# Combined Thermotherapy and Heat Shock Protein Modulation for Tumor Treatment


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## Abstract

**Introduction** Thermal therapy (hyperthermia) holds a promising treatment for tumor-affected patients particularly those with surgery intolerance. Recent advances and clinical trials for therapeutic purposes of heat shock proteins (Hsp) inhibitors and the astonishing progress in the field of nanotechnology pave the way for novel strategies for combined and effective treatment and targeting of the tumor cells. In here, we highlight the history of hyperthermia, as a therapeutic tool for tumors, and provide the state-of-the-art regarding the promising synergism between hyperthermia, HSP modulation and the targeted nanoparticles for tumor cell targeted therapy.

**Methods** A literature based collection of articles in the available search engines (PubMed and Google Scholar).

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**Results** We show the possible combination of thermal therapy together with Hsp inhibitors for treating cancers.

**Conclusions** The use of Hsp inhibitors potentiates the cytotoxic and/or anti-proliferative effects of the hyperthermia.

**Keywords** HSP · HSP inhibitors · Hyperthermia · Nanoparticles · Exosomes · Tumor treatment

## Abbreviations

| | |
|---|---|
| CRC | colorectal cancer |
| ECM | extracellular matrix |
| HIF | hypoxia inducible factors |
| HSP | heat shock protein/s |
| MRI | magnetic resonance imaging |
| MSC | mesenchymal stem cells |
| siRNA | small interfering RNA |
| SPIONs | superparamagnetic iron oxide nanoparticles |

## 1 Introduction

Thermotherapy (thermal therapy or hyperthermia) is a type of tumor treatment that were used through 5000 years of practice by physicians, surgeons, clergy, or lay people in which body tissue is exposed to high temperatures (up to 113 °F or 45 °C) [1]. Hyperthermia is a promising treatment for a wide ranges of patients particularly those with surgery intolerance [2]. Cumulative evidence showed that hyperthermia can damage and kill tumor cells with minimal injury to the adjacent normal tissues [3]. Hyperthermia is usually a regional treatment for specific tumor lesions; however, it may be used in combination with other treatments such as chemotherapy or radiation to enhance the treatment strategy [1, 4] as summarized in Fig. 1. (1) Local tumor hyperthermia potentiates the immune system response, including tumor cell attack, tumor cell surface modulation, release heat shock proteins and exosomes which possess a direct effect on immune cells and changes the tumor microenvironments [4, 5], (2) Hyperthermia makes some tumor cells more sensitive to radiation and chemotherapy and potentiate the effects of radio- and chemotherapy [6, 7].

There are several methods of hyperthermia that are currently under study, including local (skin, esophagus, rectum, and brain tumors), regional (reproductive tract, urinary tract, respiratory system, arms, legs, abdominal organs, and tumors), and whole-body (metastatic cancers) hyperthermia [8]. Reaching but not exceeding the

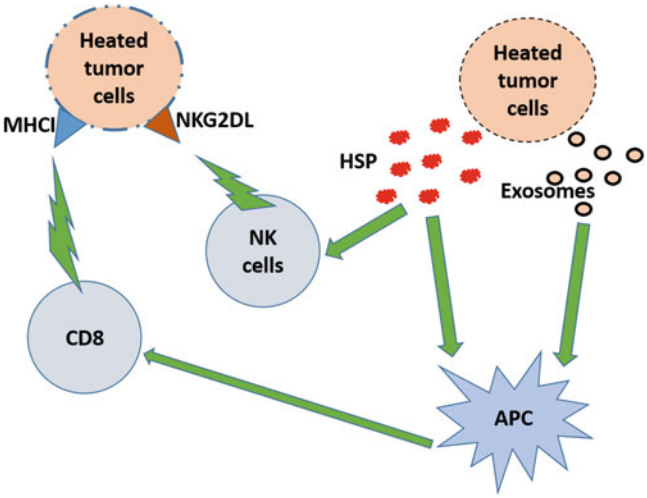


**Fig. 1 Strategies of supporting immune system through hyperthermia**. Tumor heated cells express MHC1 and NKG2DL that activate CD8+ T cells and natural killer (NK) cells, respectively, to attach the heated cells. In addition, tumor heated cells release heat shock proteins (HSP) that directly activate both NK cells and indirectly through activation of antigen presenting cells (APC) and in turn CD8+ cells. Furthermore, tumor heated cells release exosomes, which contain chemokines and activate APC cells too [4, 5]

desired temperature, of the tumor and surrounding tissue should accompany the thermal therapy. This can be achieved through CT (computed tomography) – aided insertion of needles with tiny thermometers into the treatment area to monitor the temperature [8].

Thermotolerance is a phenomenon in which cells become resistant to elevated temperatures. Thermotolerance might develop rapidly after the first heat treatment or during the thermal treatment at ~43.0 °C. Studies disclosed that thermotolerance developed in tumors and normal tissues as well [9, 10] and it is well correlated with enhanced synthesis of heat shock proteins [9, 11–13].

The kinetics of thermotolerance can be affected by various factors [10, 14]. For instance, thermotolerance is found physiologically in certain species as a form of estivation [15, 16]. It might be varied among certain cells of the same species [17–19]. Cells showed variability in thermotolerance because of the way of cell culture; cells grown in 3D compared with 2D culture showed reduced incidence of apoptosis and necrosis and a higher level of Hsp70 expression in response to heat shock [20].

