Targeting Heat-Shock Protein 90 in Cancer

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Heat-shock protein 90 (HSP90) is an important molecule chaperone associated with tumorigenesis and malignancy. HSP90 is involved in the folding and maturation of a wide range of oncogenic clients, including diverse kinases, transcription factors and oncogenic fusion proteins. Therefore, it could be argued that HSP90 facilitates the malignant behaviors of cancer cells, such as uncontrolled proliferation, chemo/radiotherapy resistance and immune evasion. The extensive associations between HSP90 and tumorigenesis indicate substantial therapeutic potential, and many HSP90 inhibitors have been developed. However, due to HSP90 inhibitor toxicity and limited efficiency, none have been approved for clinical use as single agents.

heat-shock protein 90 inhibitors molecular chaperones HSP90

1. Introduction

The HSP family is a group of molecular chaperones facilitating the folding and maturation of a group of proteins termed “client” proteins. When exposed to environmental stressors (heat, heavy metals, hypoxia, and acidosis), cells increase the expression of HSPs as an adaptive response to maintain cell homeostasis [1]. Based on molecular weights, the HSP family can be classified into HSP27, HSP40, HSP60, HSP70, HSP90, and large HSPs. Members of this family are closely linked to each other through a signal network [2].

HSP90 participates in client protein cycles differently from other chaperones. In contrast to Hsp70 and Hsp60, HSP90 does not facilitate the folding of newly synthesized proteins but mainly plays an important role in the late-stage maturation, activation, and stability of client proteins [3]. Furthermore, HSP90 interacts with a more specific client group that is highly dependent on HSP90 for activation. The HSP90 clients mostly comprises unstable signaling molecules, particularly kinases and transcription factors regulating signal transduction, protein trafficking, chromatin remodeling, autophagy, cell proliferation, and survival [1][4]. Many of these clients are oncogenic proteins considered key drivers of the occurrence and development of cancers.

2. Structure and Function of HSP90

HSP90 exists in cells in the form of a homodimer. Each monomer comprises four domains: the N-terminal domain (NTD), the middle domain (MD), the C-terminal domain (CTD), and a charged linker region of negative charges connecting the N-terminal and middle domains. The sequence of the charged linker can influence HSP90’s functions [5].

The NTD is responsible for the binding of ATP with an adenine nucleotide-binding pocket at the N-terminus. X-ray crystallography studies revealed that this binding is closely related to the Bergerat fold in the ATP binding pocket [6]. With its ATPase activity, the NTD functions as a driver of certain alterations such as the binding of client proteins. The MD is also reported to modulate HSP90 ATPase activity and binding to substrates [7]. The CTD is important for HSP90 dimerization, which is the structural basis for the normal functioning of HSP90. The CTD adenine nucleotide-binding pocket activates only when the N-terminal site is occupied [8]. The CTD is also the binding site for small molecules such as nucleotides, novobiocin, and cisplatin [1].

There are four isoforms of HSP90: HSP90α (inducible form), HSP90β (constitutive form), GRP94 (glucose-regulated protein 94), and TRAP1 (tumor necrosis factor receptor-associated protein 1). HSP90α and HSP90β share a similar structure, function, and intracellular localization in the cytoplasm. GRP94 is a glycoprotein that can protect cells from endoplasmic reticulum stress. GRP94 has a special client group, including secreted and membrane proteins including the immunoglobulin (Ig) family, Toll-like receptor, and integrins [9]. Like the cytosolic isoforms, GRP94 also contains NTD, MD, CTD and the charged linker TRAP1 is a mitochondrial isoform of HSP90 mostly located in the mitochondrial matrix. Unlike other isoforms, TRAP1 does not have the charged linker domain. TRAP1 is essential for mitochondrial homeostasis in some pathological status [10]. HSP90's normal function requires assistance from other components, and cochaperones are the most important. Cochaperones are proteins that aid HSPs in conformational cycling and the binding of substrates. Some cochaperones containing tetratricopeptide repeat (TPR) domains interact with HSP90 by binding to its C-terminal MEEVD motif. Among the most specific TPR-containing cochaperones is HSC70/HSP90-organizing protein (HOP), which can bind to both HSP90 and HSP70. HOP functions as a stabilizer of the HSP90 open conformation [4]. Other TPR-containing cochaperones include Tah1, protein phosphatase 5 (PP5), CHIP, cyclophilin 40 (CYP40), and tetratricopeptide repeat domain 4 (TTC4) [11]. In addition to that, there are also non-TPR-containing cochaperones. A well-studied non-TPR-containing cochaperone, cell division cycle 37 (Cdc37), is reported to be specific for the maturation of kinases and leads to partial inhibition of HSP90 ATPase activity [12]. Cdc37 is associated with tumor formation and progression. Other non-TPR-containing cochaperones include the Activator of HSP90 ATPase homolog 1 (activating ATPase activity of HSP90) and p23 (inhibiting HSP90 ATPase activity) [13].

