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REVIEW

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Heat shock proteins as hallmarks of cancer: insights from molecular mechanisms to therapeutic strategies

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Abstract

Heat shock proteins are essential molecular chaperones that play crucial roles in stabilizing protein structures, facilitating the repair or degradation of damaged proteins, and maintaining proteostasis and cellular functions. Extensive research has demonstrated that heat shock proteins are highly expressed in cancers and closely associated with tumorigenesis and progression. The "Hallmarks of Cancer" are the core features of cancer biology that collectively define a series of functional characteristics acquired by cells as they transition from a normal state to a state of tumor growth, including sustained proliferative signaling, evasion of growth suppressors, resistance to cell death, enabled replicative immortality, the induction of angiogenesis, and the activation of invasion and metastasis. The pivotal roles of heat shock proteins in modulating the hallmarks of cancer through the activation or inhibition of various signaling pathways has been well documented. Therefore, this review provides an overview of the roles of heat shock proteins in vital biological processes from the perspective of the hallmarks of cancer and summarizes the small-molecule inhibitors that target heat shock proteins to regulate various cancer hallmarks. Moreover, we further discuss combination therapy strategies involving heat shock proteins and promising dual-target inhibitors to highlight the potential of targeting heat shock proteins for cancer treatment. In summary, this review highlights how targeting heat shock proteins could regulate the hallmarks of cancer, which will provide valuable information to better elucidate and understand the roles of heat shock proteins in oncology and the mechanisms of cancer occurrence and development and aid in the development of more efficacious and less toxic novel anticancer agents.

Keywords Cancer, Heat shock protein, Hallmarks of cancer, Target therapy, Combination strategy, Dual inhibitors

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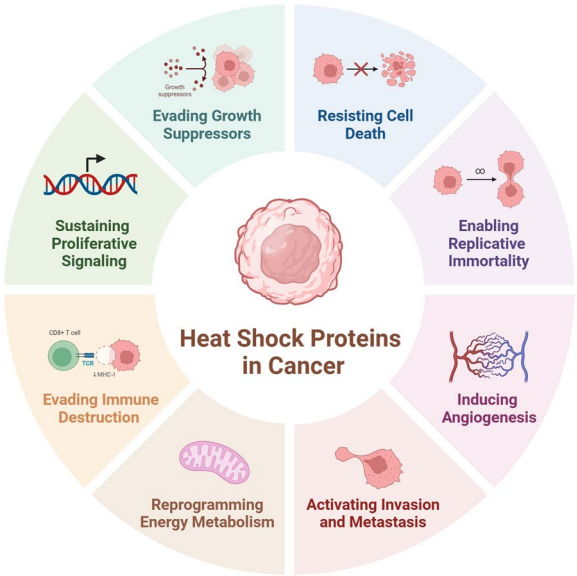
Graphic abstract



Background

Proteins are pivotal constituents within living organisms, embodying the structural and functional essence of biological entities. Their intricate three-dimensional configuration crucially influences both their structure and function. Notably, most natural proteins exhibit a folding pattern characterized by marginal stability [1, 2]. This characteristic endows proteins with the capacity to deviate structurally from their most stable state, facilitating the execution of their functions while ensuring standard protein functionality. However, the inherent drawback of marginal stability is the susceptibility of proteins to misfold. Such misfolding events can result in the impairment of normal protein function [3]. Furthermore, the accumulation of misfolded proteins has the potential to disrupt intracellular protein homeostatic networks, which gives rise to various diseases [4]. Hence, meticulous control of the protein folding process has emerged as a pivotal factor in maintaining proteome homeostasis, which thereby influences cellular and organismal health. In response to this imperative, organisms have evolved a spectrum of quality control systems. Notable among these systems are molecular chaperones [5–7], which facilitate the accurate folding of proteins, and the ubiquitin–proteasome system, which is responsible for degrading misfolded or aggregated proteins. Specifically, a molecular chaperone can be broadly defined as any protein that assists in the proper folding or assembly of other biomolecules, such as proteins, into their functionally active configurations without participating in the final structural composition [8–10].

Heat shock proteins (HSPs) are the most representative molecular chaperones [11–13]. In 1962, Ferruccio Ritossa was the first to document alterations in Drosophila salivary staining in response to heat, revealing changes in the expression of the gene encoding HSPs [14]. Twelve years later, Ursula M. Tracy et al. identified this HSP, which is overexpressed in *Drosophila melanogaster* in response to elevated temperature [15]. This family of proteins is among the oldest and most conserved in prokaryotic and eukaryotic cells, playing analogous roles across a wide range of organisms from bacteria to humans, and is characterized by a high degree of interspecies homology. Conditions of hyperthermia, hypoxia, inflammation, infection, oxidative stress, and nutrient deficiency induce the expression of HSPs [16]. HSPs respond to almost all circumstances that generate cellular stress by folding nascent peptides, unfolding and refolding misfolded proteins, assembling oligomers, degrading aberrant proteins, and transporting functional proteins [10, 12]. In summary, HSPs play a pivotal role in maintaining the stability of the intracellular environment while assisting the cell in executing specific physiological functions, such as growth, differentiation, and survival.

Cancer is a disease characterized by the breakdown of normal cell regulation and rampant cell proliferation [17]. This includes the growth of cancerous cells and the formation of nodules or polyps in solid tumors, endowing these cells with the ability to grow indefinitely, manipulate the local environment, invade nearby tissues, spread into the bloodstream, and eventually spawn a secondary tumor or metastatic clone [18, 19]. In Hanahan and Weinberg's seminal review, "Hallmarks of Cancer," several functional capabilities that human cells gain during their transition from a normal to a tumor-generating state were delineated (Fig. 1) [20]. While these functional characteristics continue to be refined [21, 22], the association of HSPs with many of them has been solidified by numerous studies. Indeed, tumor cells rely more heavily on HSPs for proliferation, differentiation, and survival than normal cells do because of the frequent misfolding of oncoproteins in cancer cells, necessitating increased chaperone activity for rectification. An expanding body of research indicates that different subtypes of HSPs exhibit trace variations in their distribution across the various stages of tumorigenesis, which is believed to correlate directly with their structural and biological roles [23–25].

In this review, HSPs are explored from the novel perspective of cancer hallmarks, diverging from the traditional approach of classifying HSPs on the basis of molecular weight reported in previous excellent reviews [24, 26–31]. Through a concise summary of the mechanisms of action of candidate compounds targeting HSPs,

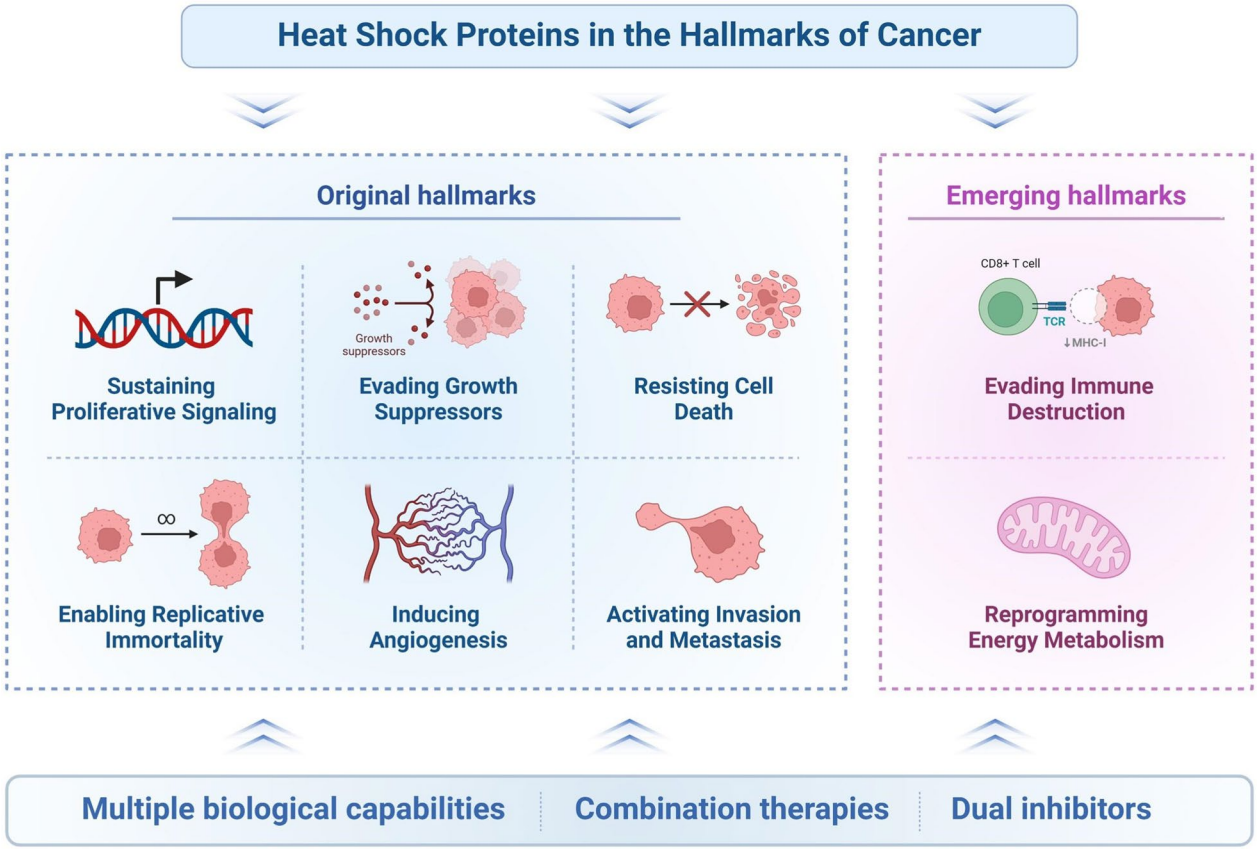


Fig. 1 HSPs have been implicated in the hallmarks of cancer and exploited for developing targeted therapeutic strategies. In 2000, Hanahan and Weinberg delineated six hallmarks of cancer, including sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis, which are described as the acquired capabilities that allow cancer cells to form malignant tumors (depicted in the figure as the "original hallmarks"). Subsequently, they introduced two emerging hallmarks in 2011 and 2022, respectively, which include reprogramming energy metabolism and evading immune destruction, as well as the acquisition of unlocking phenotypic plasticity and senescence. Among these, the roles of unlocking phenotypic plasticity and senescence in cancer are still under validation and thus not represented here. HSPs have been demonstrated to play a significant role in modulating original and emerging hallmarks

we analysed the regulatory mechanisms and subtype distribution differences of HSPs in critical stages of cancer development. Additionally, we discuss advancements in therapeutic strategies, including combination therapies and dual-target drugs. Our aim is to inspire a broader scientific community to engage in HSP research and the development of HSP-targeted therapeutics, thereby enhancing our understanding of the roles of HSPs in oncogenesis and facilitating the emergence of more precise and efficacious cancer treatment modalities.

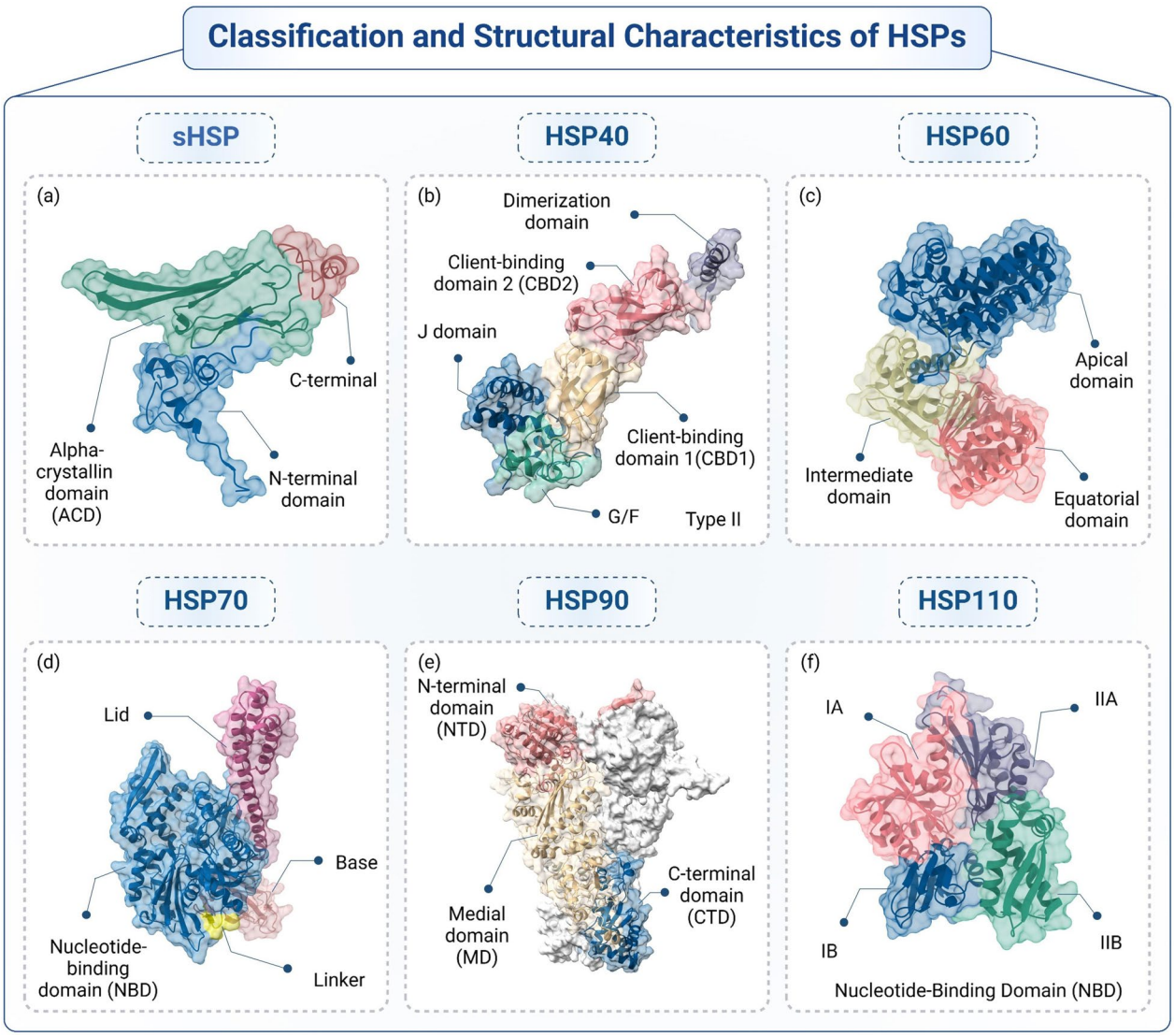


Fig. 2 Classification and structural characteristics of HSPs. **a** Structure of sHSPs (PDB ID: 2YGD) is exemplified by the monomeric structure of the human 24-meric eye lens chaperone alphaB-crystallin, which includes the C-terminal extension (red), the α-crystallin domain (green), and the N-terminal domain (NTD) (blue). **b** Structure of HSP40 illustrated by the Thermus thermophilus type B Hsp40 (PDB ID: 6PSI), comprising the J-domain (blue), the G/F-rich region (green), the C-terminal binding domain (yellow and red), and the dimerization domain (purple). **c** Structure of HSP60 (PDB ID: 7AZP) illustrated by the monomeric structure of the human mitochondrial HSPD1, consisting of the apical domain (blue), the intermediate domain (yellow), and the equatorial domain (red). **d** Structure of HSP70 (PDB ID: 4B9Q) is composed of the N-terminal nucleotide-binding domain (NBD) (blue), the C-terminal substrate-binding domain (SBD) (red), and a linker (yellow). The SBD is subdivided into a β-sheet-rich base and an α-helix-rich lid. **e** Structure of the HSP90 dimer (PDB ID: 5FWK) primarily comprises the NTD (red), the middle domain (yellow), and the C-terminal domain (CTD) (blue). **f** Structure of HSP110 (PDB ID: 6GFA) mainly consists of the NBD and an incompletely characterized SBD, with the NBD containing four subdomains: IA (red), IB (blue), IIA (purple), and IIB (green)

Classification and structural characteristics of HSPs

Researchers have classified HSPs into several families on the basis of their molecular weight: small HSPs, HSP40, HSP60, HSP70, HSP90, and large HSPs (Fig. 2). The naming convention for human HSPs adheres to the guidelines established by the Gene Nomenclature Committee of the Human Genome Organization and utilizes the Entrez gene database provided by the National Center for Biotechnology Information for their identification [32].

Small heat shock proteins (sHSPs) are a class of molecular chaperones that function by binding to unfolded substrate proteins without a dependence on ATP. The human genome encodes ten varieties of sHSPs, known as HSPB1-HSPB10. A defining characteristic of this molecular chaperone family is that it contains an alpha-crystallin domain (ACD) comprising a sequence of 80 amino acid residues [33–35] (Fig. 2a). Flanking the ACD is the less conserved N-terminal domain (NTD) and a variable C-terminal extension, which play crucial roles in oligomerization. Within the human body, sHSPs exhibit a notable degree of structural variability, ranging from dimers (HSPB6, HSPB7, and HSPB8) and heterotetramers with a defined subunit ratio (HSPB2/B3) to polydisperse coassembling oligomeric structures (e.g., HSPB1, HSPB4, and HSPB5). The exchange of subunits among these complexes is significantly accelerated by heat or other environmental stresses, facilitating interactions with nonnative intracellular proteins.

The HSP40 family constitutes the largest and most diverse subgroup within the heat shock protein family. On the basis of the presence or absence of conserved domains as defined by typical *Escherichia coli*, the HSP40 family is divided into three subclasses: DNAJA (Type I), DNAJB (Type II), and DNAJC (Type III) [36, 37]. In terms of their structure, Type I DNAJ proteins consist of an N-terminal J domain, a glycine/phenylalanine (G/F)-rich region, a cysteine-repeat (Cys-repeat) region, and a C-terminal region. Type II lacks the Cys-repeat region but features an expanded G/F-rich region (Fig. 2b). Type III DNAJ proteins differ significantly from Types I and II, as they lack both G/F and Cys-repeat regions and the placement of the J domain is irregular, as it is potentially located anywhere within the protein. The presence of the highly conserved J domain, which is composed of four α-helices that interact to provide the correct orientation for the J domain, which facilitates the cochaperone function by stimulating the HSP70 ATPase domain, is considered a defining feature of all DNAJ proteins.

HSP60 is among the most ancient and evolutionarily conserved members of the chaperone system. Structurally, HSP60 adopts a ring-shaped structure formed by two heptameric rings, with each monomer comprising three domains: equatorial, intermediate, and apical (Fig. 2c) [38–40]. The equatorial domain, which contains the ATP binding sites and forms a tight interface with adjacent monomers, is highly conserved to maintain the stability of the HSP60 ring structure. The intermediate domain bridges the top and bottom of the equatorial domain and folds into a β-sheet structure that creates a central enclosed chamber. This chamber provides an isolated environment to facilitate interactions between nonnative proteins and the HSP60 monomers. Positioned at the top of the intermediate domain, the apical domain acts like a lid, regulating the entry of substrate proteins into the chamber. Without ATP, HSP60 remains in a stable conformation as a single-ring heptamer. The binding of ATP to the equatorial domain triggers the formation of a double-ring structure, which, in conjunction with the top domain, resembles a soccer ball-like complex. Then, ATP hydrolysis induces conformational changes in the top domain, driving the folding and release of the bound protein.

HSP70 is among the most highly conserved proteins across all of biology [41]. In humans, 13 HSP70 homologues are expressed in various cellular compartments (the cytoplasm, nucleus, endoplasmic reticulum, and mitochondria), with the levels of each isoform being regulated according to cellular demands, such as growth and tissue-specific activities [42]. Structurally, HSP70 comprises two domains: a nucleotide-binding domain (NBD, approximately 35 kDa) and a C-terminal substrate-binding domain (SBD, approximately 25 kDa), connected by a flexible, disordered linker (Fig. 2d) [43]. The NBD consists of four subdomains (IA, IB, IIA, and IIB) arranged in pairs, which specifically bind to and hydrolyse ATP. The SBD is divided into a β-sheet-rich base and an α-helix-rich lid, allowing direct interactions with nonnative intracellular proteins. Notably, the disordered linker connecting the NBD and SBD is equally crucial, as it is responsible for transmitting allosteric signals and serving as a structural foundation for the functionality of Hsp70.

HSP90, a highly conserved heat shock protein, is universally expressed across all species except archaea. The human genome harbors six HSP90-encoding genes. These genes are active in various cellular compartments: inducible HSP90AA and constitutively expressed HSP90AB in the cytoplasm, GRP94 in the endoplasmic reticulum (ER), and tumor necrosis factor receptor-associated protein 1 (TRAP1) in the mitochondria [44–46]. Functionally, HSP90 predominantly exists as an ATP-dependent dimer oriented head-to-head, and its monomers are structured into three domains: the NTD, the medial domain (MD), and the C-terminal domain (CTD) (Fig. 2e) [47–49]. The NTD can bind ATP and cochaperone proteins (Hsp70, Hsp40, Hip, and Hop); the MD serves as a protease-resistant site for client protein

engagement, cochaperone interaction, and binding of the γ-phosphate of ATP; and the C-terminus features a tetramerization domain crucial for HSP90 oligomerization. Furthermore, ATP binding and hydrolysis trigger HSP90 to transition from an open to a closed conformation, a vital process for maintaining intracellular protein stability and regulating signal transduction pathways, among other biological functions.

HSP110 represents a unique category of molecular chaperones that can function independently or as a cochaperone for HSP70 [50]. HSP110 is uniquely effective in preventing the aggregation of denatured proteins, an activity called holding enzyme activity. Unlike HSP70, which shares distant homology, HSP110 lacks the characteristic ability of HSPs to assist in protein folding directly. Instead, HSP110 plays a crucial role as the primary nucleotide exchange factor (NEF) for cytoplasmic HSP70, enhancing the exchange of ADP for ATP within HSP70. Research has demonstrated the involvement of HSP110 in virtually all processes associated with cytoplasmic HSP70, including the de novo folding and refolding of proteins under stress, the dissolution of protein aggregates, and protein degradation. The unique chaperone activity of HSP110 stems from its specific biochemical and structural properties, highlighted by two identified functional domains: the NBD and the SBD (Fig. 2f) [51, 52]. The NBD, which can bind ATP, includes four subdomains (IA, IB, IIA, and IIB), with the ATP binding site located in a deep cleft at the interface of these subdomains, plays a vital role as a NEF and in enzymatic activity maintenance. In contrast, the SBD is comparatively less extensive, and its substrate binding sites and characteristics remain unknown.

In summary, although various subfamilies of HSPs perform distinct functions influenced by their structural characteristics, they share common sequence elements known as α-crystal domains. This α-crystal structure plays a crucial role in stabilizing the protein conformation through hydrogen bonds and ionic interactions, which are pivotal for the molecular chaperone activities of HSPs. These proteins play diverse enzymatic roles—including retention, folding, multivalent chelation, aggregation, and depolymerization—forming a comprehensive network that maintains cell protein homeostasis. These processes, in turn, are integral to the entire tumorigenesis process.

Targeting HSPs with inhibitors regulates acquired biological capabilities in *cancer*

In the field of oncology, HSPs have emerged as compelling therapeutic targets because of their multifaceted roles in regulating the hallmarks of cancer. This chapter delves into the intricate relationships between HSPs and these cancer characteristics, presenting both the research progress and mechanisms of action of HSP inhibitors. For clarity, this chapter is organized around each specific hallmark of cancer to elucidate the distinct interplay between each hallmark and various HSPs while reviewing advancements in HSP inhibitor research. Importantly, while certain HSPs might mechanistically connect with multiple cancer hallmarks, existing inhibitors may address only a subset of these interactions. Conversely, some HSP inhibitors exhibit activity across multiple hallmarks. This complexity underscores the dynamic nature of HSPs and the intricate process of tumorigenesis, offering a rich area for continued scientific exploration. By focusing on the mechanistic network between HSPs and the hallmarks of cancer, we aim to deepen the understanding and propel the development of novel HSP-targeted anticancer therapies, including potential combination strategies and dual-targeting inhibitors, which will be explored in subsequent chapters.

