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Immunological aspects of heat shock protein functions and their significance in the development of cancer vaccines

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The primary function of intracellular heat shock proteins (HSPs) is to protect the cell by suppressing the effects of various stress factors by either refolding misfolded proteins or blocking apoptosis. After neoplastic transformation, cells overexpress HSPs, which act as factors promoting the neoplastic process by stabilizing proteins responsible for carcinogenesis, however, HSPs can be released into the extracellular environment where they act as important modulators of the immune response. In a tumor microenvironment, extracellular HSPs are able to induce a pro- or anti-neoplastic response, using various mechanisms of affecting immune cells, The study of the role of extracellular HSPs in immunomodulation processes is a very important direction in the search for new methods of cancer treatment. This review summarizes reports on the use of HSPs in immunotherapeutic cancer strategies, in particular in cancer vaccine design.

Key words: heat shock proteins, cancer immunotherapy, vaccine

Introduction

The research conducted so far confirms the importance of heat shock proteins (HSPs) in such oncological processes as cell proliferation, infiltration and metastasis. Heat shock proteins are receiving increased attention as potential therapeutic targets. The success of anti-cancer treatment depends on the level of the body's immune protection. Heat shock proteins affect the balance between protective and destructive immune responses in the tumor microenvironment, hence the concept of using HSPs in cancer immunotherapy and designing cancer vaccines.

The innate and adaptive immune system is essential for the effective recognition and removal of neoplastic cells in the process of immune surveillance. Many previous studies have demonstrated the importance of natural killer (NK) cells, natural killer T-cells (NKT), eosinophils, αb and γδ T- and B-lymphocytes in immune surveillance [1, 2]. Studies on animal models have shown that mice deprived of any of the above-mentioned immune cell populations showed an increased susceptibility to methylcholanthrene-induced sarcomas [1].

Chemical mediators such as IFN 1, IFN-γ, IL-12, and TNF-α are equally important. In patients with immunosuppression caused by, for example, acquired immunodeficiency syndrome (AIDS), transplantation or even old age, cancer incidence is several times higher than in patients with normal immunity [3]. Kaposi's sarcoma (KS) is a neoplasm that defines the diagnosis of AIDS, as the likelihood of developing KS in people with AIDS is 175–400 times higher. Before the AIDS epidemic, the incidence of this type of sarcoma was not higher than two per million people. The second most frequently diagnosed AIDS-related cancer is non-Hodgkin's lymphoma, which is 73 times more common in these patients than in the average population [4].

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The role of HSPs in immune processes associated with cancer

Tumor recognition by the immune system is based on the expression of mutant proteins and tissue-specific antigens by the neoplastic cells, as well as overexpression of tumor-associated antigens (TAAs). One of the key factors enabling the development of a neoplastic process in the body is the tumor's ability to avoid immunological detection. This effect is achieved through:

- suppression of the major histocompatibility complex (MHC) class I expression on the neoplastic cell surface
- loss or alteration of TAA expression by neoplastic cells
- inhibition of the mechanisms of cancer cell-specific antigen recognition
- local expression of inhibitory immune molecules such as transforming growth factor (TGF) – β and Fas-ligand [5].

Given this, it is clear why scientists are interested in increasing the potential and specificity of the anti-cancer immune response.

The development of the neoplastic process is accompanied by changes in the structure and function of protein complexes and individual molecules. Protein functions are determined by its conformation (spatial structure), which depends on the functioning of heat shock proteins – molecular chaperones or stress proteins – highly conserved specialized proteins responsible for the correct folding of proteins and preventing their unwanted aggregation. HSPs help transport proteins into the cells across the membranes. HSP-molecular chaperones interact with proteins in equal amounts (stoichiometrically), therefore a huge amount of HSPs are synthesized at the time of cellular stress, forming complexes with cellular proteins. In the process of neoplastic transformation, the cell experiences oxidative stress and nutrient deficiency. We observe high expression of mutated cancer-specific proteins that have a destructive effect on the processes of cell proliferation, growth and death. This leads to high expression of HSPs [6]. Thus, intracellular HSPs play the role of cancer promoters, stabilizing the altered conformation of mutant proteins responsible for carcinogenesis [7].

