Targeting the heat shock response induced by modulated electro-hyperthermia (mEHT) in cancer

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ABSTRACT

The Heat Shock Response (HSR) is a cellular stress reaction crucial for cell survival against stressors, including heat, in both healthy and cancer cells. Modulated electro-hyperthermia (mEHT) is an emerging non-invasive cancer therapy utilizing electromagnetic fields to selectively target cancer cells via temperature-dependent and independent mechanisms. However, mEHT triggers HSR in treated cells. Despite demonstrated efficacy in cancer treatment, understanding the underlying molecular mechanisms for improved therapeutic outcomes remains a focus. This review examines the HSR induced by mEHT in cancer cells, discussing potential strategies to modulate it for enhanced tumor-killing effects. Approaches such as HSF1 gene-knockdown and small molecule inhibitors like KRIBB11 are explored to downregulate the HSR and augment tumor destruction. We emphasize the impact of HSR inhibition on cancer cell viability, mEHT sensitivity, and potential synergistic effects, addressing challenges and future directions. This understanding offers opportunities for optimizing treatment strategies and advancing precision medicine in cancer therapy.

Keywords

Heat shock response, HSF1, HSP, mEHT, Hyperthermia, Oncothermia

1. Introduction

Characterized by the uncontrolled proliferation of abnormal cells, cancer is a global health challenge that continues to impact millions of lives [1]. Indeed, cancer is the leading cause of death worldwide, accounting for nearly 10 million deaths in 2020 [2]. The complexity and adaptability of cancer cells often requires multiple approaches of treatment. In this context, adjuvant therapies like modulated electrohyperthermia (mEHT) have emerged as promising strategies to enhance the effectiveness of various cancer treatments [3,4]. mEHT utilizes controlled heat to selectively target tumor cells, and consequently activates complexes cellular responses, including the heat shock response (HSR) [3]. This intricate cellular network, which involves heat shock proteins (HSPs), can induce both protective and detrimental effects on cancer cells [5]. Initiated by the heat shock factor 1 (HSF1), the HSR protects cells from a wide range of stresses, including heat, and is crucial for cellular homeostasis [6]. Indeed, while the HSR promotes cell survival and protein homeostasis, it can also confer resistance to conventional anti-cancer therapies [7]. Thus, the modulation of the HSR holds extraordinary potential for increasing mEHT efficacy as a selective and powerful anti-tumor modality. In this review we summarize the interplay between mEHT and the HSR, exploring opportunities and challenges in cancer therapy. We focus on how the inhibition of the HSR could improve mEHT treatment effects.

2. HSF1 and the heat shock response (HSR)

The heat shock factor 1 (HSF1) is a ubiquitously expressed transcription factor that regulates the expression of chaperone genes in response to cellular stress [8]. To avoid cellular damage and protein degradation caused by a wide range of environmental stressors, organisms respond by inducing heat shock proteins (HSPs), which refold damaged proteins, consequently preserving

proteostasis [9]. This powerful adaptive mechanism is known as the heat shock response (HSR) [10]. Shortly, upon heat shock, HSF1 is phosphorylated, trimerizes, and translocates to the nucleus, where it induces chaperone gene expression by binding to the heat shock elements (HSEs) [11], promoter regions of HSPs. Consequently, transcription of HSP genes such as HSP27, HSP70, and HSP90 is activated [12]. HSPs in turn inhibit HSF1 transcriptional activity by physical interaction, creating a negative feedback mechanism for controlling the HSR [13]. Cell survival is achieved through the activation of anti-apoptotic proteins and the inhibition of pro-apoptotic proteins, a phenomenon known as thermotolerance, which enables cancer cells to withstand the effects of heat [11]. Fig. 1A illustrates the HSF1 activation. Hence, the role of HSPs is to regulate protein (re)folding, transport, translocation, and assembly under stress conditions in many normal cellular processes [5]. HSPs also help in the degradation of abnormal proteins via ubiquitinproteasome system (UPS), a process involving the post-translational conjugation of ubiquitins to proteins followed by degradation by the 26S proteasome [14]. Therefore, upregulation of HSPs increases cell survival and stress tolerance [15], not only in healthy cells under any kind of stress but also in cancer cells in which elevated expression of different members of the HSP family has been reported [16,17].

This mechanism is only possible due to the HSF1 structure that includes a multi-domain protein with distinct functional regions (Fig. 1B). Predominantly existing in a monomeric and inactive status, HSF1 comprises an N-terminal DNA-binding domain (DBD), a trimerization domain of two heptad repeat regions (HR-A and HR-B), a regulatory domain (RD), a third heptad repeat region (HR-C), and a C-terminal transactivation domain (TAD) [18].The DBD is highly conserved throughout evolution and belongs to the family of winged helix-turnhelix DBDs [19]. Once the HSR is triggered and the HSF1 homotrimer is formed, the DBD binds to the HSE [20]. The HSF1 trimerization is regulated by an oligomerization domain located next to the DBD, which contains an amphiphilic helix with hydrophobic heptad repeats HR-A and HR-B, forming a coiled coil [21]. Suppression of spontaneous HSF1 trimerization is mediated by another hydrophobic repeat, HR-C, adjacent to the carboxyl terminus of the protein [22]. Positioned at the extreme carboxyl terminus, the transactivation domain plays a crucial role in activating target genes at the transcriptional level and also regulates the extent of HSF1 activation [6]. Deletion of TAD has been shown to result in cell death during heat shock, highlighting its vital role in the survival of cells under stressful conditions [23]. Finally, the regulatory domain (RD), which undergoes post-translational modifications, is suggested to have a crucial function in detecting heat stress in humans by regulating HSF1 activity and stability [24], as its absence causes HSF1 to become transcriptionally active even in unstressed conditions [25]. This is the region for post-translational modifications (PTM), such as phosphorylation, acetylation, and SUMOylation [26].

3. The role of the HSR and HSF1 in cancer

HSF1 seems to have many roles in promoting tumorigenesis and tumor progression, as HSF1 controls many genes that may help the misleading phenotype and contribute to tumor growth [27], including genes involved in cell-cycle regulation, signaling, metabolism, adhesion and translation [28]. While HSF1 mutations are uncommon in different cancer types, frequent copy number alterations, particularly amplifications, are prevalent [29]. Indeed, many human tumor types and cancer cell lines express HSF1 constitutively at elevated levels [9,30,31], including hepatocellular carcinoma (HCC) [32,33], breast cancer [34], endometrial carcinoma [35], and oral squamous cell carcinoma (OSCC) [36], and this overexpression is related to increased malignancy and mortality.

In the malignant state, a wide variety of stressful conditions, such as hypoxia, acidity, and low glucose levels, arises from the tumor microenvironment [37]. In all these stress conditions, the cell's proteostasis network, which is responsible for the balance of protein synthesis, folding, and degradation, can become overwhelmed [38]. Therefore, cancer cells have stimulated HSR and proteasome activities due to elevated levels of constitutively misfolded proteins. At the same time, HSF1 permits cancer cells to cope with these diverse malignancyassociated stressors. In doing so, tumors reprogram their metabolism, physiology, and protein homeostasis, enabling oncogenesis [34]. The ultimate result is the facilitated cellular adaption to the malignant lifestyle [39].

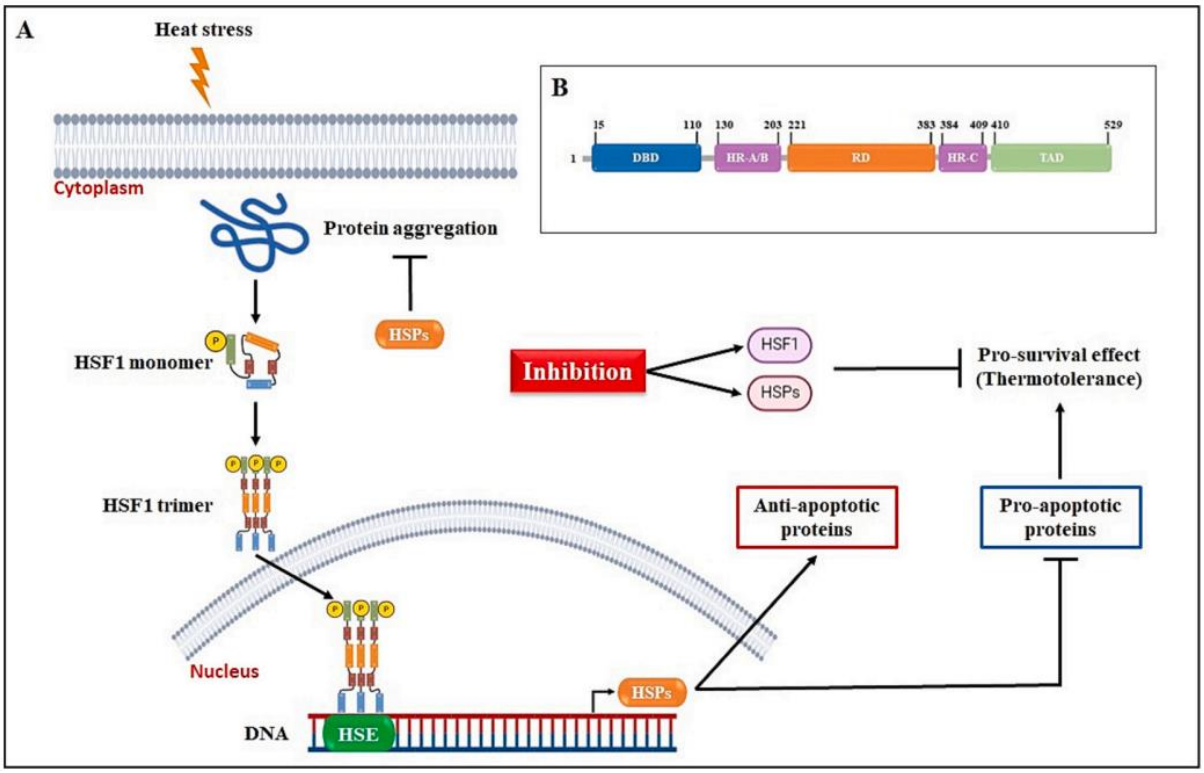


Fig. 1. Heat-induced thermotolerance and domain structure of the heat shock factor 1 (HSF1) monomer. A) Heat stress leads to aggregation of HSF1 monomer into a DNA binding homotrimer. This HSF1 trimer translocates into the nucleus where it binds to heat shock elements (HSE) in the promoter regions of HSP genes, activating the transcription of heat shock proteins (HSPs). HSPs protect (chaperone) proteins from aggregation and activate anti-apoptotic proteins and inhibit proapoptotic proteins, leading to thermotolerance. Therefore, heat-induced thermotolerance protects cells from hyperthermia-induced apoptosis. HSF1: heat shock factor 1; HSE: heat shock elements; HSP: heat shock protein; DNA: deoxyribonucleic acid; P: phosphate. Based on Ahmed et al. [11]. B) The HSF1 gene comprises a DNA-binding domain (DBD) and an oligomerization domain, denoted as HR-A/B. Under normal conditions, the HR-C domain acts to inhibit HSF1 oligomerization, maintaining it in an inactive state. HSF1 regulates transcription through the transactivation domain (TAD), and the stress responsiveness is governed by the regulatory domain (RD). Based on Anckar [6]. Created with biorender.com.

In cancer cells, HSF1 is often constitutively activated, leading to abnormal upregulation of HSPs, which confers a selective advantage to malignant cells by promoting cell survival, inhibiting apoptosis, and aiding in the development of aggressive phenotypes [40]. The oncogenic potential

of HSF1 was initially revealed by HSF1-knockout mouse models [39]. Indeed, HSF1 knockdown investigations have shed light on the crucial role of this protein in cancer growth, and the use of siRNA or genetic mutation to silence HSF1 has demonstrated a substantial reduction in tumorigenicity across multiple cancer types [41]. On the other hand, the overexpression and hyperactivation of HSF1 have been linked to poor prognosis and drug resistance in several cancer types, making it an attractive target for cancer therapy [42].

Although much less is known about the molecular mechanisms by which HSF1 regulates cell proliferation and survival in cancer cells, elevated expression of different members of the stress-inducible HSP family have been reported in a wide range of tumor types, indicating a crucial role of HSPs in tumor development [16,43]. Indeed, overexpression of HSPs have received considerable attention as prognostic biomarkers in terms of survival and response to therapy in cancer [44]. This abnormal expression of HSPs, implicated in various cancer hallmarks such as apoptosis resistance and immune tolerance, is considered a multifaceted phenomenon driven by intricate interplay between the cellular stress response, tumor microenvironment, and the unique demands of cancer cells [45]. The elevated levels of HSPs provide cancer cells with a survival advantage by promoting protein folding, stabilizing oncogenic proteins, and assisting in the proper functioning of cellular processes under stress conditions [46]. The hypoxic and nutrientdeprived tumor microenvironment induces proteotoxic stress and leads to HSPs upregulation as a cellular defense mechanism against misfolded proteins and aggregation [47,48]. Additionally, oncogenic signaling pathways, such as those driven by Myc and Ras, can transcriptionally activate HSP genes through HSF1 [27,49]. On the other hand, these chaperones play a pivotal role in the immune response owing to their unique ability to securely bind polypeptide chains. This interaction facilitates the formation of complexes between HSPs and tumor antigens [50]. These complexes serve as crucial markers, subsequently recognized by key immune cells such as monocytes, macrophages, B cells, and dendritic cells. This recognition event orchestrates a signal cascade, ultimately triggering the activation of cytotoxic T cells, a pivotal component of the anti-tumor immune response. [51]. Therefore, while HSPs are part of the cellular machinery that helps cancer cells survive stress, they can also act as allies in controlling the immune system's power to fight against cancer. This dual role highlights the complexity of HSPs' function and their potential for therapeutic interventions in cancer treatment strategies.

