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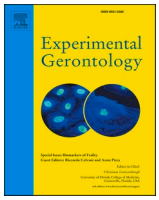

Relationship between heat shock proteins and cellular resistance to drugs and ageing

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ABSTRACT

Background and aims: Ageing is a multifactorial degenerative process which causes a decrease in the cellular capacity for repair and adaptation to external stressors. In this way, it is important to maintain the proper balance of the proteome. Heat shock proteins (HSP) will intervene in this balance, which are responsible for the correct assembly, folding and translocation of other proteins when cells are subjected to stressors. This type of protein is overexpressed in human tumor cells, while its deficit, both in function and quantity, contributes to ageing processes. The present work aims to analyze the response of cells from studies carried out in normal and tumor cells that are subjected to stressors.

Methods and results: A PubMed search was performed using the keywords "cell ageing, cell longevity, resistance, HSP, heat shock proteins, thermal shock proteins". This search generated 212 articles. Subsequently, a series of inclusion and exclusion criteria were applied to select the articles of interest to be evaluated. Normal cells subjected to external stressors at low doses increase the number of HSP, causing them to become more resistant. In addition, tumor cells expressing high levels of HSP show greater resistance to treatment and increased cell replication. HSP intervene in the cellular resistance of both normal and tumor cells.

Conclusions: In the case of normal cells, the increase in HSP levels makes them respond effectively to an external stressor, increasing their resistance and not causing cell death. In the case of tumor cells, there is an increase in resistance to treatment.

1. Introduction

Cellular ageing is due to a progressive failure in the repair and maintenance mechanisms of the cell that causes a decrease in its capacities to adapt to external stressors such as exposure to drugs, radiation, and oxidative or thermal stress. The decrease in the capacity for cellular adaptation to these factors leads to a decrease in resistance to these agents and thus to a decrease in cell survival (Rattan et al., 2004).

Ageing is a multifactorial process, 35 % being responsible for genetic origin and 65 % for environmental origin (stress, habits, etc.). There are several theories that explain cell ageing, such as the telomerase ageing theory in which this enzyme is responsible for replacing and repairing telomeres to increase the useful life of cells; the theory of oxidative stress that is due to the accumulation of free radicals that are responsible for the functional deterioration associated with ageing, leading to the suppression of the antioxidant defense system and the accumulation of lipid peroxidation products (Mercado-Sáenz et al., 2010); the theory of heat stress in which there is a gradual decrease in the response to heat shock and the repair of damage to proteins is prevented, leading to their misfolding (Calabrese et al., 2014) or the theory of the number of Hayflick divisions in which cells cannot divide indefinitely, but have a limited lifespan (Shay and Wright, 2000).

Ageing and longevity are controlled by multiple cellular and molecular signaling pathways that interact with the environment to maintain a proper balance. If any of these pathways fails due to the presence of internal or external stressors, alterations will occur in the individual's body (Hartl, 2016). Most of the cellular functions are carried out by proteins, so we must maintain an adequate balance and integrity

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of the proteome. To maintain protein homeostasis, cells must ensure that all proteins fold and assemble efficiently and preserve their functionality under a wide range of environmental and metabolic conditions (Hartl, 2016). The function of the endoplasmic reticulum can be also altered, causing the accumulation of unfolded proteins. This accumulation produces the unfolded protein response, which reduces the load of these abnormal proteins (Hetz, 2012). This response contributes to the maintenance of cell viability. Chaperones or heat shock proteins (HSP), which are responsible for the assembly, folding and translocation of other proteins, intervene in protein homeostasis. Their action in the presence of stressors is to prevent misfolding and aggregation, as well as to facilitate the folding and elimination of damaged proteins (Welch and Brown, 1996). In this way, the processes that lead to cellular ageing are avoided or slowed down (Martindale and Holbrook, 2002; Sandqvist et al., 2009). Stress-induced up-regulation of HSP promotes cell survival in the face of endogenous or exogenous challenges that have the potential to induce cell damage and death. The cellular response to unfolded proteins begins with the activation of the inositol-requiring protein 1, the protein kinase RNA-like endoplasmic reticulum kinase, and the activating transcription factor 6. These proteins control multiple processes, among which protein folding and autophagy stand out (Hetz, 2012). It has been observed that some diseases such as cystic fibrosis, Tay-Sachs disease, Marfan syndrome, among others, are caused by mutations that lead to abnormal folding of certain proteins. The role of chaperones is essential in these pathologies, since they can act by correcting abnormalities in the folding of the defective proteins (Welch and Brown, 1996). Moreover, other diseases such as amyotrophic lateral sclerosis, Parkinson's and Alzheimer are characterized by protein aggregation with age. In this regard, autophagy constitutes an additional mechanism of cellular proteostasis whose objective is to reduce protein aggregation with age, increasing the longevity. Mutations and/or knockout of some components of autophagy have been shown to reduce longevity and to induce neurodegeneration and/or dysfunction with age, depending on the type of tissue and organism (Aman et al., 2021). In addition, situations of continuous stress induced in the endoplasmic reticulum have also been related to neurodegenerative processes, diabetes, ischemia and even cancer (Hetz, 2012). In tumor cells, the basal levels of some HSP are higher, so drugs that inhibit them are being sought for use as antitumor treatment (Albakova et al., 2020).

Pardue et al. (1992) observed in aged rats subjected to hyperthermia (42 °C, 90 min) a decrease in the levels of HSP70 mRNA in the dentate gyrus granule cells and in the hippocampal pyramidal cells. In ageing, the capacity for protein homeostasis decreases as our cells and tissues grow older, leading to the accumulation of misfolded and aggregated protein species that cause dysfunction in cellular resistance by participating in aberrant interactions (Hartl, 2016). Similarly, ageing cells have a lower capacity to synthesize HSP, thus reducing their resistance to stressors and leading to cell death (Trautinger, 2001; Calderwood, 2015). To study cell resistance to stressors, human cells have been subjected to low or sublethal doses of stressors or stimuli. It has been described that these cells are capable of activating an adaptive response that increases their resistance to a more severe subsequent stress. This phenomenon is known as hormesis (López-Diazguerrero et al., 2013). Jurivich et al. (2020) found a 55 % reduction in HSF1 protein levels after exposure of murine liver cells to 42 °C for 1 h. In contrast, they also observed that sequential stress exposure increased the levels of HSF1 in young and old cells, suggesting a hormesis phenomenon induced by repetitive cycles of heat exposure.

There are a large number of hormetic agents, including radiation, heat, heavy metals, drugs, ethanol, pro-oxidants, exercise and caloric restriction (Rattan, 2004). These agents are capable to induce responses to stress, leading to an increase in HSP, antioxidant enzymes or growth factors (López-Diazguerrero et al., 2013). Thus, if the induction of stress resistance increases cell lifespan and hormesis induces stress resistance, it is most likely that hormesis produces an increase in lifespan. Hormesis describes an adaptive response to continuous cellular stressors, which represents a phenomenon in which exposure to a mild stressor confers resistance to subsequent conditions, allowing ageing to be delayed and thereby increasing cell longevity (Calabrese et al., 2014).

