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Review
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Traditional and Novel Mechanisms of Heat Shock Protein 90 (HSP90) Inhibition in Cancer Chemotherapy Including HSP90 Cleavage

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Abstract

HSP90 is a molecular chaperone that increases the stability of client proteins. Cancer cells show higher HSP90 expression than normal cells because many client proteins play an important role in the growth and survival of cancer cells. HSP90 inhibitors mainly bind to the ATP binding site of HSP90 and inhibit HSP90 activity, and these inhibitors can be distinguished as ansamycin and non-ansamycin depending on the structure. In addition, the histone deacetylase inhibitors inhibit the activity of HSP90 through acetylation of HSP90. These HSP90 inhibitors have undergone or are undergoing clinical trials for the treatment of cancer. On the other hand, recent studies have reported that various reagents induce cleavage of HSP90, resulting in reduced HSP90 client proteins and growth suppression in cancer cells. Cleavage of HSP90 can be divided into enzymatic cleavage and non-enzymatic cleavage. Therefore, reagents inducing cleavage of HSP90 can be classified as another class of HSP90 inhibitors. We discuss that the cleavage of HSP90 can be another mechanism in the cancer treatment by HSP90 inhibition.

Key Words: HSP90 inhibitors, Anti-cancer therapy, ATP binding, Acetylation, HSP90 cleavage

INTRODUCTION

Most living organisms commonly express heat shock proteins and their expression increases in response to a variety of stresses (Welch, 1993). Heat shock-induced expression of genes was first discovered in chromosomal puffing by heat shock in *Drosophila busckii* in 1962 (Ritossa, 1962). In 1974, it was first reported that the synthesis of a few proteins was enhanced by stresses such as heat shock in *Drosophila* cells (Tissieres *et al*., 1974). Heat shock protein 90 (HSP90) is a member of the heat shock protein family and functions as a molecular chaperone that supports the stability of client proteins. Typical examples of the client proteins are mutated p53, Bcr-Abl, Raf-1, Akt, human epidermal growth factor 2 (Her2/ErbB2), HIF-1α, etc. (Neckers and Workman, 2012).

HSP90 is evolutionarily conserved and has many isoforms, such as HSP90α, HSP90β, Grp94, and HSP75/TRAP1. Among these isoforms, HSP90α and HSP90β are localized in cytosol, HSP90α (major form) is constitutively expressed, and expression of HSP90β (minor form) is inducible (Sreedhar *et al*., 2004). HSP90 consists of three domains, N-terminal domain (N-domain), middle domain (M-domain), and C-terminal domain (C-domain). The N-domain has an ATP-binding pocket and ATPase activity (Prodromou *et al*., 1997). HSP90 forms a flexible dimer by interaction of C-domains. The formation and dissociation of compact dimers involving N-domains is important for the molecular chaperone activity (Rohl *et al*., 2013). Binding of ATP to the ATP-binding pocket of the N-domain promotes dimerization between the two N-domains, and the ATPase activity of the N-domain induces the hydrolysis of ATP to ADP, resulting in N-domain dissociation (Prodromou *et al*., 2000; Richter and Buchner, 2001). HSP90 requires co-chaperones, such as cdc37, Hop, p23, PP5, and Xap2, to function as a molecular chaperone. Co-chaperones interact with HSP90 and regulate ATPase activity for molecular chaperone activity of HSP90 and recruit client proteins to HSP90 (Zuehlke and Johnson, 2010; Rohl *et al*., 2013).

In most cancer cells, HSP90 and its client proteins are expressed at higher levels than normal cells. The client proteins, such as Her2/ErbB2, v-Src, Raf-1, Akt, hTERT, are important for cancer cell survival and growth (Ferrarini *et al*., 1992; Sharp and Workman, 2006; Miyata *et al*., 2013) (Table 1). HSP90 is

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Table 1. Selected client proteins of HSP90 related with tumor growth and survival

