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Current approaches to gene therapy in oncology: Construction of tumor vaccines

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Current conventional treatment of malignancies is based predominantly on the use of radio- and chemotherapy. The mentioned therapies are not directed against cancer tissue only and have severe dose-limiting toxic side effects accompanied with a suppressive effect on the patient's immune system. On the other hand, immunotherapy, and especially gene therapy, try to be more selective and less aggressive, having the purpose of triggering a specific immune response against tumor cells. Therefore, different approaches to the creation and application of gene therapy in oncology have been formed in the past few years, yet the aim of all of them is the same: to use extended knowledge about molecular mechanisms of the disease in order to devise a more specific mode of treatment. The major approach to present-day gene therapy of cancer is the generation of tumor vaccines as a possible future category of cancer treatment. The purpose of this article is to provide a brief overview on creation and potential applications of tumor vaccines as well as of some modes of gene therapy in oncology.

Key words: neoplasms-therapy; gene therapy; tumor vaccines

Introduction

Owing to unspecific activities of conventional therapies against cancer (radiotherapy, chemotherapy) a treatment of this kind is quite often accompanied with unrecoverable damage of the normal tissue. The tremendous increase of knowledge in immunology as well as the exponential development of recombinant DNA technology conditioned the renewal of interest for creation of different immunotherapies that were supposed to be more effective, more specific for tumor cells, and cause no or negligible toxic side effects. The goal of each immunomodulatory treatment is to stimulate (enhance) immune response and in this way alter the dynamics of host-tumor relationship to therapeutic advantage. At the same time, this treatment modality has to prevent the development of tumor cell resistance to such treatment, and cause no toxic deposition in the normal tissue. Therefore, for successful creation of immunotherapy, it is important first to understand the relationship between the host and specific tumor cells in order to choose the most appropriate approach. To induce tumor immunity more specifically and effectively, various methods of immunotherapy have emerged using different biological agents such as monoclonal antibodies, cytokines, tumor antigens, hormones, activated killer cells, immune T cells, DNA and others.[1-8]

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Vaccination against cancer

The idea of vaccination against tumor cells has been a distant goal of immunologist for many years, ever since 1909, when Paul Ehrlich suggested that tumors might express antigens that could be targets of immune system.[9] Certainly, at that time there was hardly anything known about tumor-associat-

ed antigens, B and T lymphocytes, antigen-specific receptors on lymphocytes, immunoregulatory cytokines etc. However, the observations that there is a difference in the velocity of tumor growth, and that some tumors stagnate for a longer period of time (even some years), indicate that organisms possess powerful regulation mechanisms (i.e. immune system) for tumor growth control.[5] And still, quite often tumor cells escape the control and do not trigger the immune response. Tumor vaccines were thus created with the intention to rebuild or retrigger the immune system and induce systemic immunity against tumor cells. For this purpose, irradiated autologous or allogeneic tumor cells, lysates of tumor cells, and occasionally, virally infected tumor cells were used as tumor vaccines. To intensify additionally the immune response, nonspecific immunostimulators (e.g. *Corynebacterium parvum* or *Bacillus Calmette-Guerin*) were added to most of the above mentioned preparations.[11–14] The basic working guide of all these experiments was to achieve an enhanced expression of MHC antigens on tumor cells and to increase the cytokine production.

Only the exponential development of molecular genetics and monoclonal antibody reagents, as well as the results of the latest investigations, provided enough information to allow speculation that diminished responsiveness or complete unresponsiveness of the immune system could be predominantly a consequence of the changes of tumor cells at the molecular level. Among the most important changes which enabled tumor cells to escape immune control researchers classified the following:[15,16]

- inadequate expression of MHC (major histocompatibility complex) antigens,
- prevention of tumor specific antigen presentation to T lymphocytes,
- absence of adhesion molecules which are important for the activation of immune system, and
- production of various factors (by tumor cells) which influence (change) the host immune system.

Gene therapy and tumor vaccines

The "gene therapy" term has become a new paradigm, associated with any kind of disease where the origin can be connected with the defined genes. Gene therapy involves a variety of new techniques for gene transfer, gene replacement, gene repair or gene deletion.