Sustaining proliferative signaling

Cell proliferation occurs when the cells in normal tissues generate new cells via division triggered by growth signals. This process replenishes ageing or dead cells, maintaining the normal structure and physiological functions of the tissue. The process is intricate and involves multiple pathways and proteins (Fig. 3). Specifically, the binding of growth factors, such as epidermal growth factor (EGF), to cell surface receptors, primarily receptor tyrosine kinases (RTKs), is pivotal [53, 54]. This binding event initiates a cascade that regulates the cell growth cycle and enables proliferation by activating downstream signaling pathways. One critical pathway, the mitogen-activated protein kinase (MAPK) signaling pathway, consists of three distinct cascades: extracellular signal-regulated kinase 1/2 (ERK1/2), c-Jun N-terminal protein kinase 1/2 (JNK1/2), and p38 MAPK [55]. Activated RTKs attract growth factor receptor-bound protein 2 (Grb2) upon ligand binding, facilitating its binding to SOS, which converts Ras-GDP to Ras-GTP, activating Ras [56]. Within the Ras pathway, Raf activation is a key step that promotes downstream MEK and ERK1/2 phosphorylation. Activated ERK then enters the nucleus to initiate transcription. The phosphatidylinositol 3-kinase (PI3K) pathway is another important pathway related to proliferation that is primarily activated by RTKs and G protein-coupled receptors (GPCRs) [57]. Activation signals lead to the recruitment of the p85 regulatory subunit of PI3K to the plasma membrane, which then activates the p110 catalytic subunit. This subunit catalyzes the conversion of PIP2 to PIP3, a second messenger. Activated PIP3 collaborates with pyruvate dehydrogenase kinase 1 (PDK1) and pyruvate dehydrogenase kinase 2 (PDK2) to activate

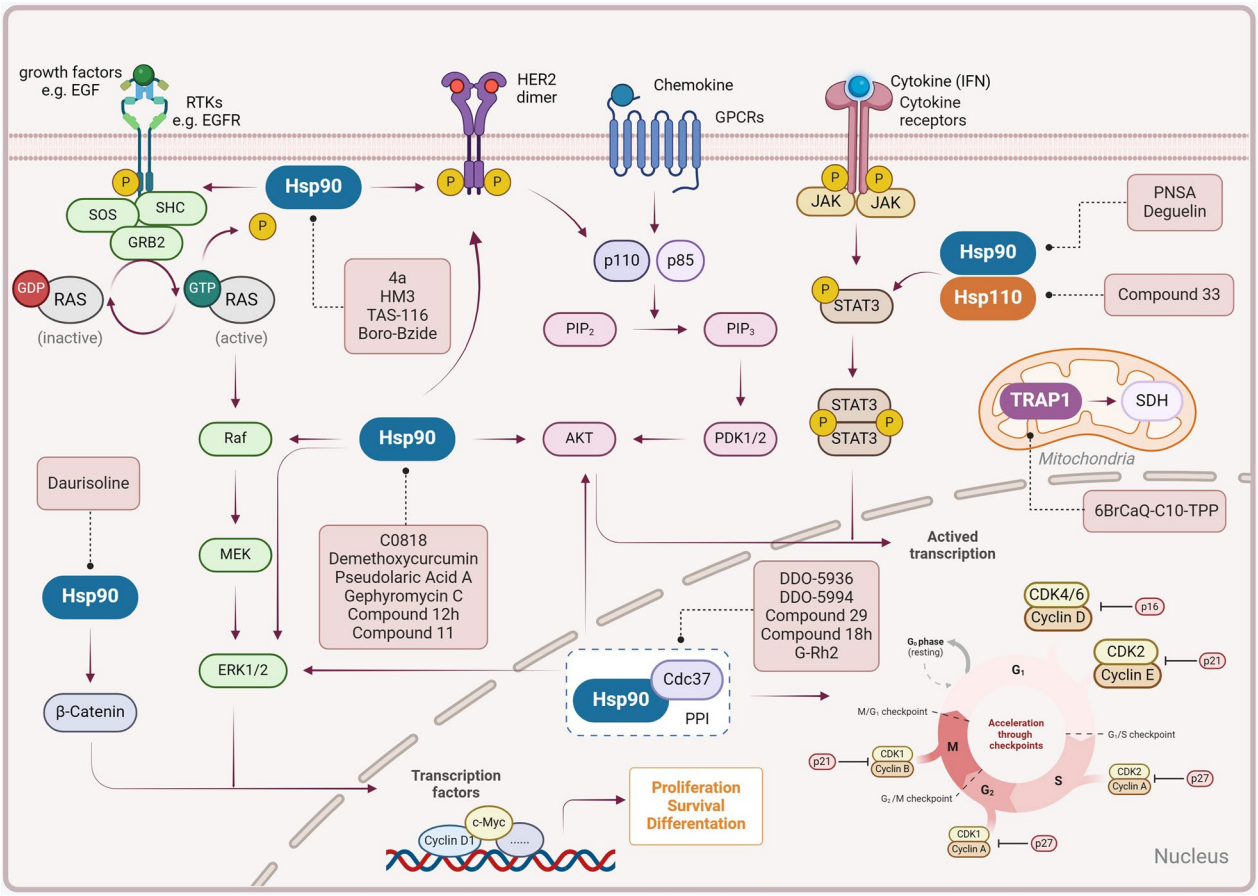


Fig. 3 Diagram of HSPs in the core proliferative signaling pathway in cancer. Upon ligand binding, receptor tyrosine kinases (RTKs) activate Ras through the adaptor proteins Grb2 and SOS, facilitating the transition of Ras from its GDP-bound to GTP-bound state. Subsequently, Raf kinase is activated, leading to the activation of MEK and ERK1/2. Ultimately, ERK translocates to the nucleus to activate transcription factors, promoting cell proliferation. The phosphatidylinositol 3-kinase (PI3K) pathway also plays a critical role in cell proliferation and is activated by signals from RTKs and G-protein coupled receptors (GPCRs), etc. Activation of PI3K results in the conversion of PIP2 to PIP3, which, in turn, activates AKT, leading to transcriptional activation and cell proliferation. Additionally, signaling pathways such as JAK/STAT and Wnt/β-Catenin activate transcription, promoting tumor growth. Cyclin-dependent kinases (CDKs) form Cyclin-CDK complexes with cyclins to regulate the cell cycle. HSP90 promotes tumor cell proliferation by stabilizing and functionally regulating various client proteins, including RAF, AKT, HER2, STAT3, etc. HSP90 also interacts with Cdc37 to co-regulate the function of multiple proteins. Moreover, HSP110 is involved in the transduction of proliferation signals in tumor cells by promoting the phosphorylation of STAT3

protein kinase B (AKT), influencing downstream targets such as NF-κB, GSK-3β, $P27^{kip1}$, and mammalian target of rapamycin (mTOR) to trigger biological responses. The PI3K pathway is not isolated but is highly crosslinked with the RAS-RAF-MEK-ERK pathway, with activated RAS directly recognizing the p110 subunit and activating PI3K [58]. Furthermore, cyclin-dependent kinases (CDKs) pair with cyclins to form Cyclin-CDK complexes that are responsible for regulating the cell cycle [59]. Signaling pathways such as Janus kinase 1 (JAK1)/signal transducer and activator of transcription 3 (STAT3) and Wnt/β-Catenin also enhance cell proliferation, ensuring its orderly progression [60, 61]. Cancer cells bypass these precise signaling mechanisms, gaining control over growth signals. This autonomy is considered the starting point of the hallmarks of cancer, which allows unchecked proliferation. Cancer cells may obtain growth factors through autocrine or paracrine mechanisms, creating a positive feedback loop for continuous growth signal release [62–64]. High levels of expressed receptor proteins increase the efficiency of these growth signals, which makes these proteins highly sensitive to limited growth signals [65, 66]. Current studies indicate that the aberrant activation of intracellular signal transduction might be the most complex mechanism involved. Some downstream signals are aberrantly expressed in cancer cells, which leads to the sustained activation of proliferative signal cascades independent of growth factors. For

example, mutant RAS can self-activate, initiating the sustained activation of downstream signaling cascades and thereby promoting transcription and proliferation [67].

Studies have demonstrated that the ability tumor cells to acquire self-sufficiency in growth signals is significantly associated with HSPs. Many proteins involved in the proliferation signaling pathway, such as epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), and their downstream components RAS, RAF, ERK, and AKT, etc., are HSP90 client proteins [68–72]. These proteins rely on HSP90 to maintain their physiological functions. Furthermore, the binding of HSP90 to the cell division cycle protein Cdc37 is crucial for the recruitment of multiple protein kinases to HSP90, facilitating the proper folding of their spatial configurations [73]. Several studies have revealed that targeting HSPs pharmacologically with small-molecule inhibitors presents a promising approach to cancer treatment (Table 1). This strategy leads to the degradation of client proteins, thereby inhibiting tumor growth. Curcumin is a naturally occurring small molecule extracted from the medicinal plant turmeric with anti-tumor property [74]. In the current study, Xu et al. designed and synthesized a novel curcumin derivative named C0818, demonstrating its potential as a therapeutic agent against liver cancer by targeting HSP90 C-terminal. Specifically, C0818 effectively inhibited the proliferation of hepatocellular carcinoma (HCC) cell lines HepG2 and Sk-Hep1. The protein levels of RAS, PI3K, and downstream signals RAF, MEK, ERK, mTOR, and AKT were decreased after C0818 treatment, whereas the proteasome inhibitors inhibited the degradation of these proteins. Further insights revealed that the reduction in RAF and AKT levels, following the suppression of HSP90 via siRNA, was not observed with C0818 treatment. This result suggested that C0818 targeted HSP90 directly and inhibited PI3K/AKT and RAS/RAF/MEK/ERK signal pathways in a proteasome-dependent manner, thus exhibiting antiproliferative effects. Detection of the expression of cell cycle-related proteins showed decreased levels of Cdc2, Cyclin B1, Cdc25c, and up-regulated levels of p21, revealing that C0818 induces cell arrest in the G2/M phase [75]. Demethoxycurcumin (DMC), another ingredient derived from turmeric, has also been reported to have anticancer effects. DMC down-regulated HER2 levels by disrupting the binding of HSP90 to HER2 and ultimately exhibited therapeutic activity against HER2-overexpressing bladder cancer cells RT4. Concurrently, DMC also reduced the levels of other HSP90 client proteins, including Raf-1, AKT, and CDK4. Significantly, combining DMC with chemotherapeutic agents, including paclitaxel and cisplatin, has been shown to improve the effectiveness of chemotherapy. This result revealed the potential of DMC as a basis for further developing targeted drugs explicitly aimed at combating HER2 overexpression [76]. Pseudolaric Acid A (PAA), a naturally occurring compound, has been identified as a novel HSP90 N-terminal inhibitor. It binds to the N-terminus of HSP90, inhibiting its chaperone function. Following treatment with PAA, a significant decrease in Hela cells in the G0-G1 phase and an increase in the G2/M phase were observed. Additionally, levels of CDK4 and AKT, which play roles in cell cycle regulation, decreased. These results indicated that PAA modulated the levels of the client proteins by inhibiting HSP90, thereby inducing cell cycle arrest in the G2/M phase and demonstrating antiproliferative activity [77]. Gephyromycin C (GC), a small molecule naturally derived from marine organisms, has also been found to act as an N-terminal inhibitor of HSP90, demonstrating significant activity against prostate cancer. By inducing cell cycle arrest in the G2/M phase, GC effectively inhibits the proliferation of PC3 cancer cells. Additionally, GC's intervention leads to the down-regulation of various HSP90 client proteins, such as AKT, checkpoint kinase 1 (CHK1), P53, CDK4, and Raf-1, suggesting that GC's anticancer effects primarily driven by its ability to inhibit HSP90 and highlighting its potential as a targeted cancer therapy [78].

He et al. recently obtained Daurisoline, an active compound from *Rhizoma Menispermi*, via high-throughput screening and conducted its biological evaluation. The experimental findings indicated that Daurisoline potentially disrupts the interaction between HSP90 and β-catenin by targeting HSP90. This action inhibits the β-catenin signaling pathway and interferes with the transcription of its downstream genes, including c-Myc and cyclin D1. Consequently, this leads to cell cycle arrest in the G1 phase and suppresses the proliferation of lung cancer cells. Furthermore, Daurisoline has demonstrated remarkable therapeutic efficacy in vitro and in vivo while maintaining a favorable safety profile. In xenograft lung cancer models, it did not significantly alter serum indicators such as alanine aminotransferase and aspartate aminotransferase, nor did it cause noticeable harm to the liver and kidneys. These findings underscore Daurisoline's promise as a viable anticancer drug candidate for additional exploration [79]. Designing and synthesizing promising small molecule inhibitors targeting HSPs based on pharmacophore modeling and other screening methods represents a significant approach to drug development [80]. Pyrimidine derivative 7t is an HSP90 N-terminal inhibitor based on scaffold hopping and is considered a promising anticancer molecule. Work has shown that 7t possesses potent antiproliferative effects in vitro across multiple cell lines, including the breast cancer cell lines MCF-7 and MDA-MB-231 and the

Table 1 Summary of HSP inhibitors regulating proliferative signals in cancer

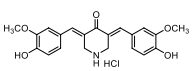
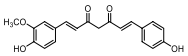
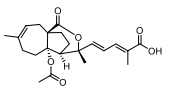
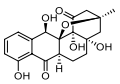
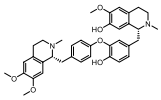
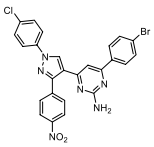
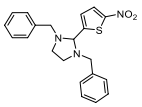
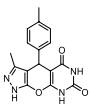
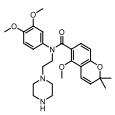
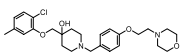
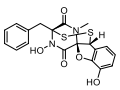
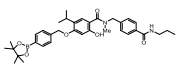
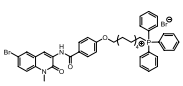
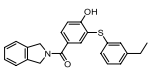
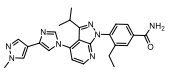
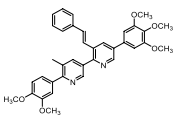
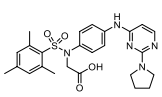
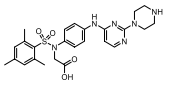
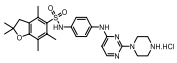
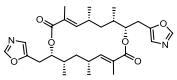
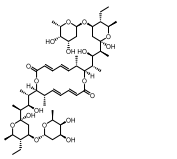
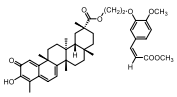
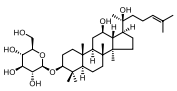
Name	Structure	Target	Hallmarks of cancer	Cancer cell line	Tumor type	Ref
C0818		HSP90	Inhibit proliferation	HepG2 (IC_{50} = 2.1 μM) Sk-Hep1 (IC_{50} = 1.9 μM)	Hepatocellular carcinoma	[75]
Demethoxycμrcumin		HSP90	Inhibit proliferation	RT4 (IC_{50} = 10.3 μM)	Bladder cancer	[76]
Pseμdolaric acid A (PAA)		HSP90	Inhibit proliferation	Hela (IC_{50} = 2.92 ± 0.09 μM)	Human cervical carcinoma	[77]
Gephyromycin C (GC)		HSP90	Inhibit proliferation	PC3 (IC_{50} = 1.79 ± 0.28 μM)	Prostate cancer	[78]
Daμrisoline		HSP90	Inhibit proliferation	A549 (10 μM) Hop62 (10 μM)	Lung cancer	[79]
Compound 7t		HSP90	Inhibit proliferation	MCF-7 (IC_{50} = 2.4 ± 0.1 μM), MDA-MB-231 (IC_{50} = 0.8 ± 0.4 μM), HCT116 (IC_{50} = 4.8 ± 0.5 μM)	Breast cancer Colon cancer	[81]
Compound 4a		HSP90	Inhibit proliferation	MCF-7 (IC_{50} = 21.58 ± 0.57 μM), A549 (IC_{50} = 31.22 ± 0.31 μM)	Breast cancer Lung cancer	[82]
HM3		HSP90	Inhibit proliferation	MCF-7 (IC_{50} = 1.28 ± 0.005 μM)	Breast cancer	[83]
Compound 57		HSP90	Inhibit proliferation	MDA-MB-231 (10 μM), 4T1 (10 μM)	Breast cancer	[88]
Compound 11		HSP90	Inhibit proliferation	Hep G2 (IC_{50} = 27.9 ± 0.7 μM), MCF-7 (IC_{50} = 44.8 ± 3.6 μM)	Liver cancer, Breast cancer	[89]
PNSA		HSP90	Inhibit proliferation	HCT-116 (0.25 μM)	Colorectal cancer	[90]
Boro-BZide (3)		HSP90	Inhibit proliferation	MCF-7 (GI_{50} = 7.63 μM)	Breast cancer	[93]
6BrCaQ-C10-TPP		TRAP1	Inhibit proliferation	MDA-MB-231 (IC_{50} = 0.008 μM)	Breast cancer	[94]
Compound 12 h		HSP90α	Inhibit proliferation	NCI-H522 (> 10 μM)	Lung cancer	[95]
TAS-116		HSP90α/β	Inhibit proliferation	SK-BR-3 (IC_{50} = 0.33 μM)	Breast cancer	[96]
Compound 33		HSP110	Inhibit proliferation	SW48 (10 μM)	Colorectal cancer	[52]
DDO-5936		HSP90	Inhibit proliferation	HCT-116 (IC_{50} = 8.99 ± 1.21 μM)	Colon cancer	[99]

Table 1 (continued)

Name	Structure	Target	Hallmarks of cancer	Cancer cell line	Tumor type	Ref
Compound 18 h		HSP90	Inhibit proliferation	HCT-116 (IC_{50} = 1.73 μM)	Colon cancer	[100]
DDO-5994		HSP90	Inhibit proliferation	HCT-116 (IC_{50} = 6.34 μM)	Colon cancer	[101]
Conglobatin A		HSP90	Inhibit proliferation	MDA-MB-231 (IC50 = 32 ± 2 μM)	Breast cancer	[102]
Elaiophylins		HSP90	Inhibit proliferation	MDA-MB-231 (IC_{50} = 0.37 μM), MIA PaCa-2 (IC_{50} = 0.26 μM)	Breast cancer Pancreatic cancer	[103]
Compound 29		HSP90	Inhibit proliferation	A549 (IC_{50} = 0.15 ± 0.03 μM)	Lung cancer	[105]
(20S) Ginsenoside Rh2		HSP90	Inhibit proliferation	HepG2 (IC_{50} = 5.02 μM)	Liver cancer	[106]

colon cancer cell line HCT116. 7t could directly bind to and exhibit significant inhibition of HSP90, inducing degradation of the HSP90 client proteins pHER2 and pERK1/2, thereby affecting the conduction of the signal pathways in which these proteins reside [81]. Another study by Wang et al. reported the anticancer potential of compound 4a containing the 1,3-dibenzyl-2-aryl imidazolidine fragment as an HSP90 N-terminal inhibitor. 4a down-regulated the expression of its client protein HER2 by binding to the ATP site at the N-terminal of HSP90, showing the most potent antiproliferative effect against cell lines MCF-7 and A549 with IC_{50} values of 21.58 μM and 31.22 μM, respectively [82]. Based on exploring the practical structure of inhibiting HSP90, the compound HM3 derived from pyrazolopyranopyrimidine is also considered an inhibitor of HSP90 N-terminal for further exploration. Preliminary activity screening showed that HM3 exhibited a significant antiproliferative effect on MCF-7 with the downregulation of the expression of HER2 by HM3 intervention [83].

Numerous small-molecule inhibitors targeting HSP90, primarily the NTD, have been developed, and some have entered clinical trials [84]. However, as research has evolved, it has become imperative to address challenges associated with the use of HSP90 N-terminal inhibitors, including the induction of pro-survival heat shock responses (HSR) and potential toxicity [85–87]. Consequently, this has led some researchers to pivot their focus toward investigating inhibitors that target the HSP90 C-terminal domain. Deguelin, a naturally occurring flavonoid, acts as a known HSP90 C-terminal inhibitor. Lee et al. crafted their design around this structure to discover an improved C-terminal inhibitor for HSP90. Compound 57 showed significant anti-tumor activity against breast cancer cell lines MDA-MB-231 and 4T1. This efficacy likely stemmed from compound 57's ability to bind to the ATP-binding pocket at the HSP90 C-terminal, inhibiting the phosphorylation levels of its client proteins AKT, STAT3, and MEK [88]. Bryant et al. identified compound 11 as a potentially promising C-terminal inhibitor of HSP90 by virtual screening based on the structures of known HSP90 inhibitors derived from neomycin. Biological evaluations revealed that compound 11 effectively inhibited the proliferation of MCF-7 cells. Further investigation into the mechanism demonstrated that treatment with compound 11 led to the degradation of multiple HSP90 client proteins, including HER2, ERα, and Raf1, in MCF-7 cells [89]. Moreover, PNSA (penisuloxazin A) was identified to target the CTD of HSP90 and inhibited the growth of HCT116 cells both in vitro and in vivo. PNSA could bind to the cysteine residues C572/C598 of HSP90 via disulfide bonds, thereby inhibiting the phosphorylation levels of its client proteins EGFR, STAT3, AKT, and ERK and inducing proteasomal degradation of these proteins while down-regulating the expression of Raf-1. Importantly, PNSA demonstrated advantages as a C-terminal inhibitor of HSP90 without triggering HSR and not inducing HSP70 expression [90].

Similar studies have focused on the problem of pan-inhibition with conventional HSP90 inhibitors, and highly selective targeting of the HSP90 subtype can reduce the possibility of adverse reactions due to over-inhibition [91, 92]. A particular study highlighted an HSP90 inhibitor, Boro-BZide (3), which achieved its antiproliferative effect on MCF-7 cells by targeting those cancer cells that exhibit higher levels of reactive oxygen species (ROS) compared to normal cells, following its conversion to BZide with an affinity for the N-terminal of HSP90α. Delightfully, post-treatment with Boro-BZide (3), the levels of HER2, EGFR, and MET decreased in MCF-7 cells, an outcome not seen in normal cells. Furthermore, Boro-BZide (3) displayed significantly less toxicity to normal breast cells than to cancer cells, in contrast to BZide [93]. Also based on structural modifications to improve selective targeting of small molecules, Vergnaud–Gauduchon's group designed and synthesized small molecule inhibitors capable of targeting TRAP1 in mitochondria based on the structure of 6-BrCaQ, a discovered HSP90 C-terminal inhibitor with anticancer activity, in which triphenylphosphonium modified 6BrCaQ-C10-TPP exhibited the antiproliferative activity in a diverse set of human cancer cells and did not induce HSR. Experiments have shown that 6BrCaQ-C10-TPP could inhibit the function of TRAP1, which was reflected in the down-regulation of the expression of its client protein succinate dehydrogenase [94]. Blagg et al. found that the compound 12 h obtained based on resorcinol derivatives showed a much higher affinity for HSP90α than HSP90β ($IC_{50} = 0.46 \pm 0.05$ μM, $IC_{50} = 22.28 \pm 2.8$ μM, respectively). In the 12 h-treated cell line, NCI-H522, the levels of HER2, AKT, and Raf-1 all showed a decreasing trend, implying that the inhibition of proliferation of NCI-H522 by 12 h might be related to the reduction of these HSP90 client proteins [95]. A study developed TAS-116, an oral selective inhibitor of HSP90α/β, based on a lead compound containing a benzamide structure. The inhibition of HSP90 by TAS-116 resulted in decreased expression of EGFR and inhibited phosphorylation of AKT and MAPK1/3, crucial signals associated with the proliferation of cancer cells. Furthermore, when TAS-116 was administered in the NCI-H1975 mouse xenograft model, it effectively controlled tumor volume without significant changes in body weight, underscoring its safety for oral use [96].

In addition to HSP90, studies have revealed that HSP110 is linked to prognostic markers for colorectal cancer. Garrido et al. analyzed the structure of HSP110 to screen for small molecule inhibitors matching it. They discovered that Compound 33 could bind to the NBD of HSP110, effectively blocking HSP110's ability to phosphorylate STAT3 and thus inhibiting colorectal cancer cell proliferation. Notably, compound 33 showed antiproliferative effects on SW480 and HCT116 cells in vitro, and it maintained these effects in vivo in mouse models without significant toxicity. This study provides a positive insight into using HSP110 inhibitors in treating colorectal cancer [52].

In addition to developing small-molecule inhibitors that directly target HSPs to interfere with pathways that favor tumor proliferation signals, researchers have also set their sights on the protein–protein interactions (PPIs) between HSPs and their co-chaperone proteins [73, 97]. Research is increasingly focusing on inhibitors of the PPIs between HSP90 and Cdc37. Cdc37 plays a crucial role by recruiting multiple kinases, enabling HSP90 to support various cellular processes [48, 98]. You and co-workers reported DDO-5936, an HSP90-Cdc37 PPI inhibitor that could bind to the E47 residue on HSP90, a critical site for its interaction with Cdc37, effectively blocking their PPI without affecting ATPase activity. Mechanistic investigations have revealed that DDO-5936 could reduce AKT and ERK1/2 phosphorylation by disrupting the HSP90-Cdc37 PPI, which was accompanied by reduced levels of CDK2/4/6, cyclin D1, and cyclin D3 alongside an increase in p21 levels, and ultimately induced Cdc37-dependent cell cycle arrest and inhibited cell proliferation in HCT-116 cells. Moreover, animal models treated with DDO-5936 exhibited no significant impairment in the function of multiple organs, further supporting the treatment's safety profile [99]. The following year, they carried out further structural optimization based on DDO-5936 and obtained the HSP90-Cdc37 PPI inhibitor 18 h with higher affinity and improved activity. 18 h selectively inhibited the expression of HSP90's kinase client proteins, CDK4 and CDK6, along with their downstream targets, cyclin D1 and cyclin D3. Additionally, 18 h has shown a reliable safety profile in in vivo bioassessments and offers the significant benefit of oral administration [100]. Based on previous studies, yet another HSP90-Cdc37 PPI inhibitor, DDO-5994, was developed. This inhibitor could bind to the previously unidentified critical hydrophobic pocket, Phe213, thus enhancing the inhibitor's affinity for the HSP90-Cdc37 PPI. In a similar vein, DDO-5994 downregulated the kinase client proteins CDK4 and CDK6, leading to cell-cycle arrest in the G0/G1 phase in HCT-116 cells [101]. Conglobatin A shows promising anticancer promise as an inhibitor of the HSP90-Cdc37 PPI. They found that Conglobatin A depleted the HSP90 client protein hypoxia-inducible factor 1α (HIF-1α) and its downstream galectin-3 (Gal3) by interfering with PPI because Gal3 is critical for stabilizing the K-Ras/MAPK signal complex and thus increasing the MAPK output to promote tumorigenesis [102]. In the same year, they also found that macrolide Elaiophylin reduced the levels

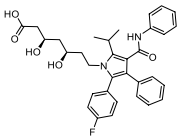
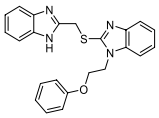
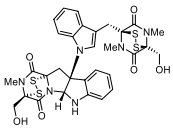
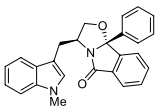
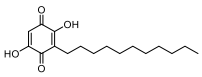
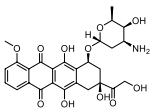
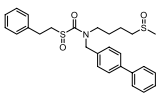
of HIF-1α and Gal3 and inhibited their proliferation by inhibiting HSP90-Cdc37 PPI in MDA-MB-231 and MIA PaCa-2 cells [103]. Additionally, prior research demonstrated celastrol's capability to disrupt the HSP90-Cdc37 PPI [104]. In this context, Chen et al. employed a molecular hybridization strategy to synthesize a series of celastrol derivatives, revealing that compound 29 exhibited enhanced inhibitory effects on the HSP90-Cdc37 PPI compared to celastrol. Treatment with compound 29 significantly reduced the levels of CDK4 and pAKT and induced cellular arrest in the G0/G1 phase in A549 cells [105]. Jin et al. reported that Ginsenoside Rh2 (G-Rh2) could directly target HSP90α and disrupt its interaction with Cdc37. In HepG2 cells treated with G-Rh2, there was a significant decrease in the protein levels of CDKs, including CDK2, CDK4, and CDK6, through a proteasome-dependent pathway, along with an increase in Cyclin D and p21 levels. Consequently, this led to cell cycle arrest in the G0-G1 phase and significantly inhibited cell growth [106]. These findings suggest that inhibiting the interaction between HSP90 and Cdc37 impacts HSP90's kinase client proteins and disrupts the signaling cascade they are involved in, demonstrating a notable effect on tumorigenesis and offering valuable anticancer benefits.

Evading growth suppressors

In addition to the induction and maintenance of progrowth signals, evading growth signal inhibition by negative feedback mechanisms is one of the hallmark capabilities acquired by tumor cells. Previous studies have shown that this negative feedback regulatory mechanism is associated primarily with the regular expression of crucial tumor suppressors such as p53, which is often referred to as the guardian of the genome because of its vital role in tumor suppression, as p53 regulates the genes responsible for cell cycle arrest and apoptosis [107]. Under typical physiological conditions, wild-type p53 (wtp53) levels are low due to the murine double minute 2 (MDM2)-mediated ubiquitin–proteasome pathway. Once a cell suffers DNA damage or internal stress, p53 undergoes posttranslational modifications, including phosphorylation and acetylation. This modified p53 then translocates to the nucleus for transcriptional activation, initiating responses such as apoptosis, cell cycle arrest, or cellular senescence to maintain cellular genetic stability and inhibit tumorigenesis. However, p53 mutations occur in approximately 50% of human malignancies. Missense mutations abolish the original cancer-suppressing function of p53, and some mut-type p53 (mutp53) variants may initiate a new gene expression program that promotes tumor growth and spread [108]. Moreover, mutp53 can form heterotetramers with wtp53, further inhibiting its function, resulting in cells exhibiting p53 loss-of-function characteristics even when some functional p53 is still present; this ultimately results in accelerated tumor formation and progression and increased resistance to treatment [109]. Retinoblastoma tumor suppressor protein (Rb) is another crucial tumor suppressor that primarily inhibits tumor cell proliferation by regulating the cell cycle. Unphosphorylated Rb binds to and inhibits the activity of the E2F transcription factor, preventing the cell from advancing from G1 phase to S phase [110]. CDKs and cyclins regulate the phosphorylation of Rb [111]. As the levels of CDKs and cyclins increase, phosphorylated Rb releases E2F, leading to the transcriptional activation of the latter and allowing the cell cycle to proceed. In many cancer types, compromised Rb function caused by the inactivation of Rb proteins often fails to appropriately control the cell cycle, facilitating the uncontrolled proliferation of tumor cells. In addition, cyclin-dependent kinase inhibitors such as p21, which can be directly activated by p53, can also directly inhibit CDKs via a negative feedback program to block cell cycle progression [112]. Progress in regulating these processes has been made with drugs (Table 2).

