ARTYKUŁ POGLĄDOWY / REVIEW PAPER
Otrzymano/Submitted: 15.01.2024 • Zaakceptowano/Accepted: 19.03.2024

HSP-70 protein – its clinical potential in the diagnosis and therapy of breast cancer

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Abstract

Breast cancer is the most common cancer among women. Its incidence increases year by year, among others due to the increase in the share of the elderly population in society. Medical progress in the field of prevention, diagnosis and treatment has improved the survival rate of women diagnosed with breast cancer, but we are still dealing with an epidemic of this disease and in the case of some of its varieties, we are almost helpless. The 70-kDa heat shock protein (HSP-70) may be an important diagnostic and prognostic factor in the case of these patients. However, its use is not limited to cancer diagnosis. Research is also being conducted to develop effective, targeted therapies based on the properties of HSP-70. This paper presents the characteristics of breast cancer and the epidemiology of this disease, as well as describes heat shock proteins (HSPs), with particular emphasis on the HSP-70 protein. Recent publications are also discussed, in which the legitimacy of protein determination in diagnostic tests was assessed, as well as the usefulness of determining its level in estimating the prognosis of patients and the purposefulness of using HSP-70 as the basis of oncological therapy. *Geriatria 2024;18:15-24. doi: 10.53139/G.20241802*

Keywords: breast cancer, HSP-70, heat shock protein, oncology

Introduction

The phenomenon of population ageing is undoubtedly the result of the progress that has been made in the field of health care at the turn of the last decades. Increasing the share of geriatric patients in the society is associated with an increase in the incidence of cancer, including breast cancer. Despite the development of many new therapeutic methods, the prognosis often remains poor, especially if the oncological patient is an elderly woman. Diagnostic and treatment methods based on HSP, including the HSP-70 protein, may become a new opportunity for patients. In order to create this review, scientific databases were searched: PubMed, Google Scholar and ClinicalKey using the keywords: HSP-70, heat shock protein, breast cancer. The most important information contained in the latest published articles were summarised, presenting the epidemiology and characteristics of breast cancer, as well as reports on the HSP-70 protein, its possible involvement in the pathogenesis of cancer and the possibility of its use in the diagnosis, assessment of prognosis and therapy of the above-mentioned disease.

Epidemiology and classification of breast cancer

The most common cancer affecting female patients is breast cancer. It is estimated that 2.3 million new cases are detected annually in the world. It is also the most common oncological cause of death among patients worldwide [1]. Poland is one of the countries where an upward trend has recently been observed in the case of new diagnoses of breast cancer [2]. This may be related to the ageing of our society, which has been observed for a long time. Extending life expectancy is undoubtedly a huge success of modern medicine, but it is also associated with an increase in the number of new cancer cases, as well as the appearance of its recurrent forms. It should not be forgotten that the oncological disease

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of elderly patients is associated with a worse prognosis and an increased probability of serious comorbidities that make therapy difficult. Often, due to the lack of guidelines tailored to the needs of seniors, older breast cancer patients are treated less effectively than younger people. In recent years, an improvement in the survival of oncological patients has been noticed, but it was definitely the smallest among geriatric patients [3]. Currently, about 80% of patients are over the age of 50, with 40% being over 65. The risk of being diagnosed with breast cancer increases by 1.5% at the age of 40, by 3% at the age of 50 and even more than 4% at the age of 70 [1]. In the coming years, we can expect a steady increase in the number of new and recurrent diseases, mainly among geriatric patients, which may be a major challenge for the healthcare system. It seems necessary to constantly search for new, effective diagnostic and therapeutic methods that would help to control the breast cancer epidemic.

In addition to advanced age and female sex, other risk factors for breast cancer can be distinguished: family history of the disease, certain genetic mutations - primarily BRCA1 and BRCA2, ethnicity - the highest probability of diagnosis in Caucasians, lack of pregnancy and breastfeeding and early menarche or late menopause, higher breast tissue density, previous history of breast cancer and a history of radiation. Among the modifiable factors that may increase the likelihood of developing the disease, we can distinguish: taking certain medications, low physical activity, smoking, alcohol consumption, high BMI, consumption of processed food and exposure to certain chemicals.