3. Roles of HSP90 in Cancer

Tumor cells are subject to multiple stressors, including altered signal pathways, mutated client proteins, hypoxia, low pH, and extra demands for nutrition. Tumor cells are highly dependent on oncoproteins involved in maintaining internal homeostasis when exposed to these hostile stressors. Reduced expression of oncoproteins or increased depletion is more lethal in tumor tissues than in normal cells. Many oncoproteins are expressed in mutated forms; therefore, their metastability and activation are more dependent on HSP90 chaperones [14]. Research on *D. melanogaster* and *Arabidopsis thaliana* model systems has demonstrated that HSP90 can function as a biochemical buffer conferring cancer cells an ability to tolerate mutated proteins and altered signal pathways [1]. Therefore, higher levels of HSP90 permit oncogenic proteins to fold properly in a hostile environment. This

overwhelming dependence of tumor cells on HSP90 is among the reasons why HSP90 is considered an ideal anticancer target [15].

HSP90 is important for multiple steps in malignant transformation and progression, including tumor proliferation, migration, invasion, antiapoptosis, immortalization, angiogenesis, and therapeutic resistance. For example, abnormal telomerase activity is observed in most human cancers but lacking in normal cells. In immortalized tumor cells, HSP90 interacts with the hTERT (human telomerase reverse transcriptase) promoter, and inhibition of HSP90 can result in decreased hTERT expression [16]. The serine/threonine kinase AKT (also known as PKB) controls key cellular processes such as proliferation and antiapoptotic effects. Inhibition of HSP90 downregulates the expression of Akt kinase and, as a result, sensitizes tumor cells to proapoptotic factors [17][18]. Focal adhesion kinase (FAK) and integrin-linked kinase (ILK) are critical promoters to cell adhesion. Inhibition of HSP90 can induce the depletion of FAK and ILK in cancer cells [19][20]. HIF-1α overexpression is reportedly linked to angiogenesis and antiapoptosis effects in cancer cells. HSP90 inhibitors can reverse the overexpression of HIF-1α (hypoxia inducible factors-1α) and simultaneously downregulate survival signaling pathways [21]. Human vascular endothelial cell growth factor (VEGF) is known to be a key player in angiogenesis via the HIF-1α/VEGF/VEGFR-2 signaling pathway. Blocking the VEGF-related pathway with an HSP90 inhibitor can suppress angiogenesis in breast cancer [22].

HSP90 is also closely related to tumor treatment resistance. For instance, therapeutic resistance to some DNA-targeted approaches is a consistent obstacle to cancer treatment. When tumor cells are exposed to treatments such as ionizing radiation or alkylation agents, the HSP family is the first line of defense to maintain DNA integrity and cell integrity [23].