Class	Client protein of HSP90	Function	References
Receptor tyrosine kinase	Her2/ErbB2	Promotes cell proliferation and inhibits apoptosis	Moasser, 2007
	EGFR mutant	Promotes cell proliferation via activation of MAPK, AKT and JNK pathway	Voldborg *et al*., 1997
	FLT3	Regulates cell survival, proliferation and differentiation	Grafone *et al*., 2012
	VEGFR	Promotes vasculogenesis and angiogenesis	Kliche and Waltenberger, 2001
Signaling molecule and Kinase	Akt	Plays a role in apoptosis, cell proliferation, transcription and cell migration	Yoeli-Lerner and Toker, 2006
	mTOR	Regulates cell proliferation, motility and survival	Guertin and Sabatini, 2007
	p38	Regulates cell proliferation, apoptosis and motility	Koul *et al*., 2013
	v-Src	Promotes formation of cancer, cell movement and proliferation	Irby and Yeatman, 2000
	Raf-1 b-Raf	Activates cell growth signaling, such as MEK1/2 and ERK1/2	Leicht *et al*., 2007
	JAK2	Promotes cell proliferation and motility	Xu *et al*., 2017
	Bcr-Abl	Promotes cell proliferation and reduces apoptosis	Quintas-Cardama and Cortes, 2009
Transcription factor	Twist1	Promotes cancer metastasis and reduces apoptosis	Puisieux *et al*., 2006
	Hif-1α	Induce cell proliferation and angiogenesis	Tiburcio *et al*., 2014
	NF-κB	Keeps cell proliferation and protects from apoptosis	Xia *et al*., 2014
	p53 mutant	Transactivates growth-promoting and oncogenic genes	Ozaki and Nakagawara, 2011
Others	Cyclin D1	Controls cell cycle	Qie and Diehl, 2016
	VDUP-1	Inhibits cell growth and metastasis and contributes to apoptosis	Kaimul *et al*., 2007
	MUC1	Prevents cell death and promotes proliferation and invasion	Nath and Mukherjee, 2014
	MMP2/9	Plays a role in invasion and angiogenesis via breakdown of extracellular matrix	Gialeli *et al*., 2011
	Survivin	Inhibits apoptosis and regulates mitosis	Mita *et al*., 2008
	Vimentin	Increases migration and invasive capacity	Satelli and Li, 2011

also involved in the transition from benign to malignant cells (Boltze *et al*., 2004). Therefore, many researchers have investigated the potential of HSP90 as a target of anti-cancer drugs (Neckers *et al*., 1999; Sharp and Workman, 2006; Modi *et al*., 2011; Dickson *et al*., 2013). As a result, several HSP90 inhibitors have been studied for use as an anticancer agent, and some clinical trials are underway. In the first part of this review, we provide an overview on traditional HSP90 inhibitors and their effects on cancers. HSP90 inhibitors hamper HSP90 function by competitively binding to the ATP binding site of HSP90, blocking the interaction with co-chaperones, or modulating acetylation. In the second part, we present a novel group of HSP90 inhibitors inducing cleavage of HSP90 and suggest that cleavage of HSP90 can be another mechanism of HSP90 inhibitors to suppress the activity of HSP90.

HSP90 INHIBITORS BLOCKING ATP BINDING

HSP90 inhibitors generally interrupt ATP binding to HSP90 and can be classified into ansamycins and non-ansamycins depending on whether they have a benzoquinone structure (Table 2).

Ansamycins

Ansamycins, including geldanamycin (GM), herbimycin A, and the macbecins, are antibiotics with anti-cancer activity and include the benzoquinone structure. These antibiotics induce death of tumor cells through HSP90 inhibition and degradation of the client proteins that are required for tumor cell survival and growth (Zhang and Zhang, 2011).

GM competitively binds to the ATP-binding pocket in the N-domain of HSP90 and inhibit chaperone activity of HSP90 via down-regulation of ATPase activity (Grenert *et al*., 1997). 17-allylamino-17-demethoxygeldanamycin (17-AAG, tanespimycin) is an analogue of GM with higher binding affinity and lower toxicity (Krishnamoorthy *et al*., 2013). 17-AAG inhibits cell proliferation and induces apoptosis via depleting HSP90 client proteins and downregulation of other downstream proteins in various types of cancer cells *in vitro* and *in vivo* (Hostein *et al*., 2001; Solit *et al*., 2002; Banerji *et al*., 2005b; Karkoulis *et al*., 2010). 17-AAG also induces cell cycle arrest in G1 phase (Solit *et al*., 2002; Karkoulis *et al*., 2010). In addition, these effects of 17-AAG in cancer cells are similar to those in glioma stem cells (Sauvageot *et al*., 2009).

17-Dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG, alvespimycin), an analogue of 17-AAG, is a more potent and water-soluble derivative. 17-DMAG binds to ATP binding site of HSP90 and inhibits ATP binding and chaperone activity of HSP90. Nuclear factor-κB (NF-κB) regulates anti-apoptotic proteins and oncogenes, such as c-FLIP, Bcl2, Mcl1, and XIAP in chronic lymphocytic leukemia (CLL). 17-DMAG