Although cancer cells have been reported to release several extracellular chaperones, the most extensively studied ones with active roles in cancer include HSP27, HSP70, and HSP90 [47]. These HSPs exhibit slight functional variations and are commonly classified based on their molecular weight. HSP27, also known as heat shock protein beta-1 (HSPB1), is among the smaller members of the heat shock protein family. Its compact size enables specific interactions with client proteins, contributing to diverse cellular functions such as cytoskeleton regulation, cell migration, and anti-apoptotic activity [52], and its overexpression has been implicated in various aspects of cancer biology [53,54]. For instance, upregulation of HSP27 by HSF1 can promote invasion and metastasis of HCC [33], and is associated with aggressive growth and resistance to chemotherapy or radiotherapy [55], consequently with poor prognosis in breast [56], ovarian [57], colorectal [58], and prostate cancers [59], while downregulation or inhibition leads to reversion of resistance [53]. HSP27 is also recognized for its significance in regulating cancer development, progression, and cell apoptosis [60]. HSP70, in turn, has critical role in protein folding, protein homeostasis, and promoting cell survival [61]. This chaperone is strongly expressed on the surface of cancer cells [62], where it might exert a dual role: intracellular HSP70, which is overexpressed in cancer cells,

promotes survival, proliferation, invasiveness, and resistance of malignant cells, while extracellularly shed or deliberated HSP70 contributes to antitumor immunity as a damage associated molecular pattern (DAMP), leading to increased cell damage [63,64]. Within cancer cells, HSP70 triggers mitotic signals, inhibits apoptosis, and suppress oncogeneinduced senescence [49]. Similarly to HSP27, HSP70 is also associated with resistance to chemotherapy and poor prognosis for a wide range of cancer patients [64], such as lung, breast, colon, liver, prostate, esophagus, and cervix [65,66]. Moreover, the upregulated HSP70 levels could potentially work as a predictive factor for both cancer diagnosis and treatment response [49]. Likewise, downregulation of HSP70 expression inhibits tumor growth and significantly promotes apoptosis, consequently increasing tumor's susceptibility to chemotherapy and radiotherapy [67].

Last, HSP90 proteins possess a significant position in fundamental process and regulatory pathways, such as apoptosis, cell cycle regulation, signaling cascades, cellular viability, as well as protein folding and degradation [68]. These essential functions are intricately managed by HSP90 proteins through their interactions with client and co-chaperones [69]. Notably, their key role extends to proteostasis maintenance, cell differentiation, and developmental processes [70]. In this context, a correlation between HSP90 overexpression and diverse cancer types has been observed and highlights the potential role of HSP90 in driving cancer progression [71]. Indeed, upregulation of HSP90 has been reported in cancer tissues compared with normal tissues in breast cancer patients [72]. This high HSP90 expression can be associated with the risk of malignant cancers that are less responsive to treatment [73], suggesting that HSP90 may contribute to cancer progression in bladder, spleen, and brain [68]. Consequently, the suppression of HSP90 through selective inhibitors like geldanamycin impedes the advancement of tumors [74]. HSP90 inhibitors, therefore, hold promise as potent and distinctive candidates for cancer chemotherapy [75]. A few HSP90 inhibitors have already been identified and have entered clinical trials [76].

4. HSR/HSF1 INHIBITION AND ITS RELATIONSHIP WITH HYPERTHERMIA

Hyperthermia is the therapy that consists of treating malignant tumors by heating the tumor area, and is based on the differential response of tumor tissue and normal-healthy tissue to heat [77]. Szasz et al. define oncological hyperthermia as “a method for killing malignant cells by controlled thermal effects, and has the potential to sensitize to complementary therapies while avoiding the destruction of healthy cells” [78]. Hyperthermia has been reported to be a clinically relevant coadjuvant for cancer treatment [79]. Many studies have demonstrated the increased drug exposure to tumor via the circulation by adding heat treatment, and hence increasing cytotoxicity of chemotherapeutic agents [80–83]. However, hyperthermia as a cancer treatment modality has been reported to be controversial [84]. The controversy arises from the challenges associated with achievement of deep heat penetration and precise heat effect, which consequently leads to the lack of selective elimination of malignant cells [85]. The ultimate result is an extensive macromolecular change that affects functions not only in tumor tissues but also in all adjacent cellular compartments, particularly when temperatures exceed 43 ◦C [86]. Additionally, an increase in temperature can boost blood flow and nutrient delivery, which potentially facilitates cancer progression leading to metastasis [87]. Nonetheless, the most relevant complication associated with the use of hyperthermia in cancer treatment is the induction of a heat stress response in cells [88,89]. This phenomenon, known as thermotolerance, is an defense mechanism of cells' susceptibility against heat-induced proteotoxicity after heat stress [16]. The mechanism of

thermotolerance is attributed to HSP production, and hampers the effects of hyperthermia [37]. This acquisition of thermoresistance against heat stress enhances cancer cell growth by preventing apoptotic cell death [11] via elevation of HSF1 [34] and HSPs [44], and reduces the hyperthermia effects in clinical treatment. Therefore, the inhibition of HSR by targeting HSF1 may sensitize cancer cells to therapies that rely on hyperthermia as a method for cancer treatment.

HSF1 is therefore considered as one of the main determinants of oncogenesis, and ablation experiments have shed lights to the role of HSF1 in cancer development. In vitro HSF1 knockdown resulted in impairment of growth, survival, invasion, migration and epithelialmesenchymal transition (EMT) of cancer cell lines, including pancreatic cancer [90,91], multiple myeloma [92], hepatocellular carcinoma (HCC) [32], colorectal carcinogenesis [93], and melanoma [94]. In turn, HSF1 knockout mouse models are proved to be remarkably resistant to a number of oncogenes [10,39,95,96]. Recently, it has been postulated that breast cancer tumors in HSF1 knockout mice, although viable, grow much slower than control tumors, suggesting that HSF1 plays a central role in cancer growth [97]. Indeed, a chemically-induced carcinogenesis model revealed that HSF1- /- mice developed fewer tumors, presented lower tumor load (total amount of cancer in the body), and longer survival, while mice-bearing functional HSF1 developed larger tumors and had shorter survival [39]. Moreover, HSF1 knockdown induce apoptosis [98], inhibit cell proliferation, and arrest cell cycle at G1 phase [93,99] in cancer cells.

HSF1 knockdown has been shown to enhance hyperthermicchemotherapy in cervical cancer [100] and to reduce proliferation and tumor size in skin [39,101], liver [98], ovarian [28], pancreatic [91], and breast [102,103] cancers. Indeed, Rossi et al. reported that HSF1 knockdown led to increased sensitivity of HeLa cells to thermochemotherapy, resulting in upregulation of apoptosis [100]. Also, the knockdown of HSF1 was associated with autophagy inhibition which increases drug sensitivity to chemotherapeutic treatment in breast cancer cells [104]. Interestingly, the knockdown of HSF1 seems to enhance cancer cell sensitivity to hyperthermia but does not have a direct influence on chemotherapy. Cancer cells sensitivity to thermochemotherapy with or without HSF1 silencing was similar regarding cell destruction [101]. In addition, the gene therapy designed to target HSF1 helped to escape thermoresistance [105–107]. McMillan et al. have demonstrated that HSF1 inactivation abolished thermotolerance in mouse embryonic fibroblasts (MEF) treated with hyperthermia, and inhibited the upregulation of HSPs, such as HSP70 [108]. Likewise, Wang and colleagues have demonstrated that functional silencing of HSF1 strongly reduced the HSP70 levels and inhibited thermotolerance in breast cancer cells, suggesting that cancer cells lacking HSP70 expression are sensitive to hyperthermia, and those expressing HSP70 may be thermotolerant [106]. Moreover, HSF1 depletion by small interfering RNA (siRNA) resulted in reduction of the constitutively high expression of HSP90 and HSP70, in breast cancer model [103]. These findings suggest that hyperthermia in combination with the inhibition of the heat shock response might be exploited for treating cancer patients.

5. Modulated electro-hyperthermia (mEHT)

Modulated electro-hyperthermia (mEHT) is a promising new adjuvant therapy form [3,4]. mEHT is a non-invasive cancer therapy applying a modulated electromagnetic field to the tumor, inducing tumor cell damage by temperature dependent- and independent mechanisms. A 13.56 MHz

radiofrequency (RF) is applied by capacitive coupling between two electrodes arranged around the tumor [109,110] (Fig. 2A).

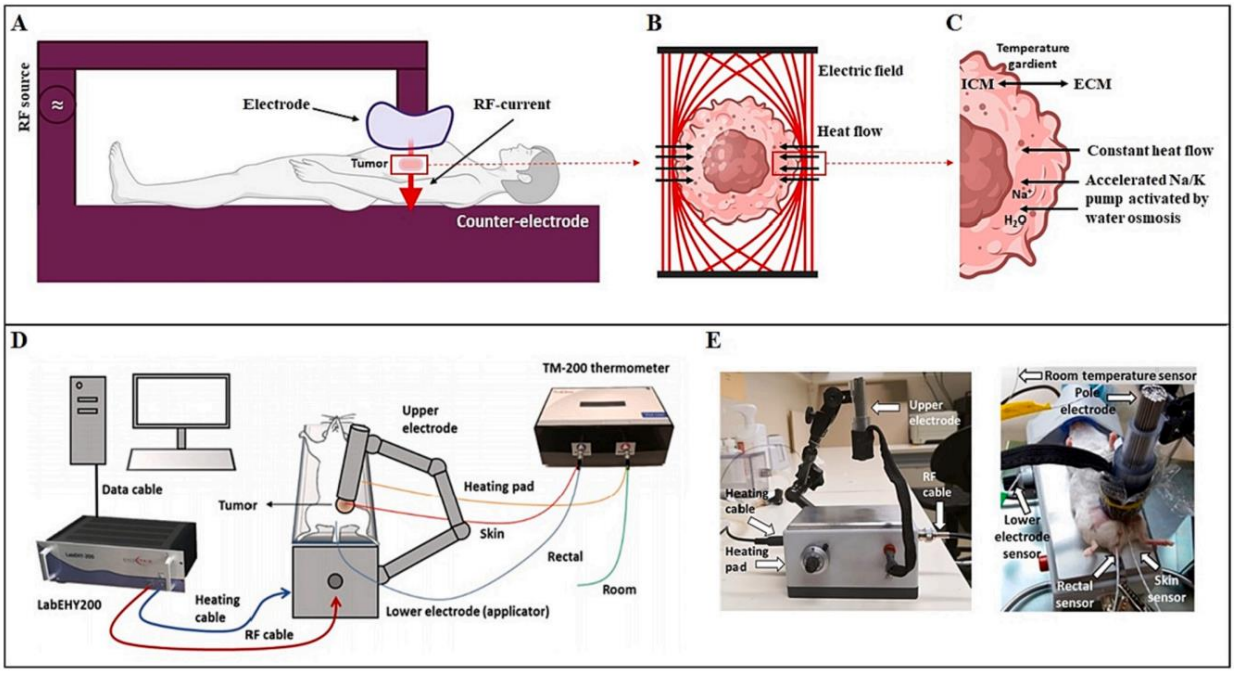


Fig. 2. Schematic illustration of modulated electro-hyperthermia (mEHT) treatment in human patients and mice. The unidirectional electric field (depicted by the red arrow) traverses the patient's body, flowing in a controlled manner from the electrode to the counter-electrode (A). This directional flow enables precise energy delivery to malignancies, particularly along cell membranes, exploiting the tendency of the electric field to follow paths of higher conductivity, such as malignant tissues. Consequently, this process induces localized heating (B). Subsequent biochemical reactions are initiated by the heat stress in the cell membrane of malignant cells. The resulting temperature gradient between extracellular and intracellular matrices induces changes in membrane potential, triggering a series of events that includes heat transfer across the membrane, elevated intracellular sodium concentration, potassium efflux, and water osmosis (C). The combined effects act synergistically and drive the induction of apoptosis. Based on Szasz et al. [85]. Created with biorender.com D) Illustration of the mEHT treatment setup LabEHY200 designed for in vivo experiments involving mice, reproduced from Schvarcz et al. [4]. E) mEHT in vivo treatment setup, reproduced from Danics et al. [3]. RF: radiofrequency, ICM: intracellular matrix, ECM: extracellular matrix, Na: sodium, K: potassium. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The energy of the RF current is selectively absorbed by tumor tissues due to several mechanisms reviewed before [111], including alterations of the cancer tissue metabolism, ion composition and the electromagnetic properties of lipid rafts [112]. The electromagnetic field induces a + 2.5 ◦C heating of the tumor compared to its surrounding [3]. The +2.5 ◦C temperature difference, significantly widens the narrow therapeutic window (ΔT: ca 1 ◦C only) achievable with conventional hyperthermia. This technique, which has been successfully applied in the clinics for over 20 years [113], differs from conventional hyperthermia methods in that mEHT creates nonhomogenous heat by increasing the temperature gradient between the intracellular/extracellular environment and the cell membrane in malignant tissues [114] (Fig. 2B). This alteration in temperature gradient affects

membrane processes, which favors signaling pathways that induce extrinsic apoptosis [113,115] rather than thermal necrosis [116] (Fig. 2C). Consequently, it induces DAMP signals that trigger immunogenic cell death (ICD) in malignant cells [117]. The temperature dependent cytotoxicity targeting cancers is thus enhanced by a synergy between the heat and the electromagnetic field [118–122].

The fundamental concept behind mEHT was the rejection of the central reliance on temperature as the primary factor. Instead, the technology focused on the core elements of power absorption, extracellular heating, and modulation, which were not dependent on temperature [123]. In fact, the modulation is able to induce non-thermal effects which enhance the cell-killing thermal effects, compared to conventional hyperthermia [118,124]. This is achieved through the promotion of immunogenic cell death and the stimulation of tumorspecific antitumoral immune responses [115]. Therefore, the resulting electromagnetic field generates irreversible cell stress [125]. Moreover, mEHT has overcome the most problematic point of hyperthermia devices. According to Roussakow, the concept of "skin sensor" in mEHT has abandoned the need of thermometry in conventional hyperthermia [123]. The mEHT electrodes induce heating only surrounding the "zone of interest", which increases selectivity of energy deposition in tumor tissues [123]. In this regard, according to Lee et al., mEHT is a promising technique that can achieve selective and effective targeting of the cancer tissue [84].