Members of the HSP70 family (HSP70 and HSC70 –also called HSPA8–) control all aspects of cellular proteostasis, such as the folding of the nascent protein chain, the importation of proteins into organelles, the recovery of proteins from the aggregation and the assembly of multiple protein complexes (Radons, 2016). Therefore, it is important to know the role of the heat shock proteins HSC70, HSP70, HSP27, HSP60 and HSP90 in human cells, in order to subsequently understand the results obtained in studies carried out in healthy human and tumor cells exposed to a certain stressor. In this way, Gutsmann-Conrad et al. (1999) reported that hepatocytes and splenocytes obtained from aged rats exhibited an age-related decrease in HSP70 expression. The observed decline with age was also found in a wide variety of tissues in rodents.

HSP increase the survival and longevity against proteotoxic stress by improving cell viability and facilitating the repair of damage to other proteins. In this sense, Siddiqui et al. (2020) found an increase in mRNA and protein (HSP47, HSP60, and HSP70) expressions after exposure of broiler fibroblasts to 41 °C for 6–72 h, also observing an increase in viability at 12 and 24 h of heat treatment. Furthermore, extracellular HSP70 has a series of cytoprotective and immunomodulatory functions, facilitating the cross-presentation of immunogenic peptides through major histocompatibility complex (MHC) antigens or in the context of acting as chaperokines or stimulators of innate immune responses (Radons, 2016). Some studies have linked the expression of HSP70 with various types of carcinoma, associating the expression of this protein with therapeutic resistance, metastasis, and poor clinical outcomes. In tumor cells, HSP70 protect cells from proteotoxic stress associated with abnormally rapid proliferation, suppress cell senescence, and confer resistance to stress-induced apoptosis, including protection against cytostatic drugs and radiation therapy (Fig. 1) (Radons, 2016). In addition, HSP27 is a protein that is located in the cell cytosol under stress-free conditions but is translocated into the nucleus when such stress is present. Its functions include microfilament stabilization, signal transduction, growth, differentiation and transformation processes, and protection against thermal and oxidative stress (Morton et al., 2009). Moreover, HSP90 participates in the folding and activation of proteins, transcription factors, and steroid hormone receptors. The stress-induced expression of HSP90 prevents the aggregation of partially unfolded proteins by maintaining these proteins in a folding state competent for refolding. Inhibition in its function delays and impairs cellular recovery to heat shock. The function of HSP60 is to facilitate the folding and assembly of proteins when they enter the mitochondria, as well as to assist in the transport of proteins across the intracellular membrane (Fig. 2) (Morton et al., 2009).

This work aims to study the response of human cells (normal and tumor) to stress-causing agents in order to evaluate the relationship that exists with ageing and with the mechanisms of cellular resistance, after exposure to drugs, heat or oxidative stress.

2. Material and methods

A PubMed search was carried out using the keywords “cell ageing, cell longevity, resistance, HSP, heat shock proteins, thermal shock proteins”. This search generated 212 articles. Subsequently, a series of inclusion and exclusion criteria were applied to select the articles of interest.

2.1. Inclusion criteria

As inclusion criteria, those articles related to cellular ageing in human cells were considered. The period of time selected was from 1999 to 2021. Thus, a total of 52 articles were obtained.

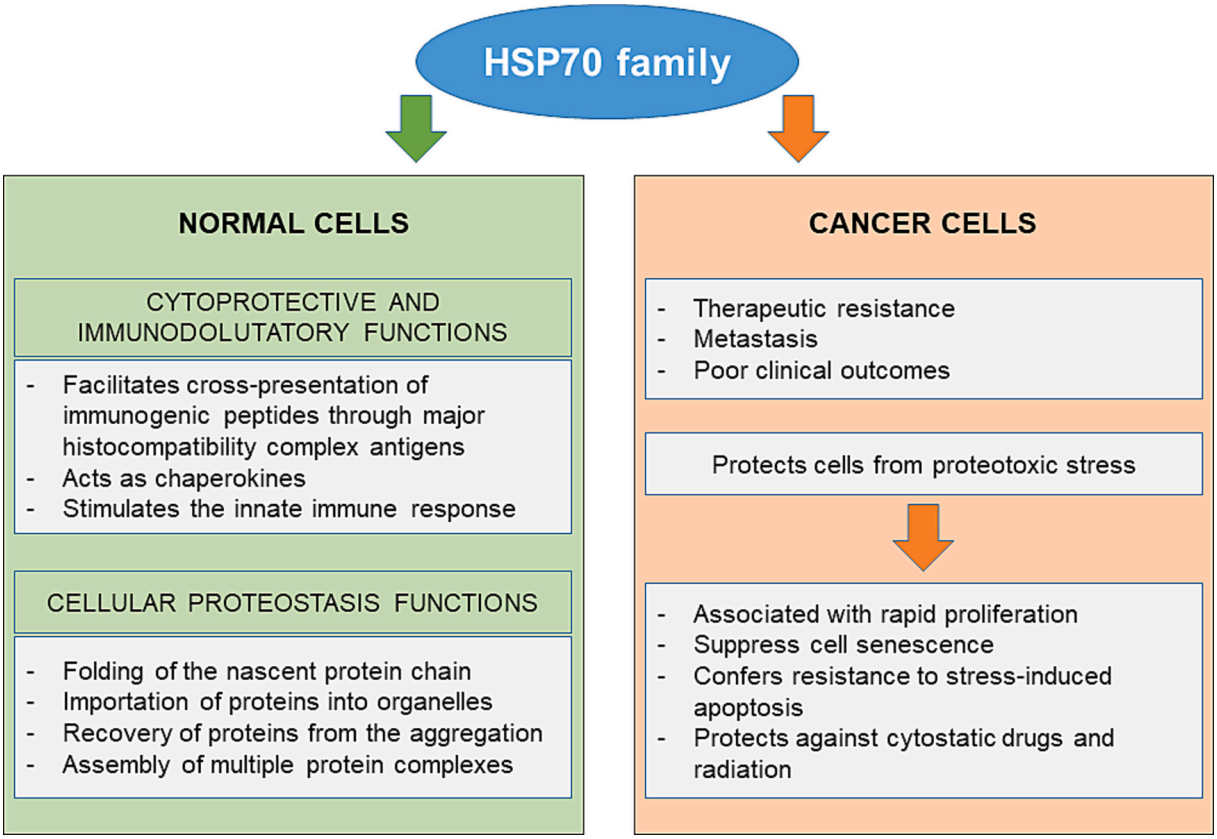

Fig. 1. Functions and effects of elevated levels of HSP70 family proteins in normal and cancer cells.

