In the current review the latest literature data on design of cancer vaccines on the basis of xenogenic tumor-associated antigens and proteins that play an important role in tumor progression have been summarized. The main benefits and disadvantages of xenogenic cancer vaccines, the results of experimental studies and clinical trials, and perspectives of use are analyzed.

Key Words: cancer, immunotherapy, cancer vaccines, tumor-associated antigens.

Up to date, a whole number of cancer vaccines (CV) has been developed which include autologic and allogenic whole-cell (genetically modified) vaccines, peptide and DNA vaccines, vaccines on the basis of dendritic cells that are used for induction of specific antitumor immune response [1–3]. However, the use of CV is limited because just for small number of tumors the spectrum of tumor-associated antigens (TAAs) has been identified. Another problem is that antigenic spectrum and expression level of this or that antigen may differ in patients bearing tumors of similar origin. Moreover, tumor cells are able escape from immune response due to immunological editing — via decreased intensity or absent expression of TAAs toward which immune response is directed [3–5].

The general problem with CVs application is the fact that TAAs are self (except viral antigens) and mostly nonmutated proteins of patient’s organism, that’s why they are weekly immunogenic for immune system [2, 4, 6]. Therefore, xenogenic TAAs compared with homologic antigens [6, 10, 11]. Firstly, xenogenic TAAs are able to involve natural immunity into anticancer response. Secondly, structural differences of xenogenic TAAs from their human analogs make them highly immunogenic and capable to induce specific antitumor reactions not only at early stages of the disease but also at late stages when patient’s organism is suffering under immunosuppressive influence of tumor and self immune system. Therefore, xenogenic TAAs compared with homologic analogs, may become more effective for prophylaxis of recurrence and metastasis, and for generation of the processes leading to destruction of advanced tumors.

Expansion of immune response on similar proteins or epitopes (epitope spreading) is an interesting phenomenon of xenogenic CV use. For example, in mice immunized with human hTRP2 protein, anti-TRP2 antibodies were found to be specific also to gp75 protein cognate to TRP2 [6].

For construction of xenogenic CVs there are used the same methodical approaches as for preparation of autologic CVs: administration of devitalized tumor cells or their fragments [10–12], or recombinant protein [13], the use of DNA-sequences [6, 14, 15], antigen-loaded dendritic cells [16, 17], etc.

There are two main ways of xenogenic CV development: the first one is directed on activation of immune system against tumor antigens — gp100 [6, 18], HER-2/neu [14, 19], acidic prostate phosphatase [16], alpha-fetoprotein (AFP) [20]; the another one allows overcome immune tolerance to proteins that play a key role in tumor progression — metalloproteinases [21, 22], endoglin [13], integrins, growth factors, and angiogenic factors [23–25], etc.

VACCINES ON THE BASIS OF XENOGENIC TUMOR-ASSOCIATED ANTIGENS

The majority of studies concerning xenogenic vaccines have been carried out on animals with melanoma — the tumor that expresses a whole number of melanoma-associated antigens. In the work of Moskaleva et al. [26] the results of working out cancer xenovaccine on the model of mouse B-16 melanoma, have been shown. An evaluation of anticancer immune response after xenovaccination of mice with...
human melanoma cells (SK-MEL-1 line) has shown that cytotoxic activity of splenocytes from immunized mice toward target melanoma B-16 cells reached its maximal level already in a week after immunization and was observed for the next 8 weeks. It has been shown that xenovaccinotherapy promoted significant antitumor effect: 80–100% growth suppression of syngenic B-16 melanoma [27] and almost without impact on spleen and thymus weights and the main hematological indexes. Also, it has been demonstrated that for xenovaccination an efficacy of use of primary human melanoma nodule cells was higher that that upon the use of transplanted SK-MEL cells.

Spontaneous melanoma in dogs has a number of patterns common with human melanoma [12]. Moreover, dogs are not inbred animals, and that is why the results of their treatment with xenogenic vaccines may be considered the most close to clinical conditions. The use of DNA-sequence coding human tyrosinase in dogs with disseminated melanoma resulted in increase of their average life span, that in some cases were accompanied with complete response — disappearance of numerous metastases in lung during nearly one year [7]. In one third of treated dogs has been registered the presence of antibodies specific to tyrosinase, has been registered that correlated (however insignificantly taking into account sampling values) with life span [7, 29]. In the work of L. M. Finocchiaro, G. C. Glikin [30] it has been shown that such xenovaccine is able to supplement successfully surgical treatment.