Degradation of mutp53 has emerged as a promising therapeutic strategy in cancer. As a family member of HSP40, DNAJA1 is pivotal in preserving mutp53 stability, a process contingent upon the farnesylation of DNAJA1. In the current study, Yang et al. demonstrated that atorvastatin treatment could transform farnesylated DNAJA1 into its unfarnesylated form through the mevalonate pathway, which impaired the interaction between mutp53 and DNAJA1, resulting in DNAJA1's inability to sustain mutp53 stability. Consequently, accumulated mutp53 was degraded, further contributing to apoptosis and cell cycle arrest in pancreatic cancer cells. DNAJA1's colocalization with p53 in the cytoplasm indicated its integral role as a chaperone protein for preventing mutp53's nuclear translocation by atorvastatin treatment [113]. In a subsequent study, a computer-assisted approach was employed to delineate the interaction domain between DNAJA1 and the mutp53^{R1175H} protein, identifying a druggable small molecule binding site within the glycine/phenylalanine region of DNAJA1. Consequently, screening efforts within drug-like libraries pinpointed a promising small molecule, GY1-22, which demonstrated significant inhibitory effects on the DNAJA1-mutp53^{R1175H} complex and led to a decrease in mutp53 protein levels via a proteasome-mediated pathway, subsequently curtailing the growth of pancreatic cancer cells driven by mutp53. Furthermore, GY1-22 treatment enhanced the expression of the wtp53-activated gene, waf1p21, indicating a partial reinstatement of the wtp53 tumor suppressor functionality [114].

Table 2 Summary of HSP inhibitors regulating growth suppressors in cancer

Name	Structure	Target	Hallmarks of cancer	Cancer cell line	Tumor type	Ref
Atorvastatin		DNAJA1	Induce growth inhibition	PO3 (40 μM)	Pancreatic cancer	[113]
GY1-22		DNAJA1	Induce growth inhibition	PO3 (IC_{50} = 28 μM)	Pancreatic cancer	[114]
Chetomin		HSP40	Induce growth inhibition	HuCCT1 (150 nM) CAL-33 (150 nM) FAMPAC (150 nM) KLE (150 nM) TOV-112D (150 nM)	Multiple cancers	[115]
SLMP53-2		HSP70	Induce growth inhibition	HepG2 (IC_{50} = 12.5 ± 0.8 μM), HCT116 $p53^{+/+}$ (IC_{50} = 8.4 ± 1.1 μM), HCT116 $p53^{-/-}$ (IC_{50} = 17.7 ± 2.3 μM)	Hepatocellular Carcinoma	[116]
Embelin		Mortalin	Induce growth inhibition	MCF-7 (IC_{50} = 10 μM)	Breast cancer	[120]
Doxorubicin		HSP60	Induce growth inhibition	NCI-H292 (IC_{50} = 80 nM)	Lung cancer	[122]
S-4		HSP90	Induce growth inhibition	SCC IC1 (7.5 μM)	Cutaneous squamous cell carcinomas	[124]

In addition to triggering the degradation of mutp53 to block mutp53-induced tumor growth, restoring the lost wild-type-like tumor suppressive function of mutp53 represents another significant anticancer pathway. Hiraki et al. screened naturally small molecules and identified Chetomin (CTM) for its capacity to inhibit tumor growth by reactivating the wild-type-like function of mutp53 both in vivo and in vitro. Through mechanistic studies, HSP40 was proposed as the target and mediator for CTM in restoring the function of mutp53. Following CTM treatment, an increased binding affinity of HSP40 for the $mutp53^{R1175H}$ variant was observed, which would potentially lead to a conformational shift of the $mutp53^{R1175H}$ protein towards a wild-type-like structure, further inducing the activation of target genes such as p21, PUMA, and MDM2 and selectively inhibiting the growth of mutant $p53^{R1175H}$ tumor cells. Recognition of the HSP40-$mutp53^{R1175H}$ complex by the wtp53-specific antibody PAb1620 demonstrated the critical role of HSP40 in reverting mutp53 to a functionally wild-type-like state [115]. Similarly, another heat shock protein, HSP70, has been implicated in the refolding and stabilizing mutp53, restoring its DNA binding and transcriptional activity. The synthesized compound SLMP53-2 exhibited growth inhibition in mutp53-expressing hepatocellular carcinoma cells by enhancing the binding capacity of HSP70 to $mutp53^{Y220C}$. SLMP53-2 treatment facilitated HSP70's assistance in refolding mutp53 to a wild-type-like conformation, sequentially activating several target genes, including those associated with cell cycle arrest (p21, GADD45), apoptosis (Bax, Killer, Survivin), ER stress (Chop), and angiogenesis (VEGF). Additionally, SLMP53-2 exhibited a synergistic effect in combination therapy with Sorafenib, enhancing the sensitivity of HCC cells to Sorafenib, which remains the sole first-line targeted therapy recommended for advanced hepatocellular carcinoma. SLMP53-2 also displayed significant antitumor efficacy in an HCC xenograft mouse model without showing considerable toxicity [116].

HSPs have been identified as forming complexes with p53, thereby inhibiting its nuclear translocation and transcriptional activation, which ultimately impedes the pathways that regulate tumor cell growth inhibition [117]. Thus, disrupting the interaction between HSPs and

p53 could serve as a strategy to re-enable p53's tumor-suppressing function. Mortalin, also known as mtHSP70, GRP75, or PBP74, is a protein in the HSP70 family, predominantly localized in mitochondria [118]. Studies have indicated that mortalin expression levels are upregulated in various cancers and are associated with tumor development, progression, and drug resistance [119]. Embelin, a natural quinone derived from the fruits of *Embelia ribes*, has been reported by Wadhwa and colleagues to reduce the binding of mortalin to p53 significantly. Molecular docking studies have shown that Embelin could form hydrogen bonds with mortalin at Val 264, Lys 265, and Thr 267 and engage in hydrophobic interactions at several sites, including Phe 250, Ser 266, and Thr 271. Concurrently, Embelin could bind with high affinity to the A and C chains of p53, forming a stable complex. These interactions obstruct the formation of the mortalin-p53 complex, allowing free p53 to translocate to the nucleus and activate transcription, thereby inducing cell cycle arrest in the G1 phase and inhibiting tumor cell growth. Notably, Embelin has demonstrated lower toxicity towards normal cells [120]. Furthermore, through virtual screening and molecular dynamics simulations, Grover's team identified two natural small molecules, DTOM and TTOM, capable of disrupting the interaction between mortalin and p53. This discovery positioned them as potential candidates for anticancer drug development. However, the execution of comprehensive biological evaluations is still forthcoming [121]. Subsequent research confirmed that Doxorubicin, a cytotoxic anthracycline antibiotic, could also inhibit tumor cell growth by disrupting the HSPs-p53 interaction. This study revealed that Doxorubicin treatment-induced post-translational modifications of HSP60 significantly increased its acetylation levels and compromised its function. Consequently, the HSP60-p53 complex dissociated, releasing p53, further inducing tumor cell cycle arrest at the G2/M phase, and activating cellular senescence through the p53-p21 pathway. Additionally, extracellular HSPs, acting as multifunctional messengers, are involved in signals between different cells, tissues, or organs. After treatment with Doxorubicin, both the dissociated and the inherently free, highly acetylated HSP60 could not reassociate with p53. These post-modified proteins underwent degradation via the proteasome system, substantially reducing HSP60 protein levels and curtailing its release into the extracellular compartment, potentially impairing signal between tumor cells and thus exerting an anti-tumor effect [122].

As another critical tumor suppressor, the hyperphosphorylation of Rb compromises its regulatory control over E2F, leading to unchecked tumor cell proliferation [123]. Therefore, exploring anticancer therapies aimed at the phosphorylation regulation mechanism of Rb represents a significant avenue of research. In the current study, Dinkova-Kostova et al. identified Sulfoxythiocarbamate S-4 as an HSP90 inhibitor that prevented the phosphorylation of Rb and thus exhibited anticancer efficacy in the human skin squamous cell carcinoma cell lines SCC IC1. Inhibition of HSP90 by S-4 resulted in significant down-regulation of the expression of its client proteins Cyclin D1 and CDK4, which inhibited the phosphorylation of Rb and the release of E2F and, in turn, induced cell cycle arrest in the G1/S phase. At the same time, S-4 treatment led to the depletion of the HSP90 client protein B cell lymphoma-2 (Bcl-2), alongside the release of cytochrome C (Cyt-C), eventually leading to apoptosis. Moreover, the level of the oncoprotein HER2 was also diminished in S-4-treated cell lines. These findings indicate that S-4 possesses multifaceted inhibitory effects on tumor cell growth, underscoring its potential for development as an anticancer drug candidate [124].

Resisting cell death

Cell death is an indispensable process in the maintenance of homeostasis and the normal functioning of organisms, as excess, damaged, or malfunctioning cells are eliminated. Functionally, cell death can be categorized into two principal types: regulated cell death (RCD) and accidental cell death [125]. Accidental cell death typically results from uncontrollable external factors, such as mechanical injury or exposure to toxic substances. Conversely, RCD, also known as programmed cell death, is governed by complex molecular mechanisms and signaling pathways, making cell death via this route susceptible to regulation through genetic manipulation or pharmacological intervention [125, 126]. Notably, RCD encompasses various subroutines, such as apoptosis, autophagy, ferroptosis, and lysosome-dependent cell death. In the context of cancer, disruptions and imbalances in these crucial cell metabolism mechanisms often lead to unchecked growth and proliferation, thereby aggravating disease severity and complicating treatment strategies. Consequently, attempting to restore RCD in cancer cells has emerged as a significant focus in contemporary cancer therapy research [127].

In recent years, HSPs have emerged as critical factors in cell survival. These proteins play pivotal roles in regulating the cellular RCD process through their chaperone activity, enabling cancer cells to develop malignant growth characteristics, notably resistance to RCD—an essential acquired hallmark of cancer cells [128]. Consequently, targeting HSPs with bioactive small molecules to modulate various RCD subroutines represents a promising strategy for cancer therapy (Table 3). This section delves into the impact of HSPs on the classical apoptotic

signaling pathway in cancer and provides an overview of recent progress in the development of small-molecule inhibitors of HSPs as anticancer agents. Furthermore, we explore cases involving other RCD subroutines, such as autophagy and lysosome-dependent cell death, to highlight their importance in the context of cancer treatment.

Targeting heat shock proteins to regulate the apoptotic pathway in cancer

Apoptosis, the earliest recognized and most classical RCD subroutine, is intricately regulated at the polygenic level to systematically induce organelle degradation or cell death without eliciting an inflammatory response [129]. Morphologically, apoptotic cells display a series of classical features, including shrinkage, chromatin condensation, membrane blebbing, nuclear collapse, and the formation of apoptotic bodies [130, 131]. Currently, apoptosis is acknowledged as a crucial mechanism for maintaining homeostasis within the internal environment, and its inhibition or resistance frequently contributes to tumorigenesis and progression [132].

Apoptosis is an intricately complex process regulated by two distinct pathways: the extrinsic pathway and the intrinsic pathway (Fig. 4) [133]. The extrinsic signaling pathway involves interactions mediated by transmembrane receptors, which are initiated by the binding of death receptors to ligands [134]. Key death receptors include tumour necrosis factor (TNF) receptors (such as TNFR1) and Fas (CD95). The binding of these receptors to their ligands, for example, Fas to FasL, leads to receptor aggregation and the subsequent formation of a death-inducing signalling complex (DISC). Critical proteins, such as Fas-associated death domain (FADD) and procaspase-8, are recruited to the DISC to activate caspase-8. Once activated, caspase-8 triggers the activation of downstream executor caspases (such as caspases 3, 6, and 7), culminating in apoptosis. In contrast, the intrinsic pathways of apoptosis are driven primarily by nonreceptor-mediated mechanisms, with a predominant role played by mitochondria [135, 136]. The Bcl-2 family of proteins, which include both proapoptotic (e.g., Bax and Bak) and antiapoptotic (e.g., Bcl-2 and Bcl-xL) members, are central to this complex process. The activation of the Bcl-2 effector proteins Bax and Bak, which can be prompted by cellular stress conditions such as DNA damage, ROS overload, or growth factor deficiency, leads to increased mitochondrial outer membrane permeability (MOMP), thus facilitating the release of proapoptotic proteins into the cytoplasm. Cyt-C, the first protein released, enters the cytoplasm and interacts with apoptotic protease activating factor-1 (Apaf-1) and procaspase-9 to form the apoptosome, within which caspase-9 is activated. This activation triggers a cascade involving downstream executor caspases, which induce apoptosis. Moreover, mitochondria release the second mitochondria-derived activator of caspases/direct IAP-binding protein with low pI (SMAC/DIABLO) and high-temperature requirement protein A2/Omi serine protease (HtrA2/Omi), which inhibit the activity of inhibitory apoptotic proteins and facilitate X-linked inhibitor of apoptosis protein (XIAP) degradation, which mediates caspase-9 degradation [137]. Importantly, these two pathways are not isolated; for example, caspase-8, in addition to directly activating effector caspases, can contribute to mitochondria-induced apoptosis by converting the BH3-only protein Bid into tBid [138]. Furthermore, the p53 gene enhances the expression of the proapoptotic protein Bax, thereby inducing apoptosis [139, 140]. Mutation of the p53 gene leads to a compromised apoptosis inhibition mechanism, enabling tumor cells to evade apoptosis.

Previous research has demonstrated that HSPs play a critical role in regulating key events of apoptosis, thereby serving as a protective mechanism against apoptosis induced under various stress conditions [26]. Consequently, HSPs are considered appropriate targets for modulating apoptotic pathways, making them a significant focus in developing innovative anticancer therapeutics. Geldanamycin (GA) was first identified as a natural inhibitor of HSP90 ATPase, sparking a wave of research into HSP90 inhibitors, with GA serving as the prototype. Alvespimycin, a semi-synthetic derivative of GA, has been shown to induce apoptosis in both imatinib-sensitive (K562) and -resistant (K562-RC and K562-RD) chronic myeloid leukemia (CML) cell lines via a mitochondria-dependent pathway. However, it also initiated HSR that led to an increased expression of HSP70 [141]. In contrast, a separate study found that 17-aminogeldanamycin (AG), another HSP90 inhibitor, effectively reduced the compensatory increase in HSP70 expression. AG demonstrated a more potent anti-melanoma effect than GA, significantly activating caspase-3/7 and inducing apoptosis in $BRAF^{V600E}$ cells. Additionally, AG alleviated ER stress, selectively reduced the activity of the inositol-requiring enzyme 1 alpha (IRE1α)-spliced X-box binding protein 1 pathway, and inhibited ERK1/2 activity, underscoring its enhanced efficacy as an HSP90 inhibitor [142].

Xu et al. employed fragment-based drug discovery to identify and refine a small-molecule HSP90 inhibitor, 4f, which demonstrated significant efficacy against nasopharyngeal carcinoma both in vitro and in vivo. The biological evaluation showed that 4f promoted the degradation of its client protein AKT by targeting HSP90, which interfered with the PI3K/AKT signaling pathway, thereby inducing apoptosis in CNE2 cells. In addition,

Table 3 Summary of HSP inhibitors regulating cell death signals in cancer

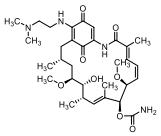
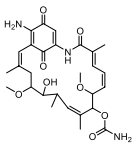
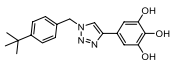
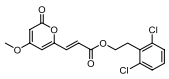
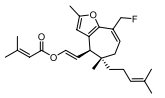
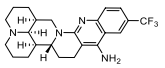
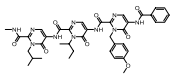
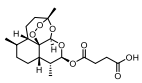
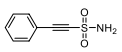
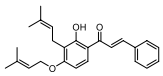
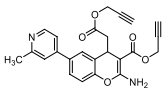
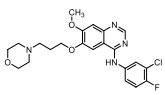
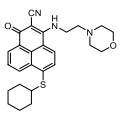
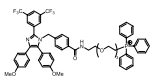
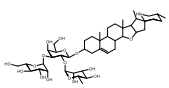
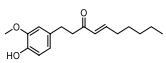
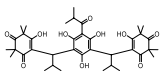
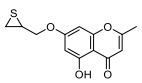
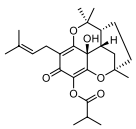
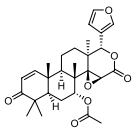
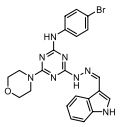
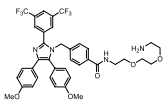
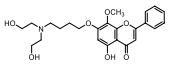
Name	Structure	Target	Hallmarks of cancer	Cancer cell line	Tumor type	Ref
Alvespimycin		HSP90	Induce apoptosis	K562-RD (IC_{50} = 44 nM) K562-RC (IC_{50} = 31 nM)	Chronic myeloid leukemia	[141]
17-Aminogeldanamycin		HSP90	Induce apoptosis	DMBC12 (0.4 μM) DMBC21 (0.4 μM) DMBC28 (0.4 μM) DMBC29 (0.4 μM)	Melanoma	[142]
Compound 4f		HSP90	Induce apoptosis	CNE2 (IC_{50} = 40 nM)	Nasopharyngeal carcinoma	[143]
Compound 5o		GRP94	Induce apoptosis	HT29 (IC_{50} = 1.07 ± 0.05 μM) HCT116 (IC_{50} = 1.88 ± 0.02 μM) SW480 (IC_{50} = 8.55 ± 0.03 μM)	Colorectal cancer	[144]
Compound 29		HSP90	Induce apoptosis	HL-60 (IC_{50} = 3.69 ± 0.37 μM)	Leukemia	[146]
Compound 22 g		HSP90	Induce apoptosis	Hela (IC_{50} = 19.6 ± 0.4 μM)	Cervical cancer	[148]
Compound 5b		HSP90α	Induce apoptosis	K562 (IC_{50} = 1.3 ± 0.3 μM)	Leukemia	[149]
Artesunate		HSP70	Induce apoptosis	4T1 (IC_{50} = 52.41 μM) MCF-7 (IC_{50} = 83.28 μM)	Breast cancer	[155]
2-phenylethynesulfonamide		HSP70	Induce apoptosis	SCC25 (20 μM) CAL27 (20 μM)	Oral squamous cell carcinoma	[156]
Compound 6		HSP70	Induce apoptosis	A2058 (IC_{50} = 11.62 ± 0.017 μM)	Melanoma	[157]
CXL146		GRP78	Induce apoptosis	HL60/MX2 (GI_{50} = 0.17 ± 0.03 μM)	Leukemia	[158]
Gefitinib		HSP70	Induce apoptosis	A549 (20 μM)	Lung cancer	[160]
S1g-6		HSP70	Induce apoptosis	BV173 (EC_{50} = 1.4 ± 0.2 μM) KCL22 (EC_{50} = 2.6 ± 0.4 μM)	Chronic myeloid leukemia	[162]
Az-TPP-O3		Mortalin	Induce apoptosis	Hela (IC_{50} = 0.61 ± 0.07 μM) HL60 (IC_{50} = 0.48 ± 0.06 μM)	Cervical cancer, Leukemia	[163]
Solasonine		Mortalin	Induce apoptosis	HepG2 (IC_{50} = 4.7 ± 0.2 μM) Hep3b (IC_{50} = 4.0 ± 0.1 μM)	Hepatocellular carcinoma	[164]
6-Shogaol		HSP60	Induce apoptosis	A549 (IC_{50} = 48.67 μM)	Non-small-cell lung cancer	[167]

Table 3 (continued)

Name	Structure	Target	Hallmarks of cancer	Cancer cell line	Tumor type	Ref
Myrtucommulone		HSP60	Induce apoptosis	HL-60 (> 10 μM)	Leukemia	[168]
J2		HSP27	Induce apoptosis	SKOV3 (IC_{50} = 17.34 μM) OVCAR-3 (IC_{50} = 12.63 μM)	Ovarian cancer	[172]
Compound 1b		HSP105	Induce apoptosis	Hela (50 μM)	Cervical cancer	[174]
Gedunin		HSP90	Induce autophagy	A549 (IC_{50} = 20 μM)	Lung cancer	[182]
A14		HSP90	Induce autophagy	A549 (IC_{50} = 0.1 μM) SK-BR-3 (IC_{50} = 0.4 μM)	Lung cancer Breast cancer	[183]
Apoptozole		HSP70	Induce LCD	Hela (IC_{50} = 5.92 ± 0.47 μM)	Cervical cancer	[163]
V8		HSP70	Induce LCD	Hut-102 (6 μM) Jurkat (6 μM)	T cell malignancy	[187]

4f was observed to reduce the protein levels of Bcl-xL, indicating its potential involvement in mitochondria-mediated apoptosis [143]. Additionally, the compound 5o, based on the 6-acrylic phenethyl ester-2-pyranone core structure, has been reported to induce apoptosis in HT-29 and HCT116 by targeting GRP94. It documented that 5o could bind to the ATP-binding pocket of GRP94 and exhibit high affinity and stability, with interactions with the residues Phe199, Asn107, and Asn162 considered crucial. Treatment with 5o resulted in the inhibition of GRP94, which leads to the dephosphorylation of the PI3K/Akt/Forkhead box O1 (FoxO1) signaling pathway and the induction of MAPKs and NF-κB p65 phosphorylation, inducing apoptosis through multiple pathways and exerting anti-colorectal cancer activity [144].

Vibsane-type diterpene compounds have been employed in treating various diseases [145]. Derivative 29, synthesized with Vibsanin C as its core structure, emerged as a novel HSP90 C-terminal inhibitor capable of inducing apoptosis in leukemia cells, specifically HL-60. Treatment with compound 29 resulted in the downregulation of Bcl-2 and the upregulation of Bax and cleaved caspase-3 in HL-60 cells, indicating that compound 29 induces apoptosis through an intrinsic, mitochondria-mediated pathway. Compound 29 also markedly suppressed tumor growth in an in vivo study using an H22 xenograft mouse model without exhibiting signs of acute toxicity. These findings underscore its potential for further research and development [146]. Matrine is an active molecule obtained from *Sophora flavescens* with pharmacological properties such as cardiovascular protection and anti-tumor effects [147]. Gao et al. developed and evaluated compound 22 g, leveraging the distinctive L-type conformation of Matrine to design a promising anticancer candidate. This compound triggered apoptosis in Hela cells, evidenced by an upregulation of Bax and cleaved Caspase-3, alongside a downregulation of Bcl-2. Molecular docking studies further revealed that 22 g interacts with multiple sites on HSP90, including Phe-138, Asn-51, Gly-137, and Gly-135, demonstrating binding solid affinity. These results underscore its potential as a novel anticancer agent and warrant further investigation [148].

To minimize the risk of inducing HSR, Kurz et al. meticulously designed a C-terminal inhibitor of HSP90 utilizing a tripyrimidine amide framework. Through a rigorous evaluation, compound 5b emerged as a compelling candidate for its ability to inhibit a broad spectrum of leukemia cell lines, including K562, KCL22, and HL60, as well as leukemia cells resistant to tyrosine

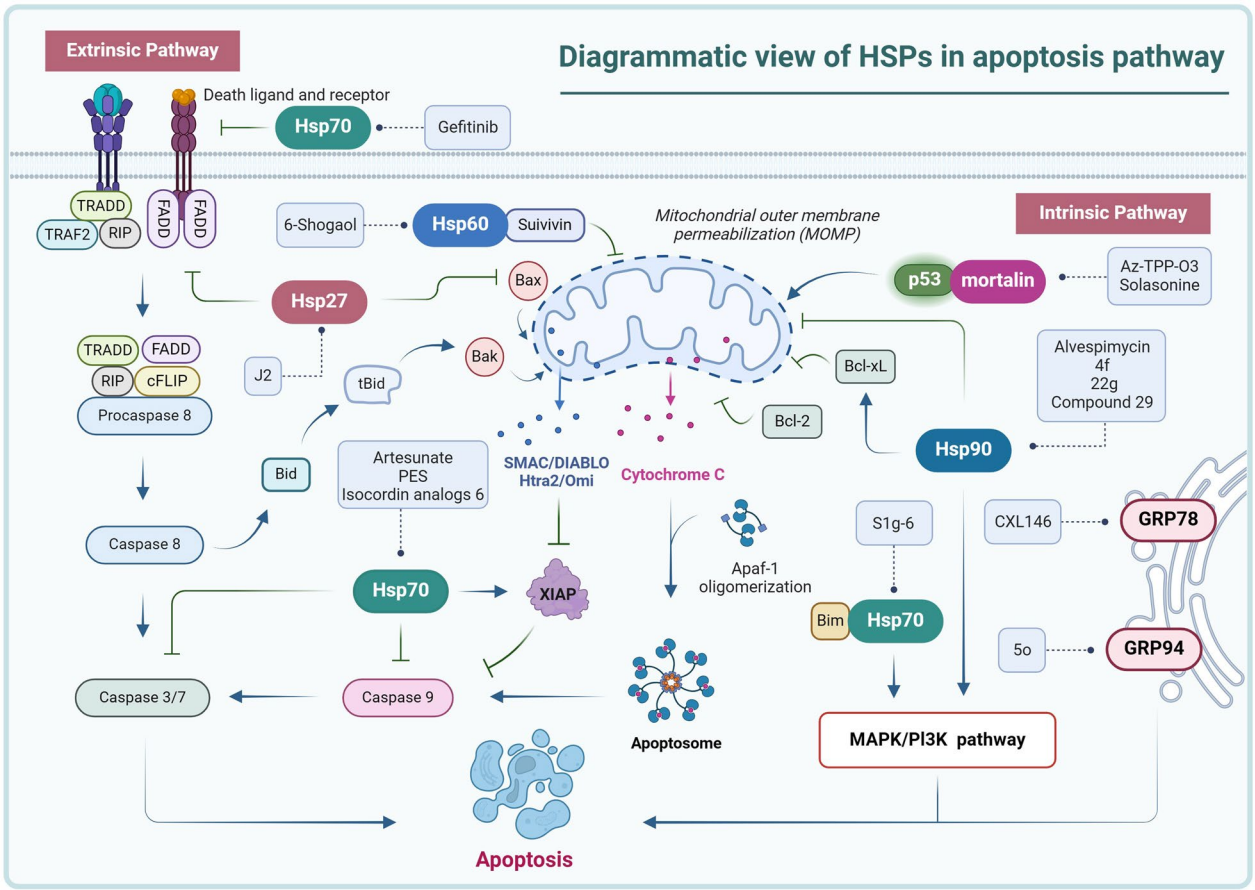


Fig. 4 Diagram of HSPs in the core apoptotic signaling pathway in cancer. Extrinsic and intrinsic pathways primarily regulate apoptosis. In the extrinsic pathway, upon ligand activation, death receptors recruit critical proteins such as FADD and procaspase-8, forming the death-inducing signaling complex (DISC). Subsequently, caspase-8 and downstream caspases-3/7 are activated, ultimately inducing apoptosis. The intrinsic pathway, also known as the mitochondrial pathway, is receptor-independent. When cells encounter DNA damage or oxidative stress, the balance of Bcl-2 family proteins shifts, with pro-apoptotic proteins (e.g., Bax and Bak) upregulated, and anti-apoptotic proteins (e.g., Bcl-2 and Bcl-xL) downregulated. These changes further lead to mitochondrial outer membrane permeabilization (MOMP) and cytochrome c release. Released cytochrome c binds to apoptotic protease activating factor-1 (Apaf-1), forming the apoptosome, activating the caspase cascade, leading to apoptosis. Additionally, mitochondrial-released proteins such as SMAC/DIABLO and Htra2/Omi can inhibit the activity of XIAP, thereby reducing the apoptotic inhibition caused by XIAP's inhibition of caspase-9. There is also a crosstalk between the intrinsic and extrinsic pathways. Caspase-8 can also cleave Bid into tBid to directly participate in mitochondria-dependent apoptosis. Furthermore, MAPK signaling, PI3K signaling, and endoplasmic reticulum stress can also induce apoptosis. HSPs have been shown to participate in the regulation of apoptotic signaling pathways through various mechanisms. HSP90 maintains the stability and function of many proteins, such as Bcl-xL and PI3K, thereby strengthening the signal transduction of its pathway and inhibiting tumor apoptosis. HSP70 regulates apoptosis through multiple pathways by modulating XIAP, caspases-3/-7/-9, Fas, Bim, and endoplasmic reticulum stability. Moreover, HSP27 has been shown to inhibit apoptosis induced by both intrinsic and extrinsic pathways by blocking the pro-apoptotic actions of Fas and Bax. Additionally, HSP60 and mortalin interact with survivin and p53 to inhibit mitochondria-dependent apoptosis, thereby promoting tumor progression

kinase inhibitors, such as K562-IMr and KCL22-IMr. 5b demonstrated stable binding to HSP90α and effectively inhibited its CTD dimerization, disrupting its chaperone activity and consequently triggering apoptosis. Concurrently, 5b induced downregulation of HSP90 client proteins within the cells it treated, including AKT, STAT5, and c-Myc. Furthermore, 5b reduced the expression of the BCR-ABL1 protein, a key mediator of drug resistance in leukemia cells, and impeded the signaling pathway it orchestrates. The findings from in vivo experiments substantiated the efficacy and safety of 5b as a prospective anti-tumor therapeutic agent [149].