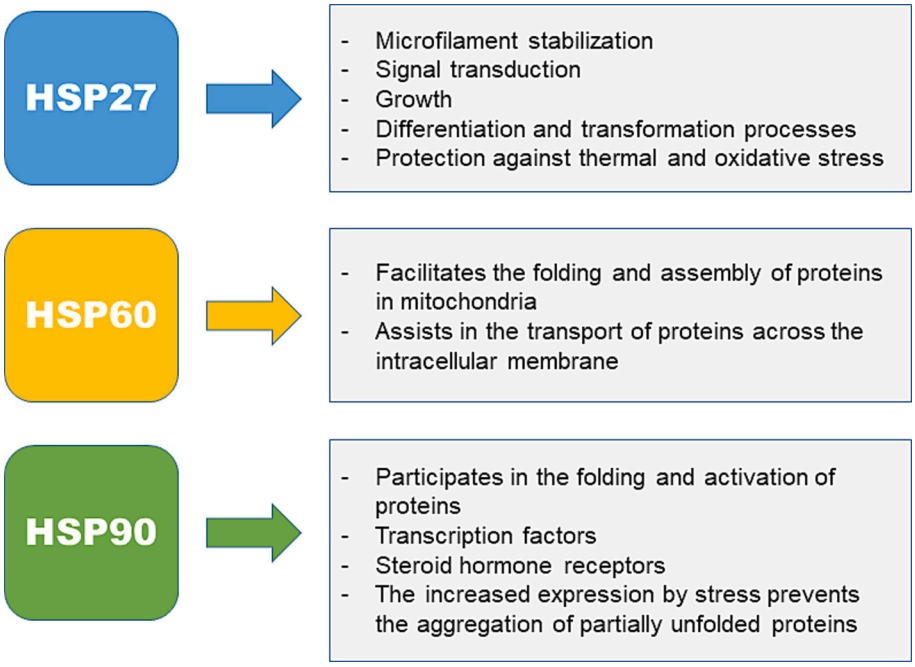

Fig. 2. Functions and effects of increased levels of HSP27, HSP60 and HSP90 proteins.

2.2. Exclusion criteria

Those articles focused on the cellular ageing of mice (*Mus musculus*), nematode (*Caenorhabditis elegans*), vinegar fly (*Drosophila melanogaster*) and yeasts (*Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, etc.) were excluded. Works about the ageing of the individual and therefore related to geriatrics, as well as those not related to cellular ageing were also excluded. In this way, 32 articles were finally left.

3. Results and discussion

The results obtained in the analyzed studies that have been published between 1999 and 2021 are presented and discussed below. The focus of this article is solely on the response of normal and tumor human cells.

3.1. Response of normal human cells to stress-causing agents and their relationship with ageing and cellular resistance

3.1.1. Exposure of cells to stressors

Fonager et al. (2002) exposed human fibroblasts obtained from the

Table 1
Response of normal human cells to different stressors.

Cell type	Stressor	HSP level	Effect	Reference
Human fibroblasts	Heat (41 °C), 1 h, 2 times a week	-HSC70: 3-fold increase -HSP70: 7-fold increase -HSP27: 4-fold increase -HSP90: 80 % decrease	-Increased cell survival -Increase cellular resistance to stress -Higher proteasome activity -Lower lipofuscin accumulation	Fonager et al., 2002
Human fibroblasts	Heat (41 °C), 1 h, 2 times a week	-Increased HSC70, HSP70 and HSP27 -HSP90 decrease	-Increased cell survival -Increase cellular resistance to stress	Rattan et al., 2004
Human T-cells	Heat (42 °C), 30 min	-66 % reduction in HSP70 transcription rates -HSF1 and SP1 DNA-binding reduced with age	-Altered molecular response to stress with age	Jurivich et al., 2005
Human fibroblasts	-Conditioning: heat (41 °C), 1 h -Resting 1 h, 2 h and 20 h. -Hypoxic stress (carbonyl-cyanide-*m*-chlorophenylhydrazone) and oxidative stress (hydrogen peroxide)	-1 h: HSP70 increases 96 % -2 h: HSP70 increases 189 % -20 h: HSP70 increases 237 %	-Increased cell survival -Increase cellular resistance to hypoxic and oxidative stress	Tandara et al., 2006
Human keratinocytes	-Control group: 37 °C -Group 1: heat (41 °C), 1 h, 2 times a week -Group 2: heat (43 °C), 1 h, 2 times a week	-Group 1: HSP: 2-fold Increase -Group 2: No alterations	Group 1: -Replicative lifespan increased 26 % -60 % increase in proteasome activity -Increased cellular metabolic rate -90 % increase in Na/K pump activity -Maintenance of youth -Maintenance of cell morphology Group 2: -No anti-ageing differences compared to controls	Rattan and Ali, 2007
Retinal pigment cells	-Geldanamycin -Radicicol	-HSP70: increases 3000 % -HSP27: increases 50 % -Does not modify HSP90 or HSC70	-Less progression to age-related macular degeneration	Ryhänen et al., 2008
Human fibroblasts	Heat (42 °C), 1–2 h	-Decrease in induction of the hsp70-reporter gene activity -Attenuated activation of HSF1 DNA-binding activity	-Oxidative modification of specific transcription factors in aged cells -Altered function and distribution of proteins with age	Lee et al., 2009
Human monocytes	Exercise 60 min with heat at 40 °C	HSP72: 58 ± 27 % increase	-Greater tolerance against thermal factors -Improved cell survival	Lee et al., 2015
Colon cells	-Heat 39.5 °C -Hypoxemia	Heat: -HSPA6: 10 % increase induced HSP70 increase Hypoxemia: -HSPA6: 12 % increase induced HSP70 increase	-Higher tolerance to stress -Improvement in the regulation of glucose metabolism	Hoter et al., 2018
Lung fibroblasts	Heat at 42 °C for 1 h	HSP40: 25,000 % increase HSP70: 100 % increase	-Improved cell survival -Binding of misfolded proteins -Protection against stress	Bozaykut et al., 2020
Colon cells	-Heat 40 °C and 42 °C -Hypoxia	HSP70 -40 °C heat only: 25 % increase -Only heat at 42°: increase of 75 % -Heat at 40 °C + hypoxia: increase of 35 % -Heat at 42 °C + hypoxia: increase of 100 % HSF1 -40 °C heat only: 25 % increase -Only heat at 42°: increase of 35 %	-Improved cell survival -Improvement in the recovery and integrity of the intestinal barrier -Higher resistance to stressors	Lian et al., 2021
Lung fibroblasts	Silicon (5–40 μg/ml) for 9 weeks	HSP60: -6 weeks: 25 % increase -7 week: 100 % increase HSP70: -6 weeks: 20 % increase -7 weeks: increase 50 % HSP90: -8 and 9 weeks: 20 % increase	-Increased cell survival -Increased resistance to stress	Stan et al., 2021

HSP: Heat Shock Protein. HSC70: Heat Shock Analog Protein (also known as HSPA8).

mammary skin of a young and healthy donor to low doses of a stressor, immersing them for 1 h twice a week in a water bath at 41 °C during their useful life and compared them with those that had not been subjected to this temperature. As a result, a three-fold increase in the levels of HSC70, seven-fold increase of HSP70 level and four-fold increase in HSP27, with respect to the control, was obtained. On the other hand, an 80 % decrease in HSP90 level was observed.