In the past few years, the role of HSP90 in the DNA repair pathway has been gradually revealed with the in-depth study of the HSP90 client group. DNA damage is mediated by members of the phosphoinositide 3-kinase (PI3K)-related kinase (PIKK) family, comprising ATR (Rad3-related), ataxia telangiectasia mutated (ATM), and DNA-PKcs (DNA-dependent protein kinase catalytic subunit). This trinity of key kinases constitutes the core of the DNA damage response [24]. When double-strand breaks (DSBs) occur, MRE11-RAD50-NBS1 (MRN), a complex that can sense DSBs, recruits HSP90 to the repair foci and activates ATM with HSP90's assistance [23]. In addition, Quanz et al. found that HSP90 is rapidly phosphorylated when DNA damage occurs, and this phosphorylation is mainly dependent on DNA-PK and ATM. Subsequently, phosphorylated HSP90 accumulates at the site of damage as a key promoter of repair [25]. Moreover, DNA-PK and ATR are identified as client proteins [26][27]. Therefore, pharmacological inhibitors of HSP90 can interfere with the DNA damage response by mediating the degradation of related proteins. Numerous in vitro and in vivo experiments have shown that HSP90 inhibitors can be used as tumor radiation sensitizers to enhance the killing effect on tumor tissues [21][28][29]. The complete mechanism by which HSP90 participates in the DNA damage repair pathway has not been entirely revealed, but the key roles of HSP90 in DNA damage repair provide perspectives for addressing the treatment resistance of some anticancer therapies.

Intriguingly, HSP90 plays a supportive role in the immune response against cancer. First, both intracellular and extracellular Hsp90 are involved in the process of antigen presentation. On the one hand, intracellular HSP90 is responsible for escorting antigenic peptides to TAP1/2 (transporters associated with antigen processing 1/2) and then into the ER (endoplasmic reticulum). In the ER, antigenic peptides are loaded onto newly synthesized MHC-I molecules. Eventually, MHC-I molecules carrying antigenic peptides are transported to the surface of tumor cells and are recognized by CD8+ T cells. On the other hand, extracellular Hsp90 secreted in the form of exosomes can bind to peptide antigens in the extracellular matrix. Then, HSP90 plus peptide antigens are recognized by HSP receptors on antigen-presenting cells (APCs) and internalized to be degraded by the proteasome. These processed peptide antigens are loaded onto MHC-II molecules in the ER and transported to the surface of APCs. Eventually, this leads to the activation of CD4+ T cells [19]. Notably, the role of HSP90 in antigen presentation has led to the research and development of HSP90-based cancer vaccines, which are not covered in detail here due to a lack of space [30].

In addition, extracellular Hsp90 is thought to be a signal for danger/damage-associated molecular patterns (DAMPs). Extracellular Hsp90 can promote the secretion of activating cytokines (IL-12) and the expression of costimulatory molecules, which potently stimulate T cells [20]. Extracellular Hsp90 also assists in the folding of receptors on immune cells such as natural killer cells and T lymphocytes [19].

4. HSP90 Combination Therapy

4.1. Chemotherapy

Taxanes are used as microtubule-targeting antitumor agents in cancer chemotherapy. Taxanes target β-tubulin in polymerized microtubules and cause mitotic arrest and apoptosis [31]. Preclinical data from different cancer cell lines and tumor xenograft models indicate that HSP90 inhibitors are synergistic with taxanes in targeting tumors. When 17-AAG and paclitaxel were combined, a significant growth inhibition effect of non-small-cell lung cancer (NSCLC) cells was observed in vitro and in vivo. The cytotoxicity of paclitaxel was enhanced 5–22-fold by 17-AAG [32]. In addition, in breast cancer with high levels of HER2 expression and amplification of AKT, Hsp90 inhibitor 17-AAG sensitized breast cancer cells to Taxol by causing the degradation of HER2 and the inactivation of Akt both in vitro and in vivo [18].

Importantly, a randomized phase II study of ganetespib combined with docetaxel (GALAXY-1) was designed to evaluate efficacy and safety in advanced NSCLC. Although this did not meet its primary endpoints, patients >6 months after the diagnosis of advanced lung adenocarcinoma showed significantly prolonged progression-free survival (PFS) and overall survival (OS) rates from this combination [33]. This finding led to the large-scale phase III trial of this combination (GALAXY-2) in patients with chemotherapy-sensitive advanced lung adenocarcinoma. Unfortunately, ganetespib synergizing with docetaxel did not improve survival in patients with advanced lung adenocarcinoma (NCT01798485) [34]. Subsequently, an research revealed the possible mechanisms for this failure. KRAS mutant NSCLC can rapidly obtain resistance to ganetespib due to bypass of ganetespib effects, inducing G2/M arrest. This acquired resistance to ganetespib then results in cross-resistance to docetaxel. Moreover,

overactivated p90RSK-CDC25C signaling is the core of G2/M arrest. Administration of p90RSK inhibitors and/or CDC25C inhibitors may reverse KRAS mutant NSCLC resistance to ganetespib [35].