Table 2. Selected HSP90 inhibitors

Target	Inhibitor	References
ATP-binding site	Ansamycin	
	Geldanamycin	Grenert *et al*., 1997
	17-AAG (Tanespimycin)	Krishnamoorthy *et al*., 2013
	17-DMAG (Alvespimycin)	Jez *et al*., 2003
	IPI-504 (retaspimycin hydrochloride)	Sydor *et al*., 2006
	Non-ansamycin	
	AUY922 (Luminespib)	Eccles *et al*., 2008
	BIIB021	Lundgren *et al*., 2009
	HSP990	Menezes *et al*., 2012
	Debio0932 (CUDC-305)	Bao *et al*., 2009
	STA-9090 (Ganetespib)	Ying *et al*., 2012
	AT13387 (Onalespib)	Woodhead *et al*., 2010
	SNX-5422 (PF-04929113)	Chandarlapaty *et al*., 2008
Deacetylation	LAQ824	Chen *et al*., 2005
	Romidepsin	Yu *et al*., 2002
	Vorinostat (SAHA)	Bali *et al*., 2005b

induces degradation of IκB kinase (IKK) and suppresses DNA binding of NF-κB. Consequently, 17-DMAG decreases transcription of NF-κB target genes and induces apoptosis (Hertlein *et al*., 2010). Her2/ErbB2 overexpression enhances cell growth and motility in breast and ovarian cancer. 17-DMAG-mediated HSP90 inactivation leads to degradation of Her2/ErbB2 (Niu *et al*., 2009). In non-small cell lung cancer (NSCLC), 17-DMAG treatment induces down-regulation of phospho-EGFR, phospho-Akt, and phospho-MAPK in EGFR-mutant cell lines than EGFR-WT cell line, and promotes cell apoptosis in EGFR-mutant cells (Kobayashi *et al*., 2012). In hepatocellular carcinoma cells, 17-DMAG induces apoptosis by degradation of survival-related proteins (Leng *et al*., 2012).

IPI-504 (retaspimycin hydrochloride) is a water-soluble analogue of 17-AAG and interacts with the ATP binding site of HSP90. IPI-504 inhibits myeloma tumor growth and has selective cytotoxicity to myeloma cancer cells compared with normal cells (Sydor *et al*., 2006). IPI-504 has anti-proliferation activity in several cancer cells through Her2/ErbB2 and Akt degradation. Tumor growth *in vivo* is also reduced by IPI-504 treatment through Her2/ErbB2 degradation (Leow *et al*., 2009). In diffuse large B-cell lymphoma (DLBCL) cell lines, IPI-504 inhibits cell growth and induces apoptosis. Among these cell lines, IPI-504-sensitive cell lines have high expression levels of phospho-Akt (Abramson *et al*., 2009).

Non-ansamycins

AUY992 (luminespib) binds to the ATP binding site in the N-domain of HSP90. AUY992 inhibits cell proliferation and induces G1/G2 cell cycle arrest and apoptosis *in vitro*. Furthermore, AUY992 inhibits growth and lung metastasis of b-Raf-mutated human melanoma tumor *in vivo* (Eccles *et al*., 2008). p23 is a co-chaperone of HSP90 and plays an important role in the activity of HSP90 and stabilization of client proteins by association with HSP90, and HSP90-p23 interaction requires ATP binding in HSP90 (Sullivan *et al*., 2002). In a Her/ErbB-overexpressing estrogen receptor (ER)-positive breast cancer xenograft model, AUY992 reduces tumor growth by inducing dissociation of HSP90-p23 interaction and degradation of client proteins (Jensen *et al*., 2008). AUY992 induces apoptosis and depletion of Akt and IKK in acute myeloma leukemia (AML) cell lines and primary AML blasts (Walsby *et al*., 2013). AUY992 also inhibits cell growth and motility in hepatocellular carcinoma and pancreatic cancer cells and reduces growth factor-mediated and angiogenesis-related protein activation and vascularization in pancreatic cancer (Moser *et al*., 2012; Cheng *et al*., 2015).

BIIB021, an orally available inhibitor of HSP90, binds to the ATP binding pocket in the N-domain of HSP90. BIIB021 has anti-proliferation activity in various tumor cell lines *in vitro* and inhibits tumor growth *in vivo*. BIIB021 also induces degradation of HSP90 client proteins (Lundgren *et al*., 2009). In CLL cells, BIIB021 induces growth inhibition and apoptosis by the mitochondrial pathway and degradation of BCR-ABL protein. In addition, the BIIB021-induced apoptosis includes an autophagic response such as the formation of autophagosome by regulating the mTOR-Ulk1 pathway (He *et al*., 2016). 17-AAG and other ansamycin derivatives are inactive in cell lines expressing P-glycoprotein (P-gp) and/or multidrug resistance-associated protein 1 (MRP-1/ABCC1). In contrast, BIIB021 is active in these cell lines. Therefore, BIIB021 may be used for therapy of tumors protected by multidrug resistant (MDR) protein (Zhang *et al*., 2010a).