The World Health Organization, based on the morphological differences of cancer cells, has distinguished 21 subtypes in the classification of breast cancer. They differ in growth rate, potential for metastasis, response to therapy and prognosis. The evaluation of the expression of several basic genes allows distinguishing 5 main types of breast cancer: luminal A, luminal B, HER2-positive, triple-negative breast cancer (TNBC) and the type with a low level of claudin (CL). Luminal breast cancers account for about 70% of all cases and are characterised by the presence of oestrogen receptors (ER) and/or progesterone receptors (PR) on the cells, which is why these are referred to as hormone-dependent cancers. Luminal A subtype breast cancers respond well to endocrine therapy. The second subtype, type B luminal carcinomas, has a slightly worse prognosis, because these are usually characterised by higher expression of genes responsible for uncontrolled cell division and less well treated with hormone therapy - often chemotherapy is used. HER2-positive breast cancer accounts for 10 to 15% of cases and grows much faster than luminal types. Before the introduction of targeted therapy targeting the HER2 receptor, the prognosis for this tumour was poor because, due to the lack of oestrogen and progesterone receptors on the cells of this tumour, it does not respond to endocrine therapy.

A heterogeneous set of breast cancers characterised by their lack of expression of HER2, oestrogen and progesterone receptors are referred to as TNBC. These cancers account for about 20% of all breast cancers, with a higher incidence in women before the age of 40. Among them the most common histological type is invasive ductal carcinoma. TNBC therapy is extremely difficult - the response to treatment is usually poor and the recurrence is common. Therefore, this type of breast cancer is associated with a very aggressive course and a poor prognosis. The last of breast cancer - CL most often lacks hormone receptors and HER2. Its expression levels of genes responsible for tumour cell adhesion, such as claudins 3,4,7, occludin and E-cadherin is low. CL makes up about 7 to 14% of all breast cancers and its prognosis is similar to TNBC. The prognosis markers which have currently been used in diagnosis:

- ER and PR - their high expression is associated with a better prognosis and enables using hormonal therapy,
- HER2 receptor - its high expression determines using targeted therapy, but at the same time reduces the chances of complete cure,
- Ki-67 and Mib1 - they provide information on the mitotic index in a given cancer, which is associated with the course of the disease
- E-cadherin – loss of its expression is related to increasing the tumor's potential to metastasize and worsening the prognosis. [1,4]. The most common cancer diagnosed in elderly women is invasive ductal carcinoma. In most cases they show an expression of estrogen and progesterone receptors. Breast tumors detected in the geriatric population are usually larger and they are often diagnosed when there is an involvement of lymph nodes. It indicates inefficiencies in cancer prevention and delay in diagnosis. The incidence of breast cancer in women over the age of 80 is decreasing, probably due to insufficient screening, including mammography. Late diagnosis of cancer in elderly

population often is associated with an advanced stage of disease. In addition, multimorbidity, poorer tolerance and treatment outcome result in increased mortality in this group compared to younger patients [5].

Heat-shock proteins

HSP can be found in all organisms and cells. Their expressions occur in relation to the heat shock response (HSR), which is a major pathway activated for alleviating cellular stress caused by, for example, radiation or chemicals. These factors result in the transcription of heat shock factor 1 (HSF-1), which activates the transcription of genes encoding various HSP. Diseases such as diabetes, cancer, dyslipidemia and neurodegenerative disorders may lead to impairment of HSR and subsequent loss of HSF-1 activation.

Some HSP are called chaperone proteins and they are responsible for many processes such as reversal of cellular stress, refolding proteins after denaturation and their unfolding, folding of protein complexes and their transport and sorting them into subcellular spaces. They are also involved in regulating the cell cycle and protecting the cells from stress and apoptosis. Under physiological conditions, they play an essential role in maintaining homeostasis by folding and spatial conformation of newly formed polypeptides as well as converting misfolded proteins into their functional forms [6].

Recent studies have shown their role in the immune response, such as antigen presentation, antigen transfer to MHC class I and II molecules, stimulation of proliferation maturation of macrophages, dendritic cells or the innate immune response by affecting several toll-like receptors. These include HSP-40, HSP-60, HSP-70, HSP-90, among others [7]. The numbers indicate their molecular weight.

