocked by anti-CD8 or anti-MHC-class-I mAb, but not by anti-CD4 *in vitro*, suggesting that the killing activity observed may result from MHC class-I dependent CD8 + CTL activity. In addition, there was no increase in NK activity against YAC-1 target cells by the sensitized spleen cells[26].

Sera isolated from the xenogeneic pigment cells-immunized mice exhibited positive staining for melanoma cells in flow cytometric analysis, showing the deposition of IgG on the tumor cells. Adoptive transfer of sera or purified Ig from immunized mice also showed the anti-tumor activity. These findings suggested that the anti-tumor immune response against melanoma tumors with xenogeneic pigment cells might be involved in both humoral and cellular immunities, including antibody and CTL response[26]. A variety of melanocyte differentiation antigens, including MART-1, gp-100, tyrosinase, TRP-1, etc. have been identified as the tumor antigens recognized by the immune system[16,17]. However, the possible epitopes response for the cross-reaction is yet to be identified.

Data show that using some whole xenogeneic homologous cells, such as proliferous endothelial cells, tumor cells or even some normal cells (melanocyte cells), can induce anti-tumor immunity. One of the advantages of using whole cells as vaccines is that all the molecules, including other un-

known molecules on the cells, may be exposed to the host immune system, which in turn makes tumor-associated or angiogenesis-associated multiple molecules targeted. So we can extend this strategy to single xenogeneic gene for induction of anti-tumor autoimmunity.

It has been known that EGFR gene encodes an Src family receptor tyrosine kinase with oncogenic potential, and often triggers a cascade of cellular biochemical events leading to cell growth and differentiation in the presence of the binding-ligands. Many human tumors, including non-small cell lung cancer, breast cancer, etc, often overexpress EGFR, and high levels of expression of this receptor are also associated with poor survival in patients with cancers[27]. So we selected single xenogeneic EGFR gene as a target molecule to test its ability of inducing the autoimmunity against the EGFR-positive tumor cells. We prepared recombinant plasmid DNA encoding human EGFR (phER) and mouse EGFR (pmER) as DNA vaccines and using an empty vector as the control. Mice immunized with phER showed both protective and therapeutic anti-tumor activities against EGFR-positive LL/2c Lewis lung cancer and MMT-06052 mammary cancer. In contrast, mice immunized with pmER or empty vector did not show the anti-tumor immunity. Sera isolated from the phER-immunized mice exhibited positive staining for EGFR-positive tumor cells in flow cytometric analysis and recognized a single 170 kD band in Western blot analysis. The subclasses of IgG1, IgG2a and IgG2b responding to EGFR-positive tumor cells were found to be elevated. There was the deposition of IgG on the tumor cells from the phER-immunized mice. Adoptive transfer of sera or purified immunoglobulins from the phER-immunized mice showed anti-tumor activity. The increasing killing activity of CTL against EGFR-positive tumor cells could be blocked by anti-CD8 or anti-MHC-class-I mAb. *In vivo* depletion of CD4 + T lymphocytes could completely abrogate the anti-tumor activity induced by phER, without detectable CTL activity and autoantibodies, whereas the depletion of CD8 + cells showed partial abrogation of the anti-tumor activity, without CTL activity, but with detectable autoantibodies. In addition, the increase in level of both IFN-γ and IL-4 was found in the supernatants harvested from spleen cells isolated from phER-immunized mice. These findings suggested that the autoreactive immune response, both humoral and cellular immunities, against the EGFR-positive tumor cells may be provoked with phER in a cross-reaction and is probably responsible for the anti-tumor activity[1)].

Similarly, we constructed recombinant plasmid DNA encoding VEGF from African xenopus as DNA vaccine to immunize mice, and the anti-tumor activity resulting from the induction of the autoimmunity against VEGF was also observed in mouse models[14].

To sum up, the observations in this review may provide a new vaccine strategy for active immunotherapy or gene therapy of cancer by the breaking of immune tolerance against the cancer cell proliferation-associated molecules or tumor angiogenesis-associated molecules in a cross-reaction between xenogeneic homologous molecules and self molecules with xenogeneic cells, peptides and genes as vaccines.

4 Problems and prospects

Most results mentioned above are mainly based on the difference between human beings and

1) Data not published.

mice, two spices having a closer evolutionary relationship. Can we extend the species to all animals, such as fruit fly, zebra fish, worms, birds or amphibians? To what extent is the difference between xenogeneic homologous genes proper and on which level (cellular, protein, peptide or gene level) will it be more prone to induce autologous immune response against tumors? A great number of questions remain open. What are the mechanisms for xenogeneic homologous genes inducing autoimmune response? How do they evolve? Which cytokines or signal pathways take part in autoimmune response against cancer? How can we effectively control the scope and degree of the responses so as to improve its specificity of anti-tumor? Which of the gene delivery method, naked DNA, DNA vaccine or virus-dependent gene therapy, will be more effective? Is heterogenous gene better than homologous one in gene therapy of cancer? What will this kind of strategy offer to therapy of autoimmune diseases, AIDS and heterogenous transplantation? And so on. All these problems are extremely vital. With the progress in related research, it is expected that inducing autoimmune response using xenogeneic homologous genes will provide a new strategy for cancer immunotherapy, and it will throw light on the treatment for other diseases[2,28]. In the meanwhile, it will have influence on application and industrialization of the achievements of Human Genome Project and other organisms' genome project, and help understand the evolutionary role of homologous genes.

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