Previous in vitro and in vivo studies have demonstrated that mEHT is more effective than traditional hyperthermia (water-bath, infrared, or RF-hyperthermia) at the same temperature [124] due to the potentiating effects of the electromagnetic field (non-temperature dependent effects) and the greater temperature difference. Fig. 2D, E illustrates the mouse setup for in vivo studies. Moreover, mEHT has been shown to enhance cell-killing effects by increasing drug uptake in cancer cells [126]. In the clinical setting, mEHT has been demonstrated to induce significant improvements in patients with breast- [127], cervical- [128], ovarian- [129], rectal- [130], and pancreatic cancer [131–133].

6. The mechanisms of cancer cell-killing by mEHT

The pathophysiological mechanisms underlying mEHT involve a combination of thermal and non-thermal effects (Fig. 3). The synergism between thermal and non-thermal effects triggers the excitation of specialized cell membrane regions, such as lipid rafts, ultimately resulting in activation of apoptotic pathways [115]. The thermal effects are achieved by selectively heating tumor tissues through the absorption of electromagnetic waves by cancer cells, which leads to increased cellular temperature [122]. These effects are, therefore, direct consequences of temperature elevation (temperature-dependent). When exposed to elevated temperatures, cells undergo several changes that influence the progression of cell cycle [134]. Application of mEHT induces irreversible cellular stress, resulting in the arrest of the tumor cell cycle and subsequent caspase-dependent programmed cell death [135,136]. The temperature elevation increases blood flow and perfusion through the target tissues, which potentially improves the efficacy of chemotherapy [137].

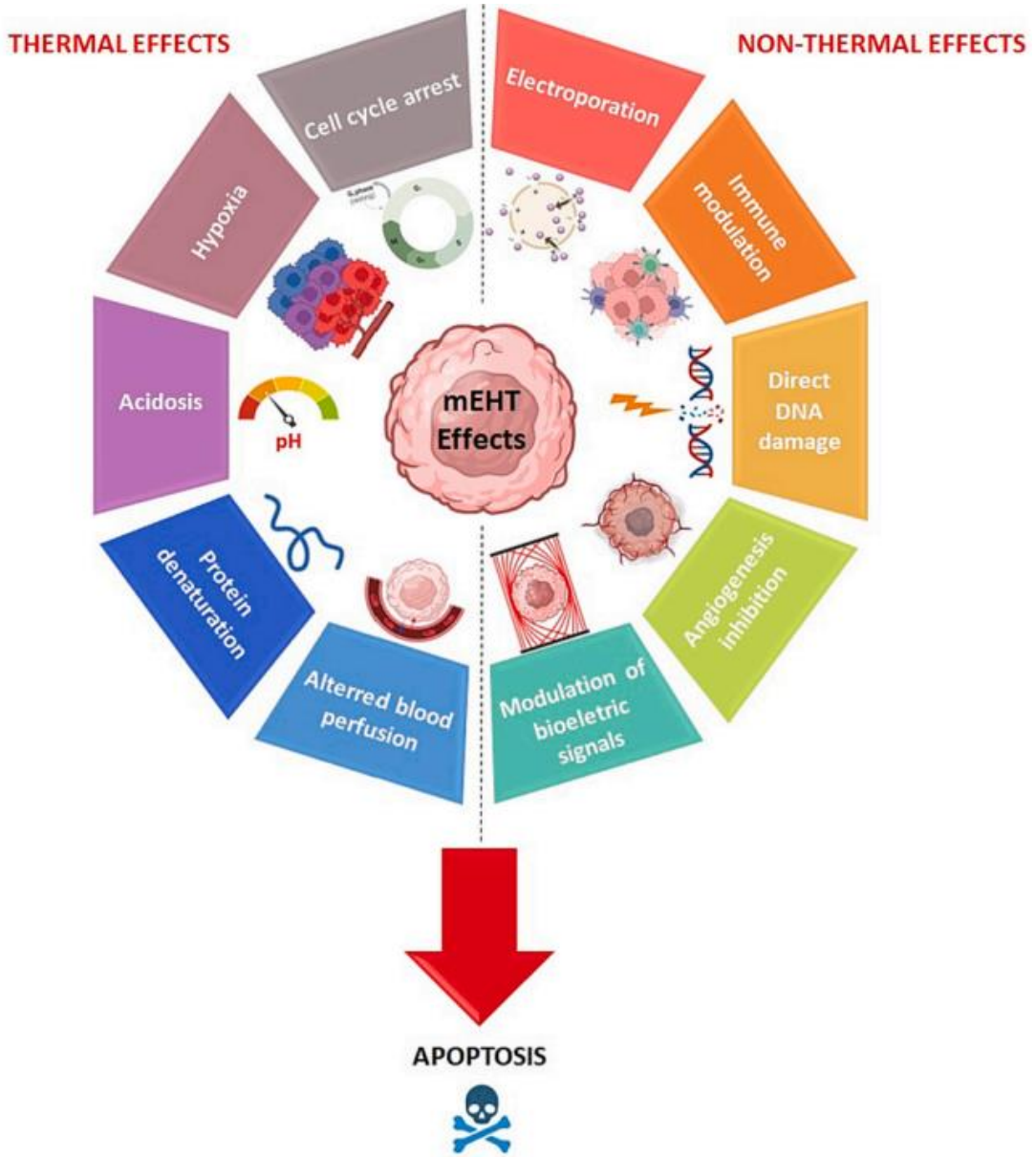


Fig. 3. Thermal and non-thermal effects of mEHT in cancer cells. Thermal effects encompass cell cycle arrest, hypoxia, acidosis, protein denaturation, and altered blood perfusion. Non-thermal effects include electroporation, immune modulation, direct DNA damage, angiogenesis inhibition, and modulation of bioelectric signals. For more details, see the text. Based on [115,130,134–141,143–150]. Created with biorender.com.

Hyperthermia can also lead to protein denaturation due to the disruption of weak bonds and interactions with the protein's structure, causing it to unfold or lose its native conformation [138]. This is the key event in the disruption of cellular homeostasis [11], and can be avoided by chaperone proteins, such as HSPs, that are able to prevent protein aggregation [139]. Furthermore, in combination with chemotherapy and radiotherapy, mEHT has shown potential in overcoming hypoxia-related resistance [130,140] and downregulating hypoxia-related target genes [139]. Finally, the rise in temperature can induce localized acidosis through elevated metabolic activity and reduced oxygen availability [141]. This harsh environment can ultimately lead to the destruction of the 'starving' tumor [136].

On the another hand, mEHT also triggers non-thermal effects that occur when the system undergoes changes in its properties under the influence of an alternating electromagnetic field, which cannot be achieved solely through heating [142], contributing to its anti-cancer properties. The non-thermal effects are primarily frequency-dependent and arise from the interaction between the biological substance and the RF-current rather than the heating process itself [143]. Indeed, the high-frequency electric fields employed in mEHT induce alterations in the electric potential across the cancer cell membranes [144]. This leads to the excitation of channels such as transient receptor potential channels (TRPs), HSPs, voltage-gated channels, and voltage-sensitive phosphatases (VSPs) [115]. These interactions subsequently engage the apoptotic signaling pathways [113]. This phenomenon also known as electroporation can enhance the uptake of certain molecules and drugs, potentially increasing the treatment effectiveness [145]. Furthermore, the conductivity and the dielectric constant in malignant tissues are higher compared to normal tissues [146]. This leads to increased energy absorption by tumors compared to the surrounding healthy tissue, raising the extracellular temperature of cancer cells and ultimately causing damage [144]. Through the electromagnetic field, mEHT is also able to induce direct DNA damage in cancer cells by several mechanisms, including the generation of reactive oxygen species (ROS) and the disruption of DNA repair pathways, which leads to genomic instability and cell death [139,147]. Moreover, previous study has confirmed that the electromagnetic field might inhibit or prevent new blood vessel formation through the inhibition of vascular endothelial growth factor (VEGF) production in breast cancer cells [148], probably via disruption of bioelectric signals that impede the formation of new blood vessels. Finally, mEHT has been proposed to induce abscopal phenomena, leading to simultaneous growth inhibition of tumors located at a distance from the site of treatment [149]. By triggering an immune response reaction, mEHT enables the body to systematically recognize and attack cancer cells, shifting the balance towards tumor suppression [150]. This is achieved through the induction of immunogenic cell death and modification of tumor microenvironment [115], leading to the activation and recruitment of immune cells, such as dendritic [151], cytotoxic T [149], and natural killer cells [152]. Additionally, mEHT may synergistically work with immune checkpoints inhibitors, which reinforce the immune response against cancer cells [153]. The immune action of checkpoint inhibitors results in abscopal effect in clinical practice [154,155].

7. mEHT and the induction of the HSR

As mentioned before, when exposed to heat shock, cells induce chaperone proteins (heat shock proteins, HSPs) that protect them from the negative effects of heat. Same phenomena is observed in cancer cells, resulting in the development of treatment resistance and the promotion of malignant processes including uncontrolled growth, reduced tumor suppression, enhanced cell survival, and the acquisition of powerful capacities for angiogenesis and metastasis [46]. As a variation method of hyperthermia, mEHT can induce heat shock response and subsequent HSPs upregulation in treated tumors. Indeed, the heat map on gene expression revealed significant induction of members of the heat shock protein family, such as HSP70 and HSP90, after mEHT treatment in a human colorectal adenocarcinoma xenograft [117]. Multiplex data using next generation sequencing (NGS), mass spectrophotometry (MS), and Nanostring confirmed the upregulation of HSP70 isoforms after mEHT treatments [4]. Corroborating the upregulation in mRNA levels, HSPs were also upregulated at the protein level [156].

The upregulation of HSP70 was also observed in a triple-negative breast cancer (TNBC) isografts treated with mEHT [3,4]. This finding was reported 24 h after the mEHT treatment and was associated with inhibition of tumor growth and proliferation. Moreover, mEHT increased more than 10-fold the extracellular HSP70 release 48 h after treatment compared to conventional capacitive coupling hyperthermia and water bath [124]. In another study, mEHT induced massive HSP70 expression not only intracellularly but also membrane-bound and extracellular HSP70 was stimulated, which can be linked to enhancement of anti-tumor immunity [157]. In fact, Kuo et al. suggested that combined mEHT therapy with curcumin and resveratrol synergistically increased the immune response and HSP70 release, hence augmenting the anti-tumor efficacy in CT26 tumors [158]. mEHT is also able to provoke HSP70 upregulation in murine colon carcinoma models [125,135,151], pancreatic adenocarcinoma [159], and melanoma xenograft [152].

Although many papers have proposed upregulation of HSPs by mEHT treatments, Andocs et al. proposed a controversial effect of mEHT in human lymphoma cells [113]. In this study, gene expression was analyzed using microarray in U937 cell line. The gene chip analysis then revealed a distinct difference in gene regulation between samples treated with water bath and those treated with mEHT at the same temperature. Notably, a highly cytoprotective gene network was activated in samples submitted to water bath treatments, resulting in upregulation of HSPs. The upregulation of HSPs ultimately prevented apoptotic cell death in this model. The same cytoprotective gene network remained silent in mEHT-treated cells [113]. This difference in pathway activation is likely attributed to the electric field effects observed in mEHT treatments [150].

A recent study has demonstrated for the first time that downregulation of HSF1 gene by CRISPR/Cas9 gene-editing tool increased sensitivity of TNBC tumors to mEHT treatments. Tumor follow-up measurements exhibited decrease in tumor volume when mEHT was applied to tumors generated from HSF1 knockdown cancer cells. This proof of concept experiment also revealed that the lentiviral construct reduced HSP70 upregulation after repeated mEHT treatments, hence decreasing heat-induced thermotolerance (data not published). Moreover, the inhibitory effect of HSPs on apoptosis is associated with its direct interaction with apoptotic molecules [160,161]. Inhibiting HSF1leads to a significant pro-apoptotic impact, accompanied by a concurrent decrease in various heat shock proteins. Thus, the already established apoptotic effect induced by mEHT [135,139,158,162] could be further enhanced when applied to HSF1 knockdown cancer cells. However, additional experiments are necessary to confirm this hypothesis.

8. Natural and synthetic HSF1 inhibitors

Besides the studies that have demonstrated successful repression of cancer growth by depletion of the HSF1 gene, a number of attempts at developing small molecule inhibitors to reduce HSF1 expression have been reported [163], but most of them are still in preclinical phase [42] (Table 1 and Fig. 4). In spite of the successful inhibition of HSF1 observed in both in vitro experiments and animal models, each inhibitor currently available for clinical use has its own set of limitations [164]. Unfortunately, for many of these compounds, the exact mechanism of action and drug specificity remains unknown [42]. Another bias comes from the fact that HSF1 carries restrictions as a target for drug development due to the absence of a clearly identifiable binding site for small molecule inhibitors, the intricate nature of its activation process, and its susceptibility to numerous

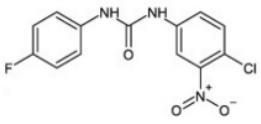
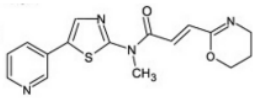
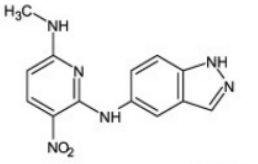
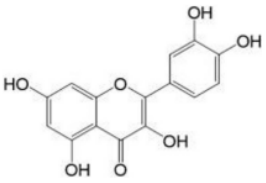
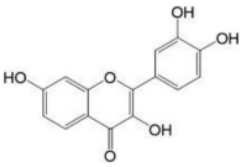
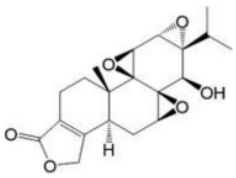
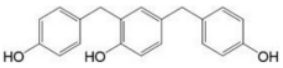
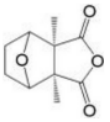
posttranslational modifications in response to different types and levels of proteotoxic stress [29]. Nevertheless, targeting HSF1 for cancer therapy might be a promising modality in cancer treatment.