In other study [12] dog melanoma 17CM98 cells transfected with DNA fragment coding human gp100, have been used for immunization. Disease stabilization has been registered in 35% of dogs, accompanied with enhancement of delayed hypersensitivity reaction on vaccine administration. There was observed no direct correlation between clinical response and specific antibody level and cytotoxic T lymphocytes (CTL) activity.

Hawkins et al. [31] have shown that immunization of mice with DNA coding human gp100, led to formation of stable antimalenoma immunity, while administration of DNA coding mouse gp100, was ineffective. It has been shown that anticancer effect of xenovaccination in this experimental model was completely independent on functional activity of CD4+ lymphocytes. In the following study [15] the authors have evaluated an efficacy of xenovaccinotherapy at post-surgical period and have shown that vaccination of mice with DNA coding human TRP-2 after excision of primary tumor prevented the development of local metastases as well as metastasis of melanoma in lung.

An ability of xenogenic DNA to overcome immune tolerance to endogenous molecules has been demonstrated also on experimental model of breast cancer. Vaccination of mice with DNA coding human HER-2/neu has induced significant antitumor immune response [14, 19]. According to the data of Gritzapis et al. [32], administration of human HER-2/neu peptide to mice was also effective, and stimulated generation of CTL that may recognize syngenic HER-2/neu-positive tumor cells.

Survivin is a protein, related to apoptosis inhibitors that similarly to HER-2/neu is hyperexpressed in cells of various tumors and could be used as target antigen for vaccine preparation. Antitumor effect of vaccination of mice by means of human survivin has been demonstrated on experimental models of glyoma, lymphoma and pancreatic cancer [33, 34]. Introduction of dendritic cells loaded with human survivin, to mice has been accompanied with induction of immune response mediated by I type T-helper cells [35].

Vaccine on the basis of recombinant rat AFP, but not mouse AFP, promoted significant increasing of life span in mice bearing Hepa1-6 hepatocarcinoma at prophylactic and therapeutic schemes of treatment. Antitumor effect depended on CD4+ and CD8+ T-lymphocytes [20].

According to the data of Gregor et al. [36], immunization of mice with human prostate-specific membrane antigen PSMA as recombinant protein or coding DNA, induced generation of antibodies able to bind with respective antigen in mice. No similar reaction was observed in response on injection of mouse PSMA cDNA. The authors supposed that the obtained results could create a basis for clinical trials of xenogenic vaccine against PSMA, in patients with prostate cancer.

Vaccination of mice with human glyoma membrane proteins (HGP) has led to suppression of glyoma growth [33]. Antitumor effect was associated with the development of immune response mediated mostly by I type T-helpers, and by infiltration of tumor tissue with CD4+ and CD8+ cells. Mouse GP introduction by the same scheme was ineffective.

High mesothelin expression is typical for pancreatic cancer. Li et al. [37] have reported that immunization of mice with human mesothelin resulted in suppression of tumor growth and this effect was associated with increased level of specific antibodies and functional activity of CTL.

Immunization of mice by means of DNA coding human B-lymphocyte differentiation antigen CD20 was favorable for longer life span of mice bearing model A20 lymphoma and induced CD8+-dependent immune response against A20 cells [38]. Meanwhile, the use of DNA-based vaccine coding mouse CD20 was ineffective.

**VACCINES ON THE BASIS OF XENOGENIC PROTEINS RELATED TO ANGIOGENESIS**

Theoretically, overcoming immune tolerance to molecules involved in angiogenesis may cause inhibition of tumor development. It has been shown that vaccination with xenogenic FGFR [23, 39], VEGF [24, 40, 41], and MMP-2 [21, 22] promotes potent immunologic control toward tumors whose development depend on these molecules. Angiogenesis is critical point for tumor growth because without sufficient blood supply tumor mass could not reach more than 2–3 mm in diameter [23].