Characterization of HSP-70

HSP-70 belongs to a subfamily of ATP-dependent chaperone proteins. It consists of two functional domains - an N-terminal nucleotide-binding domain, a C-terminal substrate-binding domain, interdomain linker and EEVD motif at the C-terminus. They are located in many intracellular spaces: in the cytoplasm, nucleus, nucleolus, endoplasmic reticulum, mitochondria, ribosomes, cytoskeleton, proteasomes and lysosomal membranes, on the cell surface as well as in the intercellular space and in exosomes. HSP-70 functions are regulated at various stages. By means of post-translational modifications of the chaperone molecule, dimerization and oligomerization, it is possible to inhibit many HSP-70 functions.

Tumour transformation involving HSP-70 can occur through its influence on: cell signalling, protein degradation, disruption of mechanisms that protect cells from transformation and as a result of its protective functions against stress-induced apoptosis. Some studies suggest that its main role in cancer transformation is to protect against oncogene-induced apoptosis. It has been proven that expression of oncogenes such as c-myc can sensitise cells to apoptosis and increased expression of HSP-70 can protect cells from programmed cell death and suppress p53 protein-dependent and oncogene-independent ageing [8].

The role of HSP-70 in the pathogenesis of breast cancer

In the course of many cancers, including breast cancer, there is an increase of HSP-70 expression. High levels of this protein correlate with a worse prognosis and faster disease progression. The research is ongoing to determine the role of HSP-70 in cancer pathogenesis. So far it has been proven that the gene encoding HSP-70 itself does not participate in carcinogenesis, but its presence is essential in this process (it is even said that cancer cells are "addicted" to HSP-70). Scientists suspect that it participates in all stages of neoplastic transformation - cancer cell proliferation, their differentiation, invasion, metastasis and cell death [9]. The role of chaperones is still unclear. Researchers predicted that the presence of chaperones facilitates the survival of cancer cells in unfavourable environmental conditions (acidic pH, low levels of oxygen and nutrients). On the other hand, when the level of oxidative stress is lowered, cancer cells still need HSP-70. This mechanism is not fully understood [10].

The role of HSP-70 in neoplastic transformation

HSP-70 participates in all stages of neoplastic transformation by interfering with cellular defense mechanisms:

Impact on immunological processes

The influence of HSPs on the immune response in the course of breast cancer is not fully understood. Increased expression of this protein has been demonstrated on the surface of cancer cells. HSP-70 is predicted to act by activating immune cells, in particular

antigen-presenting cells. [11]. In a study by Rückert et al. from 2018, the presence of 2 types of HSP-70 was found in the serum of oncological patients [12]. The first is exosomal HSP-70 released by living cancer cells, and the second, called DAMP (Danger/Damage Associated Molecular Patterns), is derived from dying cancer cells. HSP-70 secreted as DAMP has a high immunogenic potential. Stimulates both innate and adaptive immune responses. It binds to tumour antigen and is recognized by immune cells in the presence of IL-2 and IL-15. This leads to the stimulation of an immune response involving T lymphocytes (by influencing antigen activation, maturation and secretion of IFN γ), NK cells (by decreasing proliferation, migration and catalytic activity) and macrophages (by increasing the secretion of IL-6, IL-12, NO) [12]. In turn, long-term exposure of immune cells to free HSP-70 induces immune tolerance and tumour growth. Thus, it is believed that endogenous HSP-70 released from tumour cells is initially tumour suppressive, but as its expression continues to increase, disease progression occurs.

Inhibition of apoptosis and cell ageing

HSPs are involved in the mechanisms that control cell proliferation, differentiation and death. Overexpression of HSP-70 leads to an increase in cell resistance to oncogene-induced apoptosis, while a decrease in expression increases the sensitivity to induction of this process. It is assumed that HSP-70 contributes to the inhibition of apoptosis of the internal and external pathways induced by oncogenes, which results in neoplastic transformation. This is done by inhibiting the secretion of cytochrome c, caspase 3 and caspase 9 from the mitochondria, which makes the cells resistant to apoptosis [13].