As HSF1 plays a remarkable role in tumorigenesis, its knockdown may reduce the proliferation, migration and invasion of cancer cells [42,192], hence the development of HSF1 and consequently HSP inhibitors became a target of cancer research [193]. Though mEHT is able to activate a protective machinery, mainly by heat shock protein family induction, in which high expression of HSPs, such as HSP70, can protect cancer cells from cell death, the anti-tumor effect of mEHT may be enhanced by blocking the HSP-mediated defense mechanisms of cancer cells [3]. Therefore, targeting HSF1 domains with small molecules may have a favorable toxicity profile.

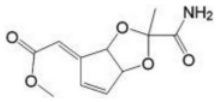
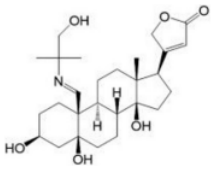
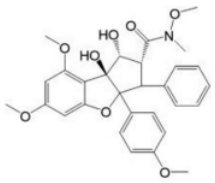
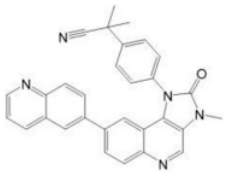
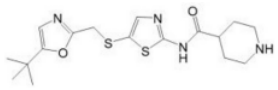
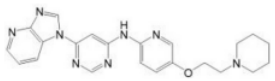
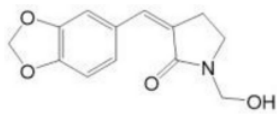
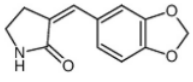
Several potential inhibitors of HSF1 have been formulated, commonly derived from either natural products or synthetic chemical structures. Recent reviews provide detailed overview of the currently available compounds, their structure and mode of action [29,42,164,194,195] (Table 1 and Fig. 4). Some of these compounds were used in combination with mEHT. Kuo et al. verified that combining curcumin and resveratrol with mEHT increased immune cell infiltration into tumors receiving this treatment [158]. In turn, HSP70 overexpression was also reported in tumors treated with combined therapy. However, the authors proposed a mechanism by which HSP70 mediates antigen-presenting cells (APCs) recruitment, leading to enhanced antitumor efficacy in CT26 tumors [158]. Resveratrol, a phenolic compound discovered in grape seeds, exerts its effectiveness by inhibiting Akt phosphorylation, leading to suppression of HSF1 activation in cancer cells [196]. Mustafi et al. proposed that resveratrol plays a role in inhibition of HSF1 translocation to the nucleus, consequently suppressing HSP70 expression [190].

Similar outcomes were presented earlier by Chakraborty et al., in which HSP70 downregulation was achieved through inhibition of HSF1 transcriptional activity mediated by obstruction of HSF1 nuclear translocation [197]. Contrarily, curcumin has been proposed to stimulate HSP expression, such as HSP70, in various cell types, including colorectal carcinoma [198] and leukemia cells [199]. This effect is most likely attributed to the activation of HSF1 [200]. Curcumin, a well-known phytochemical agent with anti inflammatory properties [201], induces HSP70 expression without compromising cellular viability in cancer cells [202]. Furthermore, curcumin has been reported to upregulate a tumor suppressor heat shock protein HLJ1 which leads to inhibition of cell invasion and metastasis in lung cancer cells [203].

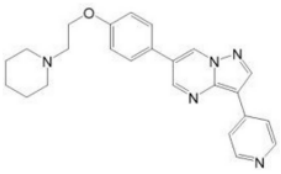
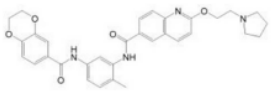
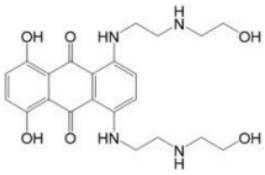
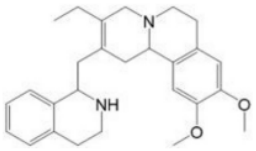
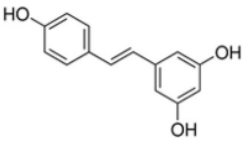
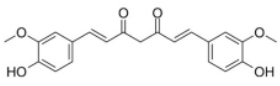
A recent paper revealed, however, that curcumin significantly decreased HSF1 expression as well as proliferation of oral squamous cancer cells [191]. This paradox sustains our perception that specific HSF1 inhibitors are needed and further pre-clinical research is essential for better understanding of the mechanisms behind these inhibitors before entering clinical trials.

Compound	Structure	Molecular Weight	Mechanism of Action	References
Direct targeted HSF1 inhibitor (DTHIB)		310	Strongly interacts with HSF1 DBD to downregulate nuclear HSF1	[165,166]
I_{HSF1}115		328	Induces Transcriptional activity inhibition through conformational change in the HSF1 DBD	[167]
KRIBB11		284	Inhibits HSF1 activity by blocking HSF1-dependent p-TEFb recruitment to heat shock genes	[168]
Quercetin		302	Inhibits HSF1-HSE complex; Reduces HSP70 through the AP-1 pathway	[169,170]
Fisetin		286	Blocks the binding of HSF1 to HSP70 promoter	[171]
Triptolide		360	Blocks the transcriptional activation of HSF1 complex on the HSP70 promoter; results in HSP90 acetylation	[172–174]
2,4-Bis(4-hydroxybenzyl) phenol		306	Degrades HSF1 protein through dephosphorylation of HSF1	[175]
Cantharidin		196	Inhibits the HSF1 binding to HSP70 promoter; blocks HSF1-dependent p-TEFb recruitment	[176]

(*continued on next page*)

Compound	Structure	Molecular Weight	Mechanism of Action	References
Stresgenin B		239	Inhibits HSP70 promoter activity	[177]
CL-43		475	Not defined	[178]
Rohinitib		521	Reduces HSF1 DBD binding affinity to HSE	[179,180]
BEZ235		469	Downregulates the inducible HSP70-encoding HSPA1A gene	[181]
SNS-032		380	Not defined	[182]
4,6-disubstituted pyrimidines		416	Inhibits HSF1 pathway indirectly through CDK9 inhibition	[182]
KNK437		247	Inhibits the HSF1 activation and the HSF1-HSE interaction; Inhibits the AKT/HSF1 pro-survival pathway	[183,184]
KNK423		217	Same as KNK437	[184]

(*continued on next page*)

Compound	Structure	Molecular Weight	Mechanism of Action	References
Dorsomorphin		399	Inhibits HSF1 nuclear translocation; Inhibits HSF1 phosphorylation	[185]
CCT251236		552	Inhibits HSF1-mediated transcriptional activity	[186]
PW3405		444	Inhibits HSF1 phosphorylation and activity	[187]
NZ28/Emunin		418	Reduces HSF1 phosphorylation and inhibits HSF1 transcriptional activity; Downregulates HSP70 *via* HSF1 inhibition	[188,189]
Resveratrol		228	Inhibits HSF1 nuclear translocation	[190]
Curcumin		368	Prevents HSF1 from binding HSE	[191]

HSF1: heat shock factor 1; DBD: DNA-binding domain; p-TEFb: positive transcription elongation factor b; HSP: heat shock protein; HSE: heat shock elements; AP-1: activator protein 1; CDK: cyclin-dependent kinase.

In vitro experiments demonstrated that quercetin and KRIBB11, two potent heat shock inhibitors, when applied in combination with mEHT treatments not only reduced breast cancer cell viability but also inhibited HSP70 mRNA upregulation normally seen in mEHT monotherapy [3]. Moreover, the mEHT + KRIBB11 synergism was also proposed to decrease the heat shock-related complement production through C4b, an acute phase protein [4]. Quercetin, a flavonoid plant pigment, is recognized for its ability to suppress the heat shock response by preventing HSF1 binding to heat shock elements (HSE) [169]. Additionally, quercetin not only suppresses the accumulation of HSP70 in tumors during combination therapy but also facilitates cell apoptosis through the HSF1 pathway [164].

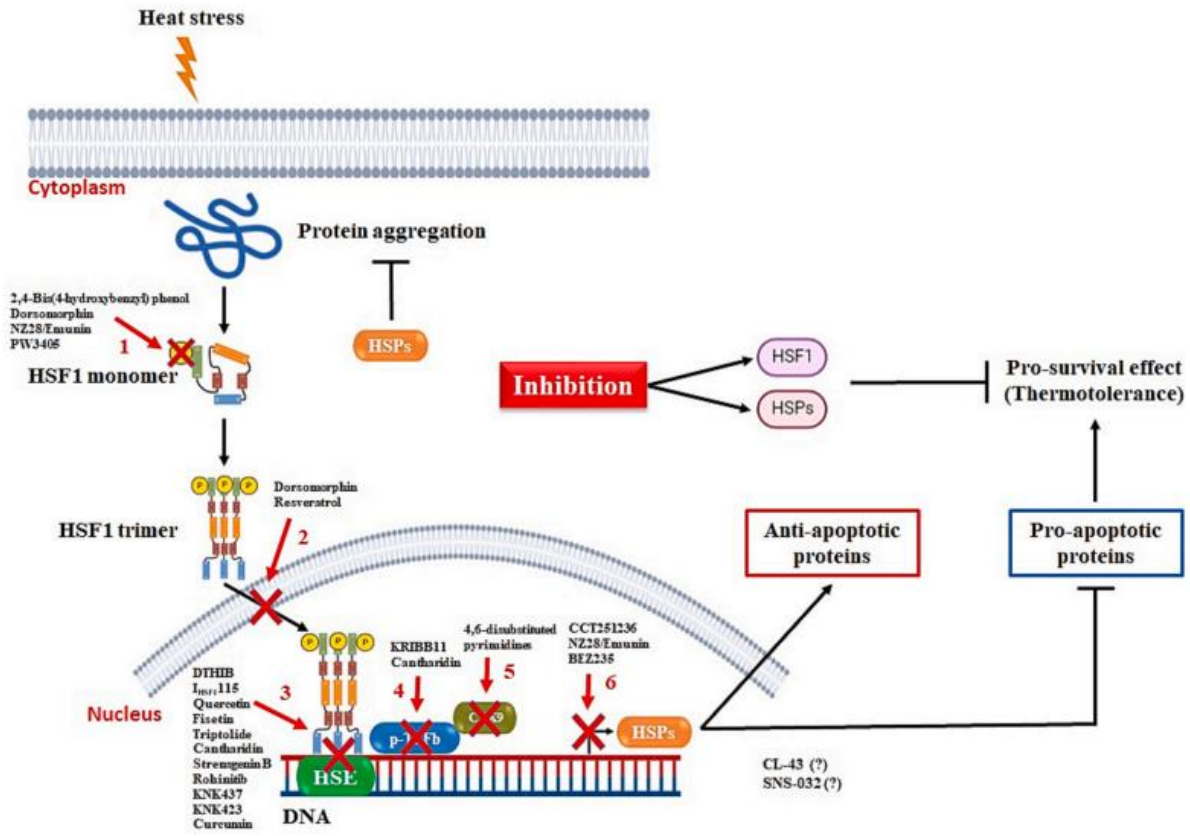


Fig. 4. Mechanism of action of HSF1 inhibitors. Various HSF1 inhibitors target distinct steps in the HSF1 pathway: 1) Emunin and PW3405 inhibit HSF1 phosphorylation, reducing its activation. 2) Dorsomorphin and Resveratrol prevent HSF1 from translocating into the nucleus. 3) Quercetin, fisetin and curcumin, suppress HSF1's ability to bind the HSE. 4) KRIBB11 and cantharidin block HSF1-dependent recruitment of the positive transcription elongation factor (p-TEFb), which impede downstream effects. 5) CDK9 inhibitors, such as 4,6-disubstituted pyrimidines, indirectly hinder HSF1 function. 6) CCT251236 and BEZ235 directly inhibit HSF1- mediated transcriptional activity. The HSF1 inhibition mechanism is still not clear for two compounds: CL-43 and SNS-032. Graphical design based on Ahmed et al. [11]. References regarding the mechanisms of each inhibitor can be found in Table 1. Created with biorender.com.

However, quercetin seems to inhibit multiple targets not limited to HSF1, such as a range of protein kinases, suggesting a non-specific mechanism of action [204]. Differently, KRIBB11 (N2 -(1H-indazole-5- yl)-N6 -methyl-3-nitropyridine-2,6-diamine), a novel synthetic chemical compound described by Yoon et al. [168], is the only commercially available HSF1 specific inhibitor. KRIBB11 exerts its inhibitory effect on HSF1 activity by disrupting the binding of positive transcription elongation factor b (p-TEFb) to the promoter region of the HSP gene [168]. KRIBB11 has been reported to inhibit HSF1 expression in pancreatic cancer [90], breast cancer [205–207] and also triple-negative breast cancer [208], bladder cancer [209], lung cancer [210], hepatocellular carcinoma [211], myeloma cells [212], glioblastoma cells [213], and leukemia model [214]. These in vivo experiments have demonstrated that KRIBB11 can reduce tumor growth without significant toxicity [168,207,209,211,212,214]. Besides HSF1 inhibition, HSPs downregulation upon KRIBB11 drug administration has also been reported [209,210,212–214]. Contrarily, Yoo et al. results were

inconsistent with those previous studies that demonstrated KRIBB11 anticancer effect through HSF1 depletion. In fact, this group failed to prove the downregulation of HSF1 and HSPs by KRIBB11, indicating that the activation of different molecular pathways by KRIBB11 depends on the application of the compound whether in a steady state or under stress conditions, such as heat shock, which could potentially result in the HSF1 activation [215]. Despite the fact that KRIBB11 seems to be highly specific to HSF1, Kang et al. suggested a possible off target effect on an anti-apoptotic protein, MCL-1, in which KRIBB11 was found to accelerate MCL-1 degradation, hence inducing apoptosis in cancer cells [216]. Finally, our recent in vivo experiment revealed synergism between mEHT and KRIBB11 in a TNBC mouse model. Simultaneously KRIBB11 administration for 8 days and four mEHT treatments demonstrated significant reduction of tumor weight with no body weight loss, and inhibition of HSP70 upregulation usually reported when tumors are treated by mEHT due to heat shock response, in both mRNA and protein levels (data not published). These results suggest that KRIBB11 might have high translational potential.