Due to the essential role of HSP in thermotolerance, we and others propose the phenomenon “anastasis” to illustrate the survival response of thermotolerant cells [19, 21–25]. Anastasis is a term coined to outline the process of cell recovery, plasticity, resilience, or cellular resurrection from the brink of cell death [24] and might be a reason for cancer cells thermotolerance [26].

## *1.1 Heat Shock Proteins in Cancer Cells*

### 1.1.1 Intracellular HSP

Overexpression of HSP is one of the key features in cancer cells which enables them to survive and develop. Several HSP including Hsp90, Hsp70, Hsp60 and sHSP perform multiple coordinated functions in tumor cells at the cellular and extracellular levels. In general, the significance of HSP in cancer comes from their implication in cancer metastasis, aggressiveness and therapeutic resistance besides their diagnostic and prognostic values [27] (Fig. 2). In this section, we briefly shed the light on the diverse oncogenic roles of major HSP such as Hsp90, Hsp70, Hsp60 and Hsp27 known in the cancer field. Hsp90, for instance, has been found to chaperone central elements along the cellular proliferation cascades which involve Erk, Src and Akt pathways [27]. In addition, it interacts with mutant oncogens and stabilizes them, thus permitting unrestricted proliferation [28, 29]. Likewise, Hsp70 plays analogous important role since silencing of Hsp70 resulted in impaired proliferation in murine mammary tumor cells [30]. Interestingly, HSP have been demonstrated to bind and stabilize the mutant p53 that is known to be mutated in more than half of cancers [31–33]. Elevated expression of Hsp90 and Hsp70 has been described in tumor cells containing mutant p53 [32, 33]. The small heat shock protein, Hsp27 (also known as HSPB1) has been shown to seriously impact the p53 mediated senescence and apoptosis [34]. These effects are of particular importance because the tumor suppression function of the wild type p53 is mostly lost upon its mutation and the mutant HSP-stabilized p53 is likely to possess oncogenic gain of function properties [35].

Another crucial aspect is the anti-apoptotic capabilities of HSP in cancer cells. These anti-apoptotic roles have been reported in many types of cancer including prostate [36], ovary [37], lung [38], liver [39], and others [27]. Hsp60, for instance, has been demonstrated to regulate apoptosis in tumor cells and its targeting by siRNA resulted in disruption of the mitochondrial function and initiation of caspase-dependent apoptosis [40]. Hsp27 and Hsp70 resist cell death *via* interacting with and inhibiting variant protein intermediates within the apoptotic pathway

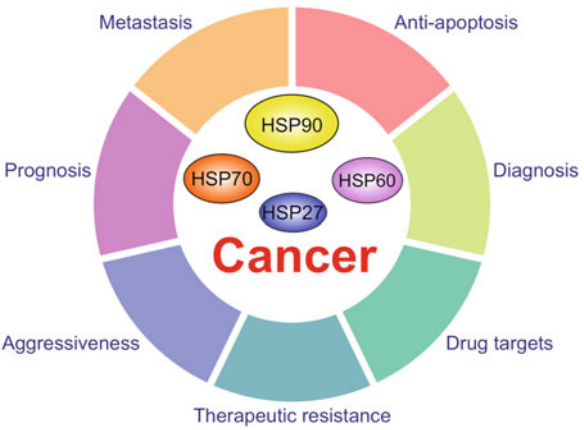


**Fig. 2 The multiple aspects of HSP significance in oncology field**. Hsp27, 60, 70, and 90 play a pivotal role in different aspects of cancer from the diagnosis till the therapeutics

[41, 42]. Hsp27 interferes with the mitochondrial release of cytochrome C and SMAC Diablo besides hampering caspases 3 and 9 activities [43–45]. It also hinders the extracellular apoptotic signals *via* inhibiting Fas, TNFα and TRAIL receptors' pathways [46]. On the other hand, Hsp70 blocks the c-Jun kinase of the programmed cell death and hampers the release of cytochrome C from mitochondria [41, 44]. Interestingly, Hsp90 has been found to inhibit cell senescence by chaperoning telomerase enzyme which is essential to recover eroded telomeres, thus prolonging cancer cell survival [47]. It is not surprising therefore, that co-targeting of more than one chaperone such as Hsp90 and Hsp70 has been beneficial in terms of better therapeutic responsiveness and enhanced sensitivity to anti-cancer drugs [48, 49]. Taken together, it seems that abundant expression of various HSP allow them to act coordinately and synergistically in order to afford an optimum conditions for cancer cell immortality [50].

It is well known that growing cancer cells develop mechanisms to support angiogenesis and satisfy their high demands for nutrients and oxygen. In this respect, Hsp90 has been demonstrated to activate and stabilize hypoxia inducible factors (HIF) which serves as a sensor of low oxygen content [51]. Stabilizing HIF1α is pivotal for stimulating the expression of vascular endothelial growth factor (VEGF) and subsequently creating the tumor capillary network and potentiating angiogenesis [52, 53].

Cancer metastasis is a complex process that characterizes malignant tumors and requires efficient HSP machinery. Overexpressed Hsp90 has been reported to chaperone focal adhesion kinase, integrin linked kinase and the receptor tyrosine kinases ErbB2 and MET [54]. Additionally, co-chaperones of Hsp90 contribute to tumor metastasis as seen in p23 which regulates metastasis in prostate cancer [55]. Similar to Hsp90, Hsp70 is likely to support MET expression and autophosphorylation in breast cancers [30, 56]. Moreover, Hsp27 has been reported to augment metastasis *via* supporting epithelial-mesenchymal transition (EMT) [57–59].