Rattan et al. (2004) obtained results similar to those previously described by Fonager et al. (2002). These authors reported that aged human fibroblasts showed increased basal levels of HSC70, HSP70 and HSP27, which caused an alteration in the maintenance of adequate structural and functional capacity. Due to this finding, the resistance of fibroblasts to heat stress at low doses was studied to assess whether the cells increased the number of HSP and thereby lead to increased survival. As indicated previously, by applying low doses of a stressor, the cell increased the number of HSP leading to increased survival. In this way, human fibroblasts were exposed to 41 °C for 1 h twice per week throughout its in vitro replicative lifespan. This stressor (thermal factor) activated HSF1, which is a chaperone transcription factor, thus increasing their number. Following this experiment, a beneficial cellular anti-ageing effect was observed. The results showed a maintenance of juvenile morphology, a reduced accumulation of damaged proteins, increased levels of various HSP, increased antioxidants and a greater resistance to ethanol, hydrogen peroxide and ultraviolet A (UVA) irradiation, as well as increased activity of the proteasome (Rattan et al., 2004). In contrast, a decrease in HSP90 was observed during cell ageing when fibroblasts were subjected to low doses of heat. This phenomenon is considered a cellular adaptive response, so that by decreasing the levels of HSP90, HSF1 is activated and leads to an increase in HSP. In addition, Jurivich et al. (2005) reported that T cells obtained from aged healthy donors showed a 66 % reduction in hsp70 transcription rates in relation to young individuals when T-cells were exposed in vitro for 30 min at 42 °C. These authors observed a reduction in the levels of HSF1 and SP1 DNA-binding with age (Table 1).

These results show that treatment with low doses of a stressor improved the functional capacity of human cells in terms of greater resistance to stress, greater ability to break down H_2O_2, increased proteasome activity, and reduced lipofuscin accumulation.

Later, Tandara et al. (2006) exposed young human fibroblasts first to heat conditioning and subsequently to hypoxic and oxidative stress factors. Each group of fibroblasts was compared with a control group that did not undergo thermal conditioning, but did undergo stressors. Young fibroblasts were preconditioned with heat by means of low doses of stressor (42 °C, 1 h) and after recovery (1, 2 or 20 h) they were treated with carbonyl-cyanide-*m*-chlorophenylhydrazone (hypoxic stress) or with hydrogen peroxide (oxidative stress) for 1 h. Subsequently, the levels of HSP70 were determined. As a result, it was obtained that in young fibroblasts the levels of HSP70 increased by 96 % when applying the stressful dose at a low level in the first hour, 189 % in 2 h and 237 % after 20 h, compared to those that were not heat-conditioned. Moreover, Lee et al. (2009) observed a decrease in the induction of the hsp70-reporter gene activity and an attenuated activation of HSF1 DNA-binding activity in senescent human fibroblasts after exposure to 42 °C, 1–2 h. These authors indicate that their results show evidence of oxidative modification of a specific transcription factor in aged cells. In this sense, they conclude that ageing affects the function, distribution and modification that these proteins undergo.

From these results, it can be concluded that previous induction by heat at low doses is beneficial for the expression of HSP70 and the subsequent increase in cellular resistance to hypoxic and oxidative stress factors, as previously reported in the studies carried out by Fonager et al. (2002) and Rattan et al. (2004).

Subsequent studies were carried out by Rattan and Ali (2007) in keratinocytes. They also used low doses of heat shock, but the effect of different temperatures was evaluated. In this report, the cells were divided into a control group, subjected to 37 °C; another group to 41 °C for 1 h twice a week; and another exposed to 43 °C for 1 h twice a week. The results showed hormonal and biochemical anti-ageing effects in those cells that had been subjected to 41 °C. Maintenance of cell morphology and vigor was also reported. It is important to indicate that an improved replicative lifespan (increased by 26 % compared to control), an increase in proteasome activity (increased by 60 %) and an increase of almost two-fold HSP level were observed. In addition, the activity of the Na—K ATPase pump increased by 90 %, producing an increase in the cellular metabolic rate. These findings show that the beneficial effect is obtained not only in one cell type but also in others whose function is different. In contrast, those cells that were exposed to 43 °C, did not show significant differences and even decreased the anti-ageing effects (Rattan and Ali, 2007) (Table 1).

The cells of the retina are chronically exposed to oxidative stress contributing to cellular senescence. These pigment cells endure such stress due to their high oxygen consumption, their exposure to high levels of phagocytosed lipid derivatives of photoreceptors, as well as their extensive exposure to light and the accumulation of photosensitizing lipofuscin.

Oxidative stress refers to progressive cellular damage caused by reactive oxygen species (ROS) and contributes to protein misfolding, accumulation of HSP proteins, and functional abnormalities of retinal pigment epithelial cells during cellular senescence and age-related macular degeneration pathology. In this sense, Ryhänen et al. (2008) did not use low-dose of heat as a stressor, but instead they used several drugs. The study carried out evaluated whether the inhibitors of the HSP90 protein, geldanamycin and radicicol, produce beneficial effects in response to oxidative stress and cytotoxicity in retinal pigment cells. These authors, after applying the drugs, studied the accumulation of HSP90, HSP70 and HSP27 proteins. The results showed that HSP90 levels were not significantly affected by geldanamycin or radicicol. However, the concentration of HSP70 protein increased considerably during all exposure times (6 to 48 h) (3000 % increase in relation to control). Both geldanamycin and radicicol caused a marked accumulation of HSP27 proteins (about 50 % more), but the effect was minor compared to the HSP70 response. There were no marked changes in the levels of HSP90, HSP70 or HSP27 in the control cells between different time points (0 h, 6 h, 24 h and 48 h). The constitutively expressed HSC70 levels were not altered in any of the treatments. This fact may explain the beneficial effect of HSP90 inhibitors, since they lead to an increase in resistance to oxidative stress and thus to a lower propensity for age-related macular degeneration (Table 1).

The fact that normal human cells are subjected to low doses of a stressor could be beneficial in vitro, since it increases the concentration of HSP70, HSC70, HSP27 and decreases the level of HSP90. These alterations increase cell resistance in vitro to oxidative, hypoxic and thermal factors, as well as increase proteasome activity, improve cell morphology and vigor, and favor a longer cell life cycle.

Recently, Hoter et al. (2018) exposed human colon cells to a heat stress factor and to hypoxic conditions. The fact of applying heat at 39.5 °C at low doses, caused the levels of the minor isoform of the HSPA6 gene to increase by 10 %. This gene is responsible for coding for HSP70, so its levels were also increased. On the other hand, colon cells subjected to hypoxic stressors produced in this case an increase in the major isoform of HSPA6 (12 % more), which again caused an increase in HSP70. These conditions produced a better response of cells to external stressors (stabilizes the conformation of proteins), as well as a better regulation of glucose metabolism.