Debio0932 (CUDC-305) binds to the ATP binding site in the N-domain of HSP90. Debio0932 induces degradation of multiple oncoproteins, such as Akt, Raf-1, and Her2/ErbB2, and inhibits cell proliferation in various tissue-derived cancer cell lines. In glioblastoma, AML, breast cancer, colorectal cancer, and NSCLC mouse models, debio0932 also induces degradation of client proteins and inhibits tumor growth (Bao *et al*., 2009).

STA-9090 (Ganetespib), a synthetic small molecule inhibitor, binds to the ATP binding site in the N-domain of HSP90 (Ying *et al*., 2012). STA-9090 induces proteasome-mediated degradation of EGFR, JAK2, and FLT3 which are essential for growth and activation of STAT, MAPK, and Akt. In addition, STA-9090 induces cell cycle arrest in G1 and G2/M phase (Proia *et al*., 2011). In Her2/ErbB2-positive breast cancer cells, STA-9090 inhibits cell proliferation, cell cycle, survival, and activation/phosphorylation of Her2/ErbB2. Furthermore,

the half-life of Her2/ErbB2 protein is decreased by STA-9090 treatment (Lee *et al*., 2018). In gastric cancer, STA9090 inhibits proliferation and induces G2/M arrest of cell cycle and apoptosis. The receptor tyrosine kinase (RTK) signaling pathway is suppressed by STA-9090 treatment (Lee *et al*., 2017). STA-9090 also inhibits cell growth and induces apoptosis in AML and NSCLC (Shimamura *et al*., 2012; Lazenby *et al*., 2015).

Heat shock protein 990 (HSP990) binds to the ATP binding site in the N-domain of HSP90. During *in vitro* experiments, HSP990 inhibits ATPase activity of TNF receptor associated protein 1 (TRAP1), a mitochondrial HSP90, by more than 90%. HSP990 inhibits the activity of HSP90 and growth of various types of cancer cell lines and has suppressive activity in most various cancer patient-derived tumors *ex vivo*. In gastric cancer, breast cancer, AML, and NSCLC mouse models, HSP990 treatment also suppresses tumor growth (Menezes *et al*., 2012).

KW-2478 binds to HSP90α with high affinity. In multiple myeloma, KW-2478 induces degradation of client proteins such as FGFR3, c-Raf, and cyclin D1, growth inhibition, and apoptosis *in vitro* and *in vivo* (Nakashima *et al*., 2010).

AT13387 (onalespib) has affinity for HSP90 by binding in the ATPase site of the N-domain (Woodhead *et al*., 2010). AT13387 suppresses proliferation and survival of cell lines from different types of tumors. In glioma cell lines, AT13387 depletes survival-related client proteins and suppresses their downstream signaling pathway. As a result, proliferation, motility, angiogenesis, and survival are decreased. In NSCLC, AT13387 shows long duration of effects *in vitro* and *in vivo* (Graham *et al*., 2012; Canella *et al*., 2017).

SNX-5422/2112 (PF-04928473) binds to the ATP binding site in the N-domain of HSP90. SNX-5422/2112 induces HSP90 client protein degradation, such as Her2/ErbB2, Akt, and cyclin D1, and inhibits cell proliferation in Her2/ErbB2-overespressing breast and ovarian cancers. In the body, SNX-5422 rapidly converts to SNX-2112, and SNX-2112 accumulates in tumors compared to normal tissues (Chandarlapaty *et al*., 2008). The growth inhibition activity of SNX-2112 is higher than 17-AAG in multiple myeloma and other hematologic tumors. In multiple myeloma cells, SNX-2112 induces apoptosis by caspase activation and suppresses Akt and ERK activation. SNX-2112 inhibits tube formation of HUVEC cells by suppression of the eNOS/Akt pathway and osteoclastogenesis of multiple myeloma cells by down-regulation of ERK/c-Fos and PU.1 (Okawa *et al*., 2009).

HSP90 INHIBITORS BLOCKING DEACETYLATION OF HSP90

The molecular chaperone activity of HSP90 is also controlled by acetylation and deacetylation of K294 and K287 in the M-domain of HSP90α and HSP90β, respectively (Bali *et al*., 2005a; Scroggins *et al*., 2007; Nishioka *et al*., 2008). Histone deacetylase 6 (HDAC6) deacetylates K294α/K287β of HSP90, and the deacetylated HSP90 acts as a molecular chaperone. Acetylation of K294α/K287β decreases the binding affinity of HSP90 to client proteins and co-chaperones (Bali *et al*., 2005a; Scroggins *et al*., 2007). In Her2/ErbB2-overexpressing breast cancer cell lines, vorinostat (also known as suberoylanilide hydroxamic acid (SAHA)) induces apoptosis via acetylation of HSP90, which dissociates Her2/ErbB2 from HSP90 and promotes polyubiquitinylation and degradation of Her2/ErbB2 (Bali *et al*., 2005b). LAQ824 induces acetylation of HSP90 in prostate cancer cells; the ATP binding activity of HSP90 is decreased as a result, and the androgen receptor is dissociated from HSP90 and degraded. Therefore, LAQ824 suppresses expression of androgen-induced prostate-specific antigen and induces anti-proliferation effects and apoptosis in prostate cancer cells. LAQ824 also reduces other HSP90 client protein levels (Chen *et al*., 2005). Romidepsin inhibits growth and induces apoptosis in wild type or mutant p53-expressing NSCLS cells. Romidepsin also reduces protein levels of ErbB1, ErbB2, Raf-1, and mutant p53, but not wild type p53. Romidepsin induces dissociation of mutant p53 and Raf-1 from HSP90, which is related with acetylation of HSP90 (Yu *et al*., 2002).