Although the idea of vaccination against tumor cells dates in the beginning of 19th century, the modern tumor vaccines represent just one of the approaches (major) to gene therapy of cancer. In other words, modern tumor vaccines are a form of gene therapy where, by use of different vectors, genes of interest are transferred into the tumor cells or into immunocompetent cells. This can be achieved by direct DNA transfer or by using viral vectors. The most prevalent nonviral techniques used for gene transfer are calcium phosphate transfection, microinjection, electroporation, liposomal gene transfer, injection of naked DNA, and receptor mediated gene transfer.[17–22] Among the biological delivery systems for gene transfer the cardinal ones are retroviral vectors, adenoviral vectors, adeno-associated virus vectors and other viral vectors.[23]

The first studies with genetically transformed tumor cells (that were used as tumor vaccines) confirmed that both classes of MHC antigens (MHC I and MHC II) play an important role in the process of triggering of the immune response and that the antitumor activity is predominantly a consequence of activation of cellular immunity.[24] Class I MHC antigens are recognized by cytotoxic lymphocytes (CD8+) and their presence is obligatory for the activation of these cells. On the other hand, class II MHC antigens (they are presented by antigen-presenting cells in the form of endosomes or lysosomes, respectively) take part in the activation of helper T lymphocytes (CD4+), cells which are classified as basic producers of different cytokines. Exactly, the defect in the helper arm (i.e. cytokine producing part) of the immune system is often the cause of inadequate immunogenicity of tumor cells: namely, the development of cellular immunity will fail in the case of inadequate cytokine production, regardless of the fact that MHC I antigens are normally expressed and active.[25]

Considering those facts, tumor vaccines were created predominately to achieve:

- enhanced production of various cytokines that participate in immune processes (IL-2, GM-CSF, IFN-α, TNF-α),
- expression of allogeneic human leukocyte antigens (HLA antigens) or
- enhanced concentration of products that are responsible for the expression or the activities, respectively, of oncogenes (e.g. of the product of p53 suppressor gene).

Therefore, depending on the manner chosen to fight tumor cells, quite a few different approaches

to creation of gene therapy and tumor vaccines have been established. This review will deal with some of them, i.e. those that have been found most promising and attractive.

Preparation of tumor vaccines with insertion of genes coding for allogeneic leukocyte antigens into autologous tumor cells

The purpose of such preparation of tumor vaccines is the transfer of genes encoding certain antigens (usually present on the surface of antigen-presenting cells) into tumor cells. B7 antigen is a molecule that normally functions as an activation molecule on antigen-presenting cells (macrophages, B lymphocytes, dendritic cells). B7 antigen represents a ligand for two types of T lymphocyte receptors i.e. for CD28 (present on CD4+ and CD8+) and CTLA4 (present only on CD8+) receptors. The role of CTL4 has not been determined yet, while on the other hand CD28 is well known to be the cardinal receptor for activation of T lymphocytes and for stimulation of cytokine production.[26] These data led to formation of a hypothesis about the transfer of a gene coding for B7 antigen into the tumor cells and about the potentials of such tumor vaccine to trigger systemic antitumor immunity. So Chen et al.,[13] as well as Townsend and Allison,[27] demonstrated that rejection of malignant melanoma cells expressing B7 ligand resulted from the activity of CD8+ T lymphocytes. Besides, in these experiments systemic immunity developed (in experimental animals) even against genetically unchanged melanoma cells (which were thus not expressing B7 ligand). On the basis of the cited studies we can conclude that tumor vaccines, created by transferring of B7 gene into autologous tumor cells, activate cytotoxic T lymphocytes and stimulate cytokine production in helper T lymphocytes, thus effectively triggering the development of systemic antitumor immunity. On the other hand, the best results with this kind of vaccines can be obtained (owing to the costimulatory mode of action of B7 on CD8+ and CD4+ T lymphocytes) only in the presence of MHC class I and class II antigens on tumor cells.