### 1.1.2 Extracellular HSP in Cancer

Despite their initial underestimation by the scientific community, the biological functions of extracellular HSP are nowadays growing dramatically. In fact, recent reports suggest that extracellular HSP are widely implicated in inflammatory and immunogenic roles [60, 61]. These observations were based on several molecular studies investigating variant HSP members both *in vitro* and *in vivo*. For instance, the secretory form of Hsp70, HSPA1A has been demonstrated to stimulate mast cells for production of tumor necrosis factor α (TNFα) and interleukin 6 (IL-6) *via* the toll-like receptor 4 (TLR4) and toll-like receptor 2 (TLR2) pathways [62–65]. HSPA1A has also been reported to induce the secretion of IL-12 from naive dendritic cells [66]. Tumor cell lines including hepatocellular carcinoma (HepG2) and murine leukemia monocytes have been described to secrete exosomes rich in HSP from different families such as Hsp60, Hsp70 and Hsp90, which enhanced the immunogenic activities of natural killer cells, macrophages and mononuclear cells [67–70]. Moreover, upon release from monocytic cell line U937, Hsp70 has been

found to stimulate the expression of matrix metalloprotease 9 (MMP-9) and augment cell motility [71]. Furthermore, extracellular Hsp70 has been demonstrated to interact with human immunoreceptors Siglec-5 and Siglec-14 trigger both anti-inflammatory and pro-inflammatory responses [72]. In colon cancer cell lines, released Hsp90β has been observed to reduce cellular adhesion and stimulate migration [73].

Clinically, several lines of evidence associate the extracellular or secretory HSP with cancer stage and progression. Hsp70 expression levels have been reported to be significantly higher in patients with liver cancer compared with control healthy group [74]. In comparison to healthy individuals, elevated Hsp70 serum levels have been detected in patients with squamous cell carcinoma [75]. High serum Hsp27 levels have been observed in many types of cancer such as epithelial ovarian cancer and were linked to tumor metastasis and progression [76, 77]. In patients with non-small cell lung cancer, measured serum levels of Hsp27 can differentiate between early and advanced stages of disease [77]. Serum Hsp90 were found significantly high in patients with cutaneous malignant melanoma compared with control subjects [78].

Collectively, it is apparent that a multitude of HSP play diverse crucial roles in the development and progression of cancer. These HSP-multifaceted functions including unlimited growth, tumor suppression prevention, increased cell survival and enhanced angiogenesis and metastasis, can therefore define the traits of cancer [50]. In accordance with these conclusions, overexpression of HSP in cancer patients has mostly been associated with poor prognosis and monitoring clinical outcome. Hence, targeting of HSP has increasingly been investigated to treat variant types of cancer.

## *1.2 Heat Shock Protein Modulation as a Target for Cancer Therapy*

Due to their pivotal roles in cancer development and metastasis, targeting HSP has been actively researched by many investigators in an attempt to treat diverse human cancers. In the following section, we summarize different targeting approaches for crucial HSP such as Hsp90, Hsp70, Hsp60 and Hsp27, especially those approaches concerning small molecule inhibitors.

### 1.2.1 Targeting Hsp90

The Hsp90 family members are the most intensely investigated HSP in relation to cancer therapeutics [27, 79]. Since Hsp90 consists mainly of N-terminal domain, middle domain and C-terminal domain, variant Hsp90 inhibitors have been interestingly found to selectively target a specific structural domain within the Hsp90 molecule (Table 1 and Fig. 3). For instance, natural inhibitors derived from *Streptomyces hygroscopicus* such

**Table 1** Common Hsp90 inhibitors used in preclinical and/or clinical cancer studies

| Class | Inhibitor | Targeting site | References |
|---|---|---|---|
| Geldanamycin derivatives | Geldanamycin (GM) | N-terminal ATP binding domain of Hsp90 | [27, 80] |
| | Tanespimycin (17-AAG, KOS-953) | | |
| | Alvespimycin (17-DMAG) | | |
| | Rataspimycin (IPI-504) | | |
| Radicicol derivatives (These compounds share common resorcinol core) | Radiciccol (RD) | N-terminal ATP binding domain of Hsp90 | [27, 80] |
| | Luminespib (NVP-AUY922) | | |
| | Ganetespib (STA-9090) | | |
| | KW-2478 | | |
| | Onalespib (AT13387) | | |
| Purine derivatives | PU3 | N-terminal ATP binding domain of Hsp90 | [27, 80] |
| | PIIB021/ CNF2024 | | |
| | PU-H71 | | |
| | MPC3100 | | |
| | CUDC-305 | | |
| Others | SNX-5422 (PF-04929113) | N-terminal ATP binding domain of Hsp90 | [81] |
| | NVP-HSP990 | N-terminal ATP binding domain of Hsp90 | [82] |
| Sulfoxythiocarbamate alkynes | STCAs | The middle domain | [83] |
| Hsp90 inhibitors with alternative mode of action | Novobiocin and KU174 | C-terminal binding domain of Hsp90 and/or disruption of Hsp90-cochaperone interaction | [80] |
| | Cisplatin | | |
| | Epigallocatechin-3-gallate | | |
| | Taxol | | |
| | Withaferin A | | |
| | Celastrol | | |
| | Gedunin | | |
| | 7-azapteridine core | | |
| New compounds | Newly identified compounds (Compound 7 and 10) | C-terminal binding domain of Hsp90 | [84] |