CLINICAL TRIALS OF TRADITIONAL HSP90 INHIBITORS FOR CANCER THERAPY

Since 17-AAG among HSP90 inhibitors first entered the clinical trial (Banerji *et al*., 2005a), many HSP90 inhibitors have entered clinical trials and are still underway. The HSP90 inhibitors that have been investigated in clinical trials are presented in Table 3. Clinical trials of HSP90 inhibitors were performed as HSP90 inhibitor monotherapy or combination therapy with other anti-cancer reagents for validation of safety, anti-cancer activity, and dosing schedule and dosage. While inhibitors of ansamycin and non-ansamycin classes have not been FDA approved, romidepsin and vorinostat among HDAC inhibitors have been FDA approved.

Another strategy that uses HSP90 in clinical trials is to suppress drug resistance using HSP90 inhibitor. Her2/ErbB2 and mutated EGFR are one of the proteins that cause drug resistance. Because Her2/ErbB2 and EGFR are clients of HSP90, clinical trials with HSP90 inhibitors were designed to overcome drug resistance by these proteins. In Her2/ErbB2-overexpressing breast cancer, 17-AAG, STA-9090, and AUY922 were used in Phase I and II clinical trials to overcome Her2/ErbB2-mediated resistance to trastuzumab (Herceptin, humanized anti-Her2/ErbB2 monoclonal antibody). The results proved that HSP90 inhibition may be used to overcome the trastuzumab resistance (Modi *et al*., 2007; Kong *et al*., 2016; Jhaveri *et al*., 2017). In addition, to overcome the resistance of EGFR mutation-positive lung cancer to erlotinib (Tarceva, receptor tyrosine kinase inhibitor), Phase I and II clinical trials using AUY922 have been performed (Johnson *et al*., 2015).

NOVEL CLASS OF HSP90 INHIBITORS INDUCING CLEAVAGE OF HSP90

Recently, it has been reported that HSP90 is cleaved by various stimuli. Cleavage of HSP90 is induced by various stimuli such as UVB irradiation (Chen *et al*., 2009), ascorbate/menadione (Beck *et al*., 2009, 2012), andrographolide (Liu *et al*., 2014), HDAC inhibitors (Park *et al*., 2015), proteasome inhibitors (Park *et al*., 2017), tumor necrosis factor (TNF) (Fritsch *et al*., 2016), a combination of gefitinib and vorinostat (Park *et al*., 2019), etc. (Table 4). Therefore, cleavage of HSP90 can be considered another mechanism of HSP90 regulation.