Stress conditions in a tumor lead to necrotic lysis of neoplastic cells accompanied by the release of HSP-peptide complexes (HSP complexes with cellular proteins) into extracellular space. Detection of HSPs in the extracellular environment suggests that HSPs perform functions other than just that of intracellular chaperones. A large number of immune cells concentrate around the site of necrosis. It has been noticed that HSP-peptide complexes, including complexes with neoplastic peptides, can be taken up by antigen presenting cells (APC) through endocytosis [8]. The absorption of the HSP70-peptide complex by APC with the participation of LOX-1, FEEL-1 and SREC-1 receptors was reported [9]. Absorbed peptides are processed by APC and participate in antigen cross-presentation. After processing, antigenic epitopes in the form of complexes with MHC class I and II are presented to T lymphocytes [10]. This results in the activation of cytotoxic T lymphocytes (CTL), which induces a cytotoxic response, and of helper T cells (Th), which, in turn, activate B lymphocytes to induce humoral response.

HSPs can be released into the extracellular space not only during necrotic disintegration, but can also be secreted in the form of extracellular milieu HSP (EX-HSP), membrane-associated HSP (mHSP) and extracellular vesicle HSP (EV-HSP) [11]. Extracellular HSPs interact with immune cells, and these interactions may have suppressive or stimulating effects [12]. The general conclusion that can be drawn from the data presented so far is that the effect of HSPs on tumor growth depends on the mechanism of their release into the extracellular space. HSPs released into the extracellular space by tumor cells in result of cellular exocytosis may have an immunosuppressive effect. They lead to immune tolerance and anergy of immune cells, creating a favorable microenvironment for invasive growth and proliferation of neoplastic cells [13, 14].

HSP60 secreted as extracellular milieu (EX-HSP60) shows immunosuppressive properties, especially in relation to CTL, participating in the increase of CD4(+), CD25 and Foxp3 cell population. It also stimulates mononuclear cells to induce the production of anti-inflammatory cytokines such as IL-10 and IL-6 by CD4(+) T lymphocytes. CD4(+) T lymphocytes stimulated in this way demonstrate immunosuppressive properties [15, 16]. It has also been established that HSP60, acting through the TLR4 receptor, stimulates B lymphocytes to secrete IL-10 and IL-6 and also stimulates the proliferation of B-lymphocytes, which acquire the ability to stimulate T lymphocytes to secrete IL-10 and TNF-α [17]. HSP60 may also induce the production of TNF-α by macrophages, promote metastatic processes through the interaction with β-catenin and enhance the transcriptional activity of cells [18].

HSP27 secreted into the extracellular space induces the differentiation of monocytes into immunotolerant macrophages. The latter produce anti-inflammatory mediators, thrombospontin-1 and IL-10, which can induce the anergy of T lymphocytes. Macrophages also demonstrate pro-angiogenic activity and participate in the formation of new blood vessels, which is one of the conditions for tumor progression [6].

Extracellular HSP70 (EX-HSP70) inhibits TNF-α-induced IL-6, IL-8 and MCP-1 production, and also inhibits the maturation of dendritic cells (DC) and cytokine secretion [19]. Furthermore, EX-HSP70 can reduce the T lymphocyte response independently of the stimulatory effect of DCs.

In most cases, extracellular vesicle HSP (EV-HSP) also exerts immunosuppressive effects. EV-HSP72 stimulates myeloid-derived suppressor cells (MDSC) and induces their suppressive activity dependent on the Stat3 pathway [13]. The immunosuppressive activity of MDSC is manifested by the secretion of IL-10, the involvement of regulatory T lymphocytes (Treg) and inhibition of CD4(+) and CD8(+) T lymphocytes.

However, the presence of HSPs in the extracellular space, especially as a result of necrotic or apoptotic tumor cell death, including destruction induced by chemotherapy or radiation therapy, may result in pro-inflammatory activation of immunocytes in both the tumor microenvironment and the entire immune system, thereby inhibiting tumor growth and metastasis. Acting as endogenous signaling factors, HSPs facilitate the functional maturation of APCs – dendritic cells and macrophages – which enhance the expression of MHC molecules and activate adaptive immune responses.

It should be pointed out that the role of EV-HSP in immunological processes is ambiguous, as they may also exhibit immunostimulatory properties (e.g. EV-HSP70 may induce chemotaxis of NK cells and enhance their cytolytic function) [20]. The immunostimulatory effect was also observed in relation to mHSP, for example mHSP70, which is able to activate the production of TNF-α by macrophages and the cytolytic activity of NK [21].