HSP-70 also mediates alternative processes of cell death - autophagy and necrosis. In contrast to healthy cells, the presence of this protein has been demonstrated in the lysosomes of cancer cells. Research results from Nylandsted et al. from 2004 proved that HSP-70 stabilises the lysosomal membrane, which allows cancer cells to avoid cell death [14]. A study by Park et al. from 2018, showed that the use of the HSP-70 inhibitor - apoptosis resulted in an increase in the permeability of the lysosomal membrane and the release of cathepsin, which led cancer cells to the path of apoptosis [15]. In turn, the work of Yaglom et al. from 2003 proved the inhibitory effect of HSP-70 on the activity of JNK kinase, which protected cancer cells against necrosis [16]. In addition, HSP-70 is involved in the protection of cancer cells against ageing induced by oncogenesis. Cell-level senescence is defined as the loss of the ability to divide due to cell cycle arrest. At the same time, metabolic activity is preserved. Senescence of tumour cells can lead to inhibition of tumour progression, which is the mechanism of action of cytostatics. It has been proven that the cellular response in the form of ageing occurs even when apoptosis mechanisms are blocked (e.g. as a result of p53 mutation, Bcl-2 overexpression or inhibition of caspases). The role of HSP-70 is to regulate the tumour suppressor protein p53 and the cell cycle kinase Cdc2. The results of Boysen et al. from 2019 showed that HSP-70 in a complex with another heat shock protein-40 kDa (HSP-40) inactivates p53 by interacting with its DNA-binding domain, which leads to the unfolded state of p53 [17]. In the case of depletion of HSP-70, there is an increase in the level of the p21 protein whose expression is dependent on p53, which leads to the subsequent activation of the ageing process. Research results of Gabai et al. from 2009 showed that knockdown of HSP-70 in breast cancer cells expressing HER2, PIK3CA or RAS oncogenes caused cell ageing [18]. The decrease in the concentration of HSP-70 in the MCF10A breast cancer cell line directed them to the ageing process by increasing the expression of p21 and reducing the concentration of survivin - an apoptosis inhibitor.

Role of HSP-70 in cancer progression

Scientific studies indicate the participation of extracellular HSP-70 in the process of tumorigenesis and angiogenesis [19].

Participation in angiogenesis

Neovascularization enables the supply of oxygen and nutrients to the tumour tissue. In addition, endothelial cells of blood vessels contribute to the further development and increase in tumour volume. HSP-70 mediates this process by participating in the activation of hypoxia-inducible factor 1 (HIF-1) which regulates the expression of the main angiogenesis-promoting factor - vascular endothelial growth factor (VEGF). Colvin et. al in the research published in 2014 showed that the knockdown of HSP-70 in the MCF-7 hormone--dependent breast cancer cell lines blocked the activation of HIF-1 under hypoxic conditions, which led to the suppression of VEGF production [10]. Another compound necessary to the angiogenesis activation is interleukin 5 (IL-5). Research by Park et. al from 2017

showed that HSP-70 intensifies angiogenesis induced by IL-5 whose secretion enables phosphorylation of the endothelial nitric oxide synthase pathway (eNOS) [20]. Additionally, the HSP may stimulate angiogenesis in a direct way, acting similarly to VEGF and leading to the formation of microvessels in vivo. In conclusion, HSP-70 induces angiogenesis through: activation of HIF-1/VEGF by tumour cells and stimulation of angiogenesis in stromal endothelial cells directly or indirectly through IL-5 secretion.

Participation in metastasis

The formation of cancer metastases is a very complicated and long-standing process. It includes mechanisms such as anoikis resistance (a form of apoptosis caused by the loss of the ability to connect to the matrix or other cells), epithelial-mesenchymal transition (EMT), cell migration, neoangiogenesis and the ability of tumour cells to grow in adjacent tissues. Results of Kluger et al. research from 2005 proved that in breast cancer models high expression of HSP-70 correlated with metastasis to lymph nodes [21]. However, when there was no metastasis, the level of this protein was much lower. Further work showed that HSP-70 inactivation was associated with reduced invasiveness and metastatic potential of breast, cervical and bladder cancer cell lines. High expression of this protein protects cancer cells from anoikis and amorphosis. This is done by changing the activity of focal adhesion kinase (FAK) and Akt kinase which participate in the above processes.