9. Conclusion and perspectives

Over the years, hyperthermia has shown promise as a cancer treatment, but it also possesses inherent weaknesses and limitations. These include challenges related to tumor depth, temperature distribution, thermal resistance and the narrow therapeutic window. Modulated electro-hyperthermia (mEHT), however, has emerged as a promising therapeutic alternative approach to conventional hyperthermia in cancer treatment, utilizing a controlled electromagnetic field to selectively target tumor cells. The localized electromagnetic exposure triggers severe and extensive cell death. However, the mEHT induced complex cellular response includes the heat shock response (HSR), which encompasses the activation of heat shock proteins (HSPs) and other molecular pathways to protect cells from damage. The stimulated HSR can promote cell survival and facilitate protein homeostasis. However, HSPs' upregulation can also confer resistance to chemo- and radiotherapy, allowing cancer cells to evade hyperthermia-induced apoptosis. Therefore, downregulation of the HSR through either HSF1 gene-knockdown or by small molecule inhibitors, such as KRIBB11, represents a significant approach for enhancing the cell/tumor-killing effect of mEHT. By targeting the key regulator HSF1, which coordinates the protective HSR, it becomes possible to impair the cellular stress response and weaken the ability of cancer cells to withstand thermal stress induced by mEHT. Inhibition of the HSR hence can disrupt protein homeostasis, compromise cellular viability, and render cancer cells more susceptible to the cytotoxic effects of mEHT. This dual therapeutic approach of combining mEHT with strategies that downregulate the heat shock response holds promise in augmenting the efficacy of mEHT as a selective and powerful anti-tumor modality. Further investigations and clinical studies, however are still necessary to optimize the application of these combined treatments and explore their full potential in clinical cancer therapy.

CRediT authorship contribution statement

Pedro Viana: Conceptualization, Writing – original draft, Supervision, Writing – review & editing. Peter ´ Hamar: Conceptualization, Writing – review & editing.

Declaration of competing interest

The authors declare no competing interests.

Data availability

Data will be made available on request.

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References

[1] S.M. Alzahrani, H.A. Al Doghaither, A.B. Al-Ghafari, General insight into cancer: an overview of colorectal cancer (review), Mol. Clin. Oncol. 15 (6) (2021) 271.

[2] H. Sung, J. Ferlay, R.L. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal, et al., Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA Cancer J. Clin. 71 (3) (2021) 209–249.

[3] L. Danics, C.A. Schvarcz, P. Viana, T. Vancsik, T. Krenacs, ´ Z. Benyo, ´ et al., Exhaustion of protective heat shock response induces significant tumor damage by apoptosis after modulated electro-hyperthermia treatment of triple negative breast cancer isografts in mice, Cancers (Basel). 12 (9) (2020).

[4] C.A. Schvarcz, L. Danics, T. Krenacs, ´ P. Viana, R. B´eres, T. Vancsik, et al., Modulated electro-hyperthermia induces a prominent local stress response and growth inhibition in mouse breast cancer Isografts, Cancers (Basel). 13 (7) (2021).

[5] C. Hu, J. Yang, Z. Qi, H. Wu, B. Wang, F. Zou, et al., Heat shock proteins: biological functions, pathological roles, and therapeutic opportunities, MedComm (2020) 3 (3) (2022) e161.

[6] J. Anckar, L. Sistonen, Regulation of HSF1 function in the heat stress response: implications in aging and disease, Annu. Rev. Biochem. 80 (1) (2011) 1089–1115.

[7] C. Mathieu, S. Messaoudi, E. Fattal, J. Vergnaud-Gauduchon, Cancer drug resistance: rationale for drug delivery systems and targeted inhibition of HSP90 family proteins, Cancer Drug Resist. 2 (3) (2019) 381–398.

[8] A. Vihervaara, L. Sistonen, HSF1 at a glance, J. Cell Sci. 127 (2) (2014) 261–266.

[9] K.E. Gumilar, Y. Chin, I.H. Ibrahim, B.A. Tjokroprawiro, J.-Y. Yang, M. Zhou, et al., Heat shock factor 1 inhibition: a novel anti-cancer strategy with promise for precision oncology, Cancers. 15 (21) (2023) 5167.

[10] L. Mendillo Marc, S. Santagata, M. Koeva, W. Bell George, R. Hu, M. Tamimi Rulla, et al., HSF1 drives a transcriptional program distinct from heat shock to support highly malignant human cancers, Cell. 150 (3) (2012) 549–562.

[11] K. Ahmed, S.F. Zaidi, R. Mati ur, R. Rehman, T. Kondo, Hyperthermia and protein homeostasis: Cytoprotection and cell death, J. Therm. Biol. 91 (2020) 102615.

[12] X. Wang, M. Chen, J. Zhou, X. Zhang, HSP27, 70 and 90, anti-apoptotic proteins, in clinical cancer therapy (review), Int. J. Oncol. 45 (1) (2014) 18–30.

[13] T. Kijima, T. Prince, L. Neckers, F. Koga, Y. Fujii, Heat shock factor 1 (HSF1)- targeted anticancer therapeutics: overview of current preclinical progress, Expert Opin. Ther. Targets 23 (5) (2019) 369–377.

[14] H.-J. Kim, H.J. Joo, Y.H. Kim, S. Ahn, J. Chang, K.-B. Hwang, et al., Systemic analysis of heat shock response induced by heat shock and a proteasome inhibitor MG132, PLoS One 6 (6) (2011) e20252.

[15] D.A. Parsell, S. Lindquist, The function of heat-shock proteins in stress tolerance: degradation and reactivation of damaged proteins, Annu. Rev. Genet. 27 (1993) 437–496.

[16] M. Rybinski, ´ Z. Szymanska, ´ S. Lasota, A. Gambin, Modelling the efficacy of hyperthermia treatment, J. R. Soc. Interface 10 (88) (2013) 20130527.

[17] C. Jolly, R.I. Morimoto, Role of the heat shock response and molecular chaperones in oncogenesis and cell death, JNCI: J. Natl. Cancer Inst. 92 (19) (2000) 1564–1572.

[18] S.W. Kmiecik, M.P. Mayer, Molecular mechanisms of heat shock factor 1 regulation, Trends Biochem. Sci. 47 (3) (2022) 218–234.

[19] M. Akerfelt, R.I. Morimoto, L. Sistonen, Heat shock factors: integrators of cell stress, development and lifespan, Nat. Rev. Mol. Cell Biol. 11 (8) (2010) 545–555.

[20] T. Neudegger, J. Verghese, M. Hayer-Hartl, F.U. Hartl, A. Bracher, Structure of human heat-shock transcription factor 1 in complex with DNA, Nat. Struct. Mol. Biol. 23 (2) (2016) 140–146.

[21] R. Peteranderl, M. Rabenstein, Y.-K. Shin, C.W. Liu, D.E. Wemmer, D.S. King, et al., Biochemical and biophysical characterization of the Trimerization domain from the heat shock transcription factor, Biochemistry. 38 (12) (1999) 3559–3569.

[22] A. Nakai, M. Tanabe, Y. Kawazoe, J. Inazawa, R.I. Morimoto, K. Nagata, HSF4, a new member of the human heat shock factor family which lacks properties of a transcriptional activator, Mol. Cell. Biol. 17 (1) (1997) 469–481.

[23] C.N. Ravarani, T.Y. Erkina, G. De Baets, D.C. Dudman, A.M. Erkine, M.M. Babu, High-throughput discovery of functional disordered regions: investigation of transactivation domains, Mol. Syst. Biol. 14 (5) (2018) e8190.

[24] R. Gomez-Pastor, E.T. Burchfiel, D.J. Thiele, Regulation of heat shock transcription factors and their roles in physiology and disease, Nat. Rev. Mol. Cell Biol. 19 (1) (2018) 4–19.

[25] S. Dayalan Naidu, A.T. Dinkova-Kostova, Regulation of the mammalian heat shock factor 1, FEBS J. 284 (11) (2017) 1606–1627.

[26] D. Kov´ acs, M. Kovács, S. Ahmed, J. Barna, Functional diversification of heat shock factors, Biol. Fut. 73 (4) (2022) 427–439.

[27] C. Dai, S.B. Sampson, HSF1: guardian of proteostasis in cancer, Trends Cell Biol. 26 (1) (2016) 17–28.

[28] C.D. Powell, T.R. Paullin, C. Aoisa, C.J. Menzie, A. Ubaldini, S.D. Westerheide, The heat shock transcription factor HSF1 induces ovarian cancer epithelialmesenchymal transition in a 3D spheroid growth model, PLoS One 11 (12) (2016) e0168389.

[29] A.M. Cyran, A. Zhitkovich, Heat shock proteins and HSF1 in cancer, Front. Oncol. (2022) 12. [30] T. Home, R.A. Jensen, R. Rao, Heat shock factor 1 in protein homeostasis and oncogenic signal integration, Cancer Res. 75 (6) (2015) 907–912.

[31] S. Jiang, K. Tu, Q. Fu, D.C. Schmitt, L. Zhou, N. Lu, et al., Multifaceted roles of HSF1 in cancer, Tumor Biol. 36 (7) (2015) 4923–4931.

[32] M. Chuma, N. Sakamoto, A. Nakai, S. Hige, M. Nakanishi, M. Natsuizaka, et al., Heat shock factor 1 accelerates hepatocellular carcinoma development by activating nuclear factor-κB/mitogen-activated protein kinase, Carcinogenesis. 35 (2) (2013) 272–281.

[33] F. Fang, R. Chang, L. Yang, Heat shock factor 1 promotes invasion and metastasis of hepatocellular carcinoma in vitro and in vivo, Cancer. 118 (7) (2012) 1782–1794.

[34] S. Santagata, R. Hu, N.U. Lin, M.L. Mendillo, L.C. Collins, S.E. Hankinson, et al., High levels of nuclear heat-shock factor 1 (HSF1) are associated with poor prognosis in breast cancer, Proc. Natl. Acad. Sci. 108 (45) (2011) 18378–18383.

[35] H. Engerud, I.L. Tangen, A. Berg, K. Kusonmano, M.K. Halle, A.M. Øyan, et al., High level of HSF1 associates with aggressive endometrial carcinoma and suggests potential for HSP90 inhibitors, Br. J. Cancer 111 (1) (2014) 78–84.

[36] J. Ishiwata, A. Kasamatsu, K. Sakuma, M. Iyoda, M. Yamatoji, K. Usukura, et al., State of heat shock factor 1 expression as a putative diagnostic marker for oral squamous cell carcinoma, Int. J. Oncol. 40 (1) (2012) 47–52.

[37] Y. Tabuchi, T. Kondo, Targeting heat shock transcription factor 1 for novel hyperthermia therapy (review), Int. J. Mol. Med. 32 (1) (2013) 3–8.

[38] V. Kohler, C. Andr´easson, Reversible protein assemblies in the proteostasis network in health and disease, Front. Mol. Biosci. (2023) 10.

[39] C. Dai, L. Whitesell, A.B. Rogers, S. Lindquist, Heat shock factor 1 is a powerful multifaceted modifier of carcinogenesis, Cell. 130 (6) (2007) 1005–1018.

[40] S.K. Calderwood, M.A. Khaleque, D.B. Sawyer, D.R. Ciocca, Heat shock proteins in cancer: chaperones of tumorigenesis, Trends Biochem. Sci. 31 (3) (2006) 164–172.

[41] J.R. McConnell, L.K. Buckton, S.R. McAlpine, Regulating the master regulator: controlling heat shock factor 1 as a chemotherapy approach, Bioorg. Med. Chem. Lett. 25 (17) (2015) 3409–3414.

[42] R.L. Carpenter, Y. Gokmen-Polar, ¨ HSF1 as a cancer biomarker and therapeutic target, Curr. Cancer Drug Targets 19 (7) (2019) 515–524.

[43] E.T.L. Soo, G.W.C. Yip, Z.M. Lwin, S.D. Kumar, B.-H. Bay, Heat shock proteins as novel therapeutic targets in cancer, In Vivo 22 (3) (2008) 311–315.

[44] D.R. Ciocca, S.K. Calderwood, Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications, Cell Stress Chaperones 10 (2) (2005) 86–103.

[45] Z. Albakova, Y. Mangasarova, The HSP immune network in cancer, Front. Immunol. 12 (2021).

[46] S.K. Calderwood, J. Gong, Heat shock proteins promote cancer: It's a protection racket, Trends Biochem. Sci. 41 (4) (2016) 311–323.

[47] L. Seclì, F. Fusella, L. Avalle, M. Brancaccio, The dark-side of the outside: how extracellular heat shock proteins promote cancer, Cell. Mol. Life Sci. 78 (9) (2021) 4069–4083.

[48] D.J. McConkey, The integrated stress response and proteotoxicity in cancer therapy, Biochem. Biophys. Res. Commun. 482 (3) (2017) 450–453.

[49] S. Yang, H. Xiao, L. Cao, Recent advances in heat shock proteins in cancer diagnosis, prognosis, metabolism and treatment, Biomed. Pharmacother. 142 (2021) 112074.

[50] J. Radons, The human HSP70 family of chaperones: where do we stand? Cell Stress Chaperones 21 (3) (2016) 379–404.

[51] E.A. Taha, K. Ono, T. Eguchi, Roles of extracellular HSPs as biomarkers in immune surveillance and immune evasion, Int. J. Mol. Sci. 20 (18) (2019) 4588.

[52] A.P. Arrigo, Mammalian HspB1 (Hsp27) is a molecular sensor linked to the physiology and environment of the cell, Cell Stress Chaperones 22 (4) (2017) 517–529.

[53] J. Wu, T. Liu, Z. Rios, Q. Mei, X. Lin, S. Cao, Heat shock proteins and cancer, Trends Pharmacol. Sci. 38 (3) (2017) 226–256.

[54] G.M. Nagaraja, P. Kaur, A. Asea, Role of human and mouse HspB1 in metastasis, Curr. Mol. Med. 12 (9) (2012) 1142–1150.

[55] M. Lampros, N. Vlachos, S. Voulgaris, G.A. Alexiou, The role of Hsp27 in chemotherapy resistance, Biomedicines. 10 (4) (2022).