Up-to-date a number of preparations — neoangiogenesis blockers have been developed and are already used in clinical practice. However, according to the opinion of some authors [24, 25], the use of CVs against angiogenesis-related molecules, has a lot of benefits,
Using Western blot analysis and ELISA assay, Luo et al. [43] have shown that vaccine on the basis of xenogenic endoglin induced CD4+ -mediated synthesis of antibodies that interacted with mouse endoglin and suppressed angiogenesis in Lewis lung carcinoma B-16 melanoma, CT26 enteric carcinoma, and Meth A fibrosarcoma models. Antiangiogenic effect could be achieved also via inactivation of soluble angiogenic molecules. It has been shown that vaccination of dogs with spontaneous sarcoma by vascular endothelial growth factor (VEGF) induced synthesis of antibodies that interacted with human and canine VEGF, and antitumor effect has been observed in 30% of animals [44].

Tumor angiogenesis could be blocked by immunization with xenogenic endothelial cells as well. It has been demonstrated in experiment that immune process against endothelial cells could cause tumor growth inhibition [8, 23, 45]. For example, the use of endothelial xenogenic human or bovine cells but not mouse (allogenic) cells treated with paraformaldehyde, was effective in various models of tumor growth at prophylactic and therapeutic schedules of treatment [45].

CLINICAL TRIALS OF XENOGENIC CANCER VACCINES

It’s necessary to note that the development of xenogenic CVs is not restricted by only experimental studies. There have been obtained preliminary clinical results demonstrating that xenovaccinotherapy may serve as effective mean for treatment of patients with melanoma, renal cancer, tumors of digestive system, lung cancer and prostate cancer [10, 11, 16, 46]. Therefore, at recent time an interest to design of polyvalent xenogenic CVs is increased. In the majority of cases positive clinical effect has been achieved, but, the best results have been registered after radical surgical treatment.

Seledtsov et al. [10, 46] have demonstrated an efficacy of the use of CV obtained from destructed cells of experimental murine tumors — B-16 melanoma and Lewis lung carcinoma, administered to patients with melanoma and colorectal cancer. Xenovaccinotherapy course included 5 subdermal injections with 1 week intervals, and 5 injections with two weeks intervals, and one dose contained the complex of membrane antigens obtained from 75 x 10^6 lyzed tumor cells. It has been shown that vaccinotherapy didn’t affect blood biochemical indexes and did not induce autoimmune reactions. Also, in the process of immunization subpopulations of blood mononuclear cells didn’t differ significantly.

The worked-out CV is safe, able to induce significant cellular and humoral immune response, and is effective for treatment of cancer patients. It has been demonstrated that being transferred in human body, xenogenic cell membranes became quickly opsonized by natural antibodies and phagocytized by antigen-presenting cells (macrophages, dendritic cells) that are capable to stimulate effectively the development of antitumor T-cell reactions. It’s necessary to note also that in the majority of vaccinated patients the significant increase of antibody levels upon xenogenic TAAs has been observed.
In blood serum of the vaccinated patients elevation of γ-IFN and IL-4 has been registered that points out the induction of cellular immune response. Interestingly, in patients with skin melanoma administration of xenogenic polyvalent vaccine has led to significant elevation of proliferative reactivity of lymphocyte toward melanoma-associated antigens. Mean survival time in 32 vaccinated patients with melanoma (stage IV) was significantly higher than that in control group (13 vs 5 months) [10, 46].

The study of clinical response on analogous vaccine in 37 patients with colorectal cancer (IV stage) has also shown significant intensity of cellular reactivity toward tumor antigens delayed skin hypersensitivity reaction, blood lymphocyte proliferative test) and increase of survival time from 7 to 17 months [11]. The authors consider reasonable to use xenogenic polyantigenic vaccine for therapy of a number of cancer types and underline that the antigenic composition of xenovaccine could be altered according to the tasks.

Prostatic acid phosphatase (PAP) is a differentiation antigen expressed by normal and malignant cells of prostate, and therefore it is not immunogenic for patient’s body. Patients with metastatic prostate cancer were multiply administered with autologic dendritic cells loaded with mouse PAP-antigen [16]. After vaccination, an increase of T-cell reactivity toward murine PAP has been observed in all patients, while to human PAP — only in 8 from 21 patients. Exactly in this group of PAP has been observed in all patients, while to human immunogenecity of xenogenic vaccines, feasibility and relatively low cost of their production open wide perspectives for their practical use.

REFERENCES