Vaccines created with insertion of genes coding for different cytokines into autologous tumor cells

Insertion of genes coding for different cytokines might play a role in "overcoming" the unresponsiveness of immune system that derives from inability for normal cytokine production which is actually a consequence of complete absence or inadequate expression of MHC II antigens. In contrast to the activities of exogenous cytokines, the cytokines produced in genetically changed autologous tumor cells mimic the activities of natural endogenous cytokines (underlie to some extent the control mechanisms of the organism), which on one hand improves their effectiveness and on the other hand minimizes their toxic side effects. When preparing tumor vaccines, different researchers introduced genes for numerous cytokines or growth factors (IL-1, IL-2, IL-3, IL-4, IL-6, IL-7, IL-10, IFN-α, TNF-α, GM-CSF and G-CSF), respectively, into tumor cells.[28–32] The effectiveness of vaccines tested on animal tumor models depended upon the type of cytokine produced by the cells, upon the abundance of cytokine synthesis and upon the type of tumor used in the study. Fearon et al. demonstrated that transfection of poorly immunogeneic mouse colon carcinoma cells with IL-2 gene results in reduction of tumorigenic potential of tumor cells and triggers the development of systemic immunity.[33] They confirmed that the phenomenon of systemic immunity results from the influence of IL-2 on CD4+ and CD8+ T lymphocytes (activation of T lymphocytes). Similar conclusions were made by Gansbacher et al. after the transduction of IL-2 gene into mouse fibrosarcoma cells (in syngeneic mice) and into human melanoma or renal carcinoma cells (in nude mice).[34,35] The fact that IL-2 triggers the development of systemic immunity through its action upon T lymphocytes was also confirmed by Rusell et al. in experiments with rat tumor model.[36] Namely, they transplanted transfected rat sarcoma cells either into syngeneic rats or into immunodeficient nude rats. The effect of vaccine in syngeneic rats with normal T lymphocyte production was highly superior to the one in nude rats. On the other hand, partly different results were obtained by Cavallo et al.[37] In agreement with other authors they demonstrated that vaccines prepared by IL-2 gene transduction are capable of challenging the immune response, which (according to Cavallo et al.) predominantly depends upon neutrophils activated with IL-2. Allione et al. created tumor vaccines with transfection of adenocarcinoma cells using genes encoding various interleukins (IL-2, IL-4, IL-7, IL-10), IFN-α, TNF-α or GM-CSF. The best antitumor protection was achieved with inoculation of tumor cells producing interleukins and IFN-, while the treatment outcome after application of tumor cells producing TNF-α was less favourable.[38] Quite interesting was also the comparison of the effectiveness

of the therapy with genetically changed cells, to therapy with tumor cells admixed with *Corynebacterium parvum*. Namely, the authors established that the antitumor activity of the mixture of tumor cells with *Corynebacterium parvum* approximated in its degree the antitumor activity of therapy with genetically modified cells. Similar results were observed by Hock et al., who demonstrated that tumor vaccines prepared by mixing of tumor cells with nonspecific immunostimulators exert an antitumor effect which is comparable to the effect of tumor vaccines created of genetically transformed cells.[39] In contrast to the authors, who achieved relatively modest results with tumor vaccines containing gene for TNF-α, Blankenstein presented encouraging outcomes (his own and of other authors) using the very same vaccines.[40] The antitumor activities of such vaccines were supposed to be based predominantly on an indirect effect mediated through stimulation of immune system and to a lesser extent on the direct antitumor effect of TNF-α. This kind of stimulation of immune system includes the activation of macrophages, as well as CD4+ and CD8+ T lymphocytes. Vaccines bearing TNF-α gene are also successful in the case of inhibited T lymphocyte production, but anyway, the presence of these cells enhances the antitumor effect of such treatment. The best protection from challenge with wild type tumor cells, as well as the most pronounced antitumor activity against formed tumors, has been ascribed to vaccines created of tumor cells bearing gene for GM-CSF. Mulligan and Pardoll studied the effectiveness of vaccines bearing genes for various individual cytokines or for combination of cytokines.[41] The most promising results were achieved with GM-CSF (in the group of vaccines bearing a gene for a single cytokine), while the most effective combination of genes for preparation of tumor vaccines comprised genes for IL-2 and GM-CSF. Dranoff et al. quite early discovered that tumor vaccines with GM-CSF gene are superior to vaccines prepared with genes encoding other cytokines in the case of stimulation of the antitumor immune response.[30] However, the activation of CD4+ and CD8+ T lymphocytes was obligatory for the development of systemic immunity also with vaccines bearing GM-CSF gene, regardless of the MHC II antigen expression on tumor cells. The effectiveness of tumor vaccines with GM-CSF gene was finally confirmed by Golumbek et al., since in their experiments not a single experimental animal immunized with the vaccine developed a tumor after challenge with highly tumorigenic wild type tumor cells.[42] The effect of vaccines with enhanced expression of GM-CSF gene is being ascribed to the stimulation of differentiation of the precursor blood cells and dendritic cells (important antigen-presenting cells for T lymphocytes).