Influence of HSP-70 on metastasis depends on which co-chaperons the protein binds to. For example, high expression of the HOP co-chaperon was shown in ovarian cancer and glioma cells in a study by Chao et al. [22]. On the other hand, in the work of Willmer et al. from 2013 was observed that the HOP inactivation stopped the formation of pseudopodia and the migration of breast cancer cells [23]. When HSP-70 binds to HOP or BAG3, tumour progression occurs. In turn, metastasis suppression occurs when HSP-70 interacts with CHIP. Extracellular HSP-70 participates in the migration and invasion of cancer cells by affecting tissue transglutaminase (tTG). During migration of the MDA-MB-23 TNBC cell Line, tTG was delivered to the cells in an HSP-70-dependent manner. HSP-70 participates in the process of cellular invasion together with other representatives of the family of HSPs. HSP-70 supports HSP-90-dependent matrix metalloproteinase-2 (MMP-2) activation. MMP-2 is an enzyme involved in the migration, invasion and metastasis of cancer cells. With inhibition of HSP-70 activity, MMP-2 activity in MDA-MB-231 cells was reduced, which limited invasion. It has been proven that extracellular HSP-70 together with other co-chaperones (e.g. HSP-90-alpha, HOP, HSOP40), increase the activity of MMP-2, which promotes the migration and invasion of breast cancer cells [24]. In addition, HSP-70 together with HSP-90 participate in the stabilisation and activation of the metastasis-promoting protein of the Wiskott-Aldrich syndrome family – WASF3. In the study of Teng et al. from 2012 showed that the motility of MDA-MB-231 cells was reduced in the case of HSP-70 knockdown [25]. The same effect was obtained in the presence of WASF3 or HSP-70 inhibitors.

The tissues surrounding the tumour also participate in the pathogenesis of breast cancer by being involved in promotion or inhibition of tumour growth. Cancer cells can modify stromal function (e.g. by secreting pro-angiogenic factors such as VEGF). Also, the stroma itself can stimulate the growth of cancer cells (with the participation of fibroblasts and tumour-associated macrophages) or destroy them through the immune system. Studies by Gabai et al. from 2016 proved that the HSP-70 presence in the stroma is necessary for tumour growth [26]. Transplantation of breast cancer cells from mice of E0771 cell sine led to the tumour development in allogeneic mice. In case of the HSP-70 knockout, no tumour growth was observed, which may be caused by reduced macrophage infiltration and a lower degree of cell migration. Inhibition of HSP-70 in the stroma presents antitumor activity even when the tumour cells themselves are resistant to inhibition. Through the above mechanisms HSP-70 participates in the formation of metastases. However, further research is needed to more accurately assess the role of this protein [27].

The use of the HSP-70 protein in the diagnosis and prognosis of patients

Breast cancer diagnosis which involves the use of the HSP-70 protein can be based on various methods. One of them is the measurement of the level of antibodies directed against this protein. In a study by Hong C.G. et al. published in 2021 evaluated the diagnostic value of six autoantibodies against tumour-associated antigens (TAAs), including HSP-70. After performing the ELISA test, it was shown that in patients suffering from breast cancer, the level of autoantibodies to HSP-70 in the serum was significantly higher than in women from the

control group. In addition, these levels in women were increased in both early and more advanced cancer stages, which may allow cancer to be detected before symptoms appear. These particular antibodies could be detected in 25,5% of women with early-stage cancer and in 25,2% of all 123 women with breast cancer, these values were statistically significant. Better results are achieved with simultaneous detection of autoantibodies BMI-1, HSP-70, NY-ESO-1 and p53, then sensitivity of the test increases to 59,6%. A correlation was also observed between the elevated level of anti-HSP-70 autoantibodies and the degree of histological malignancy, which may be a useful prognostic marker [28].

Similar results were obtained by the team of Sumazaki M. et al. In this case, they used a multi-panel test that measured the levels of seventeen antibodies, including those against HSP-70. Again, the level of serum antibodies against HSP-70 was significantly elevated compared to the control group and was considered significant ($p<0.05$). 54% of patients were seropositive for at least one antibody, and using a panel of five TAAs: p53, RalA, p90, NY-ESO-1 and HSP-70, 37% of patients with stage 0/I tumours were positive [29].

Another way may be to measure the level of HSP-70 protein itself in peripheral blood mononuclear cells (PBMC). Orfanelli T. et al. checked that among 38 women with a malignant tumour, the average protein concentration was 79.3 ng/ml, and in 8 women with a benign tumour, this value reached 44.2 ng/ml, so it was elevated. Based on these results, it can be hypothesised that the protein level can be used in the diagnosis of breast cancer [30].