The immunostimulatory properties of HSP have been studied to establish their possible use in the development of anti-cancer therapies. The first publications describing HSPs as immune regulatory molecules appeared in the 1980s. It was shown that gp96 is a carrier of TAA acting as a TAA transporter [22]. The gp96 protein in combination with tumor antigens can stimulate immune response against the tumor cells it has been isolated from. A similar ability to enhance anti-tumor immunity has been demonstrated for HSP70 and HSP90 combined with tumor peptides [23].

Several examples of the immunostimulatory effects of HSPs have been described. Extracellular HSP70 activates NK and, in particular through the CD94 receptor, stimulates their proliferation and specific migration [24, 25]. HSPs located on the surface of neoplastic cells increase their sensitivity to NK. Increased lysis of cells under the influence of NK was observed in osteosarcoma and breast cancer expressing HSP70 on the cell membrane surface [27]. When interacting with CD91, CD14, TLR4 receptors on the surface of APC, HSPs are able to induce the production of pro-inflammatory cytokines (IL-1, IL-6, IL-12, TNF). Moreover, HSPs as molecular chaperones, are capable of binding TAA and these complexes may be presented by APCs, including DCs, through MHC I and II molecules. This leads to the anti-tumor activation of CD8(+) and CD4(+) T lymphocytes, stimulation of macrophages and NK cells, as well as activation of B lymphocytes [27]. HSPs are able to stimulate the maturation and migration of DCs. In this case, they can act "independently" without forming complexes with peptides or using ATP energy, i.e., acting not only as a chaperon but also having a cytokine-like function [28, 29].

Receptor-mediated HSPs have been observed to stimulate the maturation of CD11c + DCs that enhance MHC class II expression. In addition to increased MHC class II expression, HSP-activated DCs have been found to exhibit increased CD86 expression and TNF-α and IL-12 production [30]. Moreover, nitric oxide is released by dendritic cells and macrophages during the stimulation of HSPs, namely gp96 and HSP70, which in turn leads to a cytolytic or cytostatic effect on neoplastic cells *in vivo* [31]. Chemoattraction of DC and T lymphocytes in tumors following the exposure to hyperthermia leads to the release of HSP70. It was found that DCs are activated upon contact with HSP70 released from tumor cells and that this activation is dependent on TLR4 [32]. This demonstrates the ability of endogenous heat shock proteins to stimulate DCs *via* TLR4.

As chaperones, HSPs can bind to specific receptors on DCs, contributing to the cross-presentation of their peptides [33]. Typically, the antigen interaction with APC, especially DCs, leads to its presentation in the complex with MHC class II and its subsequent recognition by helper T lymphocytes (CD4(+)) in lymph nodes. The mechanism of antigen cross-presentation lies in the ability of DCs to process and present the antigen by means of MHC class I molecules. The MHC I-antigen complex is recognized by the CD8(+) T lymphocyte receptor and activates these cells to differentiate into mature cytotoxic T lymphocytes. HSPs have the ability to bind to antigenic peptides present on tumor cells and stabilize their conformation by forming permanent complexes with them (HSP-TAA).

SRECI and LOX-1 are the two most important DC receptors that allow the cross-presentation of HSP-TAA complexes. SRECI binds to a wide variety of HSPs (HSP60, HSP70, HSP90, HSP110, gp96 and GRP170), while LOX-1 binds mainly to HSP60 and HSP70 [34]. The interaction of the HSP-TAA and MHC class I complex with the immature CD8(+) T lymphocyte receptor leads to the activation of the latter. Activated CD8(+) T lymphocytes acquire cytotoxic properties, and therefore may induce apoptosis of tumor cells in which the aforementioned HSP-TAA complexes have been formed. Cross-presentation of peptides plays an important role in immune surveillance as the bound peptide is not only protected from degradation but the efficiency of cross-presentation in DCs is also higher. Moreover, some neoplastic cells express very little neoantigens, which limits the possibility of their presentation. Thus, cross-presentation of the HSP-peptide complex widens the range of complexes available as targets for the immune system (fig. 1).