[56] B. Tˆetu, J. Brisson, J. Landry, J. Huot, Prognostic significance of heat-shock protein-27 in node-positive breast carcinoma: an immunohistochemical study, Breast Cancer Res. Treat. 36 (1) (1995) 93–97.

[57] J.P. Geisler, J.E. Tammela, K.J. Manahan, H.E. Geisler, G.A. Miller, Z. Zhou, et al., HSP27 in patients with ovarian carcinoma: still an independent prognostic indicator at 60 months follow-up, Eur. J. Gynaecol. Oncol. 25 (2) (2004) 165–168.

[58] T. Schweiger, C. Nikolowsky, P. Starlinger, D. Traxler, M. Zimmermann, P. Birner, et al., Stromal expression of heat-shock protein 27 is associated with worse clinical outcome in patients with colorectal cancer lung metastases, PLoS One 10 (3) (2015) e0120724.

[59] C.S. Foster, A.R. Dodson, L. Ambroisine, G. Fisher, H. Møller, J. Clark, et al., Hsp27 expression at diagnosis predicts poor clinical outcome in prostate cancer independent of ETS-gene rearrangement, Br. J. Cancer 101 (7) (2009) 1137–1144.

[60] S.K. Choi, H. Kam, K.Y. Kim, S.I. Park, Y.S. Lee, Targeting heat shock protein 27 in cancer: a Druggable target for cancer treatment? Cancers (Basel). 11 (8) (2019).

[61] M.E. Murphy, The HSP70 family and cancer, Carcinogenesis. 34 (6) (2013) 1181–1188.

[62] M.I.Y. Elmallah, M. Cordonnier, V. Vautrot, G. Chanteloup, C. Garrido, J. Gobbo, Membrane-anchored heat-shock protein 70 (Hsp70) in cancer, Cancer Lett. 469 (2020) 134–141.

[63] A.-L. Joly, G. Wettstein, G. Mignot, F. Ghiringhelli, C. Garrido, Dual role of heat shock proteins as regulators of apoptosis and innate immunity, J. Innate Immun. 2 (3) (2010) 238–247.

[64] Z. Albakova, G.A. Armeev, L.M. Kanevskiy, E.I. Kovalenko, A.M. Sapozhnikov, HSP70 multi-functionality in cancer, Cells. 9 (3) (2020).

[65] M.A. Vostakolaei, J. Abdolalizadeh, M.S. Hejazi, S. Kordi, O. Molavi, Hsp70 in cancer: partner or traitor to immune system, Iran. J. Allergy Asthma Immunol. 18 (6) (2019) 589–604.

[66] K. Juhasz, A.M. Lipp, B. Nimmervoll, A. Sonnleitner, J. Hesse, T. Haselgruebler, et al., The complex function of hsp70 in metastatic cancer, Cancers (Basel). 6 (1) (2013) 42–66.

[67] S. Du, Y. Liu, Y. Yuan, Y. Wang, Y. Chen, S. Wang, et al., Advances in the study of HSP70 inhibitors to enhance the sensitivity of tumor cells to radiotherapy, Front. Cell Dev. Biol. (2022) 10.

[68] B. Birbo, E.E. Madu, C.O. Madu, A. Jain, Y. Lu, Role of HSP90 in cancer, Int. J. Mol. Sci. 22 (19) (2021).

[69] Y. Miyata, H. Nakamoto, L. Neckers, The therapeutic target Hsp90 and cancer hallmarks, Curr. Pharm. Des. 19 (3) (2013) 347–365.

[70] A. Hoter, M.E. El-Sabban, H.Y. Naim, The HSP90 family: structure, regulation, function, and implications in health and disease, Int. J. Mol. Sci. 19 (9) (2018).

[71] L. Whitesell, S.L. Lindquist, HSP90 and the chaperoning of cancer, Nat. Rev. Cancer 5 (10) (2005) 761–772.

[72] F. Zagouri, T.N. Sergentanis, A. Nonni, C.A. Papadimitriou, N.V. Michalopoulos, P. Domeyer, et al., Hsp90 in the continuum of breast ductal carcinogenesis: evaluation in precursors, preinvasive and ductal carcinoma lesions, BMC Cancer 10 (2010) 353.

[73] E. Pick, Y. Kluger, J.M. Giltnane, C. Moeder, R.L. Camp, D.L. Rimm, et al., High HSP90 expression is associated with decreased survival in breast cancer, Cancer Res. 67 (7) (2007) 2932–2937.

[74] Y. Miyata, Hsp90 inhibitor geldanamycin and its derivatives as novel cancer chemotherapeutic agents, Curr. Pharm. Des. 11 (9) (2005) 1131–1138.

[75] Z.N. Li, Y. Luo, HSP90 inhibitors and cancer: prospects for use in targeted therapies (review), Oncol. Rep. 49 (1) (2023).

[76] J. Sanchez, T.R. Carter, M.S. Cohen, B.S.J. Blagg, Old and new approaches to target the Hsp90 chaperone, Curr. Cancer Drug Targets 20 (4) (2020) 253–270.

[77] Y. Cheng, S. Weng, L. Yu, N. Zhu, M. Yang, Y. Yuan, The role of hyperthermia in the multidisciplinary treatment of malignant tumors, Integr. Cancer Ther. 18 (2019), 1534735419876345.

[78] A. Szasz, Challenges and solutions in oncological hyperthermia, Thermal Med. 29 (1) (2013) 1–23.

[79] L.E. Harrison, M. Bryan, L. Pliner, T. Saunders, Phase I trial of pegylated liposomal doxorubicin with hyperthermic intraperitoneal chemotherapy in patients undergoing cytoreduction for advanced intra-abdominal malignancy, Ann. Surg. Oncol. 15 (5) (2008) 1407–1413.

[80] G. Los, O.A. Smals, M.J. van Vugt, M. van der Vlist, L. den Engelse, J.G. McVie, et al., A rationale for carboplatin treatment and abdominal hyperthermia in cancers restricted to the peritoneal cavity, Cancer Res. 52 (5) (1992) 1252–1258.

[81] P. Jacquet, A. Averbach, O.A. Stuart, D. Chang, P.H. Sugarbaker, Hyperthermic intraperitoneal doxorubicin: pharmacokinetics, metabolism, and tissue distribution in a rat model, Cancer Chemother. Pharmacol. 41 (2) (1998) 147–154.

[82] O. Dahl, Interaction of hyperthermia and chemotherapy, Recent Results Cancer Res. 107 (1988) 157–169.

[83] P.H. Sugarbaker, Laboratory and clinical basis for hyperthermia as a component of intracavitary chemotherapy, Int. J. Hyperth. 23 (5) (2007) 431–442.

[84] S.Y. Lee, G. Fiorentini, A.M. Szasz, G. Szigeti, A. Szasz, C.A. Minnaar, Quo Vadis oncological hyperthermia (2020)? Front. Oncol. 10 (2020) 1690.

[85] A. Szasz, N. Szasz, O. Szasz, Oncothermia: Principles and Practices, Springer Science & Business Media, 2010.

[86] S.-Y. Lee, G.P. Szigeti, A.M. Szasz, Oncological hyperthermia: the correct dosing in clinical applications, Int. J. Oncol. 54 (2) (2019) 627–643.

[87] G. Hegyi, G.P. Szigeti, A. Szasz, ´ Hyperthermia versus oncothermia: cellular effects in complementary cancer therapy, Evid. Based Complement. Alternat. Med. 2013 (2013) 672873.

[88] D.D. Mosser, R.I. Morimoto, Molecular chaperones and the stress of oncogenesis, Oncogene. 23 (16) (2004) 2907–2918.

[89] S.K. Calderwood, A. Asea, Targeting HSP70 induced thermotolerance for design of thermal sensitizers, Int. J. Hyperth. 18 (6) (2002) 597–608.

[90] K. Chen, W. Qian, J. Li, Z. Jiang, L. Cheng, B. Yan, et al., Loss of AMPK activation promotes the invasion and metastasis of pancreatic cancer through an HSF1- dependent pathway, Mol. Oncol. 11 (10) (2017) 1475–1492.

[91] W. Liang, Y. Liao, J. Zhang, Q. Huang, W. Luo, J. Yu, et al., Heat shock factor 1 inhibits the mitochondrial apoptosis pathway by regulating second mitochondriaderived activator of caspase to promote pancreatic tumorigenesis, J. Exp. Clin. Cancer Res. 36 (1) (2017) 64.

[92] T. Heimberger, M. Andrulis, S. Riedel, T. Stühmer, H. Schraud, A. Beilhack, et al., The heat shock transcription factor 1 as a potential new therapeutic target in multiple myeloma, Br. J. Haematol. 160 (4) (2013) 465–476.

[93] J. Li, P. Song, T. Jiang, D. Dai, H. Wang, J. Sun, et al., Heat shock factor 1 epigenetically stimulates Glutaminase-1-dependent mTOR activation to promote colorectal carcinogenesis, Mol. Ther. 26 (7) (2018) 1828–1839.

[94] Y. Nakamura, M. Fujimoto, S. Fukushima, A. Nakamura, N. Hayashida, R. Takii, et al., Heat shock factor 1 is required for migration and invasion of human melanoma in vitro and in vivo, Cancer Lett. 354 (2) (2014) 329–335.

[95] C. Xi, Y. Hu, P. Buckhaults, D. Moskophidis, N.F. Mivechi, Heat shock factor Hsf1 cooperates with ErbB2 (Her2/Neu) protein to promote mammary tumorigenesis and metastasis, J. Biol. Chem. 287 (42) (2012) 35646–35657.

[96] C. Dai, S. Santagata, Z. Tang, J. Shi, J. Cao, H. Kwon, et al., Loss of tumor suppressor NF1 activates HSF1 to promote carcinogenesis, J. Clin. Invest. 122 (10) (2012) 3742–3754.

[97] V.L. Gabai, L. Meng, G. Kim, T.A. Mills, I.J. Benjamin, M.Y. Sherman, Heat shock transcription factor Hsf1 is involved in tumor progression via regulation of hypoxia-inducible factor 1 and RNA-binding protein HuR, Mol. Cell. Biol. 32 (5) (2012) 929–940.

[98] A. Cigliano, C. Wang, M.G. Pilo, M. Szydlowska, S. Brozzetti, G. Latte, et al., Inhibition of HSF1 suppresses the growth of hepatocarcinoma cell lines in vitro and AKT-driven hepatocarcinogenesis in mice, Oncotarget. 8 (33) (2017) 54149–54159.

[99] S. Li, W. Ma, T. Fei, Q. Lou, Y. Zhang, X. Cui, et al., Upregulation of heat shock factor 1 transcription activity is associated with hepatocellular carcinoma progression, Mol. Med. Rep. 10 (5) (2014) 2313–2321.

[100] A. Rossi, S. Ciafr`e, M. Balsamo, P. Pierimarchi, M.G. Santoro, Targeting the heat shock factor 1 by RNA interference: a potent tool to enhance hyperthermochemotherapy efficacy in cervical cancer, Cancer Res. 66 (15) (2006) 7678–7685.

[101] Y. Nakamura, M. Fujimoto, N. Hayashida, R. Takii, A. Nakai, M. Muto, Silencing HSF1 by short hairpin RNA decreases cell proliferation and enhances sensitivity to hyperthermia in human melanoma cell lines, J. Dermatol. Sci. 60 (3) (2010) 187–192.

[102] L. Meng, V.L. Gabai, M.Y. Sherman, Heat-shock transcription factor HSF1 has a critical role in human epidermal growth factor receptor-2-induced cellular transformation and tumorigenesis, Oncogene. 29 (37) (2010) 5204–5213.

[103] X. Wang, D. Zhang, M. Cao, J. Ba, B. Wu, T. Liu, et al., A study on the biological function of heat shock factor 1 proteins in breast cancer, Oncol. Lett. 16 (3) (2018) 3145–3149.

[104] S. Desai, Z. Liu, J. Yao, N. Patel, J. Chen, Y. Wu, et al., Heat shock factor 1 (HSF1) controls chemoresistance and autophagy through transcriptional regulation of autophagy-related protein 7 (ATG7), J. Biol. Chem. 288 (13) (2013) 9165–9176.

[105] Y. Tabuchi, Y. Furusawa, S. Wada, K. Ohtsuka, T. Kondo, Silencing heat shock transcription factor 1 using small interfering RNA enhances mild hyperthermia and hyperthermia sensitivity in human oral squamous cell carcinoma cells, Thermal Med. 27 (4) (2011) 99–108.

[106] J.H. Wang, M.Z. Yao, J.F. Gu, L.Y. Sun, Y.F. Shen, X.Y. Liu, Blocking HSF1 by dominant-negative mutant to sensitize tumor cells to hyperthermia, Biochem. Biophys. Res. Commun. 290 (5) (2002) 1454–1461.

[107] Y. Zhang, L. Huang, J. Zhang, D. Moskophidis, N.F. Mivechi, Targeted disruption of hsf1 leads to lack of thermotolerance and defines tissue-specific regulation for stress-inducible Hsp molecular chaperones, J. Cell. Biochem. 86 (2) (2002) 376–393.

[108] D.R. McMillan, X. Xiao, L. Shao, K. Graves, I.J. Benjamin, Targeted disruption of heat shock transcription factor 1 abolishes thermotolerance and protection against heat-inducible apoptosis*, J. Biol. Chem. 273 (13) (1998) 7523–7528.

[109] C.A. Minnaar, J.A. Kotzen, O.A. Ayeni, T. Naidoo, M. Tunmer, V. Sharma, et al., The effect of modulated electro-hyperthermia on local disease control in HIV positive and -negative cervical cancer women in South Africa: early results from a phase III randomised controlled trial, PLoS One 14 (6) (2019) e0217894.

[110] G. Fiorentini, D. Sarti, C. Milandri, P. Dentico, A. Mambrini, C. Fiorentini, et al., Modulated electrohyperthermia in integrative cancer treatment for relapsed malignant glioblastoma and astrocytoma: retrospective multicenter controlled study, Integr. Cancer Ther. 18 (2019), 1534735418812691.