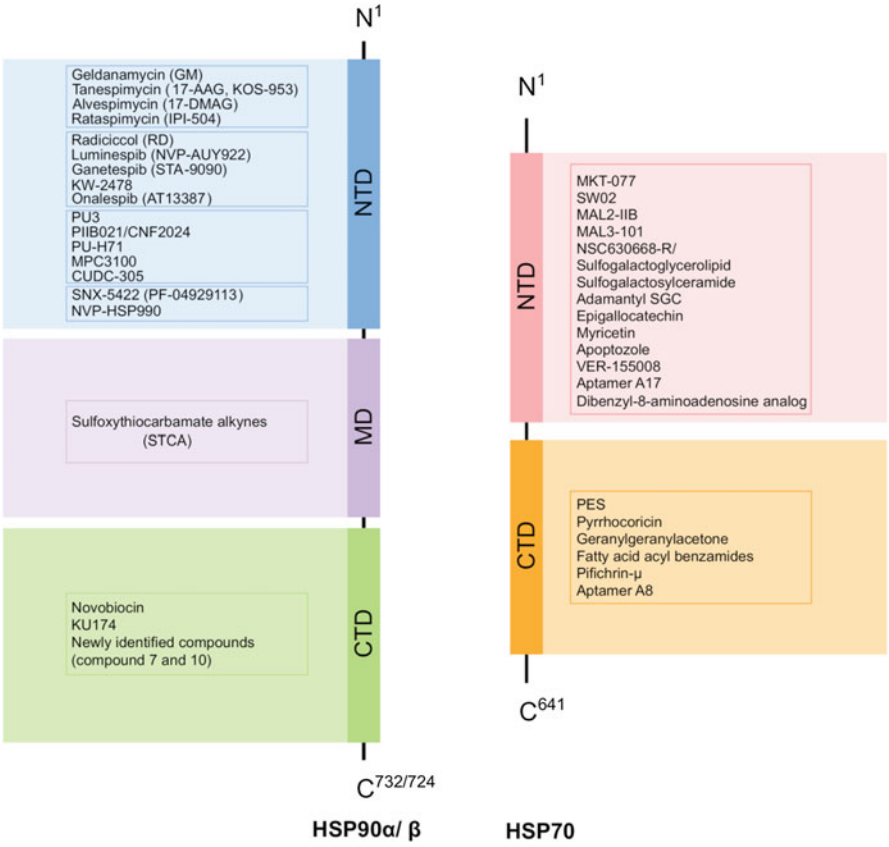


**Fig. 3 Schematic representation showing variant inhibitor molecules of Hsp90 (left panel) and Hsp70 (right panel)**. Inhibitors of HSP are grouped according to their targeting sites (*NTD* N-terminal domain, *MD* middle domain and *CTD* C-terminal domain)

as geldanamycin (GM) perform their anti-proliferative activity through association with the ATP-binding site located in the N-terminal domain of Hsp90, thus blocking its function [85, 86]. Similarly, radicicol (RD) that was primarily obtained from *Monosporium bonorden* inhibits Hsp90 *via* occupying its ATP binding pocket, subsequently hindering its ATPase activity [87]. Unfortunately due to their hepatotoxic side effects, structural instability or poor bioavailability geldanamycin and radicicol were not used in the clinic although their *in vitro* promising effects [87, 88]. Therefore, different GM analogues like 17-AAG (tanespimycin or 17-allylamino-17-demethoxygeldanamycin) and 17-DMAG (alvespimycin or 17-dimethylaminoethylamino-17 demethoxygeldanamycin) have been developed in attempt to overcome these limitations [89, 90]. Other Hsp90-inhibiting compounds, such as sulfoxythiocarbamate alkynes (STCAs), have been recently reported to target the Hsp90 middle domain *via* attacking cysteine residues and forming thiocarbamate adducts. Interestingly, the resulting conformational changes from the thiocarbamylation

process alters the chaperoning activity of Hsp90 and hinders its binding to client proteins without interference of its ATPase activity [83].

Coumarin antibiotics, such as novobiocin and its derivatives, have been described to inhibit another ATP binding site located in the C-terminal domain of Hsp90 [91]. Binding of novobiocin to the C-terminal ATP binding site disrupts the interaction of Hsp90 with many of its client proteins such as Raf-1, v-src, mutant p53 and HER2 [91]. Recent technologies, such as plasmon resonance (SPR), have been utilized to explore various Hsp90 C- terminal inhibitors among many commercially available compounds. Interestingly, these efforts enabled Terracciano and his colleagues to report newly identified compounds targeting the Hsp90 C- terminal domain and able to induce potent anti-cancer activities [84].

In addition to previous strategies, certain compounds such as celastrol and gedunin have been demonstrated to interfere with Hsp90 binding to its co-chaperones including Cdc37 and p23 [92–94]. Furthermore, other approaches aimed to inhibit the Hsp90 interaction with its client proteins [95].

### 1.2.2 Targeting Hsp70

Similar to Hsp90, various molecules have been identified to inhibit Hsp70 and currently represent powerful tools in cancer therapeutics. The majority of these compounds, summarized in Table 2 and Fig. 3, are known to target either the nucleotide binding domain (N-terminal domain) or the substrate binding domain (C-terminal domain) of Hsp70. Generally, Hsp70 inhibitors are categorized into three main groups; small molecule inhibitors, protein aptamers and antibody treatment [27].