Another chemotherapy agent, cisplatin, is used to treat several cancer types, including sarcomas, carcinomas, lymphomas, and germ cell tumors. Cisplatin interacts with DNA bases and eventually causes apoptosis. 17-AAG demonstrated synergistic anticancer activity with cisplatin in pediatric solid tumor cells (neuroblastoma and osteosarcoma) by inducing depletion of IGF1R and AKT, which are two key antiapoptotic proteins [36]. In the case of cisplatin-resistant pancreatic ductal adenocarcinoma cells (PDACs), 17-AAG sensitizes PDACs to cisplatin. The underlying mechanism of this synergism is the degradation of Fanconi anemia pathway factors by 17-AAG. As a result, the repair of DNA adducts induced by cisplatin is eliminated [37][38]. A recent study showed that ganetespib combined with pemetrexed and cisplatin was safe and effective in patients with malignant pleural mesothelioma (MPM). The combination of antifolate and platinum is the first-line treatment for MPM. Ganetespib causes the degradation of the HSP90 client thymidylate synthase to reverse antifolate resistance [39]. Furthermore, a triple combination of SNX-5422, carboplatin, and paclitaxel followed by maintenance SNX-5422 therapy showed substantial tolerance and antitumor activity against NSCLC [40].

The key role of HSP90 in the DNA damage response has been described above. HSP90 inhibitors can significantly induce depletion of essential HSP90 clients in the DNA damage response. In addition, an HSP90 inhibitor can abrogate S and G2/M cell cycle checkpoint controls by promoting the degradation of the client kinases CHK1 and WEE1 [41]. A quantitative spectrum analysis of protein expression changes after the administration of 17-DMAG revealed that DNA damage response pathways are among the most sensitive pathways at very low 17-DMAG concentrations [42]. This finding suggests that HSP90 inhibitors may selectively radiosensitize tumor cells.

HSP90 inhibitors have long been a promising solution to radiation resistance. Yin et al. reported that both BIIB021, an HSP90 inhibitor based on the purine scaffold, and 17-AAG showed anticancer effects on head and neck squamous cell carcinoma (HNSCC) xenografts as a single agent. However, xenografts treated with BIIB021 and radiation grew slower at the same time and even showed regression [43]. In a heterotopic transplantation model of colorectal cancer cells, the HSP90 inhibitor NW457 synergized with radiotherapy and induced a stronger inhibitory effect on tumor growth [28]. In another case of GBM, concurrent exposure to the HSP90 inhibitor NXD30001 and radiotherapy remarkably inhibited tumor growth and extended the median survival of tumor-bearing mice [44]. One study revealed that low, nontoxic doses of the Hsp90 inhibitor AT13387 (Onalespib) can selectively sensitize head and neck squamous cell carcinoma (HNSCC) and pancreatic cancer cells to radiotherapy, while there was no synergetic effect on normal cells [45].

4.3. Immunotherapy

Immune checkpoint blockade has received extensive attention recently due to its clinical activity in many types of human cancers. Response failures and adverse immune responses are major issues in the evolution of this immunotherapy. Some studies show that combining immune checkpoint blockade with HSP90 inhibitors may be a promising way to address these problems.

The HSP90 inhibitor ganetespib was found to potentiate the antitumor efficacy of the anti-PD-L1 antibody (STI-A1015) in mice bearing MC38 colon carcinoma tumors and B16 melanoma tumors. The mechanism contributing to this synergistic effect is that inhibition of HSP90 affects PD-L1 expression and HIF-1α, JAK2, and mutated EGFR [46]. Mbofung et al. reported that the HSP90 inhibitor ganetespib improved T-cell-mediated tumor cytotoxicity to melanoma cells. This effect of ganetespib could be explained by the upregulation of interferon response genes induced by ganetespib [47]. Moreover, in the MC-38 syngeneic mouse tumor model, ganetespib remarkably reduced the expression of immune checkpoint proteins, including PD-L1 and PD-L2 [48].