The relationship between high serum HSP-70 levels and the presence of breast cancer was demonstrated by Fahim H. H. et al. checking it by ELISA test. It was reported among 118 out of 120 patients with a malignant stage who participated in the study. In addition, in 90% of cases, immunohistochemical tests revealed the expression of HSP-70, which is most often found in the cytoplasm or nucleus of cancer tissue cells [31].

The HSP-70 protein may be helpful not only in the diagnosis of breast cancer, but also in predicting the effectiveness of therapy or determining the patient's prognosis. The level of protein may be related to the stage of the disease and the expected effect of the treatment.

In the aforementioned study, Fahim H. H. et al. the HSP-70 titer was higher in patients with malignant lesions than in patients with benign lesions. HSP-70 was significantly higher in patients with metastatic breast cancer than in patients with breast cancer without a tendency to distant metastasis. High protein levels were also associated with higher pT (primary tumour size) and more lymph nodes with metastases. It follows that a high level of expression of the HSP-70 protein has a negative impact on the patient's prognosis, as it is associated with a greater advancement of the neoplastic disease, and thus a reduced chance for the effectiveness of the therapy. This parameter may therefore be a helpful marker in determining the stage of breast cancer, and also an important element in estimating the patient's prognosis [32].

The relationship between the level of HSP-70 protein and the stage of breast cancer was also demonstrated in the study by Peterko et al. from 2022. The results of 68 patients with TNBC and 36 patients with benign breast lesions were compared. Positive expression of the HSP-70 protein in immune cells correlated with a higher stage of breast cancer, a higher degree of differentiation of cancer cells, and a greater number of lymph nodes affected by cancer metastases. Determining the level of HSP-70 may therefore be useful in the process of planning therapy in patients with breast cancer and be an important prognostic parameter [33].

Chanteloup et al. in their study from 2020, they assessed the effectiveness of determining the exosomal forms of the HSP-70 protein in peripheral blood in relation to testing the protein level directly from cancer cells. Exosomes are nanobubbles that are released into the blood by cells that have this ability. It was shown that exosomes containing HSP-70 in the membrane originated from tumour cells. This allowed them to confirm the hypothesis that the level of protein in peripheral blood exosomes is similar to the level detected in breast cancer cells using a biopsy. In addition, blood level of exosomes containing membrane HSP-70 was higher in patients with more advanced cancer and metastases present. The high level of expression of exosomal protein molecules correlated inversely with the effectiveness of therapy used in breast cancer patients. Monitoring changes in the level of exosomes with the presence of membrane HSP-70 may therefore be useful in assessing the severity of the disease, as well as being a minimally invasive method of controlling the risk of disease recurrence in patients after achieving clinical remission [34].

A study by Rothammer et al. from 2019 assessed the risk of breast cancer recurrence in patients after breast-sparing tumorectomy with adjuvant radiotherapy (RT) based on the level of HSP-70 protein expression. Patients

who had relapsed or had contralateral metastases within the first 2 years after RT had significantly higher serum HSP-70 values at the end of RT up to 6 weeks after RT compared to patients who remained disease-free, or have developed secondary endometrial cancer. An elevated level of HSP-70 in the serum could be a diagnostic indicator of an intense chronic inflammatory reaction, which was the cause of the development of a recurrence of the disease. It follows that an elevated level of HSP-70 within 6 weeks after RT may be a prognostic marker of unfavourable prognosis in patients with breast cancer [36].

These studies suggest the usefulness of determining the level of HSP-70 protein in assessing the prognosis in patients with breast cancer. The level of protein expression can be helpful in estimating the risk of disease recurrence after achieving clinical remission. This will bring many benefits related to the early detection of a potential recurrence. Assessment of the stage of cancer using the aforementioned parameter will allow for more effective and more accurate qualification of patients for appropriate types of therapy, which will increase the effectiveness of treatment of oncological patients.

Therapeutic options related to the HSP-70 protein

HSP-70 protein is of great clinical importance, primarily due to its ability to suppress apoptosis, influence on oxidative stress, stimulate the immune system and promote angiogenesis and metastasis. Its many substrate binding domains make it an attractive target for cancer therapies. For this reason, attempts have been made to develop a treatment based on HSP-70 for breast cancer, including the use of elimination or inhibition of its functions and methods of immunotherapy with the use of HSP-70 as an adjuvant.