Small molecule inhibitors such as MKT-077, an analogue of cationic rhodacyanine dye, was found to target the N-terminal ATPase domain of Hsp70 and has been tested in cancer clinical trials [99]. Other small molecule inhibitors include 2-phenylethynesulfonamide (PES) or pifithrin-μ that associates with the C-terminal domain of Hsp70 and prevents its interaction with HSP40 and other protein clients such as APAF-1 and p53 [100]. Impairment of Hsp70 function leads to misfolded protein aggregation, destabilized lysosomal membrane and apoptosis. Conversely, the natural immunosuppressive compound, 15-deoxyspergualin (15-DSG), binds to the N-terminal domain of Hsp70 and blocks its ATPase activity [101]. Second generation inhibitors such as MAL3-101 and its derivatives act on the N-terminal ATP binding domain of Hsp70 and exhibit anti-proliferative activities on cancer cell lines [102]. Notably, co-treatment of cancer cells with MAL3-101 and 17-AAG or MAL3-101 with PS-341 (bortezomib) in mouse model of melanoma showed enhanced therapeutic responsiveness [103, 104]. Interestingly, VER-155008, a compound that is derived from adenosine targeting the Hsp70 ATPase domain, was able to stimulate both caspase dependent and non-caspase dependent apoptosis in breast and colon cancer cells [105]. In addition, combination therapies including Hsp90 inhibitors such as NVP-AUY922 and VER-155008 gave better anti-cancer effects in myeloma cells [106].

**Table 2** Common anti-cancer Hsp70 inhibitors and their targeting sites [96]

| Class | Inhibitor | Targeting site | References |
|---|---|---|---|
| | MKT-077 | N-terminal ATP binding domain of Hsp70 | [97, 98] |
| Dihydropyrimidines | SW02 | N-terminal ATP binding domain of Hsp70 | [97, 98] |
| | MAL2-IIB | | |
| | MAL3-101 | | |
| | NSC630668-R/I | | |
| Sulfoglycolipids | Sulfogalactoglycerolipid | N-terminal ATP binding domain of Hsp70 | [97, 98] |
| | Sulfogalactosylceramide | | |
| | Adamantyl SGC | | |
| Flavonoids | Epigallocatechin | N-terminal ATP binding domain of Hsp70 | [97, 98] |
| | Myricetin | | |
| | Apoptozole | N-terminal ATP binding domain of Hsp70 | [97, 98] |
| Adenosine derivatives | VER-155008 | N-terminal ATP binding domain of Hsp70 | [98] |
| Protein Aptamer | Aptamer A17 | N-terminal ATP binding domain of Hsp70 | [96–98] |
| | Dibenzyl-8-aminoadenosine analog | N-terminal ATP binding domain of Hsp70 | |
| | cmHsp70.1mAb | Interact with Hsp70 epitope | |
| Small molecule inhibitor | 2-phenylethynesulfonamide (PES pifithrin-μ) | C-terminal/peptide binding domain | |
| | Pyrrhocoricin | C-terminal/peptide binding domain | |
| | Geranylgeranylacetone | C-terminal/peptide binding domain | |
| | Fatty acid acyl benzamides | C-terminal/peptide binding domain | |
| | Pifichrin-μ | C-terminal/peptide binding domain | |
| Protein Aptamer | Aptamer A8 | C-terminal/peptide binding domain | |

Protein aptamers are considered among the alternative approach targeting Hsp70. A17 was demonstrated to target the Hsp70 N-terminal ATPase domain. Moreover, combined cisplatin/A17 therapy potentiated apoptosis in cancer cell lines and efficiently inhibited tumor growth in mice models of melanoma [107]. Other targeting approaches of Hsp70 include immune based monoclonal antibodies such as cmHsp70.1, which recognizes specific membrane bound Hsp70 motif [108]. These advanced approaches have been used in clinical trials with promising anticancer results [108].

### 1.2.3 Targeting Hsp60 in Cancer

Relative to other HSP, few compounds have been known to target Hsp60 [109]. Meng and his colleagues have classified Hsp60 inhibitors according to their origin into two main groups; derivatives natural products and synthetic compounds (listed in Table 3) [109]. Based on their mode of action, Hsp60 inhibitors have been arranged into type I inhibitors, which target the ATP binding site and interfere with the Hsp60 chaperoning activities, and type II inhibitors, which comprise compounds acting through covalent association with cysteine residues within the Hsp60 molecule. However, much of the exact mechanism of action of these inhibitors are still unclear [109]. Table 3 gives an overview about the potential modulators of Hsp60 that can be used in future cancer treatments.

### 1.2.4 Targeting Hsp27 in Cancer

Hsp27 is one of the major inducible sHSP known to contribute to tumor development and malignancy. Upregulation of Hsp27 has been reported in myriad cancer types where it has been linked to poor prognosis and treatment resistance [121]. In the previous section, we briefly referred to its anti-apoptotic mechanisms as well as cancer promoting roles. Here, we present a summarized overview on the potential

**Table 3** Overview of common Hsp60 inhibitors [109]

| Class | Inhibitor | Effect | References |
|---|---|---|---|
| Natural products | Mizoribine | binds to the Hsp60 ATPase domain leading to This direct binding inhibition of the chaperone activity of the Hsp60-Hsp10 complex | [110, 111] |
| | epolactaene | Unknown mechanism of action | [109, 112] |
| | ETB (tert-butyl ester of epolactaene) | Interacts with Cys442 of Hsp60 leading to potential proximity to potential allosteric modulation of the ATP binding pocket | [112] |
| | Myrtucommulone A (MC) | Interacts directly with Hsp60 leading to aggregation and misfolding of cancer related proteins | [113] |
| | Stephacidin B | Performs anticancer activities | [114, 115] |
| | Avrainvillamide | Anticancer activities | [116] |
| Synthetic compounds | *O*-carboranylphenoxyacetanilide | Binds to Hsp60 and suppresses hypoxia-induced HIF activation | [117] |
| | Gold (III) porphyrin complexes such as A prototype gold (III) complex [Au(TPP)Cl] (10) | Though poorly understood mechanisms, it inhibits Hsp60 and performs significant anticancer activities | [118–120] |

approaches and inhibitors targeting Hsp27 in cancer therapeutic arena (summarized in Table 4).