Thus, the basic conclusions of these studies could be the following:

- even low concentrations of cytokines produced by transformed cells are capable of stimulating the antitumor immune response (comparable results were achieved after systemic high dosage cytokine therapy which is often accompanied with numerous toxic side effects);
- important role of cytokines in the process of activation of nonspecific leukocytes e.g. granulocytes and macrophages;
- cooperation between granulocytes, macrophages, lymphocytes, fibroblasts and endothelial cells represents the basis of immune reactions triggered by genetically transformed cells;
- degree of antitumor activity depends upon the tumor type, the type of cytokine produced by tumor cells, and upon the abundance of cytokine production;
- T lymphocyte activity is supposed to depend indirectly upon activation of macrophages and other antigen-presenting cells, as well as upon secondarily induced cytokines (which play an important role in the activation of T cells);
- sublethally irradiated genetically changed cells are capable of challenging the immune response, yet a less pronounced one in comparison to the immune response triggered by proliferating cells, since sublethally irradiated cells produce cytokine only during a limited period of time and because the abundance of tumor-associated antigens is insufficient;
- insertion of GM-CSF gene into tumor cells does not change their tumorigenic potential, yet cells modified in this way and afterwards sublethally irradiated, induce the development of a long lasting immune memory.

Application of tumor specific antigens as vaccines

The idea is to use specific antigens only, instead of intact tumor cells (as carriers of usually ill-defined tumor antigens), for the creation of tumor vaccines. In this case specific immunity can be enhanced (owing to the usage of specific antigens), and also whole work with gene transfection becomes sur-

plus. The basic condition for a successful application of vaccine is that the chosen antigen has to be expressed exclusively on the specific type of tumor cells and by no means on healthy normal cells. We are witnessing at present the identification of the first genes coding for human melanoma-associated antigens that are specifically recognized by autologous cytotoxic T lymphocytes. Mage-1 antigen represents an example of this kind, the antigen that cannot be found on normal cells of adults, but can be detected on approximately 50 % of human malignant melanoma cells.[43]

Insertion of genes coding for substances that make tumor cells susceptible to chemotherapeutic drugs

The use of tumor "suicide" genes offers an additional approach to the treatment of malignant disease. The idea is to modify genetically tumor cells, and to render them vulnerable to therapy with systemically delivered chemotherapeutic drugs. This kind of application of genetic engineering in cancer treatment represents gene therapy in a classical sense. Moolten et al. quite early formed an idea of transferring the classically described "suicide" gene, herpes virus thymidine kinase (HSVTK) gene, into tumor cells to make them sensitive to ganciclovir.[44,45] Their starting point was the fact that normal mammalian cells are insensitive to ganciclovir owing to incapability of kinases (present in normal cells) to phosphorylate ganciclovir into toxic metabolites. On the other hand, HSVTK phosphorylates ganciclovir and its toxic metabolites inhibit DNA polymerase, thus impeding the elongation of DNA molecule. Therefore, the accumulation of toxic metabolites interferes with DNA synthesis, resulting in apoptosis and cell death. The mechanism of action in tumor cells may be analogous to the one in virally infected cells, yet the effect of toxic metabolites spreads out also on genetically unchanged (not producing HSVTK) tumor cells – i.e. bystander effect. The exact mechanism of bystander effect remains questionable, but anyway, there is a hypothesis that toxic metabolites may be released from the cells (where they were produced) in form of lyposomes to enter genetically unchanged cells and affect them as described above. Besides, the antitumor activity also may be achieved through indirect mechanisms that include the activation of immune system. An affirmation derives from the observation that the effect of therapy with tumor vaccines (prepared with gene coding for HSVTK) followed by ganciclovir treatment is less pronounced in immunosuppressed animals (athymic nude mice).[24] Short et al., as well as Culver et al., demonstrated the effectiveness of such system on intracranial tumors in experimental animals.[46,47] Namely, they transferred *in vivo* HSVTK gene directly into tumors using vectors (fibroblasts) and afterwards treated the animals with ganciclovir. Even though they demonstrated that only a small number of tumor cells incorporated HSVTK gene, ganciclovir successfully destroyed both the transfected and the nontransfected cells.