Likewise, in the study of Lian et al. (2021) human colon cells were subjected to heat stress factors at 40 °C and 42 °C for 2 h. As a result, there was an increase of 25 % with respect to the control at 40 °C and an increase of 75 % with respect to the control at 42 °C of HSP70 and an increase of 25 % with respect to the control at 40 °C and an increase of 35 % with respect to the control at 42 °C of HSF1. Furthermore, they combined this heat stress with hypoxia by further increasing HSP levels. An increase in HSP70 was obtained at 40 °C of 35 % with respect to the

control and from 1 to 42 °C, while the increase in HSF1 at 40 °C together with hypoxemia was 75 % and 125 % at 42 °C with respect to the control. This led to an improvement in the recovery and integrity of the intestinal barrier, as well as an increase in resistance to stress. Both Hoter's and Lian's studies obtained similar results in the same cell type when heat and hypoxemia were applied at sublethal doses.

Human fibroblast cells were subjected to a mild heat stress factor (42 °C for 1 h) in the study by Bozaykut et al. (2020). An increase in HSP40 (25,000 % more than fibroblasts not subjected to heat) and HSP70 (100 % more than fibroblasts not subjected to heat) was observed. HSP40 recognized and binds unfolded proteins and HSP70 intervenes in cell repair and protection against stress. In this way, the fibroblasts experienced an improvement in protein folding, a decrease in cell death and an increase in protection against external thermal factors.

In addition, Stan et al. (2021) exposed human lung fibroblast to 5 μg/ml and to 40 μg/ml silicon for 9 weeks. At the sixth week there was an increase in HSP60 levels of 25 % compared to the previous weeks and at the seventh the increase was 100 %. Likewise, HSP70 levels increased by 20 % in the sixth week and by 50 % in the seventh, while HSP90 levels increased by 20 % from the eighth and ninth week. The increase of these HSP caused an increase in resistance to external stress, inducing an increment in cell survival.

Finally, we mention the work of Lee et al. (2015) in which the monocytes of 16 healthy men who were subjected to heat acclimatization conditions for three days were examined to measure the response of HSP. Individuals were subjected during 60 min at 50 % volume of 0_2 in an environmental chamber at 40 °C while cycling. Once the study was carried out, results were compared with a control group that had not been subjected to these conditions in such a way that an increase in HSP72 of 58 ± 27 % was evidenced compared to the control group. This level of HSP72 could probably lead to an improvement in tolerance against external thermal aggressions, increasing cell survival.

3.1.2. *Inoculation of exogenous substances*

Volloch and Rits (1999) reported the administration of ascites fluid containing an activator of HSP72 to human fibroblasts to study whether resistance to stress and cell survival is increased, after exposure to a thermal stress of 43 °C (Table 2). HSP72 is involved in protein folding, transport, and degradation, as well as in repairs of stress-induced protein damage. It also participates in cell apoptosis, in such a way that an increase in its production prevents apoptosis induced by factors that damage DNA. This effect is produced by the inhibition of the JNK protein kinase that initiates the apoptosis pathway. By blocking apoptosis, the cell can repair the induced damage providing increased resistance to stress. Mild stress confers resistance against subsequent exposure to severe stress. The results reported by these authors show an increase in the resistance to stress and an increase in the cellular longevity.

Later, Deocaris et al. (2008) evaluated whether adding glycerol to human fibroblasts produces an improvement in cellular anti-ageing functions (related to increase thermal and oxidative resistance) when these cells are subjected to an external factor. Acute exposure to 200–400 mM glycerol was found to be sufficient to induce refolding of denatured proteins, as well as to improve cell resistance to heat and oxidative stress without compromising cell viability. Furthermore, treatment of human fibroblasts with glycerol helped to maintain cell proliferation in the presence of levels of H_2O_2 (oxidative stress) that induce senescence. On the other hand, glycerol increased the activity of the proteasome and decreased the function of p53 (which is involved in cell division). These observed activities could result not only from the direct activity of glycerol, but also from a beneficial induction of the proteasome and heat shock chaperones.

Another approximation made in the study published by Wadhwa et al. (2010) reports that the HSP22 protein present in *Drosophila* was cloned in a retrovirus vector to be inoculated in human fibroblasts. These authors previously observed that HSP22 in flies was positively regulated during ageing leading to greater longevity, since it increased resistance to thermal and oxidative stress. In this study, it was found that fibroblasts possessing HSP22 showed a younger morphology and continued dividing (without entering senescence, compared to controls). On the other hand, they were found to express lower levels of beta-galactosidase leading to a longer lifespan and slowing of the ageing rate.

The administration of a recombinant human HSP70 protein to retinal pigment cells was studied to assay the resistance increase to oxidative stress (Subrizi et al., 2015). In this way, the cells were subjected to hydrogen peroxide concentrations of 1.25 mM and a 37 % decrease in IL6. An increase in cell viability of 32 % and a decrease in cell lysis of 43 % was observed. These results suggest that exogenous HSP70 provides protection against oxidative stress and that it could be a good therapeutic strategy to treat age-related macular degeneration (AMD).

On the other hand, Parseghian et al. (2016), indicate that the fact of externally inoculating a heat shock protein to human alveolar cells makes them more resistant to stressors. In this case, HSP70 was administered together with a fragment of an antibody (Fv-HSP70) and subsequently subjected to oxidative stress (H_2O_2), leading to a decrease in cell death and an increase in survival.

As shown in Table 2, the inoculation or stimulation of HSP in healthy human cells, not previously present, is beneficial to induce an increase in cellular resistance to oxidative stress and an increase in cell longevity.

Table 2
Response of healthy human cells to different stressors, after inoculating them with an exogenous substance.

Cell type	Inoculated substance	Stressor	Effect	Reference
Human fibroblasts	HSP72 activator	Heat (43 °C)	-Less apoptosis -Improved cell survival	Volloch and Rits, 1999
Human fibroblasts	Glycerol	Heat (37 °C; 45 °C) and oxidative (H_2O_2) stress	-Improved cell survival -Greater resistance to thermal and oxidative stress -Increases proteasome activity -Decreases p53 activity	Deocaris et al., 2008
Human fibroblasts	HSP22 cloned into retrovirus vector	Heat (37 °C)	-Younger morphology -They continue to divide -Longer lifespan -Slowering of the ageing speed	Wadhwa et al., 2010
Pigment cells of the retina	Recombinant HSP70	H_2O_2	-Decreases cell lysis by 43 % -Decreases IL6 by 37 % -Increases cell viability by 32 % -Less age-related macular degeneration	Subrizi et al., 2015
Human alveolar cells	Fv-HSP70	H_2O_2	-Decrease cell death -Greater survival	Parseghian et al., 2016

3.2. Response of human tumor cells to stress-causing agents and their relationship with ageing and cellular resistance

3.2.1. Heat shock proteins overexpressed in tumor cells

Heat shock factor 1 (HSF1) is an important regulator of protein quality control by inducing HSP proteins. HSF1 is considered a potential target for cancer therapy because it is overexpressed in several tumor types and its increased expression has been associated with a poor prognosis, because it modifies carcinogenesis by improving cell proliferation and survival in response to oncogenic stimuli (Im et al., 2017). Calderwood (2010) and Calderwood and Gong (2012) indicate that HSP proteins and the HSF1 factor are involved in the etiology of breast cancer. The HSP27 and HSP70 proteins increase in concentration during the transformation of healthy mammary cells into tumor cells, leading to a decrease in apoptosis and senescence. On the other hand, the HSP90 protein plays an important role in facilitating cell transformation, stabilizing the mutated and overexpressed oncoproteins found in breast tumors. This allows the activation of transformation pathways and growth stimulation in the absence of growth factors, as well as an increase in resistance to tumor treatment. HSF1 plays a role as a transformation facilitator in breast cancer (Table 3). The results obtained in the work of these authors are also reflected in the study reported by Santagata et al. (2011). Here, women with breast cancer were selected and followed for about 25 years to study how their disease progressed based on the levels of HSF1 in the cells. Thus, it was observed that women with high levels of HSF1 had greater tumor progression and lower survival.