It has been known that the plant bioflavonoid quercetin exhibits anti-cancer properties [122] *via* inhibition of heat shock response [123, 130]. Diverse anti-cancer activities of quercetin have been described in prostate, gastric, breast and oral cancers [131, 132]. Interestingly, quercetin has been demonstrated to down regulate casein kinase 2 (CK2) with consequent proteasomal degradation of Hsp27. Therefore, quercetin has been suggested to regulate Hsp27 in cancer cells [133, 134]. Another small molecule inhibitor, brivudin (RP101) has been revealed to inhibit Hsp27 through association of π-stacking with Phe29 and Phe33 of Hsp27 leading to apoptosis [124, 125]. RP101 has been used in clinical trials of pancreatic cancers where it increased the survival rates of diseased individuals [124]. In addition, in fibrosarcoma cells, combined RP101/gemcitabine treatment resulted in 30–50% reduction of invasiveness compared to gemcitabine alone [124].

An eminent strategy to target Hsp27 is the use of antisense oligonucleotide (ASO) which target Hsp27 mRNA. For instance, OGX-427 has been used in combination therapies treating prostate cancer where it remarkably reduced the tumor volume compared to monotherapies [127]. OGX-427 has been also used in phas I and phase II clinical studies of metastatic bladder and castrate-resistant prostate cancers, respectively [135]. Furthermore, treatment with OGX-427 resulted in enhanced sensitivity to radiation therapies in radiation-resistant lung as well as head and neck cancers besides reduction of tumor angiogenesis [136].

Difficulties in the application of antisense technology in vivo gave rise to new approaches that employs specific peptides to suppress the anti-apoptotic activity of Hsp27 [121]. Protein aptamers are designed in the form of short sequences of aminoacids associated with a scaffold protein. These aptamers aimed to modulate the activity of different cellular proteins, including oncogenes, transcription factors, signaling molecules, cell cycle regulators, and others [129]. The two aptamers PA11 and PA50 have been designed to specifically bind to Hsp27, disrupting its dimerization and oligomerization leading into impairment of cancer cell proteostasis. Although

**Table 4** Common Hsp27 inhibitors and their mode of action

| Class | Inhibitor | Mode of action | References |
|---|---|---|---|
| Small molecules | Quercetin | Reduces CK2 expression and increases Hsp27 degradation | [121–123] |
| | RP101 (Brivudine) | Inhibits Hsp27 through association of π-stacking with Phe29 and Phe33 of Hsp27 leading to apoptosis | [124, 125] |
| | J2 (Cross linker) | Forms a covalent bond between the cysteine of thiol groups within Hsp27 | [126] |
| Antisense Drug | OGX-427 | Antisense oligonucleotide (ASO) that targets Hsp27 mRNA | [127, 128] |
| Peptide Aptamers | PA11 | Interferes with Hsp27 oligomerization leading to dysregulated cellular proteostasis | [129] |
| | PA50 | Hampers Hsp27 dimerization resulting in disruption of Hsp27 mediated signaling | [129] |

the application of these advanced strategies looks promising in the oncology field, certain limitations remain existing in terms of the size of the investigated protein, the presence of protein complexes, RNase containing environment [121, 129].

## *1.3 Targeted Cancer Thermotherapy*

### 1.3.1 Nanoparticles in Cancer Therapy

Anticancer therapy insufficient tumor targeting, and increased side effects have directed the interest in nanomedicine for cancer therapy [137]. Nanomedicine was defined by the US National Institute of Health as 'Nanomedicine refers to highly specific medical intervention at the molecular scale for curing diseases or repairing damaged tissues, such as bone, muscle, or nerve' [137]. The nanocarriers used are (10–200) nm in size that facilitate drug uptake, fast diffusion and having a large surface area to the volume ratio [138]. Consequently, those nanocarriers with their targeting ability will be able to accumulate in the tumor site and stay longer, which increases the efficiency of the drug [138]. At the same time, they will decrease side effects and toxicity of the drug since less of the healthy tissue is exposed to it [138]. Also, nanocarriers have the potential to deliver insoluble and unstable drugs, which they can protect from degradation [138].

There are different types of nanocarriers that are named based on their composition which are: Solid Lipid, Liposomes, Micelles, Dendrimers, Polymeric, Vial, Magnetic, Carbon, and Gold carriers [137]. Those carriers are also classified into three major types of nanoparticles: one dimension, two dimensions (Carbon nanotubes), or three dimensions (Dendrimers) nanoparticles [139]. For the best results, cancer cells and biocompatibility need to be identified to select the suitable nanocarrier type that can recognize the tumor site and release the desired drug [137]. Nanocarriers can be developed to not only deliver drug but also for cancer imaging as in the case of paramagnetic nanoparticles.