Table 3. Clinical trials of anti-cancer therapy with HSP90 inhibitors

HSP90 inhibitor	Phase	Tumor type	Reference
17-AAG (Tanespimycin)	I	Relapsed or refractory acute myeloid leukemia	Walker *et al*., 2013
	II	Metastatic or locally advanced, unresectable breast cancer	Rajan *et al*., 2011
	II	Metastatic melanoma	Solit *et al*., 2008 ; Pacey *et al*., 2012
	I	Solid tumor	Tse *et al*., 2008
	I	b-Raf or NRAS mutated melanoma	Banerji *et al*., 2008
	I	Relapsed / refractory pediatric solid tumor	Weigel *et al*., 2007
	I	Refractory advanced cancer	Ramanathan *et al*., 2005
	I/II	Relapsed or relapsed and refractory multiple myeloma	Richardson *et al*., 2011
	I	Relapsed multiple myeloma	Richardson *et al*., 2010
	I	Her2/ErbB2-overexpressed breast cancer	Modi *et al*., 2007
17-DMAG (Alvespimycin)	I	Chronic lymphocytic leukemia/small lymphatic lymphoma	Maddocks *et al*., 2016
	I	Advanced solid tumor	Pacey *et al*., 2011; Jhaveri *et al*., 2012
	I	Acute myeloma leukemia	Lancet *et al*., 2010
	I	Advanced malignancies	Kummar *et al*., 2010
AUY922 (Luminespib)	IB/II	Her2/ErbB2-positive metastatic breast cancer	Kong *et al*., 2016
	II	Advanced pancreatic cancer	Renouf *et al*., 2016
	II	Gastrointestinal stromal cancer	Bendell *et al*., 2016
	I	Advanced solid tumor	Sessa *et al*., 2013; Doi *et al*., 2014; Bendell *et al*., 2015
	I/II	EGFR-mutant lung cancer	Johnson *et al*., 2015
	I/IB	Multiple myeloma	Seggewiss-Bernhardt *et al*., 2015
BIIB021	I	Advanced solid tumor	Saif *et al*., 2014
	I	Refractory metastatic or locally advanced solid tumor	Hong *et al*., 2013
	II	Gastrointestinal stromal tumor	Dickson *et al*., 2013
Debio0932 (CUDC-305)	I	Advanced cancer	Isambert *et al*., 2015
STA-9090 (Ganetespib)	I	Her2/ErbB2-positive metastatic breast cancer	Jhaveri *et al*., 2017
	II	Metastatic castrate-resistant prostate cancer	Thakur *et al*., 2016
	II	Non-small cell lung cancer	Ramalingam *et al*., 2015
	II	KRAS mutated and WT metastatic colorectal cancer	Cercek *et al*., 2014
	I	Advanced hepatocellular carcinoma	Goyal *et al*., 2015
	II	Metastatic breast cancer	Jhaveri *et al*., 2014
	II	Advanced non-small lung cancer	Socinski *et al*., 2013
	I	Solid malignancies	Goldman *et al*., 2013
HSP990	I	Advanced solid malignancies	Spreafico *et al*., 2015
KW-2478	I/II	Multiple myeloma	Cavenagh *et al*., 2017
	I	B-cell malignancies	Yong *et al*., 2016
LAQ824	I	Advanced solid tumor	de Bono *et al*., 2008
AT13387 (Onalespib)	I	Advanced solid tumor	Do *et al*., 2015; Shapiro *et al*., 2015
IPI-504 (Retaspimycin hydrochloride)	I	Gastrointestinal stromal cancer, soft-tissue sarcoma	Wagner *et al*., 2013
	II	Castrate-resistant prostate cancer	Oh *et al*., 2011
Romidepsin	II	Metastatic castrate-resistant prostate cancer	Molife *et al*., 2010
SNX-5422 (PF-04929113)	I	Refractory solid tumor	Infante *et al*., 2014
	I	Refractory solid tumor malignancies and lymphomas	Rajan *et al*., 2011
Vorinostat (SAHA)	I/II	Locally advanced breast cancer	Tu *et al*., 2014
	I/II	Metastatic breast cancer	Ramaswamy *et al*., 2012

HSP90 cleavage can be divided into enzymatic cleavage and non-enzymatic cleavage. The enzymatic cleavage generates an approximately 55 kDa fragment of HSP90 via caspase 10 activation, and the non-enzymatic cleavage generates an approximately 70 kDa fragment via chemical degradation by reactive oxygen species (ROS). There are some substances that have HSP90 cleavage activity, but it has not yet been determined whether enzymes are engaged in the process.

Chemicals and UV inducing enzymatic cleavage of HSP90

The enzymatic cleavage of HSP90 is induced by histone deacetylase inhibitors (including vorinostat), proteasome inhibitors (including MG132), and UVB irradiation. We found cleavage of HSP90 when treated with the histone deacetylase inhibitor vorinostat in leukemia cells (Park *et al*., 2015). HSP90 was also cleaved by other histone deacetylase inhibitors, sodium butyrate and valproic acid (Park *et al*., 2015). Vorinostat induces ROS generation in acute T cell leukemia cell line

Table 4. Novel class of HSP90 inhibitors inducing cleavage of HSP90

Cleavage type	Inhibitor	References
Enzymatic cleavage	Histone deacetylase inhibitors	Park *et al*., 2015
	Proteasome inhibitors	Park *et al*., 2017
	Ultra-violet irradiation	Chen *et al*., 2009
Non-enzymatic cleavage	Ascorbate/Menadione	Beck *et al*., 2009, 2012
	Oxidative stress (H_2O_2)	Castro *et al*., 2019
Others (undefined)	Tumor necrosis factor (TNF)	Fritsch *et al*., 2016
	Andrographolide	Liu *et al*., 2014
	β-Lapachone	Wu *et al*., 2016
	17-AAG (Tanespimycin)	Karkoulis *et al*., 2010
	As(III) and MMA(III)	Shen *et al*., 2008

(Ruefli *et al*., 2001). On the other hand, generation of ROS by several stimuli leads to activation of caspase and triggers apoptosis in various types of cancer cells through an extrinsic or intrinsic pathway (Kim and Chung, 2007). According to our results, ROS-induced caspase 10 activation is responsible for enzymatic cleavage of HSP90 after treatment with vorinostat (Park *et al*., 2015). Furthermore, vorinostat-induced HSP90 cleavage needs newly synthesized protein(s), and Vitamin D up-regulating protein 1 (VDUP-1) may be one of the candidate substances (Park *et al*., 2015). Vorinostat was previously reported to increase the expression level of the VDUP-1 gene (Butler *et al*., 2002), and VDUP-1 negatively regulates thioredoxin, a cellular antioxidant (Junn *et al*., 2000).