In a more recent study, combination treatment with the HSP90 inhibitor XL888 and PD-1 blockade was effective in pancreatic ductal adenocarcinoma (PDAC) models. PDAC is characterized by fibrotic stroma closely related to pancreatic stellate cells (PSCs) and cancer-associated fibroblasts (CAFs). XL888 downregulates IF-6 expression in PSCs/CAFs and directly inhibits PSC/CAF growth, thereby enhancing the efficacy of anti-PD-1 blockade [49].

4.4. Protein Kinase Inhibitors

Protein kinases are the largest single group of HSP90 clients. Several protein kinase inhibitors (PKIs) have been reported to synergize with Hsp90 inhibitors in killing tumor cells. Raf kinase is an HSP90 client; thus, HSP90 inhibition could promote the antitumor efficiency of Raf kinase inhibitors. When combining the Raf kinase inhibitor sorafenib with tanespimycin (17-AAG), clinical efficacy was observed in 9 of 12 renal cancer patients and 4 of 6 melanoma patients [50]. The addition of SCH727965 (SCH), a cyclin-dependent kinase inhibitor, to NVP-AUY922 (AUY) can induce apoptosis of osteosarcoma (OS) cells with no effect on normal osteoblasts or fibroblasts [51]. Another CDK inhibitor, dinaciclib, synergized with the novel HSP90 inhibitor HAA2020 and displayed stronger apoptotic and cell cycle control properties in acute myeloid leukemia (AML) [52].

BRAF and/or MEK inhibitors are important options for treating BRAFV600E mutant high-grade gliomas (HGGs). Nevertheless, therapeutic outcomes are often disappointing due to drug resistance. Recently, an in vitro and in vivo study reported that adding an HSP90 inhibitor may solve this problem. HSP90 inhibitors can deactivate the MAPK and AKT/mTOR pathways, which are reactivated by BRAF and/or MEK inhibitors. Therefore, HSP90 inhibitors combined with BRAF and/or MEK inhibitors induced apoptosis of HGG cells [53].

Approximately 20–30% of breast cancers are human epidermal growth factor 2 (HER2)-positive with more malignant characteristics than other breast cancer subtypes. HER2 is a member of the ErbB family of transmembrane receptor tyrosine kinases. The amplified HER2 gene induces overexpression of HER2, which leads to tumor proliferation, adhesion and aggression. HSP90 is known to modulate the tyrosine kinase activity of HER2. Once HSP90 is inhibited, HER2 cannot be folded properly and is eventually degraded. This degradation undoubtedly increases the efficacy of some HER2-targeted drugs, such as lapatinib and trastuzumab.

Lapatinib, a tyrosine kinase inhibitor inhibiting both HER2 and EGFR, has been widely used for treating HER2 (+) breast cancer patients. However, due to the acquired resistance of most patients, the prognosis is unsatisfactory. Lapatinib in combination with 17-DMAG showed a synergistic effect in suppressing cell proliferation in vitro and in

vivo [54]. Another well-known kinase inhibitor targeting HER2, trastuzumab, is a humanized anti-HER2 antibody used to treat HER2 (+) breast cancer. Similar to lapatinib, most patients eventually develop resistance to the drug within 1–2 years. In a phase I clinical trial, combination therapy showed antitumor activity and great tolerance in patients with trastuzumab-resistant HER2 (+) breast cancers [55]. A phase II trial of 17-AAG plus trastuzumab in patients with HER2 (+) metastatic breast cancer revealed that this combination had a potent anticancer effect against those patients who previously progressed by using trastuzumab.

4.5. Proteasome Inhibitors

There is a complementary effect between HSP90 inhibitors and proteasome inhibitors. When both exist simultaneously, undegraded proteins will accumulate in cells, and consequent protective mechanisms induced by such accumulation will be prevented [56].