There is also a known phenomenon of the reduction of surface molecules of the MHC class I presentation pathway in neoplastic cells, which can be used as a protective mechanism in tumor proliferation. It has been demonstrated to restore the presentation of MHC class I molecules on the cell surface after transfection with human HSP70. B16 melanoma cells with primary presentation deficiency have thus become available for recognition by CTL.

We can therefore say that the HSP-TAA complex contains not only a tumor associated antigen capable of stimulating specific immune response, but also an immunoadjuvant (in this case HSP) which is responsible for stimulating nonspecific

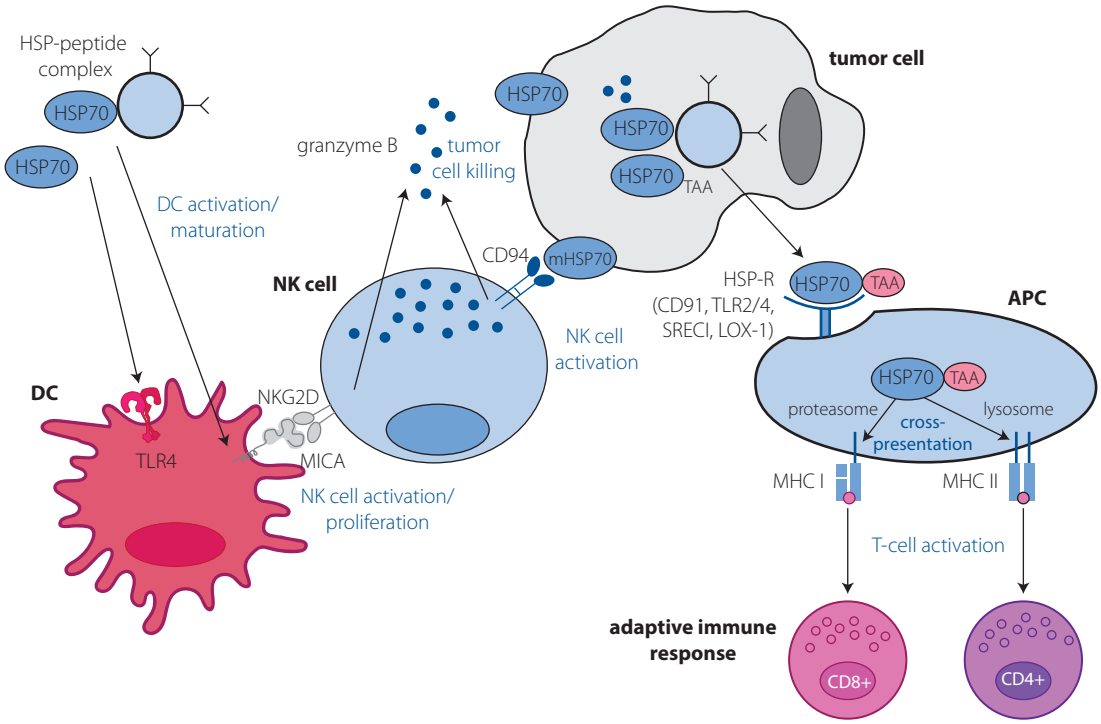

Figure 1. Antitumor immunomodulatory role of extracellular HSP70 (EX-HSP70) – [35] with modification

EX-HSP70 complexes with TAAs allowing them to be taken up by APC *via* CD91 (or other uptake receptors). EX-HSP70 provides a cross-presentation of TAA on MHC class I or II molecules, and promotes a signal cascade that activates CD8(+) and CD4(+) T lymphocytes. mHSP70 provides specific stimulation of NK-cells through the CD94 receptor. EX-HSP70 stimulates NK cells indirectly through the MICA receptor on NK cells, which binds to the NKG2D activation receptor. Activated NK cells increase the release of granzyme B, which triggers the process of perforin-independent apoptosis of cells by binding to the neoplastic mHSP70. Through binding to the TLR4 receptor of dendritic cells, EX-HSP70 stimulates their maturation and increases the expression of MICA, which, in this case, is a ligand for NKG2D

immunity. This makes the HSP-TAA complexes very promising objects for their use in the design of cancer vaccines [5]. Moreover, cross-presentation of antigens in a complex with HSP derived from a tumor of a certain haplotype, has the ability to initiate CTL upon administration of the second haplotype to the recipient [36]. This broadens the arsenal of possible tools in the technology of designing immunological cancer treatments.