[111] J.W. Zimmerman, H. Jimenez, M.J. Pennison, I. Brezovich, D. Morgan, A. Mudry, et al., Targeted treatment of cancer with radiofrequency electromagnetic fields amplitude-modulated at tumor-specific frequencies, Chin. J. Cancer 32 (11) (2013) 573–581.

[112] E. Papp, T. Vancsik, E. Kiss, O. Szasz, Energy absorption by the membrane rafts in the Modulated Electro-Hyperthermia (mEHT), Open J. Biophys. 07 (04) (2017) 216–229.

[113] G. Andocs, M.U. Rehman, Q.L. Zhao, Y. Tabuchi, M. Kanamori, T. Kondo, Comparison of biological effects of modulated electro-hyperthermia and conventional heat treatment in human lymphoma U937 cells, Cell Death Dis. 2 (2016) 16039.

[114] H.F. Alshaibi, B. Al-shehri, B. Hassan, R. Al-zahrani, T. Assiss, Modulated electrohyperthermia: a new Hope for cancer patients, Biomed. Res. Int. 2020 (2020) 8814878.

[115] A.M. Szasz, ´ G. Lor´ ant, A. Szasz, ´ G. Szigeti, The immunogenic connection of thermal and nonthermal molecular effects in modulated electro-hyperthermia, Open J. Biophys. 13 (2023) 103–142.

[116] A. Szasz, Thermal and nonthermal effects of radiofrequency on living state and applications as an adjuvant with radiation therapy, J. Radiat. Cancer Res. 10 (1) (2019) 1–17.

[117] G. Andocs, N. Meggyeshazi, L. Balogh, S. Spisak, M.E. Maros, P. Balla, et al., Upregulation of heat shock proteins and the promotion of damage-associated molecular pattern signals in a colorectal cancer model by modulated electrohyperthermia, Cell Stress Chaperones 20 (1) (2015) 37–46.

[118] G. Andocs, O. Szasz, A. Szasz, Oncothermia treatment of cancer: from the laboratory to clinic, Electromagn. Biol. Med. 28 (2) (2009) 148–165.

[119] O. Szasz, G. Andocs, N. Meggyeshazi, Modulation effect in oncothermia, Conf. Pap. Med. 2013 (2013) 398678.

[120] A. Szasz, N. Szasz, O. Szasz, Oncothermia – a new kind of oncologic hyperthermia, in: A. Szasz, N. Szasz, O. Szasz (Eds.), Oncothermia: Principles and Practices, Springer Netherlands, Dordrecht, 2011, pp. 173–392.

[121] O. Szasz, M.A. Szasz, C. Minnaar, A. Szasz, Heating preciosity¡aTrends in modern oncological hyperthermia, Open J. Biophys. 07No.03 (2017) 29.

[122] G. Andocs, H. Renner, L. Balogh, L. Fonyad, C. Jakab, A. Szasz, Strong synergy of heat and modulated electromagnetic field in tumor cell killing, Strahlenther. Onkol. 185 (2) (2009) 120–126. [123] S. Roussakow, The history of hyperthermia rise and decline, Conf. Pap. Med. 2013 (2013) 428027. [124] K.-L. Yang, C.-C. Huang, M.-S. Chi, H.-C. Chiang, Y.-S. Wang, C.-C. Hsia, et al., In vitro comparison of conventional hyperthermia and modulated electrohyperthermia, Oncotarget. 7 (51) (2016).

[125] T. Vancsik, C. Kovago, E. Kiss, E. Papp, G. Forika, Z. Benyo, et al., Modulated electro-hyperthermia induced loco-regional and systemic tumor destruction in colorectal cancer allografts, J. Cancer 9 (1) (2018) 41–53.

[126] Y.W. Tsang, K.H. Chi, C.C. Huang, M.S. Chi, H.C. Chiang, K.L. Yang, et al., Modulated electro-hyperthermia-enhanced liposomal drug uptake by cancer cells, Int. J. Nanomedicine 14 (2019) 1269–1279.

[127] T. Nagata, M. Kanamori, S. Sekine, M. Arai, M. Moriyama, T. Fujii, Clinical study of modulated electro-hyperthermia for advanced metastatic breast cancer, Mol. Clin. Oncol. 14 (5) (2021) 103.

[128] C.A. Minnaar, I. Maposa, J.A. Kotzen, A. Baeyens, Effects of modulated electrohyperthermia (mEHT) on two and three year survival of locally advanced cervical Cancer patients, Cancers (Basel). 14 (3) (2022).

[129] H.J. Yoo, M.C. Lim, S.-S. Seo, S. Kang, J. Joo, S.-Y. Park, Phase I/II clinical trial of modulated electro-hyperthermia treatment in patients with relapsed, refractory or progressive heavily treated ovarian cancer, Jpn. J. Clin. Oncol. 49 (9) (2019) 832–838.

[130] S. Kim, J.H. Lee, J. Cha, S.H. You, Beneficial effects of modulated electrohyperthermia during neoadjuvant treatment for locally advanced rectal cancer, Int. J. Hyperth. 38 (1) (2021) 144–151.

[131] G. Fiorentini, D. Sarti, V. Casadei, C. Milandri, P. Dentico, A. Mambrini, et al., Modulated electro-hyperthermia as palliative treatment for pancreatic cancer: a retrospective observational study on 106 patients, Integr. Cancer Ther. 18 (2019), 1534735419878505.

[132] G. Fiorentini, D. Sarti, G. Ranieri, C.D. Gadaleta, C. Fiorentini, C. Milandri, et al., Modulated electro-hyperthermia in stage III and IV pancreatic cancer: results of an observational study on 158 patients, World J. Clin. Oncol. 12 (11) (2021) 1064–1071.

[133] F.G. Petenyi, T. Garay, D. Muhl, B. Izso, A. Karaszi, E. Borbenyi, et al., Modulated electro-Hyperthermic (mEHT) treatment in the therapy of inoperable pancreatic Cancer patients-a single-center case-control study, Diseases. 9 (4) (2021).

[134] Z. Luo, K. Zheng, Q. Fan, X. Jiang, D. Xiong, Hyperthermia exposure induces apoptosis and inhibits proliferation in HCT116 cells by upregulating miR-34a and causing transcriptional activation of p53, Exp. Ther. Med. 14 (6) (2017) 5379–5386.

[135] T. Vancsik, G. Forika, A. Balogh, E. Kiss, T. Krenacs, Modulated electrohyperthermia induced p53 driven apoptosis and cell cycle arrest additively support doxorubicin chemotherapy of colorectal cancer in vitro, Cancer Med. 8 (9) (2019) 4292–4303.

[136] A. Szasz, Heterogeneous heat absorption is complementary to radiotherapy, Cancers. 14 (4) (2022) 901.

[137] S.-Y. Lee, J.-H. Kim, Y.-H. Han, D.-H. Cho, The effect of modulated electrohyperthermia on temperature and blood flow in human cervical carcinoma, Int. J. Hyperth. 34 (7) (2018) 953–960.

[138] J.R. Lepock, Role of nuclear protein denaturation and aggregation in thermal radiosensitization, Int. J. Hyperth. 20 (2) (2004) 115–130.

[139] T. Krenacs, N. Meggyeshazi, G. Forika, E. Kiss, P. Hamar, T. Szekely, et al., Modulated electro-hyperthermia-induced tumor damage mechanisms revealed in cancer models, Int. J. Mol. Sci. 21 (17) (2020).

[140] P.B. Elming, B.S. Sørensen, A.L. Oei, N.A.P. Franken, J. Crezee, J. Overgaard, et al., Hyperthermia: the optimal treatment to overcome radiation resistant hypoxia, Cancers (Basel). 11 (1) (2019).

[141] P. Vaupel, H. Piazena, M. Notter, A.R. Thomsen, A.L. Grosu, F. Scholkmann, et al., From localized mild hyperthermia to improved tumor oxygenation: physiological mechanisms critically involved in oncologic thermo-radio-immunotherapy, Cancers (Basel). 15 (5) (2023).

[142] C.A. Minnaar, J.A. Kotzen, T. Naidoo, M. Tunmer, V. Sharma, M.-D.-T. Vangu, et al., Analysis of the effects of mEHT on the treatment-related toxicity and quality of life of HIV-positive cervical cancer patients, Int. J. Hyperth. 37 (1) (2020) 263–272.

[143] K.R. Foster, Thermal and nonthermal mechanisms of interaction of radiofrequency energy with biological systems, IEEE Trans. Plasma Sci. 28 (1) (2000) 15–23.

[144] A. Szasz, O. Szasz, N. Szasz, Electro-hyperthermia: a new paradigm in cancer therapy, Deuts. Z. Onkol. 33 (03) (2001) 91–99.

[145] A.R. Deipolyi, A. Golberg, M.L. Yarmush, R.S. Arellano, R. Oklu, Irreversible electroporation: evolution of a laboratory technique in interventional oncology, Diagn. Interv. Radiol. 20 (2) (2014) 147–154.

[146] K. Sasaki, E. Porter, E.A. Rashed, L. Farrugia, G. Schmid, Measurement and imagebased estimation of dielectric properties of biological tissues -past, present, and future, Phys. Med. Biol. 67 (14) (2022).

[147] A. Szasz, Time-fractal modulation—possible modulation effects in human therapy, Open J. Biophys. 12 (2022) 38–87.

[148] M. Mousavi, J. Baharara, K. Shahrokhabadi, The synergic effects of crocus Sativus L. and low frequency electromagnetic field on VEGFR2 gene expression in human breast cancer cells, Avicenna J. Med. Biotechnol. 6 (2) (2014) 123–127.

[149] W. Qin, Y. Akutsu, G. Andocs, A. Suganami, X. Hu, G. Yusup, et al., Modulated electro-hyperthermia enhances dendritic cell therapy through an abscopal effect in mice, Oncol. Rep. 32 (6) (2014) 2373–2379.

[150] C.A. Minnaar, A. Szasz, Forcing the antitumor effects of HSPs using a modulated electric field, Cells. 11 (11) (2022).

[151] Y.W. Tsang, C.C. Huang, K.L. Yang, M.S. Chi, H.C. Chiang, Y.S. Wang, et al., Improving immunological tumor microenvironment using electro-hyperthermia followed by dendritic cell immunotherapy, BMC Cancer 15 (2015) 708.

[152] T. Vancsik, D. M´ ath´e, I. Horvath, ´ A.A. Varallyaly, ´ A. Benedek, R. Bergmann, et al., Modulated electro-hyperthermia facilitates NK-cell infiltration and growth arrest of human A2058 melanoma in a xenograft model, Front. Oncol. (2021) 11.

[153] S.-Y. Lee, G. Lorant, L. Grand, A.M. Szasz, The clinical validation of modulated electro-hyperthermia (mEHT), Cancers. 15 (18) (2023) 4569.

[154] M.S. Chi, M.P. Mehta, K.L. Yang, H.C. Lai, Y.C. Lin, H.L. Ko, et al., Putative abscopal effect in three patients treated by combined radiotherapy and modulated electrohyperthermia, Front. Oncol. 10 (2020) 254.

[155] M.-S. Chi, J.-H. Wu, S. Shaw, C.-J. Wu, L.-K. Chen, H.-C. Hsu, et al., Marked local and distant response of heavily treated breast cancer with cardiac metastases treated by combined low dose radiotherapy, low dose immunotherapy and hyperthermia: a case report, Therapeut. Radiol. Oncol. (2021) 5.

[156] N. Meggyeshazi, ´ G. Andocs, ´ S. Spis´ ak, T. Kren´ acs, Early changes in mRNA and protein expression related to cancer treatment by modulated electrohyperthermia, Conf. Pap. Med. 2013 (2013) 249563.

[157] B. Besztercei, T. Vancsik, A. Benedek, E. Major, M.J. Thomas, C.A. Schvarcz, et al., Stress-induced, p53-mediated tumor growth inhibition of melanoma by modulated electrohyperthermia in mouse models without major immunogenic effects, Int. J. Mol. Sci. 20 (16) (2019).

[158] I.M. Kuo, J.J. Lee, Y.S. Wang, H.C. Chiang, C.C. Huang, P.J. Hsieh, et al., Potential enhancement of host immunity and anti-tumor efficacy of nanoscale curcumin and resveratrol in colorectal cancers by modulated electro- hyperthermia, BMC Cancer 20 (1) (2020) 603.

[159] G. Forika, E. Kiss, G. Petovari, T. Danko, A.B. Gellert, T. Krenacs, Modulated electro-hyperthermia supports the effect of gemcitabine both in sensitive and resistant pancreas adenocarcinoma cell lines, Pathol. Oncol. Res. 27 (2021) 1610048.

[160] S. Takayama, J.C. Reed, S. Homma, Heat-shock proteins as regulators of apoptosis, Oncogene. 22 (56) (2003) 9041–9047.

[161] A.S. Sreedhar, P. Csermely, Heat shock proteins in the regulation of apoptosis: new strategies in tumor therapy: a comprehensive review, Pharmacol. Ther. 101 (3) (2004) 227–257.

[162] P.H. Kao, C.H. Chen, Y.W. Tsang, C.S. Lin, H.C. Chiang, C.C. Huang, et al., Relationship between energy dosage and apoptotic cell death by modulated electro-hyperthermia, Sci. Rep. 10 (1) (2020) 8936.

[163] N. Dutta, K. Pal, M. Pal, Heat shock factor 1 and its small molecule modulators with therapeutic potential, in: A.A.A. Asea, P. Kaur (Eds.), Heat Shock Proteins in Inflammatory Diseases, Springer International Publishing, Cham, 2021, pp. 69–88.

[164] Y. Chin, K.E. Gumilar, X.G. Li, B.A. Tjokroprawiro, C.H. Lu, J. Lu, et al., Targeting HSF1 for cancer treatment: mechanisms and inhibitor development, Theranostics. 13 (7) (2023) 2281–2300.

[165] B. Dong, A.M. Jaeger, P.F. Hughes, D.R. Loiselle, J.S. Hauck, Y. Fu, et al., Targeting therapy-resistant prostate cancer via a direct inhibitor of the human heat shock transcription factor 1, Sci. Transl. Med. 12 (574) (2020) eabb5647.