The proteasome inhibitor bortezomib in combination with 17-AAG was evaluated in patients with multiple myeloma (MM) in a phase I/II trial. The results showed that bortezomib plus 17-AAG was well tolerated, and bortezomib-naive patients had the highest response rates (41%) [57]. A subsequent phase III trial was suspended for nonclinical reasons.

The combination of the Hsp90 inhibitor KW-2478 with the proteasome inhibitor bortezomib showed a stronger inhibition of myeloma (MM) cell growth and synergistic antitumor efficacy in a subcutaneously inoculated human myeloma model [58]. Moreover, patients with relapsed/refractory MM showed substantial tolerance to this combination with no apparent overlapping toxicity in a phase I/II clinical trial. However, the antimyeloma activity of this combination was relatively modest [59].

4.6. Histone Deacetylase Inhibitors

Histone deacetylases (HDACs) are responsible for the deacetylation of many proteins, including Hsp90. HDAC inhibitors can induce tumor cell apoptosis, growth arrest, cell cycle arrest, senescence, differentiation, and immunogenicity and inhibit angiogenesis via different downstream cellular pathways [60]. HDAC inhibitors displays great activity in hematological tumors. However, HDAC inhibitor’s outcomes with solid tumors are not that successful as expected. On the one hand, poor pharmacokinetics of many HDAC inhibitors prevent them from accumulating to effective concentrations in solid tumors as it does in blood tumors. One the other hand, low permeability can also affect the accumulation of HDAC inhibitors. As for hematologic tumors, HDAC inhibitors are easier to reach their therapeutic concentrations and a short half-life do not affect their anticancer activity [61].

AN effective HDAC inhibitor, Panobinostat (LBH589), is thought to exhibit best anticancer activity when combined with other therapies [62]. This suggest that combination therapy may be a great strategy to address HDAC inhibitors’ limited use. When an HDAC inhibitor is administered, HSP90 is hyperacetylated. Hyperacetylation can interfere with the function of HSP90 and ultimately lead to the degradation of oncogenic client proteins [63].

4.7. Other HSP Inhibitors

Among the reasons for the limited efficacy of HSP90 inhibitor monotherapy is resistance to HSP90 inhibitors. When inhibition of HSP90 leads to downregulation of broad intracellular signal pathways, other members of the HSP family, such as HSP27 and HSP70, are upregulated for feedback, thereby neutralizing the effects induced by HSP90 inhibition [64][65]. The upregulation of HSP27 was reported to promote 17-AAG resistance by modulating glutathione (GSH) [64]. Several other studies have found this upregulation of HSP27 by HSP90 inhibition. In addition, overexpression of HSP70 can resist apoptosis induced by 17AAG [66].

Simultaneously, inhibiting several HSP molecules, including HSP90, may be the key to solving this issue. Inhibiting HSP 27 with Hsp27-specific siRNA was found to potentiate the inhibitory effect of the Hsp90 inhibitor GA on breast cancer stem-like cells (BCSCs) [65]. In another case, the HSP27 inhibitor OGX-427 enhanced the anticancer effects of the Hsp90 inhibitor PF-04929113 in castration-resistant prostate cancer (CRPC) xenografts [67]. Increased hsp70 levels are associated with antiapoptosis and limited efficacy of Hsp90-based treatments, which are realized through HSP70 inhibiting death receptor and mitochondria-initiated signaling for apoptosis. KNK437, an HSP70 inhibitor, reversed Hsp70-induced apoptosis and significantly enhanced the antileukemia activity of 17-AAG [66]. Therefore, other HSP inhibitors can be considered a solution to HSP90 inhibitor resistance in future clinical treatments.

4.8. Others

Hsp90 inhibitors also act synergistically with many other anticancer therapies, including photodynamic therapy (PDT) [68] and hormone therapy [69][70][71], via different mechanisms. Furthermore, some bifunctional drugs, such as STA-8666 (HSP90 inhibitor and topoisomerase inhibitor SN-38) [72], DHP1808 (HSP90-PI3K) [73] and MPT0G449, are essentially in combination. Generally, an Hsp90 inhibitor is a sensitizer of cancer cells to different therapies.

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