Immunological cancer treatment strategies

The search for methods of enhancing the immune response to TAA is a very dynamic area of contemporary research in oncology. The immunotherapeutic strategies developed so far can be divided into non-specific and specific. The main goal of the former is the nonspecific activation of immune responses by means of cytokines such as IL-2 [37–39], or by means of immune checkpoint inhibitors – anti-CTLA4 or anti-PD-1 drugs. Specific immunotherapy strategies can be classified as passive and active. Passive immunotherapy includes the use of:

- monoclonal antibodies against neoplastic antigens, e.g., trastuzumab [40],
- adaptive cell therapy, i.e., the transfer of *ex vivo* activated tumor infiltrated lymphocytes (TILs), and chimeric antigen receptors (CARs) [41, 42].

Cancer vaccines provide an example of active immunotherapy [43]. The main strategy of cancer vaccine design is to identify immune response targets (TAAs), to create immunogenic forms and conditions for the recognition of such antigens, and to induce proliferation and increase the activity of immunocompetent cells. Cancer vaccines can be divided into three main groups:

- cell vaccines based on the use of the whole or lysed autologous or allogeneic tumor cells and DCs modified by various *in vitro* or *in vivo* methods,
- peptide vaccines based on the identified tumor antigens; they are autologous, recombinant or synthetic vaccines based on peptides, heat shock proteins,
- genetic vaccines – this method consists in introducing DNA sequences coding for the tumor antigen to the patient.

All of these strategies have been and continue to be extensively researched and have their advantages and disadvantages. The effectiveness of the treatment depends not only on the specific medicinal preparation but also on the method of administration, dose, number of repeats as well as the nature of the TAA itself. The strategy for using cellular vaccines is to administer autologous APC preparations that act as immune activators through antigen presentation by MHC class

I and II. Dendritic vaccines provide an example of this type of therapy. During treatment with dendritic cells, monocytes are removed from the patient's blood, forced to differentiate into dendritic cells that "get acquainted" with the antigens isolated from that patient's tumor, and are then introduced into the body. Dendritic cells present TAA to cytotoxic T lymphocytes and activate them to fight cancer. Increased interest in these type of vaccines appeared after the approval of the first active cancer vaccine "Sipuleucel-T" in the treatment of patients with asymptomatic or minimally symptomatic metastatic castration-resistant prostate cancer [44]. Other approaches in cell vaccine design involve the use of whole tumor cells of autologous or heterologous origin that are pre-devitalized. In the future, they will act as immunogenic targets to stimulate specific and innate anti-tumor immunity. This vaccine provides the immune system with all potential tumor antigens in every individual patient. Another advantage of this approach is that tumor antigens and their epitopes and presentation methods do not require identification. However, these vaccines suffer from a number of disadvantages, including the difficulty of obtaining enough tumor tissue for sustained therapy and the tolerance to the patient's "own" tumor antigens of patient's immune system.

Another type of cancer vaccine consists in protein or peptide vaccines, based on the use of native antigens or specific antigen epitopes, the introduction of which stimulates an immune system response in the form of a cascade of reactions, which leads to the targeted lysis of tumor cells. These proteins/peptides induce T lymphocytes by their presentation in a complex with MHC class II. The use of peptide vaccines in oncological patients is able to activate a specific anti-tumor immune response and is not accompanied by symptoms of toxicity. The disadvantage of this type of vaccine is the lack of the possibility of significant CTL stimulation. Therefore, many protein vaccine design strategies use adjuvants to enhance the immunostimulatory properties of these vaccines.