Clinical trials and prospects

Preclinical studies have demonstrated that gene therapy represents a new and provocative mode of treatment with great therapeutic potentials. The insight into the mechanisms of growth and growth regulation of tumor cells has offered multiple potential methods for genetic intervention. Up till now, more than 100 trials with genetically altered tumor vaccines or gene therapy studies have received approval in humans. Most of them are using autologous tumor cells transfected with genes encoding different cytokines.

One of the first tumor vaccines applied in humans was Rosenberg's vaccine using tumor infiltrating lymphocytes stimulated *in vitro* with IL-2 and infusing them to the patient with malignant melanoma, along with additional IL-2.[48] In this case genetic manipulation was not included in the preparation of the vaccine, but exogenous biological response modifiers were applied to augment the immune response against tumor cells.

Another variant of creation of tumor vaccines was presented by Schirrmacher et al., who were employing a two-component human cancer vaccine. The purpose of such a vaccine was simply to challenge the immune system by inserting some viral antigens into tumor cells, thus rendering the cells much more immunogeneic. The idea was based on the analogy with virally induced tumors which are known to be the most immunogeneic tumors in humans. As the specific component (bearing specific antigens) they used the closest possible match to an individual cancer of a patient, namely autologous cancer cells from resected primary tumor or metastases. The non-lytic virus NDV (Newcastle Disease Virus) was applied as the second, nonspecific component for infection of tumor cells. In two clinical studies the vaccines were applied

postoperatively in patients with no macroscopic remnant of tumor, but with a high risk of developing recurrent disease (colorectal carcinoma and breast cancer), while in another three studies the vaccines were applied in combination with biological response modifiers to patients with remaining metastatic disease: renal carcinoma, metastatic breast carcinoma, and metastatic ovarian carcinoma.[49]

As it was postulated before, presently there are many clinical trials with tumor vaccines going on and the studies of Rosenberg and Schirrmacher are the illustrations of only two different approaches to creation of tumor vaccines. Also it is worth mentioning that lately Rosenberg modified his concept for generation of tumor vaccines by introducing genes coding for IL-2 or TNF-α into tumor-infiltrating lymphocytes.[50]

However, the transfer of preclinical knowledge and technology into clinical practice is accompanied with certain difficulties. For now the major concerns with tumor vaccines are inappropriate expression of the transferred gene, as well as frequent adverse immunological reactions of the organism against genetically transformed cells. Certainly, it would be highly desirable if gene expression could be regulated in time, quantity and place, yet with the current vectors this is impossible. Newer delivery systems should incorporate features that permit tissue/cell specific expression and allow the level of gene expression to be regulated by exogenous small molecules administered as a conventional pharmaceutical agent.

In addition, when autologous tumor vaccines are used, another group of questions, which have to be solved, comes to light. Namely, the basic term for development of human autologous tumor vaccines is to establish primary cell cultures from patient's tumor specimens. Since this is a procedure, which is labour and time consuming, there was an idea to use allogeneic cells, stably transfected with cDNA of choice, instead of autologous tumor cells.[51] Although the idea is attractive, conventional immunology still dictates that autologous cells are far better for triggering an effective MHC-restricted immune response than allogeneic cells.

Finally, we also have to bear in mind that Hock et al. prepared a potent tumor vaccine without any kind of genetic manipulation to tumor cells.[39] Namely, in his experiments sublethally irradiated tumor cells admixed with *Corynebacterium parvum* had an immunogeneic activity by all means comparable to the one of genetically transformed cells.

Conclusion

This article is dealing with a field of great importance, extremely fast developing, and extremely wide – a fact that makes every general conclusion (become) obsolete in a very short period of time. Anyway, if we try to stress the major points, we have to admit that new biological approaches to treatment of cancer are of central importance not only for the treatment, but also for understanding of some basic rules governing antigen immune recognition, cancer metastasizing, bystander effect etc. Apart from some classical methodological problems that remain to be solved before final assessment of gene therapy and tumor vaccines validity will be given, there are also some social conventions that have to be changed. Namely, quite often are attractive ideas for biotherapy of cancer received with scepticism by established oncologists, and in the majority of cases, such therapy is acceptable only for a patient who has failed every conventional treatment. Such patients are by no means the best candidates for establishing an active immune response, and studies of this kind can hardly prove the validity of immune therapy.

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