Studies have demonstrated that high expression of HSP70 in various types of cancer promotes cancer progression by inhibiting apoptosis [150]. Accumulated evidence suggests that HSP70 can inhibit the activation of Caspase-9, Caspase-8 and Caspase-3, prevent apoptosome formation by disrupting the Apaf-1 and

Procaspase-9 interaction, and interfere with the release of Cyt-C, thus participating in the regulation of extrinsic and intrinsic apoptotic pathways [151–153]. Thus, reducing HSP70 levels is a hopeful cancer treatment method that induces cancer cell death [154]. Intriguingly, a recent study suggested that Artesunate, a semi-synthetic derivative of Artemisinin, possesses anti-tumor properties in breast cancer by inhibiting the ATPase activity of HSP70. The investigation revealed that Artesunate treatment resulted in the downregulation of both Bcl-2 and HSP70 expression within 4T1 and MCF-7 cells while concurrently enhancing the expression of cleaved caspase-9, indicative of the induction of a caspase-dependent apoptotic pathway. In silico modeling via molecular docking analyses pinpointed the NBD of HSP70 as the putative binding site for Artesunate, and the interaction involved critical residues within the NBD, including Lys 72, Leu 74, and Phe 69. Furthermore, Artesunate demonstrated a favorable safety profile, exhibiting minimal cytotoxicity towards the non-malignant WI-38 cell line. These findings collectively unveil a potentially promising therapeutic avenue for Artesunate in breast cancer treatment and warrant further investigation [155]. 2-Phenylethynesulfonamide (PES), an inhibitor of HSP70 function, has been demonstrated to promote apoptosis in oral squamous cell carcinoma cell lines, SCC25 and CAL27, predominantly via the mitochondrial pathway. In PES-treated cells, there was an augmented expression of cleaved caspase-9, caspase-3, and poly (ADP-ribose) polymerase (PARP), coupled with a reduction in full-length PARP and caspase-3 levels. Simultaneously, PES downregulated the expression of HSP70 client proteins, specifically the XIAP and cellular inhibitor of apoptosis protein 1, closely associated with anti-apoptotic functions. The disruption of the interaction between HSP70 and XIAP further indicates the inhibitory effect of PES on HSP70 function. Assessments employing BALB/c mice engrafted with SCC25 cell xenografts substantiated the antitumor efficacy of PES, demonstrated by significant reductions in tumor volume and weight while maintaining stable body weight across treated subjects [156]. The Madrid group has evaluated the mechanism of action of Isocordin analogs 6 as an HSP70 inhibitor in the human melanoma cell line A2058. Results indicated that treatment with compound 6 led to decreased levels of HSP70 and the anti-apoptotic protein Bcl-2. In contrast, the pro-apoptotic protein Bax and caspase-9 levels were increased in treated cells. HSP70 has been demonstrated to bind directly to Apaf-1, thus preventing apoptosome formation by disrupting the Apaf-1 and caspase-9 interaction. However, the precise mechanisms by which compound 6 modulates HSP70 to achieve its anti-apoptotic effects require further investigation [157].

Glucose-regulated protein 78 (GRP78), a member of the HSP70 family, is localized in the ER, and its overexpression is implicated in multidrug resistance (MDR) of cancer cells. In this study, the 4H-chromene derivative CXL146 selectively reduced the protein levels of GRP78 in a dose-dependent manner in various MDR cancer cells. Employing the HL60/MX2 cell line as a model, treatment with CXL146 triggered the unfolded protein response (UPR), demonstrated by the activation of sensor proteins on the ER membrane, such as IRE1-α, protein kinase R–like ER kinase, and activating transcription factor 6. The UPR activation induced by CXL146 led to a cascade of downstream events, including the activation of ERK1/2 and JNK, which contributed to promoting apoptosis. Given that the dissociation of GRP78 from the sensor proteins above triggers UPR and shifts towards promoting apoptosis, CXL146 may target GRP78 through a similar molecular mechanism, thereby functioning as an anti-cancer agent against MDR [158].

Gefitinib, an EGFR tyrosine kinase inhibitor, represents the first small-molecule targeted therapy for lung cancer treatment. Investigations have revealed that Gefitinib could significantly enhance Fas-induced caspase-8 activation and subsequent apoptosis in A549 cells in a manner independent of EGFR and p53 status. Concurrently, Gefitinib downregulated the expression of HSP70 in A549 cells. Given that HSP70 is known to confer protection against Fas-mediated apoptosis, Gefitinib might increase sensitivity to Fas-induced apoptosis by downregulating HSP70 [159]. However, the specific relationship between gefitinib and HSP70 was not discussed, and further research is warranted to elucidate these mechanistic details [160].

Disruption of the PPIs between HSP70 and its client proteins represents a viable avenue for developing novel anti-cancer therapeutics. The Hsp70-Bim PPI has been demonstrated to inhibit apoptosis by modulating the conformation of oncogenic client proteins AKT and Raf-1 in cancer cells [161]. This study reports a novel Hsp70 inhibitor, S1g-6, which induces apoptosis in CML cell lines BV173 and KCL22 by explicitly targeting the Hsp70-Bim PPI without significant toxicity to regular tissue cell lines HEK293 and BaF3. S1g-6 selectively binds to the NBD of HSP70 within the Bim binding groove, thereby inhibiting the function of the Hsp70-Bim PPI. This action leads to downregulating AKT and Raf-1 phosphorylation and protein levels, ultimately triggering apoptosis [162]. A study by Shin et al. has developed an inhibitor, Az-TPP-O3, explicitly targeting the interaction between Mortalin and p53. Upon internalization into mitochondria, Az-TPP-O3 interrupted the Mortalin-p53 interaction, which enhanced Bax-mediated MOMP. This disruption leads to the release of Cyt-C into the cytosol,

facilitating the assembly of the apoptosome and subsequent activation of the caspase cascade, culminating in apoptosis [163]. Additionally, Solasonine, a steroidal alkaloid glycoside derived from *Solanum xanthocarpum*, has been identified to inhibit the interaction between Mortalin and p53, activating the p53 pathway to trigger apoptosis in human HepG2 and Hep3b hepatocellular carcinoma cells [164].

HSP60, a molecular chaperone predominantly localized in the mitochondria, is crucial in maintaining cellular homeostasis. The expression of HSP60 is upregulated in various cancers and confers anti-apoptotic properties that support tumorigenesis and cancer progression [40, 165]. Inhibition of HSP60 offers a novel therapeutic option for cancer treatment. The work by Zhang et al. has demonstrated that 6-Shogaol (6-SH) is a promising candidate for treating non-small cell lung cancer (NSCLC) by inhibiting HSP60. 6-SH interacts with several residues of HSP60, including Ile 150, Pro 33, and Gly 53, through hydrophobic interactions and hydrogen bonding, thereby reducing protein stability and facilitating its degradation via the proteasome-mediated pathway. There is evidence that HSP60 and survivin can form complexes that maintain survivin's stability and anti-apoptotic properties [166]. Treatment with 6-SH disrupted the mitochondrial membrane potential, accompanied by a decrease in the anti-apoptotic proteins Bcl-2 and survivin and an increase in the pro-apoptotic protein Bax. These findings suggest that 6-SH might induce tumor cell apoptosis by triggering a mitochondrial-mediated pathway [167]. Myrtucommulone A (MC) was identified as a small molecule inhibitor of HSP60. MC promoted the release of Cyt-C in HL-60 cells by inhibiting HSP60, thereby inducing apoptosis. This result is likely related to changes in mitochondrial function following HSP60 inhibition, which is accompanied by the suppression of malate dehydrogenase refolding activity, as well as the aggregation of lon protease-like protein and leucine-rich PPR motif-containing protein [168].

HSP27 plays a critical role in various stages of cancer development. It has been shown to regulate intrinsic and extrinsic apoptosis pathways by interacting with vital modulatory proteins. In the extrinsic pathway, HSP27 inhibits Caspase-3 activation and blocks the membrane translocation of death domain-associated protein (DAXX), disrupting the interaction between Fas and apoptosis signal-regulating kinase 1 (Ask-1). In the mitochondrial-mediated apoptosis pathway, HSP27 inactivates Bax, prevents Cyt-C release from mitochondria, and inhibits Caspase-9 activation, impeding apoptosome formation [169, 170]. Consequently, developing inhibitors targeting HSP27 presents a promising approach to cancer therapy. In the current study, the compound J2 was shown to inhibit the proliferation of ovarian cancer cells by inducing apoptosis through HSP27 suppression. Post J2 treatment, there was an upregulation of mRNA levels for various pro-apoptotic proteins, including TNF-α, DAXX, Ask-1, Cas-8, Caspase-3, Bax, Cyt-C, p53, Apaf-1, and Caspase-9, in SKOV3 and OVCAR-3 cells, alongside a decrease in Bcl-2 expression. Molecular docking studies indicated that J2 formed a robust interaction with the Ser82 residue of Hsp27, disrupting its phosphorylation process, a mechanism known to inhibit apoptosis [171, 172].

HSP105 is documented as one of the molecular chaperones, with studies indicating that it could inhibit apoptosis, thereby preventing cell death induced by Adriamycin [173]. Consequently, targeting HSP105 may represent a promising strategy for cancer therapy. A prenylated phloroglucinol derivative, known as compound 1b, derived from *Hypericum erectum*, has been found to decrease HSP105 expression and induce cell death in cells treated with Adriamycin. These observations suggested that compound 1b could be a valuable inhibitor of HSP105 for cancer treatment. However, the exact molecular mechanism through which compound 1b operates remains to be elucidated and calls for further detailed study [174].

Targeting heat shock proteins to regulate other RCD subroutines in cancer

Targeting heat shock proteins to regulate autophagy pathways in cancer Autophagy, a catabolic process that is both evolutionarily ancient and highly conserved, is a mechanism by which cellular homeostasis can be maintained under stress conditions by degrading damaged organelles and macromolecules and recycling their components [175, 176]. Various factors, such as nutrient deprivation, oxidative stress, DNA damage, physical stimuli, and pharmacological induction, trigger this process. Like apoptosis, autophagy is a type of programmed cell death that involves several stages: induction, phagophore nucleation, phagophore expansion, cargo recognition and sequestration, autophagosome–lysosome fusion, degradation, and recycling [177]. The regulation of autophagy involves multiple signaling pathways and pivotal proteins. mTOR, comprising mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), is a critical negative regulator that is inhibited by the PI3K/AKT/mTOR pathway. Inhibition of mTORC1 leads to activation of the unc-51-like kinase 1 (ULK1) complex, which is integral for another crucial regulatory factor, Beclin-1 [178]. The activated ULK1 complex phosphorylates autophagy-related gene 14 (ATG14), enhancing the interaction between Beclin-1 and vacuolar protein-sorting 34 (VPS34) and facilitating autophagic progression. This interaction also activates the PI3K complex, promoting the synthesis of PI3P

and expansion of the phagophore membrane. In addition, light chain 3 (LC3) is a marker protein for autophagy; converting LC3-I to LC3-II is essential for autophagosome formation and the initiation of related processes [179]. Autophagy interacts with apoptosis, as these processes share specific essential proteins and signaling pathways such as the PI3K/AKT pathway, suggesting that their regulation may affect cell death independently or synergistically [180]. Furthermore, the interaction between Beclin-1 and Bcl-2 through its BH3 domain modulates autophagy inhibition [181]. Autophagy has been identified as a viable target in cancer therapy, with inhibitors of certain HSPs showing anticancer efficacy to treat tumors by regulating autophagy.

Gedunin, an HSP90 inhibitor, is recognized for its therapeutic potential in treating lung cancer by modulating autophagy and apoptosis. Studies reveal that Gedunin suppressed autophagy and enhanced apoptosis in A549 lung cancer cells by inhibiting HSP90α and HSP90β. This process led to decreased levels of the client protein EGFR and its downstream PI3K/AKT signaling pathway. Crucially, the inhibition of HSP90 by Gedunin disrupted its association with the Hsp90/Beclin-1 complex, altering the interaction between Beclin-1 and Bcl-2. Thus, the mRNA levels of Beclin-1, pivotal for autophagy initiation, were reduced due to the loss of HSP90-mediated stabilization and were degraded via the ubiquitin–proteasome pathway, inhibiting autophagy. The expression of the Atg5-12 complex, essential for autophagosome formation, and the autophagy marker protein LC3 were also reduced. Moreover, autophagy inhibition by Gedunin triggered ER stress and the UPR, culminating in mitochondria-mediated apoptosis marked by the downregulation of the anti-apoptotic protein Bcl-2 and the upregulation of caspase-3. Notably, Gedunin was safer for normal lung cells than the anticancer drug 5-fluorouracil (5-FU) [182]. In related research, a novel HSP90 N-terminal inhibitor, A14, has shown antitumor activity in HER2-overexpressing SK-BR-3 cells and EGFR-overexpressing A549 cells by inducing the degradation of HSP90 client proteins and autophagy. A14 led to concentration-dependent increases in the autophagy marker LC3-II and decreases in client proteins such as HER2, AKT, RAF-1, CDK4, and CDK6, with minimal impact on the non-client protein ERK. However, further studies are needed to elucidate the detailed mechanism by which HSP90 inhibition promotes autophagy [183].

Targeting heat shock proteins to regulate lysosome-dependent cell death in cancer Lysosome-dependent cell death (LCD) is a form of programmed cell death closely linked to the functions of lysosomes [184]. These crucial organelles, equipped with various hydrolases, degrade cellular waste and macromolecules, maintaining cellular homeostasis under normal conditions through membrane integrity preservation and hydrolase containment. Under stress conditions, such as oxidative stress and pathogen infection, lysosomal membrane permeabilization (LMP) occurs, releasing hydrolases such as cathepsins, which damage the structures and functions of cells, ultimately leading to cell death. Notably, HSP70 is found in the lysosomes of tumor cells but seldom in normal cells, where it stabilizes the lysosome by binding to its membrane, thus inhibiting LCD [185, 186]. Inhibiting HSP70 may promote LMP and tumor cell death [185].

In experiments with Hela cells treated with Apoptozole (Az), Az translocated to the lysosomes, induced LMP, and released cathepsins by targeting lysosomal HSP70. This mechanism facilitated the cleavage of the pro-apoptotic protein Bid into tBid and led to Bcl-2 degradation. These events activated Bax, induced MOMP, and released Cyt-C, culminating in cell death. Later stages of Az treatment saw further promotion of cell death as Az, released from compromised lysosomes, blocked the interaction between HSP70 and Apaf-1. Moreover, lysosomes are pivotal in autolysosome formation during autophagy, providing additional energy support to tumor cells. Az treatment increased LC3-II and p62 levels and autophagosome accumulation, indicating an impairment of the autophagy process [163]. Compound V8, a novel lysosome-targeting HSP70 inhibitor used in T-cell malignancy treatment, rapidly accumulates in lysosomes and binds directly to lysosomal HSP70. This binding could inhibit acid sphingomyelinase (ASM), an enzyme vital for lipid metabolism and lysosomal stability. ASM inhibition disrupted lipid metabolism and accumulated sphingomyelin, causing LMP and cathepsin release, thus triggering LCD. Furthermore, V8 impaired lysosomal function by reducing lysosomal acidity, enzymatic activity of acid phosphatase, and cathepsin expression, thereby inhibiting autophagy. In vivo studies supported the anticancer efficacy of V8 and demonstrated its low toxicity, underscoring its potential as a targeted therapy candidate [187].

In summary, cancer treatment strategies focused on inducing cell death have been extensively explored. The cases outlined here emphasize the critical role of promoting programmed cell death in cancer therapy and the unique value of HSPs in modulating the process of cell death.

Enabling replicative immortality

Limitless replicative potential is a hallmark of tumor cells, distinguishing them from normal cells. Unlike normal cells, which undergo senescence and cell death after a finite number of divisions, tumor cells exhibit continuous division and proliferation, thereby facilitating tumor

growth. Prior research has underscored the critical role of telomeres in enabling ongoing cell proliferation [188]. Positioned at the noncoding terminal regions of chromosomes, telomeres primarily safeguard the chromosome ends from end–to-end fusion, thus preserving genomic stability and integrity. Since DNA sequences at the chromosome ends are incompletely replicated and lost during each cell cycle, telomere length is considered a "timer" for how many more cell divisions can take place. In tumor cells, where cell division occurs more frequently, telomeres are consumed more rapidly. Consequently, telomere elongation is fundamental to achieving replicative immortality in tumor cells.

Telomere elongation is regulated by telomerase, a DNA polymerase that appends telomeric repeat sequences to chromosome ends [189, 190]. Human telomerase reverse transcriptase (hTERT), which serves as the catalytic subunit, translocates to the nucleus, where in conjunction with the telomerase RNA component (TERC), it forms the active site of the telomerase complex [191]. The presence and activity of hTERT are prerequisites for the functional operation of the telomerase complex.

Research has shown that HSP90 and its cochaperone p23 are associated with the activity of hTERT. HSP90 and p23 interact directly with hTERT to form an HSP90-p23-hTERT complex, facilitating a favorable conformational change during the nuclear translocation of hTERT [192]. Concurrently, immunophilin FK506-binding protein (FKBP) 52 connects the HSP90-hTERT heterocomplex to the dynein-dynactin motor complex, enhancing the nuclear import of hTERT along microtubules and its subsequent assembly with TERC into an active telomerase complex [193]. Conversely, HSP70 and the cochaperone C-terminus of HSC70-interacting protein (CHIP) bind to the inactive form of hTERT [194]. This binding event inhibits the nuclear translocation of hTERT by causing p23 dissociation and facilitates the ubiquitination and proteasomal degradation of hTERT by CHIP, reducing its cytoplasmic concentration and inhibiting telomerase assembly and activation. In addition, HSPs indirectly influence telomerase activity by modulating the phosphorylation of a client protein, Akt [195, 196]. The HSP90 inhibitor GA has been shown to degrade hTERT through the proteasome pathway, inhibiting the assembly and normal function of the active telomerase complex [197]. The depletion of functional HSP90 also leads to rapid telomere shortening and apoptosis induction [198, 199]. Thus, the critical reliance of telomerase and its components on HSPs suggests that targeting these proteins may offer a viable strategy for suppressing telomerase activity and treating cancer.

To date, studies focusing on directly reducing telomerase activity by heat shock protein inhibitors have yet to be reported [200]. However, there have been a few instances in which heat shock protein inhibitors used with other anticancer agents have been shown to indirectly modulate telomerase activity [201–203]. Furthermore, nanoparticle delivery systems containing the HSP90 inhibitor 17-allylamino-17-demethoxygeldanamycin (17-AAG) have demonstrated potential in eradicating residual breast cancer cells and preventing local tumor recurrence by diminishing telomerase activity and reducing HSP90 mRNA expression levels [204].

Inducing angiogenesis

Angiogenesis, defined as the formation of new blood vessels from the existing vasculature, is a crucial step in the invasion and metastasis of solid tumors [205, 206]. Angiogenesis provides tumor cells with oxygen and the necessary nutrients and facilitates the removal of metabolic waste, thus supporting tumor growth and migration. Hypoxia is widely recognized as the primary inducer of angiogenesis (Fig. 5a) [207]. Under hypoxic conditions, the activity of prolyl hydroxylase domain-containing proteins and factor-inhibiting HIF (FIH) is reduced, resulting in the decreased degradation of HIF through the proteasome pathway [208]. Consequently, HIF-α accumulates rapidly, translocates to the nucleus, and forms a heterodimer with HIF-1β. This complex then interacts with the coactivators p300/CBP and pyruvate kinase type.

M2 (PKM2) and binds to the hypoxia response element in gene promoters to initiate transcription, which leads to the release of various proangiogenic factors. These factors promote endothelial cell proliferation and the formation of new blood vessels. Among the well-known proangiogenic factors are vascular endothelial growth factor (VEGF) and its receptors, particularly VEGFR2, which are essential for VEGF-induced signal transduction in endothelial cells [209]. Layered binding to VEGFR2 triggers phosphorylation and activation of the receptor, facilitating several downstream signaling pathways, including the AKT, Src, FAK, p38 MAPK, and ERK1/2 pathways, and enhancing endothelial cell proliferation, migration, and vascular permeability. Additional secreted angiogenic signals, such as fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), angiopoietin-1 (Ang-1), and angiopoietin-2 (Ang-2), further regulate angiogenesis and complement VEGF signaling [205]. For example, PDGF is vital for pericyte and microvascular maturation, enhancing pericyte migration towards and around blood vessels and the production of a connective tissue matrix, thus supporting the formation of new blood vessels [210]. Moreover, PDGF and FGF also influence cell migration and adhesion, thereby improving the stability of the vascular wall, including pericytes and smooth muscle cells. Additionally, other

HIF-1-induced gene products, such as matrix metalloproteinases (MMPs), facilitate endothelial cell migration by degrading the extracellular matrix (ECM) and releasing associated growth factors, whereas specific integrins, such as αvβ3, mediate the attachment of proliferating endothelial cells to the provisional ECM, supporting endothelial cell invasion [211–213].

Research has demonstrated that HSPs are essential in tumor cell angiogenesis. The transcription factor HIF-1α requires HSP90 to enhance its stability at the protein level and inhibit its degradation via the proteasome [214–217]. HSP90 inhibition results in the degradation of HIF-1α and a subsequent reduction in the protein levels of its downstream effector, VEGF, thereby exhibiting an anti-proliferative effect [218]. Tumor growth and invasive metastasis depend on angiogenesis, which is a multi-step process; therefore, inhibiting any stage of this process can impede tumor progression. Given the critical role of HSPs in tumor angiogenesis, targeting these proteins presents a viable strategy for modulating angiogenesis in cancer therapy (Table 4). A work by Zhang et al. reported the anti-angiogenic properties of a Garcinia xanthones derivative, compound 25, which modulated the expression of its client protein HIF-1α by inhibiting HSP90, thereby suppressing angiogenesis in human umbilical vein endothelial cells (HUVECs). This compound also demonstrated significant anticancer activity in various cancer cells, including MCF-7, SK-BR-3, and PC-3, suggesting its potential as a small molecule with anti-angiogenic activity in cancer therapy [219]. Furthermore, Lee's team has explored deguelin's structural optimization and structure–activity relationships to identify potent anticancer small molecules. They discovered that a deguelin derivative, compound 5, exhibited anti-angiogenic effects by binding to the C-terminus of HSP90, which disrupted its chaperone interaction with HIF-1α, leading to the destabilization of HIF-1α and a consequent reduction in the expression of downstream target genes such as VEGF and Insulin-like growth factor 2 (IGF2). In vivo studies confirmed that compound 5 effectively inhibited angiogenesis in H1299 cells [220]. Additionally, compound 25, derived from structural modifications of deguelin, also demonstrated inhibitory activity against HIF-1α and antitumor effects in H1299 cells, with molecular docking results indicating that it targets HSP90 at residues Lys615 and Asn622. These findings suggest the potential of compound 25 to regulate HIF-1α levels as an HSP90 C-terminal inhibitor, although further studies are required to elucidate the detailed mechanism of action [221]. In related research, curcumin and its analogs, acting as HSP90 inhibitors, have shown anti-angiogenic effects. Specifically, the curcumin analog UBS109 was found to downregulate HSP90 expression in MIA PaCa-2 and PANC-1, affecting HIF-1α levels and decreasing the expression of VEGF, Ang-1, Ang-2, and transforming growth factor β (TGF-β). Both in vitro and in vivo evaluations confirmed that UBS109 significantly inhibited angiogenesis [222].

A work by Pang et al. has investigated the anticancer mechanisms of the chemotherapeutic agent β-lapachone. Their study indicated that β-lapachone induced cleavage of HSP90 through a redox cycling mechanism, distinct from the proteasomal degradation typically associated with traditional HSP90 inhibitors, in NQO1-expressing A549 and PC-3 cell lines. This process led to the degradation of crucial HSP90 client proteins, including RIP, AKT, and VEGFR2, via the proteasomal pathway. The efficacy of β-lapachone in inhibiting angiogenesis was confirmed by comparing the vascular length and angiogenic area in both in vitro and in vivo models. Significantly, β-lapachone has also demonstrated favorable safety profiles. These findings offer insights into the potential use

(See figure on next page.)