On the other hand, in the study published by Wadhwa et al. (2010), the HSP22 present in *Drosophila* was cloned in a retrovirus vector, which was inoculated in human cancer cells (in addition to the healthy fibroblasts that we already examined previously). These cancer cells were transferred to mice to study how they evolved in the presence of a stressor and compared to a control group that did not express HSP22. Breast cancer, lung cancer, and osteosarcoma cells showed increased migration, independent growth, the formation of more tumors, and increased resistance to treatment.

As can be observed in these studies, the increase in the concentration of HSP in tumor cells produces an increase in the capacity for replication, migration, and drug resistance.

Jin et al. (2016) observed that overexpression of mortalin (which is a type of HSP70) in breast tumor cells was significantly correlated with the histological grade (tendency to ductal subtype), the clinical stage (greater stage III) and lymph node metastasis in relation to those breast cancers that did not express mortalin. In addition, to further assess the progression of breast cancer where mortalin levels were greatly increased, they studied disease-free survival and overall survival rate in 155 cases of women with breast cancer. Thus, the patients who overexpressed mortalin had a lower disease-free survival and overall survival than those who had low expression of it.

Chatterjee and Burns (2017) indicate that HSP and HSF1 factor are involved in the etiology of breast cancer. Their levels were measured, finding an increase in HSP27, HSP40, HSP70, HSP90 and HSF1. This overexpression leads to increased multidrug resistance, inhibition of apoptotic cell death, and acceleration of tumor progression (Table 3). Furthermore, the presence of very high levels of HSP90 resulted in a ductal type carcinoma.

Recently, Hoter and Naim (2019) reported the different HSP that are overexpressed in ovarian cancer and their implication in its development. HSP90 levels are shown to be elevated causing an increase in tumor progression, as well as the ability to metastasize. On the other hand, the overexpression of HSP70 induces an increase in tumor growth and HSP60 a decrease in the overall survival. All of these HSP previously discussed produce an increase in resistance to the treatment. Finally, note that HSF1 levels are also high, implying an unfavorable prognosis for the patient.

On the other hand, in the article published by Xu et al. (2020), the levels of HSP expressed in lung adenocarcinoma cells were studied, where an increase in HSP90 was evidenced. These tumors have a worse prognosis in patients where HSP levels are higher, as well as an increase in resistance to treatment and an increase in cell growth.

When a cell is subjected to thermal stress, the heat shock factor 1 (HSF-1) is activated by phosphorylation and enters the cell nucleus, where it binds to the heat shock element (HSE) and to DNA. This union activates the transcription of HSPs, especially HSP27, HSP60, HSP70 and HSP90, thus increasing the number of chaperones in the cytoplasm; which inhibit protein aggregation. On the other hand, HSPs activate cellular metabolism. HSP60 activates the tricarboxylic acid (TCA) cycle, HSP70 activates glycolysis via the phosphoinositide 3-kinase/protein

Table 3
Heat shock proteins that are overexpressed in human tumor cells and their relationship with cell resistance.

Cell type	Overexpressed HSP	Effect	Reference
Breast cancer cells	HSP70, HSP27, HSP90 and HSF1	-Decrease apoptosis and senescence -Stabilization of oncoproteins -Increased cell growth -Increased drug resistance	Calderwood, 2010; Calderwood and Gong, 2012
Breast cancer cells, osteosarcoma and lung cancer	HSP22 cloned in retroviral vector and inoculated	-Increase in migration -Independent growth -Formation of more tumors -Increased resistance to treatment	Wadhwa et al., 2010
Breast cancer cells	HSF1	-Increased cell growth -Decreased cell survival -Increase in drug resistance	Santagata et al., 2011
Breast tumor cells	Mortalin (HSP70)	-Increased cell growth -Decreased survival -Increased drug resistance -Increased metastasis -Major tumor stage	Jin et al., 2016
Breast tumor cells	HSP70, HSP27, HSP90, HSP40 and HSF1	-Decrease apoptosis and senescence -Increased tumor progression -Increased drug resistance	Chatterjee and Burns, 2017
Ovarian cancer cells	HSP90, HSP70, HSP40 and HSF1	-Increased tumor progression -Increased metastasis -Decreased survival -Increased tumor growth	Hoter and Naim, 2019
Lung adenocarcinoma cells	HSP90	-Increased cell growth -Decreased survival -Increased drug resistance	Xu et al., 2020

kinase B (PI3K/AKT) signaling pathway; while HSP90 also activates glycolysis by the cMyc, PI3K/AKT and hypoxia-inducible factor 1α (HIF-1α) signaling pathways. On the other hand, HSPs modulate the action of pyruvate kinase 2 (PKM2) and succinate dehydrogenase (SDH) enzymes, increasing the synthesis of pyruvate and fumarate, respectively (Fig. 3) (Kabakov et al., 2020; Le Breton and Mayer, 2016; Morimoto, 2011; Yang et al., 2021).

3.2.2. *Exposure of tumor cells to heat shock proteins inhibitors*

The observation of an increase in resistance to drugs used in cancer chemotherapy has led to more in-depth studies on the relationship between exposure to HSP inhibitor drugs and induction of resistance.

The HSP90 protein is essential to ensure the survival of some tumor cells, so its inhibition is an important target for some drugs. Chen et al. (2013) identified that the inhibition of HSF1, highly expressed in hepatocellular carcinoma, induces an inhibition of HSP90; which leads to laying the foundations for possible combined treatments.

In this sense, Hu et al. (2015), evaluated the response of osteosarcoma cells to different drugs. These authors studied the effect of telomerase inhibition with imetelstat alone and in combination with the HSP90 inhibitor, alvespimycin (Table 4). Osteosarcoma is the most common primary bone cancer in children and adolescents, accounting for approximately 3 % of malignancies in people younger than 20 years of age. With modern treatment approaches, survival estimates for patients with localized and metastatic osteosarcoma are 70 % and 20 %, respectively. For the study, they applied imetelstat twice a week for 14 to 36 weeks on three cell lines. In all of them there were a 50 % telomerase inhibition compared to the control and a reduction in cell growth, as well as a decrease in the formation of osteosarcoma colonies. However, complete growth detection was not manifested. On the other hand, when imetelstat was applied in combination with alvespimycin, it was observed that telomerase inhibition was 13 % greater than when imetelstat was administered alone, accelerating telomere shortening. In this case, cell growth arrest was produced.