### 1.3.2 SPIONs

Magnetic Iron oxide nanoparticles have many applications compared to other nanoparticles used in diagnosis, treatment, and treatment monitoring. Superparamagnetic iron oxide nanoparticles (SPIONs) have a smaller size compared to iron oxide nanoparticles (IONP). Those particles have a size between (20–150) nm and have a more complicated synthesis than large IONP [140–142]. They have two structural compositions either they have a magnetic particle core (Magnetite $Fe_3O_4$, or Maghemite $\gamma$-$Fe_2O_3$), that differ in their physical properties, coated with a biocompatible polymer [143, 144]. Or they can be composed of a porous biocompatible polymer where they get precipitated inside the pores [144]. SPIONs have an important role in biomedical applications [141]. For example, they are being developed for an advanced

magnetofection, which is a transfection method, and magnetic resonance imaging (MRI) [141].

SPIONs have great superparamagnetic behavior, chemical stability, high saturation magnetization, and appropriate biocompatibility for therapy [141]. Therefore, when SPIONs are used for drug delivery they will have a long blood retention time, biodegradability and low toxicity, which increase the efficiency and decrease side effects of the drug in patients [140]. Also, they have been involved in magnetic hyperthermia-based cancer therapy for their ability to enhance competency, which generates localized heat under a fluctuating magnetic field [141]. Thus, SPIONs have more advantages than other nanoparticles to be used in drug targeting or magnetic hyperthermia for colorectal cancer therapy [141].

### 1.3.3 Synthesis Approaches

Two approaches are used in nanomaterial synthesis: a bottom-up approach, or top-down approach. In the bottom-up approach, nanoparticles used as the building blocks for complex nanostructures and have a better chance of producing structures with less defect [143]. While the top-down approach uses larger initial structures to attain nanostructures. Additionally, SPIONs synthesis methods are subdivided into 3 general types: physical, chemical, or biological [145]. Ninety percent of the methods used in their synthesis are chemical, while the 7% physical and 3% biological methods [146]. Because chemical approaches in synthesis have more direct procedures and fast product collection. Thus, those methods are the route that will be used for mass production of therapeutic nanoparticles in the future.

### 1.3.4 Chemical Synthesis: Co-precipitation

Co-precipitation is the most used chemical method especially in biomedical applications [143]. This method requires the usage of Fe (II) salt in aqueous, to a base solution in the presence of oxidant [147]. Like using iron chloride ($FeCL_3$) with ferrous sulfate ($FeSO_4$). Further, Ammonia ($NH_3$) is usually added as a precipitating agent. The advantages of this method are that it is simple, cheap, and convenient [143]. Thus, it enables rapid large-scale production [147]. Yet, the nanoparticles product morphology form aggregations. The particles are of a large size with poor crystallization and high oxidation capability. Affecting factors of the products when using this technique are the concentration of cations, the presence of counter ions, and the pH of the solution [148]. Additionally, using anionic surfactants as dispersing agents or coating agents like proteins or starches can stabilize the product particles [148].

### 1.3.5 SPIONs Enhancement: Surface Functionalization

SPIONs are not stable in the aqueous environment, so they would aggregate and precipitate [138]. Therefore, a coating is required to add stability to the nanoparticles in liquid [138]. The coating can be achieved in two main approaches either during the synthesis process, or post-synthesis coatings [140]. Also, depending on the type of application those nanoparticles will be used for, the coating type will differ to provide the most stable interactions. The coating of SPIONs is similar to the coatings used for enhancement of IONPs. For example, in an application for drug delivery, the IONPs need to be coated with different moieties, which can eliminate their aggregation in blood [140]. Polyethylene glycol PEG is one of the most used in coatings for IONPs, that can be implemented in SPIONs as well [137]. Because PEG has a high solubility, biocompatibility, stability, prolonged blood circulation time, and allows bioconjugation for modifications with various functional groups [140]. However, SPIONs PEG-coated has limited binding sites available for they have a small size which limits their conjugation surface [140]. Another example, Dextran coating is used in applications for MRI imaging using IONPs, which also can be carried out on SPIONs [140]. Because it stabilizes the magnetic nanocrystals by overcoming their weak ligand-particle interactions and their easy detachment; Since they provide a cross-link using hydrogen bonds in between the iron oxide and dextran-based, which are reversible [140]. The cross-linking changes the IONPs size and stabilize the product which helps in providing a sufficient signal for MRI imaging. Hence, MRI imaging using IONPs depends on the morphology of the IO crystals [140].

### 1.3.6 Targeting

Advantages of SPIONs makes them good candidates for drug delivery and targeting. Pharmacokinetic profile for SPIONs is important to evaluate their biotransformation in the body in ADME parameters (absorption, distribution, metabolism, and excretion) [141]. The profiling will give information on how those nanoparticles can be used in drug delivery and targeting. The targeting of drug-containing nanoparticles can be achieved by three major approaches which are either passive targeting, active targeting, or triggered drug targeting [138]. In passive targeting, depends on the utilization of permeability enhancement, which works indirectly in specifying the tumor site [137]. While, in active targeting, it depends on the targeting of overexpressed receptors on the cancer cell surface, therefore, it targets directly to the tumor site [137]. Triggered drug targeting in the case for SPIONs, where they are targeted to the tumors by using an external magnetization on the tumor site [140]. Since the drug-coated SPION magnetic ability will enable them to move toward tumor location [149]. Multiple new researches are being conducted to investigate and implement SPIONs magnetic targeting as a new advantageous therapy which would increase the specificity of the drugs and decrease the side effects of drugs on patients. Recently, a study has used SPIONs magnetic properties

in response to an external magnetic field for targeting to a specific site [150]. They have synthesized citric acid-capped SPIONs linked to the anticancer drug, doxorubicin, by noncovalent interactions [150]. They have observed an associated drug release and a significant cellular uptake after the magnetic targeting, with low cytotoxicity [150]. Further, releasing the drug at the specific location is dependent on the effect of internal or external stimuli like the concentration of the particles and the external magnetization effect, for the case of SPIONs [149]; Where the drug can be released by dissolution, diffusion, or vehicle rupture [140].