Fig. 5 Illustration of HSPs in the Other Hallmarks of Cancer. **a** HSPs in inducting angiogenesis. Under hypoxic conditions, hypoxia-inducible factor degradation is inhibited. Accumulated HIF-α translocates to the nucleus, activating transcription that leads to the secretion of multiple pro-angiogenic factors (e.g., VEGF, IGF) and downstream signaling pathways, thereby promoting vascular growth. HSP90 contributes to angiogenesis by enhancing the protein stability of HIF-α, preventing its degradation, and modulating the activity of proteins such as STAT3 and AKT. Additionally, GRP78 has been implicated in vascular growth. **b** HSPs in activating tumor invasion and metastasis. Signaling pathways (e.g., EGF, FGF) activate downstream transcription factors regulating EMT, leading to loss of epithelial polarity and increased mobility. Multiple HSPs, including HSP90, HSP70, HSP40, and HSP27, promote the EMT process by modulating transmembrane receptor tyrosine kinases (e.g., EGFR, FGFR), transcription factors (e.g., TWIST1, Slug), and calcium-dependent adhesion proteins (e.g., E-cadherin, N-cadherin). **c** HSPs in reprogramming energy metabolism. Under aerobic conditions, normal cells metabolize glucose via glycolysis in the cytoplasm to produce pyruvate, which then undergoes oxidative phosphorylation in the mitochondria to generate energy. Under anaerobic conditions, oxidative phosphorylation in normal cells decreases, and glycolysis produces energy. In tumor cells, regardless of oxygen, there is a tendency to produce energy through glycolysis, known as aerobic glycolysis. HSPs regulate critical factors involved in the metabolism of tumor cells. Indeed, HSP90 achieves metabolic reprogramming in tumor cells by interacting with various proteins, including PKM2, HK2, and PFKP. TRAP1 stabilizes HIF-1α by inhibiting SDH, promoting tumor progression. HSP60 supports tumor metabolism via DLST and mitochondrial integrity. **d** HSP90 in evading immune destruction. Dendritic cells take up extracellular antigens in the cell. The internalized antigens bind with HSP90 and are then released into the cytoplasm, ultimately degraded through the proteasome pathway

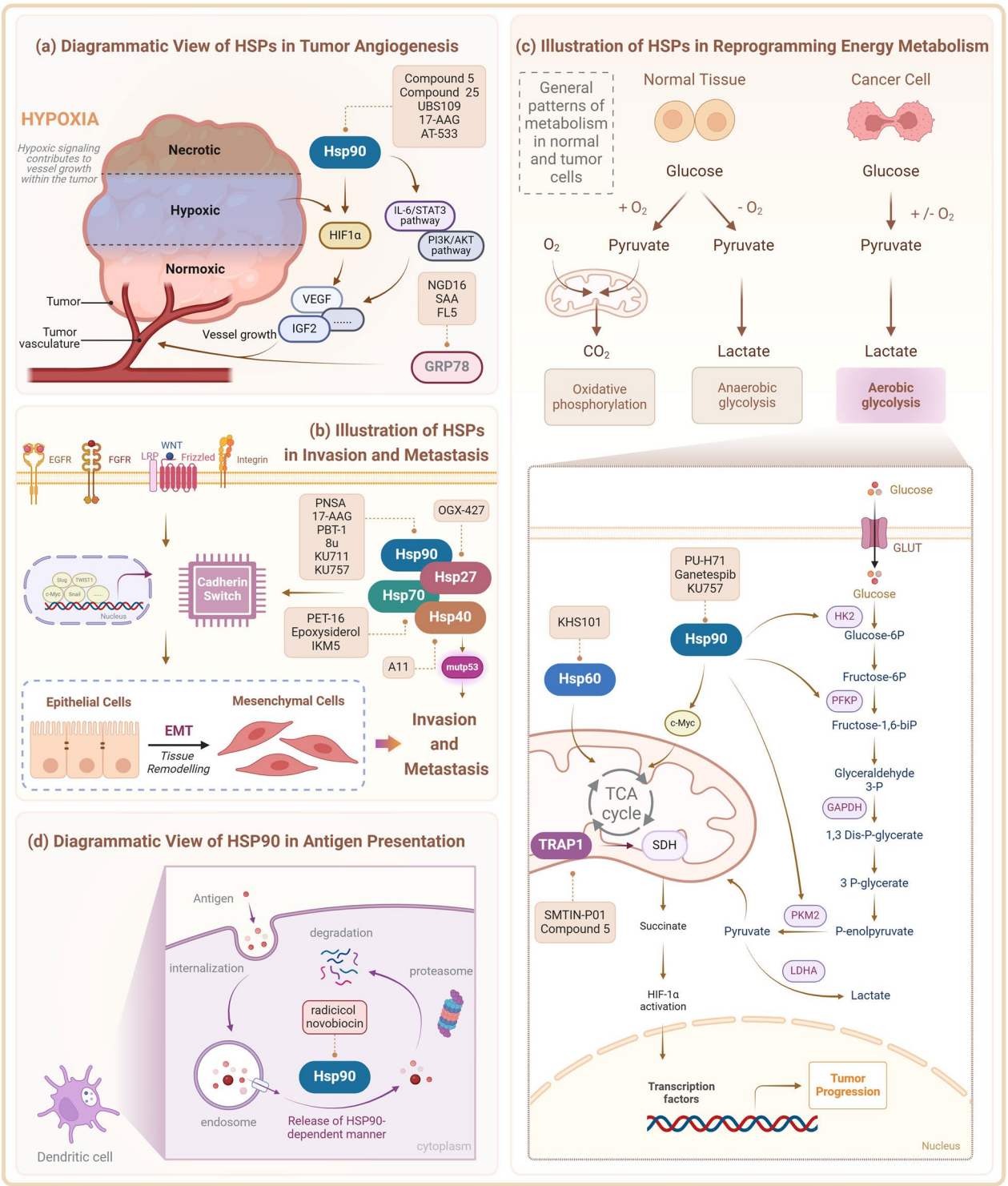
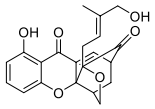
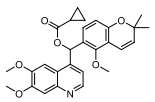
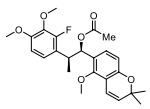
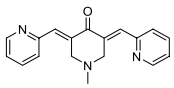
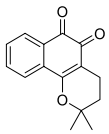
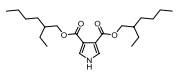
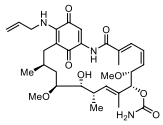
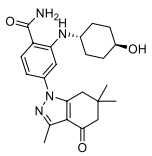
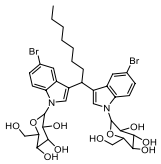
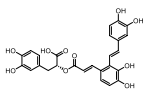
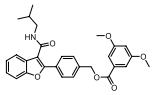


Fig. 5 (See legend on previous page.)

of β-lapachone in cancer therapy [223]. Heat shock factor 1 (HSF-1), a conserved transcriptional regulator in eukaryotes, is pivotal in regulating the HSR. Modulating HSF-1 levels via HSP90 has been demonstrated to exert anti-angiogenic effects. Similarly, direct inhibition of HSF-1 yields comparable effects and, to a certain extent, modulates HSP90 levels [224, 225]. A pyrrole derivative, TCCP, has been identified for its potential to inhibit

Table 4 Summary of HSP inhibitors regulating angiogenesis in cancer

Name	Structure	Target	Hallmarks of cancer	Cancer cell line	Tumor type	Ref
Compound 25		HSP90	Inhibit angiogenesis	MCF-7 (IC_{50} = 2.89 ± 0.18 μM), PC-3 (2.16 ± 0.36 μM)	Breast cancer Human prostate cancer	[219]
Compound 5		HSP90	Inhibit angiogenesis	H1299 (IC_{50} = 10 μM), HRMECs (100 μM)	Non-small-cell lung cancer	[220]
Compound 25		HSP90	Inhibit angiogenesis	H1299 (IC_{50} = 20 μM) HRMECs (0.1 μM)	Non-small-cell lung cancer	[221]
UBS109		HSP90	Inhibit angiogenesis	MIA PaCa-2 (0.25 μM), PANC-1 (0.25 μM)	Pancreatic cancer	[222]
β-lapachone		HSP90	Inhibit angiogenesis	A549 (6 μM) HUVECs (6 μM)	Lung cancer	[223]
TCCP		HSP90 HSP70	Inhibit angiogenesis	MDA-MB-231 (> 25 μM)	Breast cancer	[226]
17-AAG		HSP90	Inhibit angiogenesis	LPS863 (IC_{50} = 0.5 μM), 14GS-076 (IC_{50} = 0.5 μM)	Liposarcoma	[228]
AT-533		HSP90	Inhibit angiogenesis	MCF-7 (0–50 μM) MDA-MB-231 (0–50 μM) HUVECs (10 nM)	Breast cancer	[229]
NGD16		GRP78	Inhibit angiogenesis	PC-3 (IC_{50} = 0.8 μM)	Prostate cancer	[232]
Salvianolic acid A		GRP78	Inhibit angiogenesis	DLD1 (30 μM) HCT-116 (30 μM)	Colon cancer	[235]
FL5		GRP78	Inhibit angiogenesis	786-O (IC_{50} = 10 μM) HUVECs (EC_{50} = 1.514 μM	Renal cancer	[236]

angiogenesis. In breast cancer cells MDA-MB-231, TCCP inhibited both the expression and nuclear translocation of HSF-1, subsequently reducing the expression of HSP90 and HSP70 to varying degrees and exhibiting anti-tumor activity. The proposed underlying mechanism involved the phosphorylation of HSF-1, which might lead to AKT inactivation and altered expression of HSPs. Additionally, the phosphorylation of ERK1/2 appeared to contribute to these effects. While initial studies have revealed the anti-angiogenic potential of TCCP, detailed investigations into the specific mechanisms are still required [226].

In addition to exploring novel compounds, re-investigating the diverse mechanisms of action of known HSP inhibitors has become a focal point. The first HSP90

inhibitor to enter clinical trials, 17-AAG, has been extensively studied for its potential mechanisms in cancer therapy, particularly its effects on tumor angiogenesis [227]. Treatment with 17-AAG significantly reduced the phosphorylation levels of STAT3 in liposarcoma cells LPS863 and 14GS-076. STAT3, a well-recognized transcription factor, initiates the expression of various oncoproteins, such as VEGF, and triggers cascade reactions. This treatment also decreased the expression of multiple angiogenic factors, including VEGF, interleukin-6 (IL-6), IL-8, and VEGFR2, with IL-6 notably acting as an upstream factor stimulating STAT3 activity in tumor cells. These modifications in angiogenic factor levels further delineated the role of 17-AAG in blocking angiogenesis in liposarcoma. Besides, 17-AAG enhanced antitumor effects both in vitro and in vivo by modulating the AKT along with IL-6/STAT3/VEGF pathways [228]. Another study highlighted the efficacy of the known HSP90 inhibitor AT-533 in breast cancer treatment via its anti-angiogenic effects. In breast cancer cell lines MCF-7 and MDA-MB-231, AT-533 lowered HSP90 activity, diminishing HIF-1α activity. This reduction impaired the HIF-1α/VEGF/VEGFR-2 signaling pathway, impacting several downstream signaling factors, including ERK1/2, FAK, and AKT, and ultimately demonstrating its potential to inhibit breast cancer angiogenesis and exert anticancer activity. Notably, AT-533 showed superior anti-angiogenic effects compared to the classic HSP90 inhibitor 17-AAG in vitro [229].

Research has demonstrated that GRP78 promotes angiogenesis, and the targeted knockdown of GRP78 can significantly impede the vascular growth of tumor cells without markedly affecting normal cells [230, 231]. NGD16, a novel *N*-glycosylated derivative of Diindolylmethane, has been shown to downregulate the expression of GRP78 in PC-3 and HUVEC cells, consequently diminishing their angiogenic potential. Treatment with NGD16 led to decreased expression of pro-angiogenic proteins VEGF2 and MMP-9 in these cell lines while simultaneously increasing levels of the angiogenesis inhibitor tissue inhibitor of metalloproteinase-1 (TIMP-1). The expression levels of these proteins are strongly correlated with GRP78, which regulates the MMP-9 and VEGF-A response cascade. Remarkably, NGD16 demonstrated anti-angiogenic effects comparable to those of the tyrosine kinase inhibitor sunitinib. Still, it also offered better bioavailability and fewer toxic side effects, thus highlighting its potential as a tumor therapy via inhibition of HSPs. Although the precise mechanism by which NGD16 suppresses GRP78 is not fully understood and appears not to involve changes in mRNA levels, further investigation is necessary [232]. GRP78, traditionally recognized for its intracellular localization, has also been identified in the extracellular space, where it safeguards endothelial cells and facilitates tumor-associated angiogenesis within the tumor microenvironment [233, 234]. This extracellular activity of GRP78 suggests that targeting secreted GRP78 could represent an innovative strategy in cancer therapy. Additionally, Salvianolic acid A (SAA), identified through computational drug design methods including molecular docking, has been found to inhibit the secretion of GRP78 in cancer cells by binding to the specific residue K633 on GRP78 and promoting its degradation via the lysosomal pathway. This effect is observed in DLD1 and HCT-116 cells treated with SAA, leading to reduced vascular growth. Notably, the selective absence of secreted GRP78 in normal cells renders the anti-angiogenic action of SAA tumor-specific, a feature corroborated by favorable safety profiles in mouse models [235]. Furthermore, a small molecule compound, FL5, has emerged as a candidate with potential antitumor activity through selective inhibition of GRP78. Studies indicate that FL5 targets csGRP78 on the surface of 786-O cancer cells, demonstrating cytotoxic effects. FL5 also inhibits the activity of HUVEC cells expressing csGRP78, suggesting its broader anti-angiogenic properties and potential application in cancer treatment [236].

Activating invasion and metastasis

Tumor invasion and metastasis are highly complex, multistep phenomena that are often termed the invasion–metastasis cascade (Fig. 5b) [237, 238]. This process encompasses several stages, including epithelial–mesenchymal transition (EMT), alterations in cell adhesion and motility, matrix remodelling, local invasion, extravasation, and micrometastasis formation [237]. EMT involves the transformation of epithelial cells from a polarized, immotile state to a mesenchymal phenotype, which is characterized by the loss of their inherent polarity and adhesion characteristics and increased mobility and invasiveness [239, 240]. EMT is a pivotal mechanism in tumor progression that is regulated by an array of critical molecules and signaling pathways, including the TGF-β pathway, the EGF/EGFR pathway, and the Wnt/β-catenin pathway [241–243]. TGF-β is a key regulator of EMT, as it facilitates this transition via both Smad-dependent and Smad-independent pathways and their downstream signals [244–246]. Concurrently, E-cadherin has been recognized as a marker of EMT, with its upregulation considered an antagonist of invasion and metastasis; however, increased expression of *N*-cadherin and vimentin serves as an initiator in most malignancies [247, 248]. Additionally, activation of the Wnt signaling pathway significantly contributes to EMT induction [249]. The canonical Wnt/β-catenin pathway impairs β-catenin degradation, leading to the accumulation and translocation

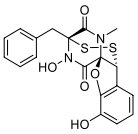
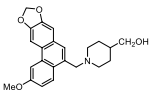
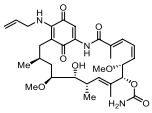
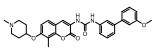
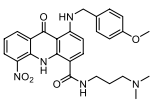
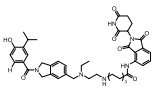
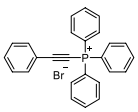
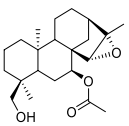
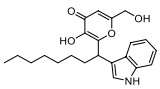
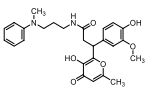
of β-catenin to the nucleus. Once there, β-catenin forms a complex with lymphoid enhancer factor/T-cell factor (LEF/TCF), initiating the transcription of downstream target genes, such as c-Myc, Cyclin D1, Snail, and Slug. In addition to these signaling entities and cytokines, the protease system is crucial in EMT. Key enzymes, including matrix metalloproteinases (e.g., MMP-2 and MMP-9) and urokinase, degrade collagen and fibronectin in the extracellular matrix, facilitating tumor cell migration [250].

The complexity of tumor cell invasion and metastasis mechanisms has been extensively studied, and critical proteins involved have been identified as primary targets for anti-cancer drug development. HSPs are central to tumor invasion and metastasis, serving as chaperones for critical regulatory factors [251–254]. Consequently, targeting HSPs to inhibit these processes has become a focal point in contemporary research, yielding promising results (Table 5). PNSA, a C-terminal inhibitor of HSP90, has demonstrated significant anti-tumor efficacy in the HCT116 cell xenograft model [90]. Research by Li and colleagues revealed that PNSA dose-dependently inhibited the invasion and metastasis of breast cancer cells. Post-treatment, EMT in MDA-MB-231 cells was reversed, evidenced by increased E-cadherin and decreased *N*-cadherin, vimentin, c-Myc, MMP-2, and MMP-9 levels. PNSA also reduced the phosphorylation of ERK and AKT and the protein levels of β-catenin and its upstream receptors, EGFR and FGFR, via HSP90 inhibition without upregulating the protective HSP70. Further studies suggested these protein alterations were linked to proteasomal degradation following HSP90 inhibition. Additionally, PNSA curbed the invasive capabilities of trastuzumab-resistant breast cancer cells, JIMT-1, warranting further investigation as a potential breast cancer treatment [255]. Phenanthrene-based tylophorine derivative-1 (PBT-1) was initially identified as a potential anti-tumor agent for lung adenocarcinoma [256]. Subsequent research by Yang et al. explored PBT-1's direct targets and its role in attenuating lung adenocarcinoma. PBT-1 inhibited metastasis in lung adenocarcinoma cells, CL1-5, potentially by binding to HSP90 in these cells to suppress Slug expression, thereby enhancing E-cadherin expression and reducing AKT phosphorylation. In vitro and in vivo experiments confirmed PBT-1's ability to suppress the invasive and metastatic capabilities of CL1-5 and reduce filopodia formation. Importantly, PBT-1 displayed minimal toxicity, though further research is necessary to elucidate its specific binding mode with HSP90 and the complete anti-metastatic mechanism [257]. As an established HSP90 inhibitor, 17-AAG remains integral in various cancer research studies. It has been shown to inhibit tumor sphere formation in diverse cancer cell lines, including ovarian cancer lines A2780, SKOV3, and renal cancer lines RCC4 and A498. Mechanistic insights suggested that 17-AAG might inhibit TWIST1 transcription and reverse EMT by disrupting the HSP90-STAT3 interaction, thereby reducing migration and invasion. However, the detailed mechanisms remain to be further elucidated [258]. Cohen et al. reported that HSP90 C-terminal inhibitors, KU757, also demonstrated anti-migration and anti-invasion properties against various head and neck squamous cell carcinoma (HNSCC) lines. Treatment with KU757 inhibited the EMT process in cell lines MDA-1986, UMSCC 22B, and UMSCC 22B-CIS [259].

The HSP90 isoform, HSP90α, is currently being investigated as a potential target for inhibiting tumor metastasis. An acridone derivative, compound 8u, has been demonstrated to directly bind to HSP90α, leading to a reduction in its expression and consequently inhibiting the invasion and migration of liver cancer cells HepG2. Treatment with 8u resulted in decreased HSP90α levels, increased E-cadherin expression, and reduced expression of β-catenin, *N*-cadherin, vimentin, Slug, and Snail. The inhibition of the transcription factors Snail and Slug partially mitigated their repressive effects on E-cadherin, supporting the anti-metastatic role of 8u. Furthermore, it has been observed that the PI3K/AKT signaling pathway might contribute to the anti-metastatic effects of 8u in liver cancer, with silencing of HSP90α markedly lowering phosphorylated AKT levels [260]. Recognizing the critical role of HSP90α in tumor migration and metastasis and addressing the toxicity limitations of pan-HSP90 inhibitors, Wu et al. have developed a potent and selective HSP90α degrader, compound X10g, utilizing proteolysis-targeting chimera technology. Preliminary studies indicated that X10g exerted anti-tumor effects against various breast cancer cell lines by targeting the ASP292 residue of HSP90α, displaying low toxicity and favorable pharmacokinetic properties. These findings suggested that the selective degradation of HSP90α by X10g represented a novel approach for breast cancer treatment through anti-invasion therapy [261].

Melanoma is characterized by high invasiveness, and it has been demonstrated that HSP70 is highly expressed in this cancer type. Murphy et al. investigated the therapeutic potential of the HSP70 inhibitor PET-16 in melanoma. They discovered that pFAK acts as a novel client protein of HSP70. Inhibition of HSP70 by PET-16 led to decreased pFAK levels, thereby diminishing the metastatic capabilities of melanoma. In vivo, experiments in mice supported this finding, which confirmed the anti-migration, invasion, and metastatic effects of PET-16 on melanoma [262]. In a separate study, Epoxysiderol was identified as a new modulator of Hsp70. This compound

Table 5 Summary of HSP inhibitors regulating invasion and metastasis in cancer

Name	Structure	Target	Hallmarks of cancer	Cancer cell line	Tumor type	Ref
PNSA		HSP90	Inhibit invasion and metastasis	MDA-MB-231 (IC_{50} = 0.86 μM)	Breast cancer	[255]
PBT-1		HSP90	Inhibit invasion and metastasis	CL1-5 (1.32 μM)	Lung adenocarcinoma	[257]
17-AAG		HSP90	Inhibit invasion and metastasis	SKOV3 (0.5 μM) A2780 (0.5 μM) OVC203 (0.5 μM)	Ovarian cancer	[258]
KU757		HSP90	Inhibit invasion and metastasis	MDA-1986 (1 μM) UMSCC 22B (1 μM) UMSCC 22B-*cis* (1 μM)	Head and neck Squamous Cell Carcinoma	[259]
Compound 8u		HSP90α	Inhibit invasion and metastasis	HepG2 (> 1 μM)	Hepatocellular carcinoma	[260]
X10g		HSP90α	Inhibit invasion and metastasis	MDA-MB-231 (IC_{50} = 51.48 ± 4.42 μM), MDA-MB-468 (IC_{50} = 16.46 ± 3.54 μM), MCF-7 (IC_{50} = 8.93 ± 4.36 μM)	Breast cancer	[261]
PET-16		HSP70	Inhibit invasion and metastasis	1205Lu (1 μM) B16-F10 (3 μM) WM852 (1 μM)	Melanoma	[262]
Epoxysiderol		HSP70	Inhibit invasion and metastasis	HeLa (IC_{50} = 20.2 ± 0.9 μM)	Cervical cancer	[264]
IKM5		GRP78	Inhibit invasion and metastasis	MCF-7 (IC_{50} = 0.150 ± 0.095 μM) MDA-MB-231 (IC_{50} = 0.209 ± 0.265 μM)	Breast cancer	[266]
A11		DNAJA1	Inhibit invasion and metastasis	CAL33 (IC_{50} = 13.8 μM), KHOS/NP (IC_{50} = 16.6 μM), Huh7 (IC_{50} = 20.7 μM)	Multiple cancers	[267]

inhibited the ATPase activity of Hsp70 by binding to it, notably affecting its function, especially on the plasma membrane of HeLa cells. Given that HSP70 expression on the plasma membrane is associated with tumor metastasis promotion, Epoxysiderol might also serve as an anti-metastatic agent for cancer treatment [263, 264]. Recent studies have also highlighted the inhibition of GRP78 as a strategy to suppress cancer cell migration and invasion [265]. An indolylkojyl methane analog, IKM5, was found to reduce GRP78 expression in various breast cancer cell lines, including MDA-MB-231, MDA-MB-468, and MCF7, thereby reducing their invasive potential. IKM5 not only directly bound to GRP78, reducing its levels, but also disrupted the interaction between GRP78 and the negative regulator of MMPs, TIMP-1. This interaction was mediated by upregulation of the tumor suppressor protein prostate apoptosis response 4(Par-4), which reduced MMP-2 and MMP-9 levels, decreased vimentin, and increased E-cadherin expression in treated MDA-MB-231 cells. In vitro experiments supported these mechanisms and the therapeutic potential of IKM5, with preliminary assessments indicating reliable safety and favorable pharmacokinetic properties, suggesting further exploration of IKM5 as a cancer treatment option [266].

Iwakuma and colleagues investigated a compound, A11, which targeted the HSP40 family member DNAJA1 and significantly curtailed cancer cell metastasis. Their findings indicated that A11 could bind to DNAJA1, promoting its proteasomal degradation. This interaction reduced levels of conformationally altered mutp53,

thus exhibiting anti-metastatic activity. In cancer cells expressing DNAJA1 and conformational mutp53 and treated with A11, there was a noticeable inhibition in filopodia formation and migration capacity. Importantly, A11 did not significantly impact cells with wild-type p53 or DNA-contact mutp53, highlighting its specificity. Thus, A11 emerges as a promising HSP40 inhibitor for treating cancers that express conformational mutp53 [267].

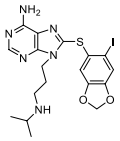
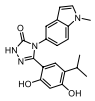
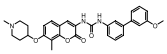
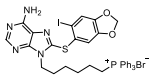
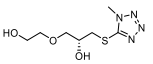
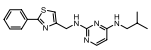
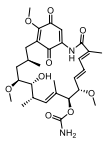
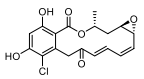
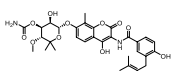
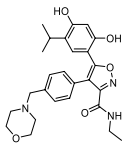
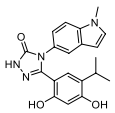
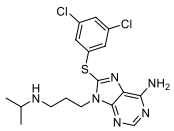
Additionally, the overexpression of HSP27 in liver cancer cell lines, has been linked to enhanced cancer progression [268]. OGX-427, an HSP27 inhibitor, was explored to assess its anti-cancer efficacy. Both in vitro and in vivo studies demonstrated that OGX-427 treatment reduced HSP27 levels in MHCC97H and HCCLM3 cells, decreasing their migration and invasion capabilities. Mechanistically, OGX-427 inhibited the phosphorylation of AKT, leading to the blockade of the AKT signaling pathway, reflected in the downregulation of ITGA7 and MMP2 genes, which are closely related to HSP27-mediated invasion and metastasis in liver cancer. These findings underscore the potential of OGX-427 as a therapeutic agent for mitigating invasion and metastasis in hepatocellular carcinoma [269].

Reprogramming energy metabolism

Reprogramming energy metabolism has been increasingly recognized as an emerging hallmark of cancer (Fig. 5c) [21]. Under normoxic conditions, normal cells mainly perform glycolysis in the cytoplasm and oxidative phosphorylation in the mitochondria to meet energy demands. Conversely, oxidative phosphorylation is curtailed under anaerobic conditions, with glycolysis becoming the predominant energy source. Unlike normal cells, cancer cells prefer glycolysis for energy generation even under aerobic conditions, a phenomenon known as the Warburg effect or aerobic glycolysis [270]. This preference is often viewed as overflow metabolism, where cancer cells excessively consume nutrients to support their energy needs for uncontrolled proliferation and metastasis [271]. In addition, cancer cells rely on increased fatty acid synthesis and glutamine metabolism in addition to aerobic glycolysis to fulfill their energy requirements [272, 273]. Recent research has shed light on several metabolic determinants crucial for cell proliferation, including nutrient uptake, macromolecule biosynthesis, and electron carrier regeneration, which involve numerous enzymes and metabolic pathways [271]. These results align with the definition of metabolic reprogramming by Hanahan and colleagues as an emerging hallmark—much of which it is related to the roles of proteins. For example, the uptake of nutrients, which is primarily initiated by RTKs, is regulated through the PI3K/AKT signaling pathway, and the RTK downstream signaling protein RAS can also augment glycolysis by modulating glucose transporter 1 (GLUT1) [274–277]. Additionally, the growth demands of tumor cells necessitate the de novo synthesis of essential macromolecules, which is often regulated by transcriptional and signaling pathways controlled by factors such as MYC and mTOR [278, 279]. These insights highlight the importance of protein-driven metabolic reprogramming in cancer and the potential for targeting specific metabolic pathways with protein inhibitors.