We also found that MG132, a proteasome inhibitor, induces HSP90 cleavage through a mechanism similar to that of vorinostat treatment (Park *et al*., 2017). Although the mRNA level of VDUP-1 was not changed after treatment with MG132, the protein level of VDUP-1 was significantly increased. E3 ubiquitin ligase Itch-mediated ubiquitination and proteasomal degradation is involved in the regulation of VDUP-1 protein level (Zhang *et al*., 2010b). Therefore, MG132 may up-regulate the VDUP-1 protein level by inhibiting the proteasome activity and blocking the proteasomal degradation of VDUP-1. Another antioxidant glutathione was also decreased by MG132 treatment. In short, MG132 also induces HSP90 enzymatic cleavage via generation of ROS and subsequent activation of caspase 10.

UVB irradiation induces HSP90 cleavage by activating the Fas/Fas ligand axis (Chen *et al*., 2009). In this case, Fas ligand secretion and Fas expression were increased by UVB irradiation. Caspase 8 was activated by interaction with the FADD domain of active Fas, and HSP90 was cleaved by caspase 8-mediated active caspase 10. Importantly, apoptosis of cells increased when HSP90 was down-regulated and cells harboring mutation on the cleavage site of HSP90 showed better survival compared with control cells upon UVB irradiation. Therefore, it is likely that the HSP90 cleavage is not merely a side effect of caspase activation but an essential process for the regulation of apoptosis.

Chemicals inducing non-enzymatic cleavage of HSP90

In leukemia cells (K562 cell line), ascorbate/menadione (asc/men) treatment induces HSP90 cleavage with a molecular weight of approximately 70 kDa. The HSP90 cleavage is selectively induced in tumor cells, but not in normal cells. The asc/men-induced HSP90 cleavage triggers degradation of HSP90 client proteins, including Bcr-Abl, and the cleavage of HSP90 is induced by ROS generation and inhibited by antioxidant (Beck *et al*., 2009). The cleavage of HSP90 is induced by Fenton reaction in the presence of redox-active iron. The cleavage site in this reaction is between I131 and G132 in HSP90α and I126 and G127 in HSP90β (Beck *et al*., 2012).

In Jurkat cells, oxidative stress (H_2O_2) induces iron-dependent HSP90 cleavage with a molecular weight of approximately 70 kDa, and the HSP90 cleavage inversely correlates with cell proliferation. Cleaved HSP90 accumulates as aggregates in an insoluble form, and actin also aggregates in sequence. Using a cell-free *in vitro* model, it was proved that the cleaved HSP90 gains another function to directly induce aggregation of actins (Castro *et al*., 2019).

Others inducing cleavage of HSP90

TNF induces HSP90 cleavage depending on Cathepsin D (CtsD). TNF treatment in U937 (human myeloid leukemia) cells induced HSP90 cleavage in a time-dependent manner, which was blocked by treatment with pepstatin A (PepA), a CtsD inhibitor. In this case, HSP90 was observed to be cleaved into 60 kDa and 40 kDa fragments and it was concluded using the mutagenesis technique that the 465th tyrosine residue may be the target of HSP90 cleavage. Apoptosis of U937 cells expressing mutant HSP90 (Y465W) by TNF was decreased compared to control U937 cells (Fritsch *et al*., 2016).

Andrographolide (andro), a diterpenoid lactone isolated from *Andrographis paniculata*, has anti-inflammatory activity and inhibits cell transformation through v-Src degradation (Liang *et al*., 2008). When temperature-sensitive v-Src-expressing cell line (ts-v-Src; RK3E cell line) was treated with andro, 40 kDa fragments of HSP90 were also detected with v-Src degradation. Andro-induced HSP90 cleavage was related with a decrease of v-Src and cell apoptosis. In this phenomenon, andro-mediated ROS generation plays an important role in HSP90 cleavage and v-Src suppression. Furthermore, in leukemia cells, andro-induced HSP90 cleavage was correlated with BCR-ABL down-regulation and apoptosis (Liu *et al*., 2014).