### 1.3.7 Magnetic Field-Induced Hyperthermia

We have stated that SPIONs have been recently involved in inducing thermal therapy that is generated by localized heat under a fluctuating magnetic field [141]. Hence, this feature makes SPIONs to be considered as therapeutic agents without the addition of functional moieties [151]. The increase in temperatures >42 °C changes many of the functional and structural proteins [151]. This procedure causes cellular necrosis. The application of an external magnetic field with a specific alternative frequency and current, depending on the SPIONs shape and size, will cause an increase in the kinetic energy of those nanoparticles [151]. Hence, they ultimately will heat up and increase the temperature of the surrounding region [151]. Moreover, based on multiple studies, it has been observed that temperatures were retained in normal tissue, whereas elevated temperatures and loss of the extracellular matrix (ECM) were observed in tumor tissue. Additionally, the loss of the ECM increased drug diffusion into tumor cells. Also, the magnetic hyperthermia effect can be used for triggering drug-release. This can be achieved by coating the drug with a thermal sensitive label [151]. Additionally, new cancer studies have found that cancer tissue is penetrated by mesenchymal stem cells (MSC) [141]. Thus, current research is investigating the possibility to combine MSC with SPIONs by endocytosis [141]. Therefore, the applied magnetics hyperthermia will be more specific since the MSC are placed in between tumor cells [141]. In summary, SPIONs have several therapeutic applications that can be developed for personalized combination therapies for CRC patients.

## *1.4 Combined HSP Targeting with Hyperthermia*

The approach for HSP targeting in combination with hyperthermia was elegantly described in the work of Ito et al. [152, 153]. Firstly, they developed in situ vaccination for tumor treatment. Tumor cells will be a target for immune system *via* release of Hsp70-tumor antigen complexes at the tumor site, and the recruitment of immune effector cells, including APC, to the tumor subsequently occurred as a consequence of the inflammation. Hyperthermia was selectively induced in the tumor cells by the mean of magnetite cationic liposomes (MCL). Based on their

results, they proposed that intracellular hyperthermia by means of MCL is an in situ vaccination therapy for cancer [152]. Moreover, they confirmed the results by Hsp70 gene therapy; human Hsp70 gene was introduced in the cells by cationic liposomes and hyperthermia was induced by exposing the MCLs to an alternating magnetic field for 30 min. The temperature at the tumor (melanoma) reached 43 °C and was maintained by controlling the magnetic field intensity. The combined treatment strongly arrested tumor growth over a 30-day period, and complete regression of tumors was observed in 30% of the treated mice [152]. Lately, Ito et al. targeted Hsp90 through geldanamycin concomitantly with thermosensitive ferromagnetic particles-mediated hyperthermia. Results showed HSP inhibitor exerted an antitumor effect by increasing the cells' susceptibility to hyperthermia in both *in vitro* and *in vivo* models [153]. Similarly, Vriend et al. [154] showed that treatment with a single, short course, with a relatively low dose of Hsp90 inhibitor (Ganetespib) potentiated the cytotoxic as well as radio- and chemosensitizing effects of hyperthermia and reduced the thermotolerance in cervix cancer cell lines. Moreover, it has been demonstrated that Hsp70 inhibition in combination with magnetic fluid hyperthermia generated a synergistic effect and could be a promising target to enhance magnetic fluid hyperthermia therapeutic outcomes in ovarian cancer [155]. In addition, the co-inhibition of Hsp70/Hsp90 with quercetin plus 17-DMAG significantly increased apoptosis in hyperthermia-treated cancer cell HNE1 both *in vitro* and *in vivo* as well as synergistically sensitized nasopharyngeal carcinoma cells to hyperthermia [48].

## 2 Conclusions

A number of challenges must be overcome before hyperthermia can be considered a standard treatment for cancer [3, 7]. Many clinical trials are being conducted to evaluate the effectiveness of hyperthermia in combination with other therapies for the treatment of different cancers. There are numerous challenges in creating the most effective SPION that is ideal for its intended application. Those obstacles include finding the suitable particle morphology, coating, and determining the best concentration with the lowest toxicity for the most effective therapy [156]. Moreover, the ability to target the nanoparticles to the tumor location with low specificity is difficult since the SPIONs depend on probe-based delivery for targeting or applying an external magnetic field. Likewise, problems in drug delivery includes: (1) the probability that modifications to conjugate the drug to the nanoparticle might change its properties, (2) drug distribution in the body, (3) releasing the drug to enzymatic digestive organelles, like endosomes and lysosomes, that causes drug digestion and decrease its effect, and (4) the probability of losing the magnetization of SPIONs when it undergoes a large number of coatings and a large number of chemical reaction [140]. Moreover, one of the recent challenges is found in the ability of the SPIONs to cross the blood-brain barrier (BBB) and target glioblastoma cells at the same time [157]. Also, oral administration of SPIONs, which is more preferred by

patients, was found to have a lower therapeutic efficiency compared to direct injection in the bloodstream [157]. Thus, challenges in drug administration need to be considered in new drug development [157]. In addition, research with HSP inhibitors together with SPIONs targeted different HSP such as Hsp90, Hsp70, and others would prevent resistance as well as potentiate the cytotoxic and/or antiproliferative effects of the hyperthermia.

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