Tumor cells undergo metabolic reprogramming that is characterized by modifications in the activity and expression of numerous metabolic enzymes, culminating in alterations in proteostasis. Consequently, HSPs, which serve as chaperones, are pivotal in maintaining cellular proteostasis and significantly influence tumor cell metabolism. Recent investigations have elucidated the association between HSP90 and cellular energy metabolism, notably the mitochondrial HSP90 family member TRAP1 [280]. TRAP1 plays a crucial role in regulating critical metabolic pathways, facilitating tumor metabolic rewiring by enhancing the pentose phosphate pathway and gluconeogenesis, which support tumor survival under conditions such as hypoxia and nutrient scarcity, thereby promoting tumor cell proliferation [281, 282]. TRAP1 also maintains mitochondrial function, which is crucial for mitochondrial metabolism [283, 284]. As a molecular chaperone, TRAP1 influences the activities of its client proteins, cytochrome c oxidase (COX) and succinate dehydrogenase (SDH), which regulate the expression of GLUT1 [285]. In summary, TRAP1 significantly contributes to energy metabolism in tumor cells from multiple aspects. Additionally, HSP90 interacts with various metabolic enzymes, including glyceraldehyde-3-phosphate dehydrogenase (GAPDH), PKM2, and GLUT1, to facilitate metabolic reprogramming and sustain cell proliferation [286–288]. Studies have also focused on the role of HSP90 in the interaction between metabolism and epigenetics; although this subject has not yet been thoroughly explored, the significant contributions of HSP90 to cellular metabolism and the metabolic reprogramming of cancer cells have been substantiated [289]. As part of the HSP70 family, the ER-resident protein GRP78 adapts to adverse environments and sustains tumor cell proliferation by modulating GLUT1 and PKM2 expression under glucose deficiency [290, 291]. Concurrently, the important role of HSP60 in proteostasis control underscores its potential as a target for anticancer metabolic therapy [292]. Although few studies have linked HSP40 with cellular metabolism, the association of HSP40 with the PKM2 isoform renders it unstable, consequently reducing the levels of its downstream molecule, PDK1, which adversely affects the survival of cancer cells [293]. The

Table 6 Summary of HSP inhibitors regulating energy metabolism and immunity in cancer

Name	Structure	Target	Hallmarks of cancer	Cancer cell line	Tumor type	Ref
PU-H71		HSP90	Inhibit energy metabolism	OCI-LY1 (0.5 μM) OCI-LY7 (0.5 μM)	B-cell lymphomas	[295]
Ganetespib		HSP90	Inhibit energy metabolism	HN6 (0.1 μM) HN12 (0.1 μM)	Head and neck squamous cell carcinoma	[296]
KU757		HSP90	Inhibit energy metabolism	WRO-LvR (IC_{50} = 1.619 ± 0.12 μM	Thyroid cancer	[297]
SMTIN-P01		TRAP1	Inhibit energy metabolism	MDA-MB-231 (2 μM), Hela (2 μM), SK-OV3 (2 μM), H460 (2 μM)	Multiple cancers	[298]
Compound 5		TRAP1	Inhibit energy metabolism	sMPNST (100 μM)	Malignant peripheral nerve sheath tumor	[299]
KHS101		HSPD1	Inhibit energy metabolism	GBM1 (7.5 μM)	Glioblastoma	[300]
Geldanamycin		HSP90	Induce immune destruction	McCAR (1 μM) ARP (1 μM)	/	[306]
Radicico		HSP90	Induce immune destruction	BMDCs (25 μM)	/	[309]
Novobiocin						
AUY-922		HSP90α	Induce immune destruction	MDA-MB-231, TC-1, PC3, H1299, HCT116, A375	Multiple cancers	[313]
Ganetespib		HSP90	Induce immune destruction	THP-1 (> 10 nM)	Murine colon adenocarcinoma	[314]
PU-WS13		HSP90β1	Induce immune destruction	H1299 (50 μM) MV4-11 (75 μM)	Lung cancer Leukemia	[315]

associations between HSPs and cancer cell energy metabolism collectively affirm the potential of HSPs as effective targets for anticancer metabolic therapy. Reports have also indicated that inhibitors targeting HSPs may negatively impact energy metabolism in cancer cells, thereby potentially aiding the development of novel anticancer metabolic therapies (Table 6).

For instance, PU-H71, an inhibitor of HSP90, has been found to suppress glycolysis in the lymphoma cell lines OCI-LY1 and OCI-LY7, as evidenced by a reduction in glucose uptake and lactate secretion. Concurrently, decreased basal oxygen consumption suggested compromised mitochondrial respiration. Further investigation revealed that PU-H71 might disrupt MYC-induced metabolic programs by targeting HSP90, with MYC supporting the energy metabolism in cancer cells [294]. This finding suggested a novel antitumor mechanism of PU-H71 through metabolic reprogramming [295].

Similarly, Ganetespib, another well-studied HSP90 inhibitor, also positively affected the metabolism of cancer cells. Ganetespib inhibited the protein levels of key glycolytic enzymes PKM2 and phosphofructokinase platelet (PFKP) by suppressing HSP90, thereby inhibiting the energy metabolism of cancer cells. After treatment with Ganetespib, the levels of PKM2 and PFKP in HNSCC cell lines HN6 and HN12 were decreased, and this process was proven to be HSP90-dependent. Ultimately, both cell lines significantly reduced glycolytic flux and glucose uptake. Notably, a strong correlation was observed between HSP90 gene expression and the expression of these glycolytic enzymes, supporting the continued exploration of HSP90 inhibitors in cancer metabolism research [296]. Another study emphasized the impact of the HSP90 C-terminal inhibitor KU757 on lenvatinib-resistant thyroid cancer cells, demonstrating that treatment led to reduced protein levels of glycolysis-related enzymes lactate dehydrogenase A (LDHA), hexokinase 2 (HK2), PKM1/2 and decreased expression of genes LDH, PKM2, HK2. Consequently, glycolytic rate and capacity in the drug-resistant WRO-LvR cells were suppressed [297]. These findings underscore the potential of HSP90 inhibitors in targeting cancer metabolism and expand the possibilities for HSP90 as an anticancer target.

Kang et al. developed a small molecule inhibitor, SMTIN-P01, targeting mitochondrial chaperonin TRAP1 in cancer cells. This compound, a conjugate of the HSP90 inhibitor PU-H71 with triphenylphosphonium, transformed the pan-HSP90 inhibitor into a specific inhibitor and exhibited enhanced cytotoxicity compared to PU-H71 alone. SMTIN-P01 was observed to preferentially accumulate within mitochondria, leading to mitochondrial membrane depolarization in HeLa liver cancer cells. While the direct impact of SMTIN-P01 on the energy metabolism of HeLa cells was not explicitly described, the findings suggested that the conjugate could interfere with cancer cell energy metabolism, considering TRAP1's role in regulating oncogenic metabolic pathways [298]. Additionally, another study emphasized the importance of selectively targeting TRAP1 to disrupt cancer cell metabolic networks. This research introduced selected allosteric inhibitor 5 of TRAP1 that restored TRAP1-dependent inhibition of SDH activity by disrupting the interaction between TRAP1 and SDHA, which enhances HIF-α stability. Both in vitro and in vivo evaluations demonstrated that allosteric inhibitors targeting TRAP1 could effectively suppress its oncogenic potential and prevent tumor formation [299].

In the ongoing investigation of KHS101, a promising agent for treating glioblastoma (GBM), researchers have found that KHS101 induced cytotoxicity in cancer cells by disrupting their energy metabolism pathways. Specifically, KHS101 could selectively bind to the mitochondrial chaperone protein HSPD1, leading to the aggregation of HSPD1 and its client proteins and disrupting the protein network centered on HSPD1. This interference affected vital enzymes, including aldolase, fructose-bisphosphate A, aldolase A, involved in glycolysis regulation; dihydrolipoamide S-succinyltransferase (DLST) in the tricarboxylic acid (TCA) cycle; and ATP synthase F1 subunit alpha (ATP5A1), which is crucial for oxidative phosphorylation. Consequently, this results in a selective impairment of mitochondrial bioenergetics and glycolysis in GBM cells. The demonstrated efficacy and safety of KHS101 in xenograft models underscored its potential as a valuable anticancer drug, warranting further investigation [300].

The case mentioned above exemplifies the potential of targeting HSPs to disrupt cancer cell energy metabolism, a promising strategy for cancer treatment. Nevertheless, the intricacy of cellular metabolic networks and the similarity between the metabolic pathways of cancerous and normal cells present significant challenges in developing antitumor metabolic agents. Continued mechanistic studies and exploring potential small molecules are crucial to advancing treatment strategies in oncology.

Evading immune destruction

Immune evasion is a critical mechanism by which cancer cells evade detection and destruction by the host immune system, facilitating their proliferation and metastasis [21, 301, 302]. This process employs various strategies: modification of tumor antigens to evade immune recognition; creation of an immunosuppressive microenvironment that impairs immune cell functionality; activation of immune checkpoint pathways to inhibit immune responses; manipulation of major histocompatibility complex (MHC) molecules to impede effective antigen presentation; and immune editing, wherein tumors undergo a selective process under immune pressure that leads to the survival of cells with augmented immune evasion capabilities. Collectively, these mechanisms contribute to unchecked tumor growth, which presents substantial challenges in cancer therapy and underscores the need for therapeutic approaches that can overcome or bypass these immune evasion strategies [303–305]. HSPs help tumor cells manage intrinsic and extrinsic environmental stresses, such as hypoxia and chemotherapeutic exposure, by preventing the aggregation of misfolded proteins, thereby increasing tumor cell survival. Additionally, HSPs can alter tumor-immune system interactions by modulating immune cell functions and the microenvironment, further contributing to tumor immune evasion (Table 6).

In the assessment of the effects of Hsp90 inhibition on human T lymphocytes and natural killer cells, Munshi et al. discovered that Hsp90 inhibition led to the irreversible downregulation of crucial antigens such as CD3, CD4, and CD8, co-stimulatory molecules including CD28 and CD40L, and αβ receptors on T lymphocytes. Additionally, it activated receptors like CD2, CD11a, CD94, NKp30, NKp44, NKp46, and KARp50.3 on NK cells. These findings indicated that Hsp90 inhibition altered T lymphocytes and NK cells' expression profiles and functional activities of antigens, resulting in immunosuppression [306]. This concept was supported by further studies, which proposed that HSP90 inhibition might impair immune effector functions, including those of T cells, NK cells, and potentially B cells [307]. Additionally, Lee et al. reported that the expression levels of GRP78 might influence the susceptibility of tumor cells to T-cell-mediated immune responses, and inhibiting GRP78 could enhance T-cell immune surveillance and rejection of tumors [308].

In antigen presentation, the Hsp90 family plays an integral role. Udono and colleagues demonstrated that it experiences a reduction in cross-presentation and priming capabilities in HSP90α lacking dendritic cells (DCs) and mice. Moreover, in DCs deficient in HSP90α or treated with HSP90 N-terminal inhibitor radicicol and HSP90 C-terminal inhibitor novobiocin, there is a diminished translocation of antigens from endosome to the cytoplasm, which is mediated by HSP90 (Fig. 5d). These results affirmed the essential role of HSP90α in the cross-presentation function of DCs [309]. Research by Shastri et al. uncovered large, C-terminally extended protein degradation intermediates in the antigen processing pathway, which are associated with the molecular chaperone Hsp90α. Using siRNA technology to reduce Hsp90α expression, they noted the disappearance of these intermediates and a reduced presentation of peptide-loaded MHC I complexes on the cell surface [310]. Moreover, inhibition of HSP90 has been shown to downregulate MHC II-mediated antigen presentation by antigen-presenting cells to both endogenous and exogenous peptides [311]. Additionally, a study by Blum et al. indicated that the HSP70 family member HSC70 also participated in the MHC II antigen presentation pathway [312]. Collectively, these studies suggested that HSPs play critical roles in both MHC I and MHC II antigen presentation, illustrating that inhibition of HSPs can significantly impact immune responses across various therapeutic modalities.

HSPs are being explored in terms of their role in modulating immune checkpoints. Kim et al. demonstrated that inhibition of HSP90 with AUY-922 sensitized immune-resistant tumors to adoptive T cell transfer and programmed death 1 (PD-1) blockade, reactivating the immune cycle of tumor-reactive T cells. Specifically, targeting HSP90A enhanced the efficacy of immune checkpoint blockade (ICB) therapy by reversing the immunoresistance of tumor cells to cytotoxic T lymphocyte mediated killing, transitioning the immunophenotype from non-T cell-inflamed to T cell-inflamed tumors. This result indicated that HSP90A signaling is a promising target for combination therapies with immunotherapy [313]. Additionally, research by Lairson and colleagues found that an HSP90 inhibitor, Ganetespib, significantly reduced the surface expression of programmed death ligand-1 (PD-L1) through mechanisms involving the regulation of master transcription factors, specifically STAT-3 and c-Myc and also affected the expression of other checkpoint proteins, such as PD-L2 [314]. Zhang et al. reported that the expression of HSP90B1 in most tumors negatively correlates with CD8 + T cell infiltration. Notably, the HSP90B1 inhibitor PU-WS13 effectively inhibited the proliferation of cancer cells in leukemia and solid tumors and significantly reduced the expression of the immune checkpoint protein PD-L1 on the surface of these cells [315].

In summary, over the past decade, the potential of HSPs as targets for immunological interventions has been progressively revealed due to the rapid expansion of the field of immunotherapy [307, 316]. Notably, tumor immunology that leverages HSPs has experienced significant advancements, including the development of anticancer vaccines and the use of molecular chaperone-based immunotherapy combined with immune checkpoint inhibitors [317–320]. Indeed, gaining a deeper understanding of the mechanisms through which HSPs facilitate tumor immune evasion will certainly enhance the design of more effective immunotherapeutic strategies for clinical application.

Targeting HSPs with inhibitors regulates multiple acquired biological capabilities in *cancer*

HSPs function as molecular chaperones by interacting with client proteins to modulate protein stability and facilitate various physiological processes. HSPs regulate a diverse array of proteins with different roles, including transmembrane tyrosine kinases (e.g., EGFR and HER), the metastable signaling protein AKT, mature signaling proteins (such as p53), and cell cycle regulatory factors (e.g., CDK4 and CDK6) [24]. These client proteins are integral to the complex signaling pathways involved in all tumor development phases. Owing to the intricate and extensive interactions among these signaling molecules, HSPs contribute to tumor progression due to their multifaceted roles and through crosstalk among multiple critical biological processes. Consequently, targeting HSPs could offer a spectrum of anticancer effects, such

as inhibiting cell growth and proliferation, promoting cell death, reducing cell migration and invasion, and impacting energy metabolism [31, 321]. This section describes advances in the development of small-molecule inhibitors that target HSPs to exert multifaceted anticancer effects. These persistent research endeavors continue to inspire innovative approaches for creating small-molecule inhibitors targeting HSPs and advancing cancer treatment.

HSP90 is an extensively studied and characterized member of the heat shock protein family, pivotal in stabilizing numerous client proteins. Crucially, targeting HSP90 disrupts multiple biological pathways critical in tumor development, offering a broader therapeutic impact than conventional single-target approaches. This strategy also potentially reduces the development of drug resistance in tumor cells. Developing small molecule inhibitors that target HSP90 to modulate multiple signaling pathways has yielded promising outcomes. Naturally derived and synthetically designed small molecule inhibitors have demonstrated significant therapeutic potential. For instance, cordycepin, derived from Cordyceps, exhibits antibacterial, anti-inflammatory, antiviral, and antitumor properties [322]. Studies by Eberhart et al. indicated that cordycepin affected the low adenosine deaminase uveal melanoma cell line by inhibiting cell growth, inducing apoptosis, reducing tumor migration, and decreasing colony formation. Molecular docking studies suggested that cordycepin could bind to the HSP90 N-terminal, competing with ATP, leading to increased protein ubiquitination, particularly of the HSP90 client protein Akt, and affecting the protein levels of HIF-1α, Akt, ERK, and EGFR [323]. Theaflavin-3-gallate, identified as a natural HSP90 inhibitor, has been evaluated as a potential breast cancer treatment. It inhibited cell proliferation, cell cycle progression, metastasis, invasion, and induced DNA damage and cancer cell death, with MMP9 and VEGFA pathways playing crucial roles in its anticancer effects. However, the specific molecular mechanisms of theaflavin-3-gallate in conjunction with HSP90 inhibition remain to be entirely determined [324]. Furthermore, Saucerneol (SN), derived from Saururus chinensis, has shown efficacy in treating nasopharyngeal carcinoma (NPC). Gu et al. reported that SN notably arrested the cell cycle in the HONE1 cell line by reducing CDK2 and CDK4 levels and inhibited proliferation and metastasis by selectively binding to GRP94, thereby disrupting the PI3K/AKT/mTOR pathway. In mouse models, SN exhibited significant anti-NPC activity with low toxicity [325].

Rational drug design is a viable approach for developing novel small-molecule inhibitors. SNX2112, an HSP90 inhibitor previously identified for its anti-proliferative effects in cancer cells, has been explored as a potential therapeutic agent in various tumors [326]. Advanced papillary renal cell carcinoma (PRCC) treatment needs standardized, practical methods, leading researchers to investigate potential therapeutic candidates [327]. In studies, PRCC cells treated with SNX2112 displayed inhibited growth, cell cycle arrest, and induced apoptosis. Additionally, a reduction in the levels and phosphorylation of several HSP90 client proteins, including MET, AKT, and ERK1/2, was observed [328]. Cao et al. clarified the crystal structure of the HSP90-SNX2112 complex via X-ray diffraction, demonstrating the effective binding of SNX2112 to the N-terminal ATP-binding site of HSP90, thus inhibiting its chaperone function. Preliminary pharmacological assessments showed that SNX2112 effectively inhibited proliferation, arrested the cell cycle, and induced apoptosis in NSCLC cells [329]. Despite its potential, GA, a previously explored drug, did not progress to clinical trials due to significant toxicity. Yu et al. improved the structure of GA using Discovery Studio and total synthesis techniques, resulting in a more potent anti-tumor molecule, compound 1, with enhanced activity and reduced toxicity. Compound 1 induced cell cycle arrest and apoptosis, with a notable decrease in the expression of HSP90 client proteins such as EGFR and IGFR and their downstream pathways AKT and ERK1/2, which are crucial in tumor progression [330]. Compound C4, featuring a 2-aniline thiazole structure, was screened for its efficacy against NSCLC. Experiments indicated that C4 could selectively target HSP90, leading to the degradation of its client proteins β-Catenin and Bcl-2 and exerting effects such as inhibiting cell proliferation, metastasis, and inducing apoptosis [331]. Catechol derivatives as HSP90 inhibitors have shown limited anti-cancer capabilities, necessitating further structural modifications. Liou's team explored derivatives with an open-ring structure, identifying 6b as possessing diverse anti-cancer functions with minimal to no ocular toxicity. 6b was effective in arresting the cell cycle, inhibiting migration, and inducing apoptosis in A549 lung cancer cells. Treatment with 6b reduced levels of various HSP90 client proteins and disrupted AKT signaling [332]. In another study, a novel quinoline-catechol HSP90 inhibitor, compound 11, was evaluated. Immunoblotting experiments indicated that treatment with compound 11 decreased the expression of EGFR, SRC, FAK, and Rb in prostate cancer cells, substantiating its role in modulating client protein levels and inducing cell cycle arrest and apoptosis [333].

The nuclear factor of activated T cells (NFAT) is a critical mediator in T-cell activation, regulating the expression of numerous genes implicated in cancer [334]. Zhou and colleagues demonstrated that compound YZ129 modulated NFAT levels and exhibited therapeutic

potential against GBM. Their mechanistic studies indicated that YZ129 interacted with HSP90, inhibiting the HSP90-calcineurin-NFAT signaling pathway. This interaction suppressed NFAT nuclear translocation and downregulated the expression of pro-angiogenic and matrix remodeling genes VEGFA and MMP2. YZ129 has also been shown to inhibit cell proliferation and migration, induce cell cycle arrest, and promote apoptosis in GBM treatment. Furthermore, YZ129 affected other signaling pathways, including the RTK/PI3K/AKT/mTOR pathway. However, further comprehensive studies are necessary to confirm YZ129's potential as a novel therapeutic agent for GBM [335]. The SRC family of proteins, encoded by proto-oncogenes, are non-receptor tyrosine kinases essential in acute lymphoblastic leukemia (ALL) development. The lymphocyte-specific SRC family kinases (SFK) are client proteins of HSP90. Inhibiting HSP90 might disrupt SRC kinase stability in ALL, presenting a viable therapeutic strategy. Indeed, treatment with the HSP90 inhibitor NVP-BEP800 has suppressed SRC kinase activity and phosphorylation levels. Notably, NVP-BEP800 use in ALL cell lines reduced cancer cell proliferation, increased apoptosis, and delayed tumor growth in mouse models [336].

To overcome the limitations of traditional N-terminal HSP90 inhibitors, Seo and colleagues developed the HSP90 C-terminal inhibitor SL-145 and examined its mechanisms of action in triple-negative breast cancer (TNBC). SL-145 appeared to modulate signaling pathways involving HSP90 client proteins such as AKT, MEK, ERK, and STAT3 by reducing their expression, thus demonstrating anti-tumor activity. Significantly, downstream targets of STAT3, including MMP-2/MMP-9, integral to angiogenesis in TNBC, were also affected. Furthermore, SL-145 has been shown to disrupt the tumor stem-like characteristics in TNBC, associated with tumor invasion and metastasis, as confirmed through in situ syngeneic xenograft tumor models and cell migration assays. Notably, comparisons of SL-145 with HSP90 N-terminal inhibitors highlighted that SL-145 did not induce an HSR and exhibited no significant hepatorenal toxicity [337]. Similarly, the ansamycin analogs KU711 and KU758, as HSP90 C-terminal inhibitors, targeted and suppressed the proliferation of breast cancer stem cells, impacting TNBC invasion and migration. This effect was evidenced by reduced levels of cancer stem cell and EMT markers. KU711 and KU758 also altered the levels and phosphorylation of proteins in tumor development signaling pathways such as mTOR, AKT, and CDK4/6, inhibiting cell growth, migration, EMT, and induced apoptosis in TNBC. Notably, KU711 and KU758 outperformed the HSP90 N-terminal inhibitor 17-AAG in anti-cancer activity, with KU711 also demonstrating non-toxicity to liver and kidneys and a higher overall survival rate post-treatment, affirming its safety [338]. Furthermore, NCT-80, a compound based on the structure of deguelin, has been developed to target the C-terminal of HSP90. This interaction inhibited the binding of HSP90 to its client protein STAT3, reducing STAT3 stability and disrupting the Wnt signaling pathway, known for its role in resistance to HSP90 inhibitors. Given the significance of the STAT3 and Wnt pathways in EMT and cancer stem-like characteristics, NCT-80 has been evaluated for its effects on these processes. Studies show that NCT-80 can inhibit migration, colony formation, and cancer stem-like proliferation of NSCLC cells, exhibiting good safety at therapeutic doses in mouse models [339].

With an evolving understanding of HSP90's functions and mechanisms, researchers are increasingly focusing on developing small molecule inhibitors that disrupt the PPIs between HSP90 and its client proteins [44]. Cdc37, a co-chaperone of HSP90, assists in recruiting a variety of kinases to form HSP90-Cdc37-kinase complexes, essential for HSP90's chaperoning roles [48]. Several studies have reported that small molecule inhibitors targeting the HSP90-Cdc37 interaction exhibit significant anti-cancer potential [340]. DDO-6600, a covalent inhibitor of HSP90, disrupts the PPI between HSP90 and Cdc37, leading to reduced levels of various client protein kinases and demonstrating anti-cancer effects such as inhibition of growth, migration, invasion, cell cycle arrest, and apoptosis induction in different cancer cell lines in vitro. Additionally, in vivo, studies of DDO-6600 have confirmed its therapeutic efficacy [341]. In the same year, Tubocapsenolide A (TA) was identified as another covalent inhibitor of HSP90 that targeted the C-terminal of HSP90, inhibiting its interaction with Cdc37. This inhibition led to increased degradation of several kinase client proteins of HSP90, such as EGFR, AKT, and CDK4. Consequently, TA has been shown to inhibit growth and colony formation, induce cell cycle arrest, and trigger apoptosis in various cancer cell lines [342]. Additionally, it has been reported that okicamelliaside (OCS), a molecule with anti-cancer properties, disrupted the stability of the HSP90-Cdc37 complex, leading to a decrease in protein levels and phosphorylation of AKT and ERK1/2. OCS has demonstrated promising anti-cancer effects, including inhibition of cell growth, reduced migration, and induction of apoptosis in tumor cell lines such as HeLa and A549, along with low toxicity [343].

Amidst the extensive research on small molecule inhibitors targeting HSP90, the role of other HSP family members in tumor development is increasingly recognized as crucial. Therefore, investigating the roles of different small molecule inhibitors in cancer processes is vital for advancing tumor therapy and discovering new

anti-cancer drugs. SHetA2, a sulfur-containing compound currently in Phase I clinical trials approved by the National Cancer Institute, is being tested for treating cervical, ovarian, and endometrial cancers [344]. SHetA2 targeted HSP family proteins such as Grp78, Hsc70, and mortalin, disrupting mitochondrial oxidative phosphorylation and glycolysis in endometrial cancer cell lines. Moreover, it interfered with the interaction between mortalin and inositol trisphosphate receptor, inhibiting energy metabolism, protein synthesis, and overall metabolism in cancer cells, thereby suppressing cell growth, migration, invasion, and cell cycle progression. SHetA2 also demonstrated low toxicity and, when combined with paclitaxel, might reduce the dosage of the latter. These findings underscore SHetA2's potential as an anti-cancer drug [345]. Pifithrin mu (PES), targeting HSPA1A within the HSP70 family, has been modified with triphenylphosphine to selectively target mitochondria for anti-cancer effects. AP-4-139B has been shown to inhibit metastasis and invasion in colorectal cancer cells. Treatment with AP-4-139B also generally reduced the expression of client proteins such as AKT and EGFR. Notably, the treatment significantly enhanced immune cell infiltration into tumors, inducing immunogenic cell death [346]. Furthermore, KNK437 has been identified to inhibit the level of DNAJA in colorectal cancer cells, thereby inhibiting proliferation and suppressing migration. These anti-cancer effects might be associated with the involvement of the DNAJA1-CDC45 pathway [347]. In fact, HSP27 is increasingly recognized as a biomarker and therapeutic target in various cancers. Gleave et al. have identified that the anti-parasitic drug Ivermectin could bind to HSP27, exhibiting preliminary anti-cancer activity. Ivermectin impacted cancer-related pathways mediated by HSP27, such as those involving EGFR and androgen receptor, potentially enhancing the efficacy of anti-EGFR and anti-AR drugs in lung and prostate cancers, respectively. Moreover, Ivermectin treatment led to reduced cell migration in these cancers. Current research is exploring the binding mode of Ivermectin with HSP27 and its potential mechanisms of action, with further studies needed to investigate additional biological effects [348].

Small-molecule inhibitors that target HSPs to regulate multiple key biological processes in tumor progression have demonstrated promising therapeutic potential, which has greatly enhanced the understanding of tumor biology and expanded the options available for cancer treatment. However, given the complexities of the signaling pathways involved in cancer development, the detailed mechanisms of these small-molecule inhibitors require further clarification.

Combination therapies with HSP inhibitors

Manipulating multiple targets to improve the therapeutic efficacy and safety of drugs has attracted increasing interest from those in the pharmaceutical field. Strategies that employ combination therapies provide a holistic approach to cancer treatment by concurrently modulating various signaling pathways [349, 350]. The convergence of small-molecule inhibitors with anticancer agents frequently results in synergistic outcomes, rendering combination therapy particularly potent, especially against complex or advanced malignancies that show a limited response to monotherapy. Furthermore, combination therapy offers significant promise in mitigating drug resistance [351, 352]. Cancer cells often develop resistance to single agents due to genetic or epigenetic modifications, undermining the therapeutic impact of the agent [353]. Employing diverse small-molecule inhibitors with anticancer agents may produce enhanced antitumor effects by leveraging multiple mechanisms to impede or prevent the adaptation of the cancer cells to treatment, thus delaying or circumventing the onset of drug resistance. HSPs, which are overexpressed in various cancers and have been implicated in several biological functions—including assisting cancer cells in evading apoptosis, enhancing their survival, and facilitating their invasion and migration—constitute crucial targets for combination therapy [254]. Research indicates that small-molecule inhibitors directed at HSPs exhibit synergistic effects when combined with various drugs and increase the sensitivity of cancer cells to anticancer agents (Table 7). Thus, combination therapy targeting HSPs may offer a sustainable therapeutic option for cancer management.