The basic principle of using genetic vaccines is to introduce mRNA or DNA sequence coding for the neoplastic antigen to the patient [45]. The sequence is placed in a plasmid and controlled by a promoter. When the vaccine is administered, the body cells that have absorbed the DNA synthesize the encoded protein. Then it is transported to the nearest lymph node, where it induces a specific immune response [46]. There are several options for the delivery of genetic vaccines. Viral vector-based cancer vaccines are considered a subtype of genetic vaccines. Viral vectors such as adenoviruses [47], pox or avipox viruses [48], some *herpes* viruses, and the like are used to create viral cancer vaccines. The virus in the vaccine is attenuated and contains a nucleotide sequence encoding the tumor antigen. The advantages of these vaccines include high transgene expression in infected cells, high immunostimulatory capacity and relative ease of production [49]. A drawback of using viral vectors is their ability to elicit an antiviral immune response to the vector. Similar advantages and disadvantages exist when using bacterial vectors, in particular in the case of intracellular bacteria *Listeria monocytogenes* [50]. This type of vaccine allows the attraction of the endogenous presentation of the encoded antigens by MHC class I. Stimulation of the CTL *via* the endogenous presentation pathway is a very desirable feature of active anti-cancer therapy since a stable CTL response is essential for anti-tumor immunity. HSPs are used as antigens, chaperones or adjuvants of DNA or peptide based vaccines [51]. It has been demonstrated that specific immunostimulation is induced for a wide range of antigens (including HER2/neu, mucin-1, E7, AFP, MAGE-3, gag, survivin and PSCA) with HSP70-mediated DNA vaccines [52–54].

Despite the significant anti-tumor activity of various immunotherapeutic strategies demonstrated in preclinical studies, the efficacy required in the drug registration processes has not been obtained. Research is still ongoing. The involvement of immunologically active HSPs is one of the investigated cancer immunotherapy strategies.

Design and application of vaccines based on HSP

Research on the use of anti-cancer properties of HSPs began in the mid-1980s [55]. The first trials involved vaccinating mice with attenuated tumor cells [56]. This enabled immune reaction against live cancer cells, but only in relation to allogeneic neoplasms. In the next stage, researchers started searching for molecules in neoplastic cells that may be responsible for the development of immunity. Tumor cell lysates were biochemically fractionated and the individual fractions were tested for their ability to vaccinate mice and generate an immune response against live tumor cells of the same type. It was shown that the fractions capable of inducing an immune response contained HSPs [57, 58]. HSPs obtained from autologous tumor tissue turned out to be associated with tumor-specific antigens, forming the so-called "antigenic imprint" of the tumor. The immunoadjuvant properties of HSPs are based on two mechanisms – the ability to induce an adaptive cytotoxic response of T lymphocytes to TAA in combination with HSPs and non-specific stimulation of immune cells. The development of HSP-based cancer vaccines is based on four main assumptions:

- HSPs obtained from other organisms act as classical foreign antigens, eliciting an immune response against their non-conservative epitopes,
- HSPs are able to elicit an immune response in the case of autologous administration in the absence of tolerance of the host's immune system to them,
- HSPs can cause the development of an immune response against a specific protein in the presence of cross-reactivity between HSPs and the protein,
- HSP-TAA are able to stimulate a specific immune response against the antigens included in the complex, while an immune response to HSPs will not develop.

The last of the described mechanisms determines the direction of the development of HSP-based vaccines that can be used in the prevention and treatment of various conditions, including infectious and neoplastic diseases[59]. Such vaccines were initially demonstrated to be effective in animals (e.g., in the treatment of liver cancer in rats [57]), and in the mid-1990s, studies of HSP-based vaccines were initiated in cancer patients.

The active ingredient in such vaccines is not a single HSP, but HSP-TAA complexes. There are two variants of such vaccines: recombinant cancer vaccines obtained by *ex vivo* formation of a complex using HSP and/or recombinant peptides, and cancer vaccines obtained by isolating HSP-TAA from a patient's tumor tissue that contains a specific tumor antigen set. The use of linked HSP-TAA complexes in the development of vaccines increases the ability of APCs to present TAA through MHC class I and II with subsequent activation of CD8(+) and CD4(+) T lymphocytes.

The ability of HSP or HSP-TAA complexes to induce antitumor immunity is dose dependent. Low doses of HSP-TAA complexes are effective in stimulating an anti-tumor immune response, while high doses do not, and may even be immunosuppressive. High doses of gp96-peptide complexes induce immunological tolerance, hence the attempts to use them in the treatment of autoimmune diseases [60, 61].

Currently, HSPs are being studied as immunostimulatory molecules in various therapeutic models. The promising results have been obtained in animal models of tumor growth. Extracellular HSP70 derived from the L1210 leukemia cell was used to immunize DBA/2 mice. The specific activation of CTL was found, which inhibited the growth of the implanted tumor [62]. These results have been confirmed in animal models of colon cancer and melanoma. An increased expression of HSP70 in the exosomes of the hyperthermically treated tumor cells was detected. The immune response in animals with cancer after the introduction of HSP70-enriched allogeneic exosomes was significantly higher than when using exosomes derived from cells without prior hyperthermia. As a result, increased IgG2a and IFN-γ production and tumor regression were observed [63].