[166] Q. Dong, Y. Xiu, Y. Wang, C. Hodgson, N. Borcherding, C. Jordan, et al., HSF1 is a driver of leukemia stem cell self-renewal in acute myeloid leukemia, Nat. Commun. 13 (1) (2022) 6107.

[167] N. Vilaboa, A. Bor´e, F. Martin-Saavedra, M. Bayford, N. Winfield, S. Firth-Clark, et al., New inhibitor targeting human transcription factor HSF1: effects on the heat shock response and tumor cell survival, Nucleic Acids Res. 45 (10) (2017) 5797–5817.

[168] Y.J. Yoon, J.A. Kim, K.D. Shin, D.S. Shin, Y.M. Han, Y.J. Lee, et al., KRIBB11 inhibits HSP70 synthesis through inhibition of heat shock factor 1 function by impairing the recruitment of positive transcription elongation factor b to the hsp70 promoter, J. Biol. Chem. 286 (3) (2011) 1737–1747.

[169] N. Nagai, A. Nakai, K. Nagata, Quercetin suppresses heat shock response by Down-regulation of HSF1, Biochem. Biophys. Res. Commun. 208 (3) (1995) 1099–1105.

[170] W. Yang, M. Cui, J. Lee, W. Gong, S. Wang, J. Fu, et al., Heat shock protein inhibitor, quercetin, as a novel adjuvant agent to improve radiofrequency ablation-induced tumor destruction and its molecular mechanism, Chin. J. Cancer Res. 28 (1) (2016) 19–28.

[171] J.A. Kim, S. Lee, D.-E. Kim, M. Kim, B.-M. Kwon, D.C. Han, Fisetin, a dietary flavonoid, induces apoptosis of cancer cells by inhibiting HSF1 activity through blocking its binding to the hsp70 promoter, Carcinogenesis. 36 (6) (2015) 696–706.

[172] S.D. Westerheide, T.L.A. Kawahara, K. Orton, R.I. Morimoto, Triptolide, an inhibitor of the human heat shock response that enhances stress-induced cell death*, J. Biol. Chem. 281 (14) (2006) 9616–9622.

[173] X.-J. Li, Z.-Z. Jiang, L.-y. Zhang, Triptolide: progress on research in pharmacodynamics and toxicology, J. Ethnopharmacol. 155 (1) (2014) 67–79.

[174] S. Ganguly, T. Home, A. Yacoub, S. Kambhampati, H. Shi, P. Dandawate, et al., Targeting HSF1 disrupts HSP90 chaperone function in chronic lymphocytic leukemia, Oncotarget. 6 (31) (2015) 31767–31779.

[175] T. Yoon, G.-Y. Kang, A.-R. Han, E.-K. Seo, Y.-S. Lee, 2,4-Bis(4-hydroxybenzyl) phenol inhibits heat shock transcription factor 1 and sensitizes lung cancer cells to conventional anticancer modalities, J. Nat. Prod. 77 (5) (2014) 1123–1129.

[176] J.A. Kim, Y. Kim, B.M. Kwon, D.C. Han, The natural compound cantharidin induces cancer cell death through inhibition of heat shock protein 70 (HSP70) and Bcl-2-associated athanogene domain 3 (BAG3) expression by blocking heat shock factor 1 (HSF1) binding to promoters, J. Biol. Chem. 288 (40) (2013) 28713–28726.

[177] H. Akagawa, Y. Takano, A. Ishii, S. Mizuno, R. Izui, T. Sameshima, et al., Stresgenin B, an inhibitor of heat-induced heat shock protein gene expression, produced by Streptomyces sp. AS-9, J. Antibiot. (Tokyo) 52 (11) (1999) 960–970.

[178] A.D. Nikotina, L. Koludarova, E.Y. Komarova, E.R. Mikhaylova, N.D. Aksenov, R. Suezov, et al., Discovery and optimization of cardenolides inhibiting HSF1 activation in human colon HCT-116 cancer cells, Oncotarget. 9 (43) (2018) 27268–27279.

[179] T. Agarwal, N. Annamalai, A. Khursheed, T.K. Maiti, H.B. Arsad, M.H. Siddiqui, Molecular docking and dynamic simulation evaluation of Rohinitib — Cantharidin based novel HSF1 inhibitors for cancer therapy, J. Mol. Graph. Model. 61 (2015) 141–149.

[180] S. Santagata, M.L. Mendillo, Y.C. Tang, A. Subramanian, C.C. Perley, S.P. Roche, et al., Tight coordination of protein translation and HSF1 activation supports the anabolic malignant state, Science. 341 (6143) (2013) 1238303.

[181] J. Acquaviva, S. He, J. Sang, D.L. Smith, M. Sequeira, C. Zhang, et al., mTOR inhibition potentiates HSP90 inhibitor activity via cessation of HSP synthesis, Mol. Cancer Res. 12 (5) (2014) 703–713. [182] C.S. Rye, N.E. Chessum, S. Lamont, K.G. Pike, P. Faulder, J. Demeritt, et al., Discovery of 4,6-disubstituted pyrimidines as potent inhibitors of the heat shock factor 1 (HSF1) stress pathway and CDK9, Medchemcomm. 7 (8) (2016) 1580–1586.

[183] S. Yokota, M. Kitahara, K. Nagata, Benzylidene lactam compound, KNK437, a novel inhibitor of acquisition of thermotolerance and heat shock protein induction in human colon carcinoma cells, Cancer Res. 60 (11) (2000) 2942–2948.

[184] M. Koishi, Yokota Si, T. Mae, Y. Nishimura, S. Kanamori, N. Horii, et al., The effects of KNK437, a novel inhibitor of heat shock protein synthesis, on the acquisition of thermotolerance in a murine transplantable tumor in vivo, Clin. Cancer Res. 7 (1) (2001) 215–219.

[185] N. Li, T. Wang, Z. Li, X. Ye, B. Deng, S. Zhuo, et al., Dorsomorphin induces cancer cell apoptosis and sensitizes cancer cells to HSP90 and proteasome inhibitors by reducing nuclear heat shock factor 1 levels, Cancer Biol. Med. 16 (2) (2019) 220–233.

[186] M.D. Cheeseman, N.E. Chessum, C.S. Rye, A.E. Pasqua, M.J. Tucker, B. Wilding, et al., Discovery of a chemical probe Bisamide (CCT251236): an orally bioavailable efficacious Pirin ligand from a heat shock transcription factor 1 (HSF1) phenotypic screen, J. Med. Chem. 60 (1) (2017) 180–201.

[187] D. Zhang, B. Zhang, Selective killing of cancer cells by small molecules targeting heat shock stress response, Biochem. Biophys. Res. Commun. 478 (4) (2016) 1509–1514.

[188] N. Zaarur, V.L. Gabai, J.A. Porco Jr., S. Calderwood, M.Y. Sherman, Targeting heat shock response to sensitize cancer cells to proteasome and Hsp90 inhibitors, Cancer Res. 66 (3) (2006) 1783–1791.

[189] D. Schilling, A. Kühnel, S. Konrad, F. Tetzlaff, C. Bayer, J. Yaglom, et al., Sensitizing tumor cells to radiation by targeting the heat shock response, Cancer Lett. 360 (2) (2015) 294–301.

[190] S. Banerjee Mustafi, P.K. Chakraborty, S. Raha, Modulation of Akt and ERK1/2 pathways by resveratrol in chronic myelogenous leukemia (CML) cells results in the downregulation of Hsp70, PLoS One 5 (1) (2010) e8719.

[191] T. Liu, T. Long, H. Li, Curcumin suppresses the proliferation of oral squamous cell carcinoma through a specificity protein 1/nuclear factor-κB-dependent pathway, Exp. Ther. Med. 21 (3) (2021) 202.

[192] S.J. Kim, S.C. Lee, H.G. Kang, J. Gim, K.H. Lee, S.H. Lee, et al., Heat shock factor 1 predicts poor prognosis of gastric Cancer, Yonsei Med. J. 59 (9) (2018) 1041–1048.

[193] S. Chatterjee, T.F. Burns, Targeting heat shock proteins in cancer: a promising therapeutic approach, Int. J. Mol. Sci. 18 (9) (2017).

[194] B. Dong, A.M. Jaeger, D.J. Thiele, Inhibiting heat shock factor 1 in cancer: a unique therapeutic opportunity, Trends Pharmacol. Sci. 40 (12) (2019) 986–1005.

[195] C. Sharma, Y.H. Seo, Small molecule inhibitors of HSF1-activated pathways as potential next-generation anticancer therapeutics, Molecules. 23 (11) (2018).

[196] M. Velayutham, A.J. Cardounel, Z. Liu, G. Ilangovan, Discovering a reliable heatshock Factor-1 inhibitor to treat human cancers: potential opportunity for phytochemists, Front. Oncol. 8 (2018) 97.

[197] P.K. Chakraborty, S.B. Mustafi, S. Ganguly, M. Chatterjee, S. Raha, Resveratrol induces apoptosis in K562 (chronic myelogenous leukemia) cells by targeting a key survival protein, heat shock protein 70, Cancer Sci. 99 (6) (2008) 1109–1116.

[198] Y.-C. Chen, S.-H. Tsai, S.-C. Shen, J.-K. Lin, W.-R. Lee, Alternative activation of extracellular signal-regulated protein kinases in curcumin and arsenite-induced HSP70 gene expression in human colorectal carcinoma cells, Eur. J. Cell Biol. 80 (3) (2001) 213–221.

[199] M.-H. Teiten, S. Reuter, S. Schmucker, M. Dicato, M. Diederich, Induction of heat shock response by curcumin in human leukemia cells, Cancer Lett. 279 (2) (2009) 145–154.

[200] K. Kato, H. Ito, K. Kamei, I. Iwamoto, Stimulation of the stress-induced expression of stress proteins by curcumin in cultured cells and in rat tissues in vivo, Cell Stress Chaperones 3 (3) (1998) 152–160.

[201] H. Hatcher, R. Planalp, J. Cho, F.M. Torti, S.V. Torti, Curcumin: from ancient medicine to current clinical trials, Cell. Mol. Life Sci. 65 (11) (2008) 1631–1652.

[202] K.E. Dunsmore, P.G. Chen, H.R. Wong, Curcumin, a medicinal herbal compound capable of inducing the heat shock response, Crit. Care Med. 29 (11) (2001) 2199–2204.

[203] H.-W. Chen, J.-Y. Lee, J.-Y. Huang, C.-C. Wang, W.-J. Chen, S.-F. Su, et al., Curcumin inhibits lung cancer cell invasion and metastasis through the tumor suppressor HLJ1, Cancer Res. 68 (18) (2008) 7428–7438.

[204] L. Whitesell, S. Lindquist, Inhibiting the transcription factor HSF1 as an anticancer strategy, Expert Opin. Ther. Targets 13 (4) (2009) 469–478.

[205] A. Yallowitz, A. Ghaleb, L. Garcia, E.M. Alexandrova, N. Marchenko, Heat shock factor 1 confers resistance to lapatinib in ERBB2-positive breast cancer cells, Cell Death Dis. 9 (6) (2018) 621.

[206] T. Yang, C. Ren, C. Lu, P. Qiao, X. Han, L. Wang, et al., Phosphorylation of HSF1 by PIM2 induces PD-L1 expression and promotes tumor growth in breast cancer, Cancer Res. 79 (20) (2019) 5233–5244.

[207] R.L. Carpenter, S. Sirkisoon, D. Zhu, T. Rimkus, A. Harrison, A. Anderson, et al., Combined inhibition of AKT and HSF1 suppresses breast cancer stem cells and tumor growth, Oncotarget. 8 (43) (2017) 73947–73963.

[208] V. Tandon, R. Moreno, K. Allmeroth, J. Quinn, S.E. Wiley, L.G. Nicely, et al., Dual inhibition of HSF1 and DYRK2 impedes cancer progression, Biosci. Rep. 43 (1) (2023).

[209] M. Huang, W. Dong, R. Xie, J. Wu, Q. Su, W. Li, et al., HSF1 facilitates the multistep process of lymphatic metastasis in bladder cancer via a novel PRMT5- WDR5-dependent transcriptional program, Cancer Commun. (Lond.) 42 (5) (2022) 447–470.

[210] S. Lee, J. Jung, Y.J. Lee, S.K. Kim, J.A. Kim, B.K. Kim, et al., Targeting HSF1 as a therapeutic strategy for multiple mechanisms of EGFR inhibitor resistance in EGFR mutant non-small-cell lung cancer, Cancers (Basel). 13 (12) (2021).

[211] Z. Shen, L. Yin, H. Zhou, X. Ji, C. Jiang, X. Zhu, et al., Combined inhibition of AURKA and HSF1 suppresses proliferation and promotes apoptosis in hepatocellular carcinoma by activating endoplasmic reticulum stress, Cell. Oncol. 44 (5) (2021) 1035–1049.

[212] J.H.L. Fok, S. Hedayat, L. Zhang, L.I. Aronson, F. Mirabella, C. Pawlyn, et al., HSF1 is essential for myeloma cell survival and a promising therapeutic target, Clin. Cancer Res. 24 (10) (2018) 2395–2407.

[213] P. Antonietti, B. Linder, S. Hehlgans, I.C. Mildenberger, M.C. Burger, S. Fulda, et al., Interference with the HSF1/HSP70/BAG3 pathway primes glioma cells to matrix detachment and BH3 mimetic–induced apoptosis, Mol. Cancer Ther. 16 (1) (2017) 156–168.

[214] C. Ishikawa, N. Mori, Heat shock factor 1 is a promising therapeutic target against adult T-cell leukemia, Med. Oncol. 40 (6) (2023) 172. [215] K. Yoo, H.H. Yun, S.Y. Jung, J.H. Lee, KRIBB11 induces apoptosis in A172 glioblastoma cells via MULE-dependent degradation of MCL-1, Molecules. 26 (14) (2021).

[216] M.-J. Kang, H.H. Yun, J.-H. Lee, KRIBB11 accelerates Mcl-1 degradation through an HSF1-independent, Mule-dependent pathway in A549 non-small cell lung cancer cells, Biochem. Biophys. Res. Commun. 492 (3) (2017) 304–309.