Combination of HSP inhibitors and protein kinase inhibitors

Protein kinases are enzymes that catalyze the phosphorylation of proteins, and phosphorylation of proteins is involved in many critical signaling pathways [354]. Dysregulation of these enzymes can disrupt cellular functions, leading to diseases, particularly cancer [355, 356]. Consequently, protein kinase inhibitors are crucial in antitumor drug development, with many such inhibitors, including EGFR and MEK inhibitors, already approved for clinical use.

Gefitinib, an EGFR-tyrosine kinase inhibitor (TKI), treats NSCLC [357]. However, post-treatment mutations in EGFR and related genes often confer resistance to Gefitinib [358]. Xu et al. reported that a novel curcumin-derived HSP90 inhibitor, C086, combined with Gefitinib, significantly improved antitumor effects in vitro in A549

Table 7 Summary of the combination of HSP inhibitors and other anti-cancer agents

Compound 1	Compound 2	Hallmarks of cancer	Cancer cell line	Tumor type	Ref
C086	Gefitinib	Inhibit proliferation, induce apoptosis	A549 NCI-H1975	Non-small cell lung cancer	[359]
NA49	Gefitinib	Inhibit proliferation, induce cell death	HCC82, NCI-H165, NCI-H1975	Lung cancer	[360]
Luminespib	Osimertinib	Inhibit proliferation	PC9, PC9-OR	Non-small cell lung cancer	[362]
IPI504	HHT	Inhibit proliferation, induce apoptosis	MV4-11, MOLM-13, THP-1, Kasumi-1	Acute Myeloid Leukemia	[363]
17-DMAG	Lapatinib	Inhibit proliferation	LR-SKBR3 LR-BT474	Breast cancer	[364]
PU-H71	Trametinib	Inhibit proliferation	KPC4662, MiaPaca2, Panc-1, Capan-2, HPAC-1, SW1990, HPAF, AsPC1	Pancreatic ductal adenocarcinoma	[365]
17-AAG	$Rictor^{-/-}$	Inhibit proliferation, induce apoptosis, inhibit migration and invasion	PC12, HEK 293 T	Pheochromocytoma	[366]
HAA	Dinaciclib	Inhibit proliferation, induce apoptosis	HL60	Acute myeloid leukemia	[367]
17-AAG	Belinostat	Inhibit proliferation, induce apoptosis, inhibit migration and invasion	MDA-MB-231	Triple-negative breast cancer	[369]
Gamitrinib	Panobinostat	Inhibit proliferation, induce apoptosis, inhibit metabolism	U87, LN229, T98G	Glioblastoma	[370]
Quercetin	Vorinostat	Induce apoptosis	Hut78	Cutaneous T-cell lymphoma	[373]
Onalespib	Cisplatin	Inhibit proliferation	MIA Paca-2 Panc-1	Pancreatic ductal adenocarcinoma	[379]

Table 7 (continued)

Compound 1	Compound 2	Hallmarks of cancer	Cancer cell line	Tumor type	Ref
WGA-TA	Cisplatin	Induce apoptosis, inhibit migration and invasion	MDA-1986, UMSCC-22B	Head and neck squamous cell carcinoma	[380]
NK16s	Cisplatin	Induce cell death	NCI-H460, A549	Non-small cell lung cancer	[381]
NDNB1182	Immune checkpoint blockade (Nivolumab and Ipilimumab)	Inhibit proliferation	Pten-CaP8, RM1, Myc-CaP, DU145	Prostate cancer, breast cancer	[384]
XL888	PD-1-targeted antibodies (Clone RMP1-14)	Inhibit proliferation	Panc02, MT-5, KPC-Luc	pancreatic cancer	[385]
PU-H71	Prodigiosin	Inhibit proliferation, induce apoptosis, inhibit angiogenesis	MDA-MB-231	Triple-negative breast cancer	[388]
17-AAG	Salinomycin	Inhibit proliferation	A549, H1975	Non-small cell lung cancer	[389]
DPB	3-MA	Induce apoptosis	A549	Non-small cell lung cancer	[390]
S1g-6	S1	Induce apoptosis	K562, U937, H23, HL-60	Chronic myeloid leukemia Lymphoma Lung cancer	[391]
shGRP78	Artesunate	Induce ferroptosis	AsPC-1, PaTU8988	Pancreatic cancer	[392]
Kuanoniamine C	Bortezomib	Induce cell death	U2OS, Saos-2	Osteosarcoma	[393]
Ganetespib	Talazoparib	Induce cell death	OVCAR-3, OC-1, OC-16	Ovarian carcinoma	[394]

and NCI-H1975 cells, showing enhanced growth inhibition, cell cycle arrest, and apoptosis induction compared to single-agent treatments. This combination also downregulated the EGFR signaling pathway by inhibiting HSP90's chaperone function, which is beneficial for NSCLC treatment [359]. Similarly, in addressing Gefitinib resistance, Lee et al. explored a small molecule of HSP27 inhibitor, NA49, in combination with Gefitinib. This regimen demonstrated synergistic anticancer activity in EGFR-WT and EGFR-Mut cells, with dependency on HSP27 expression levels. In vivo studies confirmed enhanced tumor suppression, highlighting this combination as a potential strategy to counteract Gefitinib resistance [360]. Osimertinib, another EGFR-TKI, is used for NSCLC with the EGFR T790M mutation but also faces resistance challenges [361]. Jordi and colleagues investigated the combination of the HSP90 inhibitor Luminespib with Osimertinib, noting enhanced inhibitory effects on cell proliferation and colony formation in EGFR-mutated NSCLC cell lines. Protein blot analysis showed that Luminespib reduced the expression of resistance-associated proteins, such as EGFR, MET,

AKT, and STAT3, promoting further exploration of this combination for treating EGFR-resistant NSCLC [362]. Homoharringtonine (HHT), an anti-leukemic agent, also shows limited efficacy alone. Research by Xu's team into combining HHT with the HSP90 inhibitor IPI504 for treating FLT3-ITD(+) acute myeloid leukemia (AML) demonstrated that this combination induced apoptosis and cell cycle arrest in FLT3-ITD(+) AML cells through the synergistic inhibition of Hsp90 client proteins FLT3 and its downstream factors, with more potent antitumor effects than monotherapy [363]. Lapatinib, a tyrosine kinase inhibitor, has been linked with poor prognosis due to acquired resistance. The combination of the HSP90 inhibitor 17-DMAG with lapatinib significantly inhibited phosphorylation of HER2, EGFR, Akt, and ERK in resistant LR-BT747 cells and exhibited synergistic tumor suppression in mouse xenograft models, suggesting a viable approach to overcoming resistance in clinical settings [364].

Leach's team performed in vivo experiments using a syngeneic orthotopic pancreatic ductal adenocarcinoma (PDAC) xenograft mouse model, exploring many drug combination treatments to identify a practical therapeutic approach for PDAC. Their findings revealed that the concurrent administration of the HSP90 inhibitor PU-H71 and the MEK inhibitor Trametinib significantly suppressed tumor growth. This inhibition was achieved by overcoming the activation of resistance pathways typically induced by MEK inhibitors alone, such as the PI3K/AKT/mTOR signaling axis, thereby enhancing the efficacy of Trametinib. This study substantiated the potential of dual inhibition of HSP90 and MEK as a promising therapeutic strategy for PDAC [365]. Further contributions to cancer therapy involve the utilization of protein kinases and HSPs. For instance, Zhong et al. demonstrated that dual inhibition of mTORC2 and HSP90 could produce a profound antitumor effect in pheochromocytoma by disrupting Akt signaling [366]. Similarly, Abdalla et al. proposed a combination therapy targeting CDKs and HSP90, suggesting its potential effectiveness in treating AML [367]. These studies underscore the ongoing exploration and application of kinase and HSP inhibitors in diverse cancer treatment regimens.

Combination of HSP inhibitors and histone deacetylase inhibitors

The dynamic equilibrium between histone acetylation and deacetylation is pivotal for regulating chromosomal structure and gene transcription [368]. Inhibiting histone deacetylases (HDACs) enhances the transcription of antitumor factors by modulating acetylation levels, thus exerting anticancer effects on tumor-associated signaling pathways. Zhang et al. documented the antitumor impacts of combining the HSP90 inhibitor 17-AAG with the HDAC6 inhibitor Belinostat in the TNBC cell line MDA-MB-231. They observed that this combination augmented apoptosis, induced cell cycle arrest, and suppressed migration and invasion in MDA-MB-231 cells [369]. In a related study, Siegelin et al. explored the therapeutic potential of jointly inhibiting TRAP1 and HDACs in treating GBM. Their findings suggested that this combination therapy provided substantial antitumor benefits over monotherapy, as demonstrated in both in vitro assays and a mouse GBM patient-derived xenograft model. The mechanism of action might involve multiple pathways, including disruption of the electron transport chain through TRAP1 inhibition and modulation of the expression of Bcl-2 family proteins to induce apoptosis and affect the energy metabolism of GBM cells [370]. Furthermore, although HDAC inhibitors treat cutaneous T-cell lymphoma, resistance to these agents remains challenging. Prior research has linked the overexpression of HSP72 with resistance to HDAC inhibitors [371, 372]. Fujii and colleagues demonstrated that the HSP72 inhibitor Quercetin could potentiate apoptosis induced by the HDAC inhibitor Vorinostat in Hut78 cells expressing high levels of HSP72. This enhancement is achieved by inhibiting HSP72 expression and amplifying both endogenous and exogenous caspase pathways [373]. This study presented a potential strategy to overcome resistance to HDAC inhibitors in cancer therapy.

Combination of HSP inhibitors and metal complex

Metal complexes have been extensively explored as anticancer agents, among which platinum complexes are the most extensively investigated and utilized in clinical settings [374–376]. A prominent class member, Cisplatin treats various malignancies, including ovarian, testicular, lung, and head and neck cancers [377, 378]. It exerts its anticancer effects primarily by binding to DNA and impeding DNA synthesis, thereby inducing tumor cell apoptosis. Nonetheless, cisplatin therapy is associated with significant adverse effects, such as organ toxicity and drug resistance. Consequently, researchers have explored combining Cisplatin with other therapeutic agents. Notably, the combination of Cisplatin and the HSP90 inhibitor Onalespib has demonstrated synergistic effects in cisplatin-resistant PDAC, as reported by the Dobbelstein team. This synergy arose from HSP90 inhibition, which impeded the repair of platinum–DNA adducts by diminishing Fanconi anemia factors, thus reinstating the sensitivity of drug-resistant PDAC cells to Cisplatin [379]. Besides, PPI inhibitors targeting HSP90 have shown potential. Cohen et al. evaluated trichostatin A (WGA-TA), an HSP90 chaperone complex inhibitor,

in combination with Cisplatin in HNSCC treatment. WGA-TA disrupted the interaction between HSP90 and its co-chaperone cdc37, inhibiting the PI3K/Akt/mTOR pathway, a critical mechanism associated with cisplatin resistance and HNSCC progression. The combined therapy significantly reduced EMT-associated proteins such as e-cadherin and components of the eukaryotic translation initiation complex and enhanced the cleavage of PARP, indicating inhibited tumor cell growth, migration, invasion, and induced apoptosis [380]. Furthermore, Lee's team reported the potential of a novel HSP27 inhibitor, NK16, which, when used with Cisplatin or paclitaxel, enhanced cytotoxicity in NSCLC through abnormal HSP27 dimerization and increased apoptosis markers PARP and caspase-3 [381].

Combination of HSP inhibitors and other antitumor agents

Tumor immunotherapy stands at the forefront of cancer treatment, with immune checkpoint inhibitors making significant progress [382]. However, as immunotherapy directly targets the human immune system, it is imperative that we carefully consider the potential immune-related adverse effects it may induce and evaluate the reliability of its therapeutic efficacy [383]. To enhance treatment outcomes, researchers have begun to explore combination therapy strategies to optimize immunotherapy, with some studies mainly focusing on the role of HSPs. For instance, Lu and co-workers investigated an HSP90β inhibitor, NDNB1182, in conjunction with ICB therapy as an anticancer approach. Their findings in syngeneic mouse models of prostate and breast cancer demonstrated that NDNB1182 augmented the effectiveness of the checkpoint blockade therapy [384]. However, the precise mechanisms underlying these effects warrant further investigation. Additionally, a study by Lesinski et al. described the substantial cytotoxic impact of the HSP90 inhibitor, XL888, on pancreatic stellate cells or cancer-associated fibroblasts. In related mouse models, XL888 was shown to enhance the effectiveness of anti-PD-1 therapy, as evidenced by increased infiltration of CD8 + and CD4 + T cells and elevated levels of immune response-related genes [385]. In conclusion, the integration of HSP inhibitors with immunotherapy represents a promising therapeutic strategy for cancer treatment.

Antibiotics are conventionally utilized for antibacterial purposes; however, ongoing research reveals that certain antibiotics may exhibit anticancer properties [386, 387]. Furthermore, integrating HSP inhibitors with specific antibiotics has been reported to constitute a novel antitumor therapy potentially. Anwar et al. demonstrated that combining Prodigiosin and the HSP90 inhibitor PU-H71 led to pronounced anticancer activity in MDA-MB-231 human breast cancer cells. This regimen significantly increased the transcriptional levels of pro-apoptotic genes Bax, caspase-3, caspase-8, and caspase-9 and decreased the expression of oncogenic pathways components mTOR, EGFR, and VEGF, potentially through the downregulation of HSP90α. Additionally, in silico predictions suggested favorable absorption and activity of Prodigiosin and PU-H71 in vivo, underscoring the potential of this combination for further exploration as an anticancer strategy [388]. In related findings, Lin et al. observed that the HSP90 inhibitor 17-AAG augmented the cytotoxic effects of Salinomycin in NSCLC cell lines A549 and H1975 by suppressing the expression of thymidine phosphorylase [389].

The regulation of programmed cell death represents a compelling strategy in cancer therapy, underscored by notable advancements. Given the critical role of HSPs within the regulatory networks of programmed cell death, targeting both HSPs and the underlying mechanisms of cell death may offer substantial therapeutic potential. Su et al. described a novel approach that combines a new HSP90 inhibitor, DPB, with an autophagy inhibitor 3-MA in treating A549 lung cancer cells. This combination augmented apoptosis by suppressing autophagy, thereby increasing PARP cleavage induced by DPB [390]. Additionally, Bcl-2 family proteins, central to the apoptotic pathway, also facilitated innovative combination therapies. A new HSP70 inhibitor, S1g-6, used alongside the Bcl-2 inhibitor S1, interfered with the Bcl-2-HSP70 interaction through Bim inhibition, promoting mitochondria-dependent apoptosis in various cancer cell lines [391]. Furthermore, the knockdown of GRP78 enhanced the effect of the ferroptosis inducer artesunate in pancreatic cancer cells, highlighting the potential for future investigations into GRP78 inhibitors as part of a combined regimen with artesunate for cancer treatment [392].

Bortezomib, a proteasome inhibitor, is employed in treating osteosarcoma, and the downregulation of GRP78 expression enhances the cytotoxicity of Bortezomib-induced cell death in U2OS cells. Namba et al. discovered a naturally derived active compound, Kuanoniamine C, that could reduce GRP78 expression via the p53 pathway without triggering ER stress. Their studies showed that combining Kuanoniamine C with Bortezomib augmented the anticancer activity by further downregulating GRP78 [393]. Additionally, combining the PARP inhibitor Talazoparib and the HSP90 inhibitor Ganetespib disrupted the expression of proteins integral to the DNA damage and cell cycle checkpoints in ovarian cancer cells. This combination enhanced the sensitivity of these cells to Talazoparib, thus partially overcoming the limitations associated with monotherapy [394].

The cases discussed herein involve small-molecule inhibitors that target various cellular functions. Notably, HSP inhibitors can be combined effectively with drugs that target these diverse functions, with each combination regimen demonstrating unique advantages and therapeutic potential. These results underscore the pivotal role of HSPs in combinatorial cancer therapy.

Dual inhibitors targeting HSPs for *cancer* therapy

Significant advancements have been made in combination therapies, and the development of molecular entities capable of modulating multiple targets has increasingly become a focal point in drug discovery. In 2004, Morphy et al. systematically reviewed strategies for developing multifunctional ligands and introduced the concept of "designed multiple ligands" for drug development [395, 396]. Since then, drug development has transitioned into a new era characterized by a shift towards multitargeted drugs. As single chemical entities, these multitargeted agents offer multiple advantages, including enhanced therapeutic efficacy, decreased risk of drug–drug interactions, and more predictable pharmacodynamics and pharmacokinetics [397–400]. Moreover, the ability of a single multifunctional molecule to modulate different targets simultaneously may facilitate disease treatment through multiple mechanisms, potentially offering a novel strategy to mitigate off-target effects and diminish drug resistance. This approach is particularly advantageous for treating multifactorial diseases such as cancer.

HSPs are recognized as potential targets for cancer therapy because of their chaperone properties, which allow for client protein modulation and the regulation of multiple associated pathways. The ability of HSPs to impact various biological pathways due to their chaperone characteristics presents opportunities for developing dual-targeted inhibitors. This challenging endeavor could lead to significant advances in the development of small-molecule dual-target drugs that target HSPs. Carefully designed chemical entities may enhance the promiscuity of drug molecules, facilitating the precise selection of critical biological pathways in the disease process and potentially reducing the side effects associated with modulating the central role of HSPs in terms of physiology or pathology [401]. Currently, the development of dual-target inhibitors has focused primarily on HSP90, utilizing hybrid strategies, virtual screening, computer-aided drug design, and mechanistic studies of natural or synthetic compounds, among other approaches [402]. This section highlights the dual-target inhibitors that simultaneously target HSP90 and crucial protein signals, such as HDAC6, tubulin, topoisomerase II (Topo II), and PI3K. We also explore isolated cases involving dual-target inhibitors of HSP27 (Table 8).

Dual inhibitors of HSP90 and HDAC6

The interaction between HDAC6 and HSP90 in cancer has attracted significant interest. HDAC6 serves as a deacetylase for HSP90, and its inhibition results in hyperacetylation and subsequent inactivation of HSP90 [403, 404]. Simultaneously, HSP90 inhibition led to degradation of HDAC [405]. Preclinical evidence has demonstrated that combining HSP90 inhibitors with pan-HDAC inhibitors can synergistically benefit various cancers [406, 407]. Given the high degree of correlation and overlapping signaling networks in cancer, developing novel small molecule drugs that simultaneously target both HSP90 and HDAC may offer a promising approach to cancer therapy.

Liou and colleagues have focused on developing dual-target inhibitors targeting HSP90/HDAC, designing and synthesizing a series of novel hydroxamic derivatives based on the structure of the HDAC inhibitor SAHA and the active fragment resorcinol of the HSP90 inhibitor. Biological evaluation of these compounds, particularly compounds 20, revealed significant sub-G1 phase cell accumulation leading to substantial cell death in the H1975 cell line. Concurrently, these compounds effectively induced apoptosis, activating apoptosis-related proteins, including caspase-3, caspase-8, caspase-9, PARP, and γH2AX. Additionally, the protein levels of HSP90 client proteins such as EGFR, SRC, FAK, and Rb were downregulated in treated cells. Cytotoxic agents, such as IFN-γ, trigger the expression of PD-L1 on the surface of tumor cells, leading to T-cell function inhibition and tumor cell immune evasion [408]. Encouragingly, compounds 20 was shown to reduce the expression levels of IFN-γ-induced PD-L1 in a dose-dependent manner in H1975 cells [409]. Encouraged by these findings, Liou et al. developed another Isoindoline scaffold-based compound 17 as a dual inhibitor of HSP90 and HDAC6. This compound demonstrated potent inhibitory activity against HSP90α and HDAC6, with IC_{50} values of 46.8 nM and 4.3 nM, respectively, and exhibited growth-inhibitory effects on various cancer cell lines, including A549 (GI_{50} = 0.76 μM) and EGFR-resistant H1975 (GI_{50} = 0.52 μM). Molecular docking analysis demonstrated that the 2,4-dihydroxy-5-isopropylbenzoyl functionality of compound 17 forms hydrogen bonds with HSP90 residues N51, D93, and T184, and hydrophobic interactions with residues F138 and T184. Additionally, the *N*-(2,3-dihydro-1H-isoindol-5-yl)formamide motif forms hydrogen bonds with residues K58 and G108, and together with the octyl linker—which interacts with residues A55 and M98—situated at the periphery of HSP90's binding site. The hydroxamic acid moiety also binds to residues E47, N51, and G137 of HSP90, showing that compound 17 targets HSP90 and inhibits its function

Table 8 Dual inhibitors targeting HSPs for cancer therapy

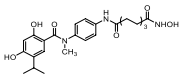
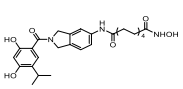
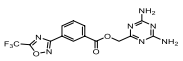
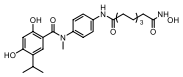
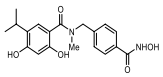
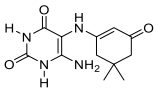
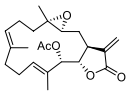
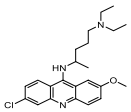
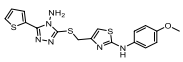
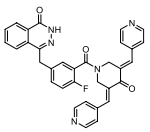
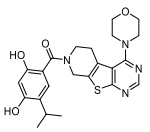
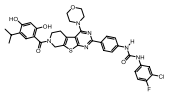
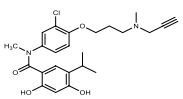
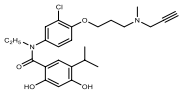
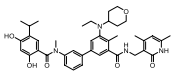
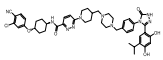
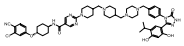
Name	Structure	Target	Hallmarks of cancer	Cancer cell line	Tumor type	Ref
Compound 20		HSP90/HDAC	Inhibit proliferation, induce apoptosis	H1975 (GI_{50} = 0.69 μM), A549 (GI_{50} = 0.77 μM), HCT116 (GI_{50} = 0.83 μM)	Lung cancer Colorectal cancer	[409]
Compound 17		HSP90/HDAC6	Inhibit proliferation, induce apoptosis	H1975 (GI_{50} = 0.52 μM), A549 (GI_{50} = 0.76 μM)	Human non-small cell lung cancer	[410]
Compound 10		HSP90/HDAC6	Inhibit proliferation	MCF-7 (5 μM)	Breast cancer	[411]
MPT0G449		HSP90/HDAC	Inhibit proliferation, induce apoptosis	HL-60 (IC_{50} = 0.19 ± 0.04 μM), MOLT-4 (IC_{50} = 0.11 ± 0.03 μM)	Acute leukemia	[413]
Compound 6e		HSP90/HDAC6	Inhibit proliferation, induce apoptosis	H1975 (GI_{50} = 1.7 μM)	Human non-small cell lung cancer	[414]
Compound 3a		HSP90/Topo II	Inhibit proliferation, induce apoptosis	HepG-2 (IC_{50} = 14.31 ± 0.83 μM), A-549 (IC_{50} = 30.74 ± 0.76 μM), MCF-7 (IC_{50} = 27.14 ± 1.91 μM),	Hepatocellular carcinoma Lung cancer Breast cancer	[420]
13-Acetoxysarcocrassolide		HSP90/Topo II	Inhibit proliferation, induce apoptosis	Molt4 (13.34 μM)	Acute lymphoblastic leukemia	[421]
Quinacrine		HSP90/Topo II	Inhibit proliferation, induce apoptosis, inhibit migration	K562 (20 μM), A549 (20 μM), HL-60/MX2 (20 μM), HL60 (20 μM)	Acute myeloid leukemia, Lung adenocarcinoma, Chronic myeloid leukemia	[422]
Mortaparib		Mortalin/PARP1	Inhibit proliferation, induce apoptosis, inhibit migration and invasion	Hela (5 μM) SKOV3 (5 μM)	Cervical cancer Ovarian cancer	[429]
Compound 4		HSP90/PARP	Inhibit proliferation	MCF-7 (IC_{50} = 0.97 ± 0.13 μM)	Breast cancer	[430]
Compound 8 m		Hsp90/PI3Kα	Inhibit proliferation, induce apoptosis, inhibit migration and invasion	B16 (IC_{50} = 1.06 μM)	Melanoma	[434]
Compound 17o		Hsp90/mTOR	Inhibit proliferation, induce apoptosis, induce autophagy	SW780 (IC_{50} = 0.16 ± 0.03 μM), J82 (IC_{50} = 0.36 ± 0.03 μM)	Bladder cancer	[441]
Compound 4-b		HSP90/MAO A	Inhibit proliferation	GL26 (IC_{50} = 0.73 ± 0.06 μM), U251R (IC_{50} = 0.84 ± 0.25 μM)	Glioblastoma	[445]
Compound 4-c		HSP90/MAO A	Inhibit proliferation	GL26 (IC_{50} = 0.49 ± 0.28 μM), U251R (IC_{50} = 0.26 ± 0.10 μM)	Glioblastoma	[445]
Compound 7		HSP90/EZH2	Inhibit proliferation induce apoptosis	Pt3R (IC_{50} = 1.015 μM)	Glioblastoma	[449]

Table 8 (continued)

Name	Structure	Target	Hallmarks of cancer	Cancer cell line	Tumor type	Ref
WCA-814		HSP90/AR	Inhibit proliferation, induce apoptosis	22Rv1 (IC_{50} = 26.5 ± 4.4 nM), LNCaP (IC_{50} = 171.2 ± 34.8 nM)	Castration-resistant prostate cancer	[454]
SQA-710		HSP90/AR	Inhibit proliferation, induce apoptosis	22Rv1 (IC_{50} = 34.3 ± 11.9 nM), LNCaP (IC_{50} = 73.8 ± 9.4 nM)	Castration-resistant prostate cancer	[454]

effectively. Moreover, compound 17 meets general structural requirements for HDAC6 inhibitors: it has a cap that obstructs the binding site, a zinc-binding group (ZBG), and a linker connecting them. The 2,4-dihydroxy-5-isopropylbenzoyl and *N*-(2,3-dihydro-1H-isoindol-5-yl)formamide motifs serve as the cap, forming hydrogen bonds with residues S564, F566, and I569. The hydroxamic acid extends into HDAC6's catalytic site as the ZBG, coordinating with the zinc ion. The linker traverses HDAC6's hydrophobic channel, forming hydrophobic interactions with residue F620. These findings suggested that compound 17, due to the structural similarity between HSP90 and HDAC6 binding sites, acts as a dual-target inhibitor, effectively targeting both to inhibit their activity. Western blot analysis confirmed compound 17 induced a dose-dependent increase in acetylated α-tubulin and triggered the degradation of HSP90 client proteins Akt and STAT3. Compound 17 effectively induced apoptosis in H1975 lung cancer cells, evidenced by activating multiple apoptosis-related proteins caspase-3, caspase-8, caspase-9, PARP, and γH2AX. Notably, the downregulation of PD-L1 expression in 17 treated cells highlights its potential in tumor immunotherapy [410].