HSP90 is necessary for the folding and stability of proteins, which are essential for cell growth, differentiation and survival. Due to this characteristic and the fact that HSP90 is expressed at higher levels in cancerous tissues, inhibition of HSP90 can overcome cell growth signals and drug resistance in many cancers (Daunys et al., 2019).

Along the same lines, Daunys et al. (2019), studied the response of tumor cells to an HSP90 inhibitor in combination with standard treatment, but in this case, they used pancreatic tumor cells. Pancreatic cancer is one of the most aggressive and deadly cancers. After diagnosis, patients have a 5-year survival rate of only 8.5 %. Such a low survival rate is mainly due to the disease being diagnosed in late stages and rapidly progressing to the lymphatic system and local organs. Furthermore, the increasing resistance of pancreatic cancer to available chemotherapy is a serious problem. Daunys et al. (2019), studied the combination of anticancer agents that are used as a treatment in pancreatic cancer in combination with drugs that inhibit HSP90 (such as ICPD47 and ICPD62). They also determined whether mild hyperthermia could increase the activity of HSP90 inhibitors. These authors observed that combinations of HSP90 inhibitors and anticancer agents have a synergistic anticancer effect in pancreatic cell lines, with the most potent combinations being ICP47 with GEM and ICP47 with 5FU.

On the other hand, mild hyperthermia has shown an increase in the effect of ICP47 in several lines of pancreatic cancer. Similar results were shown in the study by Ito et al. (2009), where hyperthermia of 43 °C

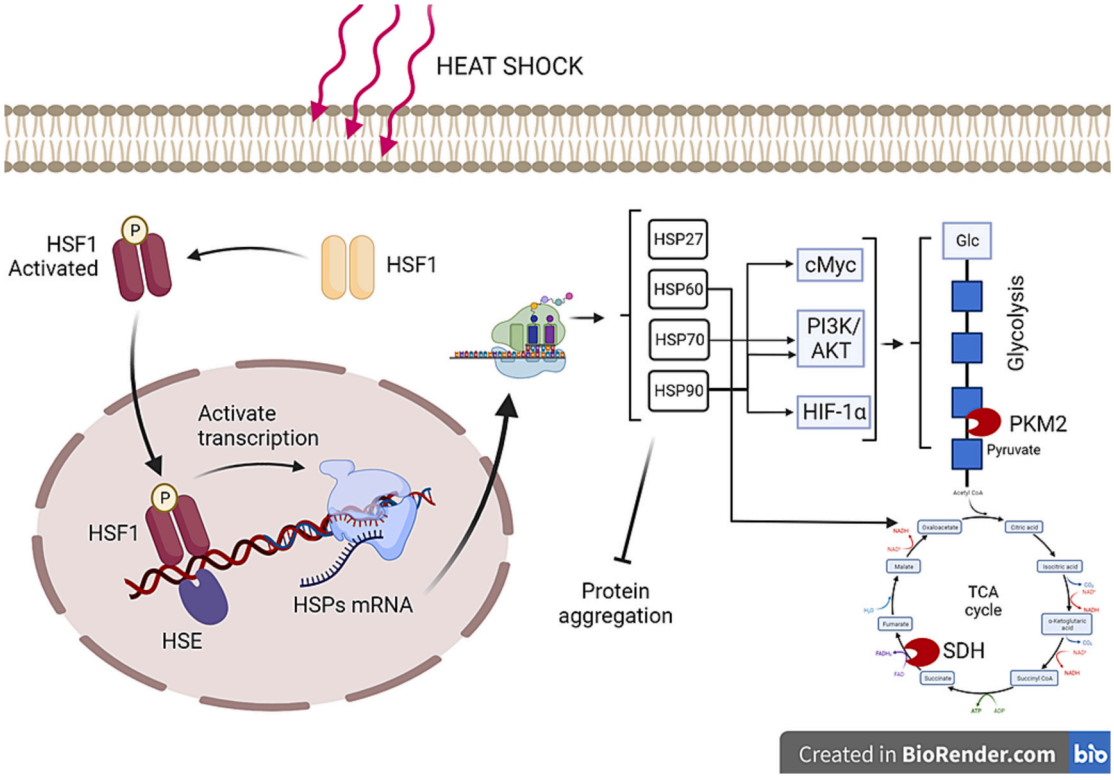

Fig. 3. Response of cancer and aged cells to heat stress. When a cell is subjected to thermal stress, the heat shock factor 1 (HSF-1) is activated by phosphorylation and enters the cell nucleus, where it binds to the heat shock element (HSE) and to DNA. This union activates the transcription of HSPs, especially HSP27, HSP60, HSP70 and HSP90, thus increasing the number of chaperones in the cytoplasm; which inhibit protein aggregation. On the other hand, HSPs activate cellular metabolism. HSP60 activates the tricarboxylic acid (TCA) cycle, HSP70 activates glycolysis via the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) signaling pathway; while HSP90 also activates glycolysis by the cMyc, PI3K/AKT and hypoxia-inducible factor 1α (HIF-1α) signaling pathways. On the other hand, HSPs modulate the action of pyruvate kinase 2 (PKM2) and succinate dehydrogenase (SDH) enzymes, increasing the synthesis of pyruvate and fumarate, respectively. Figure created with BioRender.com

Table 4
Response of human tumor cells to different HSP inhibitor drugs.

Cell type	Stressor	Inhibition	Effect	Reference
Melanoma cells	-Low heat dose at 43 °C + geldanamycin (HSP90 inhibitor)	HSP90	-Increased effect of geldanamycin in combination with low doses of heat (1.8 times)	Ito et al., 2009
Osteosarcoma cells	-Imetelstat -Imetelstat + alvespimycin	-Imetelstat: telomerase -Alvespimycin: HSP90	Imetelstat: -Telomerase inhibition (50 % compared to control) -Reduction of cell growth -Decrease formation of osteosarcoma colonies -Not complete growth arrest. Imetelstat + alvespimycin: -Higher inhibition of telomerase (13 %) -Complete arrest of cell growth	Hu et al., 2015
Glioblastoma stem cells	-Temozolamide + BIS inhibitor (small interfering RNA)	HSF1	-Lower expression of HSF1 -Decrease resistance to treatment	Im et al., 2017
Malignant pleural mesothelioma cells	-Ganetespib -Ganetespib + pemetrexed + cisplatin	HSP90	-Cell sensitization -Increased senescence (70 % higher when the three drugs are combined) -Drug resistance decrease	Di Martino et al., 2018
Pancreatic tumor cells	-GEM and 5FU (common anticancer) in combination with ICPD47 and ICPD62 (HSP90 inhibitors) -Heat at low doses	HSP90	Drugs: -Anticancer synergistic effect. Lower resistance to treatment Drugs + Low dose heat: -Increase the effect of ICP47	Daunys et al., 2019
Pancreatic tumor cells	Ganetespib + 5FU + external irradiation	HSP90	-Reduces tumor proliferation -Decreases tumor survival -Lower treatment resistance	Nagaraju et al., 2019
Colon cancer cells	DCZ5248	HSP90	-Inhibition of both HSP90 and late stage autophagy that leads to cell cycle arrest and apoptosis -Potent antitumor activity -Induces degradation of HSP90 client proteins (CDK4, CDK6, AKT, and RAF1) -Induces vacuole formation, LC3 II conversion and p62 upregulation	Chen et al., 2021

increased the activity of the HSP90 inhibitor, geldanamycin, 1.8 times more in human melanoma cells, compared to the effect of hyperthermia and the compound alone.