In a study in the J558 myeloma model, the effectiveness of stimulating an anti-tumor immune response with exosomal forms of HSP70 was tested. The J558 myeloma cell line that produced the transgenic form of membranę-bound HSP70 in the exosome (mHSP70-EV) was developed, and the efficacy of these exosomes was tested compared to the exosomes from heat-shocked tumour cells expressing cytoplasmic HSP70. Exosomes released from these cells were used to immunize mice. mHSP70-EV significantly stimulated cytotoxic CD4(+) type 1 (Th1) and CD8(+) T lymphocytes, specifically activated NK cells, which significantly exceeded the effects of HSP70-EV [64].

The ability of NK cells to specific activation and damage mHSP70-positive tumor cells has also been demonstrated in animal models of lung cancer and glioblastoma. Combination therapy consisting of NK cells activated ex vivo with the natural HSP70 peptide (TKD) and a low dose of IL-2 (TKD/IL-2) was demonstrated. The adaptive transfer of TKD/IL-2 *ex vivo* activated murine NK cells resulted in inhibition of tumor growth and improved survival of the animals. This regimen therapy was well-tolerated. The antitumor activity was associated with a massive infiltration with CD8(+) T and NK cells in both tumor models and a decreased in PD-1 expression in immune effector cells [65].

Recent reports concern the use of immunotherapy using recombinant HSP70 in CT-26 Colon cancer and B16 melanoma models. The introduction of recombinant HSP70 to the tumor cel stimulates the transport of endogenous HSP70 to the extracellular space of the tumor, leading to a rapid activation of the immune response. The immunomodulatory effect of HSP70-bearing exosomes was manifested by CD8(+) activation, the accumulation of antitumor cytokines and the activation of NK cells, which had a positive effect on the reduction in tumor growth rate and elevation of life span in mice [66].

Furthermore, HSP70 enriched exosome derived from immune cells may also be of interest in anticancer immunotherapy. Scientists investigated the therapeutic effect of macrophage-derived HSP70 enriched exosome in the WEHI-164 fibrasarcoma model both *in vitro* and *in vivo*. Heat shock has been shown to increase the expression of membrane-bound HSP70 in macrophage-derived exosomes. In addition, the immunization of animals with these exosomes reduces the number of tumor cells, indicating a potential immunoadjuvant role of HSP70 in cancer immunotherapy [67].

In all the above-mentioned studies, the researchers showed that HSPs play an important role in anticancer immunity. At present, achievements in the field of HSP-based oncoimmunology are widely integrated into the phase of clinical trials. A study of the safety and efficacy of the antitumor vaccine based on the HSP-96 peptide complex (HSPPC-96), prepared from tumor specimens of patients with metastatic melanomas, was conducted. Activation of the immune response to HSP-96 related peptides was observed in patients receiving the vaccine weekly for 4 consecutive weeks. The overall survival of patients who showed an immune response was 82%. Moreover, the toxicity of the vaccine was very low [68]. Other studies confirm the effectiveness of the HSP-96 vaccination. Phase I and II clinical trials were conducted to investigate the efficacy of the HSPPC-96 vaccine in patients with recurrent glioblastoma multiforme. The study involved 41 patients. The primary endpoint was overall survival of 6 months. Studies have confirmed that the HSPPC-96 vaccine is safe and deserves further research [69].

Studies have shown that Vitespen, an autologous tumor derived heat shock protein gp96 peptide complex vaccine, has shown positive results in phase III clinical trials in patients with melanoma and renal cell carcinoma. It has been observed that Vitespen elicits a major MHC I mediated immune

response in many types of cancer, as well as a clinical response in patients with early stage disease. In addition, the vaccine has a relatively low incidence of side effects [70, 71]. Another study investigated the safety, immunogenicity and clinical efficacy of an autologous vaccine of leukocyte-derived HSP70 peptide complexes in patients with chronic myeloid leukemia. Treatment with the vaccine was performed in conjunction with imatinib mesylate. Clinical responses were observed in 13 of 20 patients and were significantly correlated with the activation of immune responses, including an increase in the frequency of CML-specific IFN-γ producing cells and IFN-γ secreting NK cells. In addition, there were no side effects, indicating the safety of this vaccine [72].