In the same year, Rastelli et al. employed ligand-based and structure-based approaches to devise and screen a series of dual-target inhibitors that concurrently target HSP90 and HDAC6, followed by biological evaluations. The in vitro findings revealed that these compounds exerted anti-proliferative effects on MCF-7 cells through multi-target interactions. Among these, candidate compound 10 was particularly notable for its efficacious targeting of HDAC6 at multiple binding sites. Within HDAC6, the zinc ion in the active site coordinated with 5-(trifluoromethyl)-1,2,4-oxadiazole, and its phenyl ring engaged in π-π stacking interactions with the Phe620 and Phe680 residues. The 2,4-diamino-s-triazine moiety was also positioned near His500, Pro501, and Leu749, extending towards the solvent-exposed region atop the HDAC6 cavity. Although compound 10 showed limited inhibitory activity against HSP90, its phenyl ring was close to Leu103, Leu107, and Phe138, with the 5-(trifluoromethyl)-1,2,4-oxadiazole extending into a small pocket comprising Phe22, Gly108, Trp162, and Phe170. These detailed structural observations provide valuable insights for future structural optimization efforts [411]. MPT0G449, an HSP90/HDAC6 dual-effect inhibitor designed using the same strategy as before to treat acute leukemia. This study demonstrated that MPT0G449 exhibited significant anti-cancer activity in HL-60 and MOLT-4 cell lines. The compound induced cell cycle arrest at the G2 phase and initiated apoptosis in acute leukemia cells through intrinsic and extrinsic caspase-mediated pathways. MPT0G449 also promoted the degradation of aurora A, a mitotic serine/threonine kinase essential for mitosis and meiosis, by inhibiting HSP90 [412]. Additionally, treatment with MPT0G449 led to the downregulation of several oncogenic signaling pathways, including AKT/mTOR, STAT3/5, and MEK/ERK in xenograft models, thus suppressing tumor growth and inducing apoptosis in tumor cells [413].

Building on previous promising and positive explorations, a novel hybrid compound, 6e, was synthesized as an HSP90/HDAC6 dual inhibitor. The resorcinol moiety and carbonyl oxygen of compound 6e form hydrogen bonds with the carboxyl side chain of D93 and the hydroxyl side chain of T184 in HSP90α, as well as with multiple water molecules. Additionally, the isopropyl group on the resorcinol ring fits snugly within a hydrophobic pocket formed by residues L107, F138, V150, and W162. The hydroxamate group not only forms hydrogen bonds with the main and side chains of residue N106 of HSP90α but also coordinates with the zinc ion in HDAC6 and further forms hydrogen bonds with residues H573 and Y745. Meanwhile, the benzene ring engages in π-π interactions with F583 and F643, projecting into the hydrophobic channel, while the 2,4-dihydroxybenzoyl moiety acts as a capping group at the edge of the HDAC6 binding site, forming hydrogen bonds with residues H614 and L712. The biological evaluation demonstrated that 6e showed high affinity and selectivity for HSP90α and HDAC6, with IC_{50} values of 61 nM and 106 nM, respectively, and exhibited potent inhibitory activity against the H1975 cell line, with a GI_{50} of 1.7 μM. Treatment with 6e led to the downregulation of HSP90 client proteins such

as HER2, EGFR, MET, and AKT and induced apoptosis in a dose-dependent manner in H1975 cells, as evidenced by the cleavage of caspase-3, caspase-8, and PARP, thereby inhibiting their proliferation. In vivo, experiments in an H1975 xenograft mouse model confirmed that the dual-targeting inhibitor 6e exhibited promising anti-tumor effects characterized by delayed tumor growth. Notably, during the in vivo biological evaluations, 6e did not display the ocular toxicity often associated with HSP90 inhibitors in preclinical or clinical trials. These findings suggested that 6e warrants further investigation as a potential therapeutic for NSCLC [414].

Dual inhibitors of HSP90 and Topo II

Topoisomerases within the nucleus play a critical role in relaxing DNA supercoiling, thereby regulating essential processes such as DNA transcription, replication, and gene expression [415, 416]. Inhibition of these enzymes can disrupt DNA replication, induce DNA breaks, and ultimately result in the death of tumor cells [417]. There is evidence suggesting that HSP90 inhibitors can impede DNA repair mechanisms [418]. Importantly, HSP90 and Topo II share structural similarities in that they have an N-terminal region belonging to an ATP-binding superfamily, the GHKL family, and share unconventional ATP-binding folds [419]. Thus, compounds exhibiting dual inhibitory activity against topoisomerases and HSP90 will likely demonstrate enhanced potency as anti-tumor agents.

In this study, a hybrid compound 3a, based on the cyclohexenone-pyrimidine scaffold, was synthesized and evaluated as a dual-targeted inhibitor against tumorigenesis, simultaneously targeting Hsp90 and Topo II. The investigations revealed that compound 3a exhibited notable anti-proliferative effects across various tumor cell lines, including HepG-2, A-549, and MCF-7, and demonstrated significant inhibition of Hsp90 and Topo II in preliminary in vitro assays. In HepG-2 cells, compound 3a induced cell cycle arrest at the G1-S phase and activated receptor or mitochondria-dependent apoptosis pathways by upregulating caspase-3 gene expression. Molecular docking studies suggested that 3a formed hydrogen bonds with Thr27 and Gln365, the key amino acids at the ATP-binding site of Topo II, respectively, and formed a stacking effect at Tyr144. At the same time, 3a and the key amino acids Asp93 and Asn51 of HSP90 can also form hydrogen bonding and hydrophobic interaction with residues Phe138. These effects promoted the stable binding 3a to these targets and consequently inhibiting their activity and function. Additionally, an evaluation of the geometric properties of 3a through density functional theory indicated favorable oral bioavailability, underscoring its potential as an anticancer agent [420]. Concurrently, Liu et al. investigated the cytotoxic effects of another compound, 13-acetoxysabine (13-AC), as a dual inhibitor of Topo II and HSP90 in leukemia cells. Molecular docking revealed that 13-AC could bind to the active sites of HSP90 and Topo II through hydrogen bonds and hydrophobic interactions at several sites, including residues Asn51, Lys112, Phe138, Ser149, Ser148 and Asn150, etc. Both in vitro and in vivo studies demonstrated that 13-AC induced apoptosis in Molt4 cells via oxidative stress and HSP90 inhibition, a process associated with PARP and caspases cleavage, phosphatidylserine externalization, and mitochondrial membrane potential disruption, without causing adverse effects on the liver and kidneys in mice [421].

In the context of drug repurposing, Huang et al. identified quinacrine (QA), an anti-malarial drug, as a potential dual-target inhibitor of both Topo II and HSP90. Comparison of the active domains of Topo II and Hsp90 revealed significant overlap in their β-fold, with the center of the ATPase regions positioned close to the magnesium ion. Several key amino acids, such as Asn91 and Asn120 of Topo II, overlap highly with Asn51 and Asp93 of HSP90, respectively. These findings suggested structural similarities between the N-terminal ATPase domains of Topo II and Hsp90. Further study demonstrated that QA inhibited Hsp90 by interacting with its N-terminal ATP-binding site and caused downregulation of HSP90 and associated client proteins, such as Akt, in A549 cells. Notably, compared to 17-AAG, an increase in QA concentration led to a significant downregulation rather than upregulation of the expression of Hsp70 and Hsp90, indicating that QA did not induce the HSR. It is also substantial that QA inhibited the activity of Topo IIα without disrupting DNA cleavage, positioning QA as a catalytic inhibitor with limited DNA intercalating activity, as opposed to a toxic inhibitory effect. Biological assessments indicated that QA exhibited broad anti-proliferative effects and retained efficacy in drug-resistant cell lines such as MCF-7/ADR and HL-60/MX2. Besides, QA effectively inhibited the proliferation and migration of A549 cells and induced apoptosis in HL-60 cells in a dose-dependent manner. However, due to the complex conformational changes associated with dynamic ATP depletion in Topo II, whether QA binds directly to the ATP-binding site of Topo II remains unconfirmed. Despite the disparity in the sizes of the ATP-binding sites of Topo II and Hsp90, essential hydrogen bonds are formed between Asn120 of Topo II and QA and between Asn106 of Hsp90 and QA, providing critical insights for future structural optimization [422].

Dual inhibitors of HSPs and PARP1

PARP1, a member of the PARP protein family involved in DNA repair and genomic integrity maintenance, is recognized as a critical target for cancer therapy due to its vital roles in nuclear processes such as transcription regulation and inflammation [423–425]. Nonetheless, alternative DNA repair mechanisms can restore DNA damage induced by PARP inhibitors [426, 427]. Given that HSPs, as molecular chaperones, can stabilize client proteins conferring resistance to PARP inhibition, a concurrent inhibition strategy of PARP and HSPs may synergistically improve the effectiveness of PARP inhibitors [428]. Wadhwa's team reported the dual inhibitor of mortalin and PARP1, Mortaparib, for cervical and ovarian cancer treatment. Mortaparib interacted with mortalin and activated p53 by inhibiting mortalin transcription, leading to p53-mediated cancer cell growth arrest and apoptosis. Besides, mortalin inhibition and the resulting ATP reduction led to mitochondrial dysfunction, further causing PARP1 inactivation and triggering apoptotic signaling. Mortaparib also suppressed PARP1 and disrupted the mortalin-PARP1 interaction, significantly reducing the levels of PARP1, mortalin, and the mortalin/PARP1 complex in treated cells. Moreover, Mortaparib-treated cells displayed inhibited cancer cell migration, metastasis, and angiogenesis, with no apparent toxicity in animal models [429]. Leveraging the potential of dual targeting mortalin and PARP in cancer therapy, Wu et al. designed and synthesized a series of molecular hybrids of the PARP-1 inhibitor Olaparib (Ola) and the HSP90 inhibitor C0817, a curcumin derivative. Preliminary evaluations indicated that compound 4 exhibited potent activity against MCF-7 human breast cancer cells, at least 200-fold more potent than Ola. Compound 4 could bind to HSP90 and reduce the expression of breast cancer 1. By inhibiting PARP and downregulating HSP90, compound 4 attenuated the DNA damage response, thereby inducing a synergistic lethal effect on cancer cells [430].

Dual inhibitors of HSPs and other oncoproteins

PI3K is an intracellular kinase in numerous essential signaling cascades [431, 432]. The PI3K/AKT signaling pathway, a classic route of intracellular signal transduction, responds to extracellular cues to regulate cellular survival and function and is vital in cancer development and progression [433]. Thus, PI3K has emerged as a significant target for anticancer drug development. He et al. focused on identifying multitarget small molecule inhibitors of HSP90 and its oncogenic client proteins, and they have synthesized the inaugural HSP90/PI3K dual-target inhibitor, 8 m, utilizing chemical structure fragments from representative Hsp90 and PI3Kα inhibitors. 8 m acted via the PI3K-AKT pathway and HSP90 client protein-associated signaling pathways, markedly reducing the expression of client proteins such as Cdc37, CDK4, EGFR, B-Raf, and C-Raf, and concurrently decreasing c-Myc, ERK1/2, and JNK levels. Additionally, biological assays demonstrated that 8 m effectively inhibited melanoma cell migration, invasion, and proliferation and induced apoptosis and cell cycle arrest. This study offers insightful contributions to developing Hsp90/PI3Kα dual-target inhibitors for cancer therapy [434]. Moreover, the mTOR protein, a highly conserved serine/threonine kinase downstream of PI3K, is critical in cell proliferation, survival, metabolism, autophagy, apoptosis, and migration processes [435, 436]. The PI3K/Akt/mTOR signaling pathway, often overactivated in cancers, is linked with tumor growth and development, positioning mTOR as a crucial antitumor target [437, 438]. Recent advances demonstrate the synergistic anticancer effects of combined HSP90 and mTOR inhibitors across various cancers, promoting the development of single molecules that target both HSP90 and mTOR [439, 440]. The team designed and synthesized a novel series of thieno[2,3-d]pyrimidine derivatives with resorcinol and morpholine groups, targeting both HSP90 and mTOR. Initial biological evaluations indicated compound 17o exhibited potent inhibitory activity against HSP90 and mTOR, significantly reducing proliferation in SW780 bladder cells. Post-treatment with 17o, the expression of Hsp90 client proteins such as CDK4, C-Raf, and CDC2 markedly declined. Additionally, 17o induced autophagic cell death and apoptosis via the mitochondrial pathway and reduced phosphorylation of mTOR and AKT, thus demonstrating antitumor activity by inhibiting the hyperactivated PI3K/AKT/mTOR pathway [441].

Studies have indicated that monoamine oxidase A (MAO-A) inhibitor could impede the progression of GBM and other cancers [442–444]. Shih and colleagues aimed to identify dual inhibitors of MAO-A and HSP90 to enhance the efficacy of GBM treatments. This research synthesized a series of compounds by integrating the pharmacophore of HSP90 inhibitors, specifically 4-isopropyl catechol, with the MAO-A inhibitor clorgyline. It assessed the inhibitory effects of these compounds on MAO-A and HSP90, revealing that compounds 4b and 4c demonstrated significant activity against both temozolomide-sensitive and temozolomide-resistant glioblastoma cells. Subsequent in vitro and in vivo analyses indicated that these compounds might suppress HER2, phosphorylated AKT, and enhance HSP70 expression by inhibiting MAO-A and HSP90, thereby inhibiting the growth of human GBM and various other cancer cell lines. Compounds 4b and 4c decreased PD-L1 expression in GBM cells, suggesting their potential as immune checkpoint inhibitors. These findings contribute to developing potential therapeutic agents for GBM and

other malignancies [445]. Key evidence suggested that EZH2 is involved in the tumorigenesis and progression of GBM, with the combination of the EZH2 inhibitor Tazemetostat and the HSP90 inhibitor STA9090 showing promising therapeutic potential [446–448]. Building on this foundation, a study by Liou et al. reported the design of a novel dual-target EZH2/HSP90 inhibitor, compound 7, through a molecular hybridization strategy that integrates the pharmacophores of Tazemetostat and STA9090. Structure-based molecular docking revealed that compound 7 interacts with the ATP binding pocket of HSP90 and the methylation site of EZH2, consistent with the binding sites of inhibitors STA9090 and Tazemetostat. In vitro activity assays indicated that compound 7 inhibited both EZH2 and HSP90, with IC_{50} values of 6.29 nM and 60.1 nM, respectively. This dual inhibition led to significant inhibitory activity against TMZ-resistant Pt3-R cells, with an IC_{50} of 1.015 μM. Furthermore, compound 7 induced cell cycle arrest at the M phase, increased the expression of apoptosis-related genes and enhanced the accumulation of ROS by disrupting redox homeostasis in mitochondria. Importantly, in vivo experiments demonstrated that compound 7 exhibited significant anti-GBM effects, effectively suppressing tumor growth derived from TMZ-resistant GBM cells and showing the ability to penetrate the blood–brain barrier, providing strong evidence for its potential as an anti-GBM agent [449].

Ligand-directed conjugates are promising for selectively targeting tumor cells and minimizing systemic toxicity [450, 451]. ARs are effective targets in treating castration-resistant prostate cancer (CRPC) [452, 453]. Accordingly, a study by Qin et al. reported the development of small-molecule conjugates, WCA814 and SQA-710, composed of an AR antagonist and an Hsp90 inhibitor, for treating CRPC. These conjugates could bind to overexpressed extracellular Hsp90 (eHsp90) on CRPC cell surfaces, inducing endocytosis and concentrating the drugs in tumor cells LNCaP and 22Rv1. The internalized conjugates inhibited AR function through their antagonist component and decreased AR protein levels by inhibiting Hsp90, effectively suppressing AR signaling. In vivo, studies in 22Rv1 and LNCaP xenograft models showed that these compounds had enhanced inhibitory efficacy compared to single AR antagonists or Hsp90 inhibitors. Notably, WCA-814 specifically accumulated in CRPC cells without causing significant organ damage, indicating good tolerance and potential for improving therapeutic indices while reducing systemic toxicity. This study underscores the benefits of ligand-directed therapeutic conjugates in cancer therapy and offers insights into the future development of such active molecules [454]. Additionally, Nimesulide, known for its anti-inflammatory properties, exhibits antitumor activity [455]. Using Nimesulide as a scaffold, Jaragh-Alhadad et al. developed analogs targeting tubulin and HSP27 for treating female cancers. Preliminary evaluations in SKOV3 and SKBR3 cell lines showed that these analogs inhibited tubulin and HSP27 at micromolar concentrations, though further studies are required to elucidate their mechanisms of action [456].

All these findings demonstrate the potential of dual-target HSP inhibitors in recent cancer therapy advancements, providing a valuable reference for developing new drugs to enhance efficacy and address challenges like drug resistance.

Conclusions

Here, we presented a comprehensive review of the pivotal roles of HSPs in the hallmarks of cancer, encompassing the development of a series of HSP inhibitors and the study of their respective mechanisms of action. On the one hand, this review highlights the involvement of HSPs in various characteristic processes of cancer, such as sustaining proliferative signaling, evading growth suppressors, and resisting cell death. On the other hand, this review also highlights the efforts and challenges scientists face in developing targeted HSP inhibitors. Although some drugs targeting HSPs have been approved for clinical use, such as ruxolitinib, a JAK inhibitor that effectively reduces the expression of HSP70 and HSP90, specific HSP inhibitors have not received widespread approval from global regulatory agencies, including the United States Food and Drug Administration (FDA), with the exception of pimitespib, which is marketed in Japan [457]. Furthermore, this review highlights the multiple biological effects of HSP-targeted inhibitors in cancer therapy, which not only helps elucidate the necessity of developing inhibitors that bind specifically to HSPs, including subtype-selective and function-selective inhibitors but also highlights the potential of using HSP inhibitors in combination with other therapeutic agents or developing multitargeted drugs for cancer treatment. As presented in this review, combination treatment plans involving HSP inhibitors and protein kinase inhibitors, HDAC inhibitors, or metal complexes, among other anticancer drugs, have been explored. More importantly, HSP-based dual-target inhibitors offer great potential for enhancing therapeutic efficacy and reducing resistance, providing researchers with ample opportunities for innovation.

By analyzing the research reports presented in this review, we can conclude that HSPs are involved in nearly every aspect of the hallmarks of cancer. As our understanding of tumor hallmarks and HSPs deepens, the relationships between them will become more distinct and

explicit. Notably, different classes of HSPs exhibit different distribution patterns in various tumor hallmark processes, likely due to the differences in spatial distribution and biological functions among HSP types. For example, HSP90 is involved primarily in regulating signal transduction pathways and stabilizing and activating various signal transduction proteins, such as kinases and receptors; thus, HSP90 participates in almost all stages of cancer development. In contrast, HSP60 is localized mainly in the mitochondria and is involved in the proper folding and assembly of mitochondrial proteins, contributing to hallmark processes such as evading growth suppression, resisting cell death, and metabolic reprogramming. Additionally, there is an imbalance in the scope and depth of research on different types of HSPs, which has led to the more comprehensive and detailed understanding of HSP90 and HSP70, whereas other types of HSPs await further investigation. These underexplored areas may obscure the yet-to-be-elucidated relationship between HSPs and cancer hallmarks. When targeting HSPs for cancer therapy, the development of combination treatment strategies and dual-target inhibitors has emerged as a significant opportunity, with the following primary advantages. (1) Overcoming drug resistance: Combination therapy or dual-target modulation can target HSPs and other tumor-related pathways simultaneously, reducing the occurrence of drug resistance. (2) Increased therapeutic efficacy: Targeting HSPs or their related pathways can help overcome the compensatory mechanisms of tumor cell growth systems, increasing the vigor and practicality of treatment due to the differences and complementarities in the roles of different HSPs in tumorigenesis and development. (3) Reducing toxic side effects: Previous studies have shown that some individual HSP inhibitors may cause significant side effects. Combination therapy or dual-target inhibitors can mitigate these side effects while exerting synergistic antitumor effects. (4) Precision medicine: Different types of tumors and those at different stages have varying degrees of reliance on HSPs. Combination therapy strategies and dual-target inhibitors can be individually tailored according to the characteristics of the tumor and HSP expression, improving treatment specificity.

In the future, it is imperative to delve deeply into the mechanisms by which different HSPs participate in the hallmarks of cancer in terms of tumor cell proliferation, invasion, metastasis, angiogenesis, and immune modulation, as this review provides a theoretical foundation for the development of new precision drugs and therapeutic strategies. Given the differential distribution of HSPs regarding the hallmarks of cancer, the further formulation of personalized HSP-targeted treatment plans on the basis of the expression profiles and functional characteristics of HSPs in tumors of different types and in different stages, as well as the discovery of biomarkers that can predict the efficacy of HSP-targeted therapies, will significantly advance the field of precision medicine. Furthermore, enhancing the chemical structures of current HSP inhibitors is imperative. Such refinements should encompass the incorporation and precise control of chiral structures [458, 459], late-stage saturation [460], and conformational optimization [461], among other modifications. Emphasis should be placed on improving the specificity, selectivity, stability, and bioavailability of dual-targeted or multitargeted drugs. Additionally, there should be concerted efforts to explore novel heat shock protein-targeted therapies, such as targeted protein degraders [462, 463], allosteric inhibitors [464–466], and covalent inhibitors [341, 467], along with advanced drug delivery systems [468]. Moreover, unprecedented opportunities will arise with the rapid development of artificial intelligence technology, especially in terms of a detailed understanding of the microstructures of HSPs and high-throughput screening of related targeted drugs. On this basis, special attention must be given to the precise actions of drug molecules on various HSP subtypes or isoforms [469], which is crucial for improving drug–target specificity, reducing toxic side effects, and enhancing specific drug functions. Finally, owing to the molecular chaperone characteristics of HSPs, expanding the exploration on their mechanisms of action and therapeutic potential in autoimmune diseases and neurodegenerative diseases will be beneficial for broadening the research and application scope of HSPs.

In summary, future research on HSP-related cancer therapies should focus on elucidating their mechanisms of action, developing efficient inhibitors with low toxicity, other drug modalities, and new delivery systems and combination therapy strategies. Additionally, promoting personalized precision therapy and clinical translation and expanding the application of HSP-targeted drugs to other diseases will be paramount. Achieving these goals will require close cooperation and collaborative innovation across various domains, including basic research, drug development, and clinical application, to provide more effective and safer treatment options for cancer patients.

Abbreviations

ACD	Alpha-crystallin domain
AKT	Protein kinase B
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
Ang	Angiopoietin
Apaf-1	Apoptotic protease activating factor-1
Ask-1	Apoptosis signal-regulating kinase 1
ASM	Acid sphingomyelinase
ATG14	Autophagy-related gene 14
ATP5A1	ATP synthase F1 subunit alpha

Bcl-2	B cell lymphoma-2
Cdc37	Cell division cycle 37
CDK	Cyclin-dependent kinase
CHIP	C-terminus of HSC70-interacting protein
CHK1	Checkpoint kinase 1
CML	Chronic myeloid leukemia
COX	Cytochrome c oxidase
CRPC	Castration-resistant prostate cancer
CTD	C-terminal domain
Cyt-C	Cytochrome C
DAXX	Death domain-associated protein
DCs	Dendritic cells
DISC	Death-inducing signaling complex
DLST	Dihydrolipoamide S-succinyltransferase
ECM	Extracellular matrix
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
eHsp90	Extracellular Hsp90
EMT	Epithelial–mesenchymal transition
ER	Endoplasmic reticulum
Erα	Estrogen receptor α
ERK	Extracellular signal-regulated kinase
FADD	Fas associated death domain
FGF	Fibroblast growth factor
FGFR	Fibroblast growth factor receptor
FIH	Factor-inhibiting HIF
FKBP	FK506-binding protein
FoxO1	Forkhead box O1
Gal3	Galectin-3
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GBM	Glioblastoma
GLUT1	Glucose transporter 1
GPCR	G protein-coupled receptor
Grb2	Growth factor receptor bound protein 2
GRP78	Glucose-regulated protein 78
GSK-3β	Glycogen synthase kinase-3β
HCC	Hepatocellular carcinoma
HDACs	Histone deacetylases
HER2	Human epidermal growth factor receptor 2
HIF-1α	Hypoxia inducible factor-1α
HK2	Hexokinase 2
HNSCC	Head and neck squamous cell carcinoma
HSF-1	Heat shock factor 1
HSPs	Heat shock proteins
HSR	Heat shock response
hTERT	Human telomerase reverse transcriptase
HUVECs	Human umbilical vein endothelial cells
ICB	Immune checkpoint blockade
IGF2	Insulin-like growth factor 2
IL-6	Interleukin-6
IRE1α	Inositol-requiring enzyme 1 alpha
JAK	Janus kinase
JNK1/2	C-Jun N-terminal protein kinase 1/2
LCD	Lysosomal-dependent cell death
LDHA	Lactate dehydrogenase A
LEF	Lymphoid enhancer factor
LMP	Lysosomal membrane permeabilization
LC3	Light chain 3
MAO-A	Monoamine oxidase A
MAPK	Mitogen-activated protein kinase
MD	Medial domain
MDM2	Murine double minute 2
MDR	Multidrug resistance
MHC	Major histocompatibility complex
MMPs	Matrix metalloproteinases
MOMP	Mitochondrial outer membrane permeability
mTOR	Mammalian target of rapamycin
mTORC	Mammalian target of rapamycin complex
mutp53	Mut-type p53
NBD	Nucleotide-binding domain
NEF	Nucleotide exchange factor
NFAT	Nuclear factor of activated T cells
NF-κB	Nuclear factor-κB
NPC	Nasopharyngeal carcinoma
NSCLC	Non-small cell lung cancer
NTD	N-terminal domain
Par-4	Prostate apoptosis response 4
PDAC	Pancreatic ductal adenocarcinoma
PDGF	Platelet-derived growth factor
PDK	Pyruvate dehydrogenase kinase
PD-1	Programmed death 1
PD-L1	Programmed death ligand-1
PFKP	Phosphofructokinase platelet
PIP2	Phosphatidylinositol-4,5-biphosphate
PIP3	Phosphatidylinositol-3,4,5-triphosphate
PI3K	Phosphatidylinositol 3-kinase
PKM2	Pyruvate kinase typeM2
PPIs	Protein–protein interactions
PRCC	Papillary renal cell carcinoma
Rb	Retinoblastoma tumor suppressor protein
RCD	Regulated cell death
ROS	Reactive oxygen species
RTK	Receptor tyrosine kinase
SBD	Substrate-binding domain
SDH	Succinate dehydrogenase
SFK	SRC family kinases
sHSPs	Small heat shock proteins
STAT3	Signal transducer and activator of transcription 3
TCA	Tricarboxylic acid
TCF	T-cell factor
TERC	Telomerase RNA component
TGF-β	Tumor growth factor β
TIMP-1	Tissue inhibitor of metalloproteinase-1
TKI	Tyrosine kinase inhibitor
TNBC	Triple-negative breast cancer
Topo II	Topoisomerase II
TRAP1	Tumor necrosis factor receptor-associated protein 1
TNF	Tumor necrosis factor
ULK1	Unc-51-like kinase 1
UPR	Unfolded protein response
VEGF	Vascular endothelial growth factor
VPS34	Vacuolar protein-sorting 34
wtp53	Wild-type p53
XIAP	X-linked inhibitor of apoptosis protein

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Declarations

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Not applicable.

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Competing interests
The authors declare no competing interests.

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