Another study by Nagaraju et al. (2019) in pancreatic tumor cells using ganetespib as an HSP90 inhibitor showed a reduction in tumor proliferation and survival when combined with the chemotherapy 5FU and radiation. A reduction in HSF1 was also observed.

Glioblastoma multiforme is the most aggressive brain tumor. Glioblastoma stem cells often develop resistance to temozolamide, this being the conventional drug used in glioblastoma therapy after surgery, causing tumor recurrence. In addition, glioblastoma has elevated levels of HSF1. The cell death suppressor (BIS) is an HSF1 target gene that promotes cell survival in normal and neoplastic cells. BIS transcription is regulated by HSF1 through two main elements, thermal and oxidative stressors. As a consequence of the depletion or decrease of BIS, a decrease in HSF1 and an alteration in cell resistance are produced, thereby decreasing tumor survival. Im et al. (2017), investigated the resistance of glioblastoma to treatment with temozolamide by adding a small interfering RNA to produce a decrease in BIS (cell death suppressor gene). This study evaluated the response of glioblastoma cells subjected to this interfering RNA that resulted in BIS depletion and, together with temozolamide, resulted in lower resistance to treatment and lower cell survival (Table 4).

Mesothelioma is a rare tumor that occurs in the lung pleura. This tumor is characterized by pharmacological resistance to its first-line treatment, because tumor cells become tolerant to stress due to a rearrangement in the secretome (a set of proteins found in the extracellular space). Di Martino et al. (2018), studied the response of chemoresistant malignant mesothelioma cells by adding an HSP90 inhibitor, such as ganetespib, alone and in combination with the usual treatment (pemetrexed + cisplatin). These authors observed that when administering ganetespib alone a chemosensitization of tumor cells was produced. In addition, when adding this drug together with pemetrexed + cisplatin, the cellular senescence was produced (70 % more than when administering ganetespib alone), thereby reducing resistance to the first-line treatment.

Chen et al. (2021) found that DCZ5248 compound induced the inhibition of both HSP90 and autophagy at late stage, leading to cell cycle arrest and apoptosis. This fact makes this substance to have a powerful antitumoral activity. The mechanisms involved are the degradation of HSP90 client proteins (CDK4, CDK6, AKT, and RAF1), the vacuole formation, LC3 II conversion and p62 upregulation (Table 4).

There is evidence of the relationship between telomere length and cell survival, such that, in each cell division, telomeres shorten until they reach a critical point where cell enter senescence. Cancer cells avoid this problem by activating the telomerase enzyme that adds nucleotides to telomeres and allows their unlimited proliferation (Albakova et al., 2020). Telomerase activity is regulated, in part, by heat shock proteins. HSP90, which participates in the assembly of telomerase, plays a key role in the folding and function of TERT (the gene that encodes the catalytic component of the telomerase enzyme complex) (Albakova et al., 2020). For this reason, HSP90 is found at higher levels in tumor cells.

All of these studies (Table 4) show an increase in resistance to treatment and an increase in cell survival. This response is related to an increase in the heat shock protein HSP90, since it participates in the assembly of telomerase, causing telomeres not to be shortened and leading to a longer cell life. When an HSP90 inhibitor is administered together with the usual treatment of this type of tumors, a decrease in cell survival and resistance to the antineoplastic treatment was observed.

Recent scientific and clinical data show that HSP90 inhibitors strongly affect the survival of cancer cells and their use in combination with anticancer agents makes it possible to postpone cellular resistance to chemotherapy (Daunys et al., 2019).

3.3. *Future perspectives*

Finally, it is important to highlight the great progress that is being made to treat those tumors that are resistant to antineoplastic drugs. It

would be very useful the creation of vaccines with HSP. Tumor cells have a resistance to being eliminated by the immune system because they present antigens on the cell surface in the absence of a stimulating signal, so that lymphocytes are not activated. Vaccination with antigenic materials obtained from the tumor is one way to reverse this resistance and activate cytotoxic T lymphocytes. Studies in this area aim to achieve a population of lymphocytes that respond to the tumors presented by the host, in which T cells obtained could be capable of entering tumor vessels and lymphocytes activated to destroy cancer cells (Murshid et al., 2008).

Nowadays, HSP vaccines undergoing clinical trials have been shown to be safe and effective in treating various types of tumors. Additionally, these vaccines can be used in combination with other therapies, such as ionizing radiation.

An example of these techniques began with the clinical trial conducted by Mazzaferro et al. (2003) in which twenty-nine patients with colon cancer and liver metastases received the HSPPC-gp96 vaccine (a heat shock protein and an autologous peptide complex, obtained from these autologous liver metastases). Gp96 is an endoplasmic reticulum stress response protein that is undergoing clinical trials for the treatment of cancer. This protein has shown promising results, such as the induction of antitumor immunity and the presentation of benefits for patients when administered as part of a multidose regimen.

In the study by Mazzaferro et al. (2003), T cell response was measured before and after vaccination. As a result, it was obtained that vaccination with HSPPC-gp96 is safe and causes a significant increase in the response of T cells against colon cancer. This shows a lower resistance of tumor cells to be eliminated by T lymphocytes.

Future advances in HSP-based immunotherapy will be enhanced by understanding the mechanisms by which HSP peptide complexes induce innate and adaptive immunity to tumor cells and target the destruction of primary and metastatic cancer cells (Murshid et al., 2011).

4. Conclusion

After having collected the results of multiple studies carried out in both healthy human and tumor cells, we can reach the conclusion of the importance of HSP in cell resistance when they are subjected to various stressors.

The fact of subjecting human cells to an external stressor (oxidative, thermal, hypoxic, pharmacological) at low or sublethal doses is beneficial, since an increase in the level of HSC70, HSP70 and HSP27 is produced, although they decrease the level of HSP90. This means that when they are again subjected to a higher dose stressor, they respond appropriately and cell death does not occur. Therefore, they increase the resistance to the stressors.

On the other hand, the fact that some HSP are overexpressed in different tumor cells (HSF1 and HSP90), it causes an increase in drug resistance and an enhancing in cell growth and migration. Due to this fact, therapies that inhibit HSP90 are being sought to decrease resistance to treatment and cell proliferation.

Finally, the development of autologous HSP vaccines for the treatment of cancer could be a breakthrough for those tumors that are resistant to treatment.

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Isabel C. Peinado-Ruiz: Methodology, Formal analysis, Investigation, Data curation, Writing – original draft. **Antonio M. Burgos-Molina:** Methodology, Investigation. **Francisco Sendra-Portero:** Methodology, Investigation. **Miguel J. Ruiz-Gómez:** Conceptualization, Methodology, Investigation, Data curation, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

No data was used for the research described in the article.

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