The concept of vaccines based on this protein has been also proposed, using its ability to stimulate T-lymphocytes. Their creation involves the extraction of the HSP-70-peptide complex from tumour cells, purification and reinsertion into the body of patients in the form of a vaccine stimulating the immune system through the MHC class I pathway and targeted destruction of cancer cells containing specific antigens [8]. In addition, the effectiveness of many HSP-70 inhibitors has been tested, including: inhibitors targeting the substrate binding domain, nucleotide binding domain, HSP-70-HSP-40 complex inhibitors, HSP-70-NEF complex inhibitors or allosteric HSP-70 inhibitors. However, the monotherapies described so far have shown unsatisfactory therapeutic ratios (toxicity/efficacy), and the only HSP-70 inhibitor evaluated in a human clinical trial as an anticancer drug, in the context of advanced or recurrent gynaecological cancers, is SHetA2 (sulphur heteroarotinoid A2). Therefore, there is a need for more research and more drug development. Researchers are looking to improve outcomes by combining the use of inhibitors with standard chemotherapeutic agents or targeted therapies. It is assumed that they can improve the anticancer potency of HSP inhibitors even at lower concentrations, and thus reduce their side effects [36-38].

Development of a correlated treatment targeting intracellular signalling pathways, to increase efficacy, reduce side effects and avoid drug resistance, is also an important issue. In other words, combination therapies of several HSP inhibitors are tested. For example, preclinical studies have attempted to combine HSP-70 inhibitors with HSP-90 inhibitors or cisplatin. However, tests are still needed to evaluate their effectiveness [39].

The importance of the influence of the HSP-70 protein on the previously known and used anti-cancer therapies should also be indicated, and more precisely its role in the generation of resistance to anti-cancer drugs. An example of this is the importance of HSP-70 in the body's response to the drug adriamycin (ADR), which is widely used in postoperative adjuvant chemotherapy for breast cancer patients, especially those at high risk of recurrence. Despite its clinical importance, patient resistance to this drug turns out to be a major challenge. Multiple mechanisms have been described to undermine the effectiveness of ADRs, including drug target mutation, apoptosis disorder, altered signalling, impaired glucose metabolism, and other drug interactions. In addition, there have been studies showing the influence of HSP proteins on the previously mentioned resistance. In a study by Weizi Hu et al. intercellular transport of HSP-70 has been shown to contribute to the transmission of ADR resistance. Thus, it has been proven that this protein affects the effectiveness of adriamycin treatment [40].

In conclusion, many studies indicate the potential of the HSP-70 protein in the development of anti-cancer therapies, which are becoming a promising starting point for the development of effective methods of treating breast cancer. However, there is a need to conduct further research and to continue learning about the properties of HSP-70, and to develop methods that take into account its different isoforms and correlation with other HSPs.

Discussion

Breast cancer, as already mentioned in this paper, is a serious and frequent problem for patients' health, which is why various activities and research are undertaken not only in terms of understanding the pathomechanisms of the disease, but also various therapeutic approaches. The ideal therapy would be the one that brings therapeutic success with a low risk of side effects, that is highly available and its use is low enough to be used in general clinical practice. However, this is very unlikely to create such a therapy, although many clinical trials are currently conducted. The latest methods of breast cancer treatment in the phase of clinical trials include gene therapies, variants of photothermal therapy, the use of immunotherapy and new drugs (also in combination with chemotherapy) [41-47]. The results of numerous studies and the development of so many new therapeutic methods make it necessary to compare data on innovative therapies in terms of their effectiveness and side effects. Therefore, therapy using HSP-70 in the treatment of breast cancer needs to be more thoroughly investigated and compared to determine whether its use may bring greater therapeutic benefits with a lower risk of side effects compared to other new therapies.

It is also important that research is being conducted on the use of HSP-70-based therapies in other disease entities, such as chronic myelogenous leukaemia, colorectal cancer, non-small cell lung cancer or hepatocellular carcinoma. In clinical trials, therapy of oncological diseases with the use of HSP-70 turned out to be well tolerated by patients [36], in contrast to therapies based on HSP-90, the use of which may be associated with hepatotoxicity, fatigue, nausea, diarrhoea, muscle pain and retinal dysfunction [48]. In addition, there are also reports on other HSPs that could potentially be prognostic markers and an element of breast cancer therapy, such as HSP-20 [49] or HSP-60 [50], however, clinical trials should be conducted to evaluate their clinical effectiveness.

Conflict of interest
None

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