The novel anti-cancer drug β-Lapachone (β-lap) induces HSP90 cleavage in NAD(P)H:quinone oxidoreductase-1 (NQO1)-expressing lung and prostate cancer cells and HUVEC cells. The cleavage of HSP90 is induced by β-lap-mediated ROS generation, and the cleavage of HSP90 and down-regulation of client proteins are restored by antioxidant treatment. NQO1-mediated activation of β-lap triggers a futile cycle, and NQO1-depedent quinones cannot cleave HSP90, unlike β-lap. Therefore, the futile re-

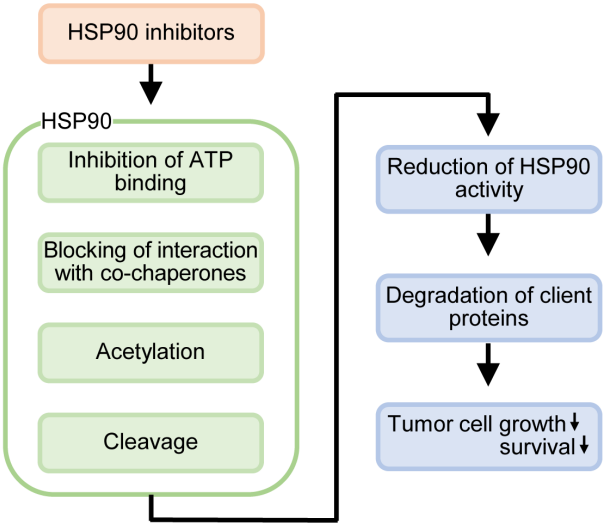

Fig. 1. Diagram of HSP90 inhibitor-mediated tumor cell suppression. HSP90 inhibitors are known to inhibit the binding of ATP, block binding to co-chaperones and regulate acetylation to inhibit the activity of HSP90. In addition, the cleavage of HSP90 discussed in this paper appears to suppress the activity of HSP90. Inhibition of HSP90 by HSP90 inhibitors reduces chaperone activity and inhibits growth and survival of tumor cells through degradation of the HSP90 client proteins.

dox cycle of β-lap may generate ROS, and the chemical structure of β-lap is a critical factor for HSP90 cleavage (Wu *et al*., 2016).

As described earlier, 17-AAG inhibits HSP90 by binding to the ATP-binding pocket of HSP90. In human urinary bladder cancer cells, 17-AAG induces cell apoptosis and down-regulation of client proteins, and the cleavage of HSP90 was also examined (Karkoulis *et al*., 2010). The detailed action mechanism involved in 17-AAG has not been defined yet.

Arsenic compounds arsenite (As(III)) and monomethylarsonous acid (MMA(III)) induce ROS-mediated apoptosis and HSP90 cleavage. It was reported that the NADPH inhibitor, diphenyleneiodonium chloride (DPI), inhibit As(III)-induced apoptosis and that HSP90 cleavage is also reduced by DPI treatment. In addition, JNK inhibitor, SP600125, blocks HSP90 cleavage, whereas the ERK inhibitor PD98059 does not. Therefore, As(III) and MMA(III) induce HSP90 cleavage via NADPH and JNK activation, thereby inducing cell apoptosis (Shen *et al*., 2008).

CONCLUSIONS

HSP90 is a molecular chaperone that supports folding and stabilization of the client proteins. Likely because many client proteins of HSP90 are required for cancer cell survival and growth, most cancer cells express HSP90 more highly than normal cells (Ferrarini *et al*., 1992; Sharp and Workman, 2006; Neckers and Workman, 2012; Miyata *et al*., 2013). Various inhibitors of HSP90 have been studied as anticancer drugs and clinical trials are underway. Most of the traditional inhibitors target ATP binding or deacetylation of HSP90, and thereby block the molecular chaperone activity of HSP90, resulting in degradation of the client proteins and increased cell death in cancer cells. Recently, several studies including ours have shown that the cleavage of HSP90 is correlated with degradation of client proteins and directly or indirectly affects the survival and growth of cancer cells (Shen *et al*., 2008; Chen *et al*., 2009; Liu *et al*., 2014; Fritsch *et al*., 2016; Wu *et al*., 2016). In addition, HSP90 cleavage inducing reagents, such as β-lap and vorinostat (Ramaswamy *et al*., 2012; Tu *et al*., 2014; Park *et al*., 2015; Wu *et al*., 2016), are also used for anti-cancer therapy. Therefore, it can be carefully postulated that chemicals inducing HSP90 cleavage may have anticancer activity. Taken together, the present results show that HSP90 cleavage may be another mechanism of HSP90 inhibitors and targeting of HSP90 cleavage is potentially another strategy for cancer chemotherapy (Fig. 1). We are presently screening chemicals that can induce HSP90 cleavage and are planning to verify whether they have anticancer activity when used alone or in combination. The results can be helpful to understand the mechanism of HSP90 inhibition in several aspects and may provide novel candidate drugs for cancer therapy.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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