Encouraging results from the phase II vaccine trials, based on a heat shock protein fused to sequences from the oncogenic E7 protein of HPV-16 in woman with high-grade cervical intraepithelial neoplasia was obtained. Four injections of HPV-16 HSP E7 fusion protein were given 3 weeks apart. Complete regression of intraepithelial neoplasia was observed in 35% of women and was correlated with the immune response [73].

DNA-HSP65, a DNA vaccine encoding the 65 kDa heat shock protein *Mycobacterium leprae* (HSP65), was tested in phase I clinical trials of hsp65 DNA in patients with advanced head and neck cancer. 42% of patients showed disease stability or regression following immunization. DNA-HSP65 induced some degree of immunostimulation with no evidence of pathological autoimmunity [74].

Was reported of a phase I clinical trials to evaluate the safety and immunogenicity of a therapeutic human papillomavirus administered to women with HPV-16 + cervical intraepithelial neoplasia (CIN)2/3. In the above study it was applied HPV-16 DNA vaccine [a plasmid expressing a Sig-E7-detox]-heat shock protein 70 fusion protein. Complete histologic regression occurred in 33% individuals. This vaccine was safe and well tolerated [75].

In this study, researchers examined a vaccination strategy using dendritic cells (DC) loaded with apoptotic and necrotic cell bodies derived from autologous tumors. Using this approach, clinical and immunologic responses were achieved in 33% patients with relapsed indolent non-Hodgkin's lymphoma (NHL). The achievement of clinical and immunological response was significantly associated with the degree of surface expression of calreticulin and HSP90 in DC antigenic cargo [76].

Other authors in phase I clinical trials tested a strategy for treating patients with of colon and lung cancer patients, with *ex vivo* heat shock protein 70-peptide-activated, autologous natural killer cells. After stimulation, the activity of NK cells against HSP70 membrane-positive colon carcinoma cells was enhanced in 10 of 12 patients [77].

Activation of CTL against neoplastic cells has been demonstrated through administering dendritic cells transfected with HSP70 mRNA (HSP70-DC) to patients with hepatocellular carcinoma associated with hepatitis C virus [78]. HSP105 peptide vaccines used in patients with colorectal cancer and esophageal cancer showed the ability to induce an immune response in phase I studies [79]. Cellular vaccines, the effect of which is related to overexpression of HSP70, have shown immunostimulatory effects in models of glioblastoma and ovarian carcinoma [80, 81]. Preparations designed with the use of pure HSP70 protein turned out to be active when tested in the B16 glioma and melanoma model [82]. Recombinant chaperones are an alternative source of HSPs for the development of cancer vaccines based on immunogenic peptides. When delivered to the tumor, recombinant HSP70 increases the sensitivity of cancer cells to the cytolytic activity of lymphocytes, reduces the level of immunosuppressive T regulatory cells and lowers the production of IL-10 [83]. The use of HSP70-TAA complexes has an immunostimulatory effect in models of leukemia, lung and ovarian cancer [84]. In addition, HSP70 in complex with antigenic peptides such as the Melan-A, MAGE-A1, tetanus toxin and influenza HA protein has been used to stimulate an antigen-specific immune response [85].

Attempts are also being made to combine HSP70-based vaccines with other anti-cancer drugs, such as immune checkpoint inhibitors, which researchers believe may improve efficacy. Intratumoral HSP70 injections have also been used in conjunction with local hyperthermia, irradiation or cationic magnetite liposomes [86, 87].

Conclusions

In recent years, the potential of HSP as an immunotherapeutic tool has been gaining more and more recognition. The influence of HSPs on the functioning of the immune system, manifested in particular by the activation of dendritic cells, increased activity of T lymphocytes, NK cells and increased antigenic presentation of TAA, allows the use of these proteins as therapeutic targets in oncology, including the development of cancer vaccines. A number of studies have demonstrated the anti-cancer efficacy of HSP-based vaccines, setting directions for further research. It should be noted that the safety and efficacy of cancer vaccines also depend on the route of administration, dose and vaccination schedule. Combining vaccines with other treatments can improve their effectiveness.

Conflict of interest: none declared

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