Biomacromol. J., Vol. 3, No. 1, 5-17, July 2017.

Biomacromolecular Journal
www.bmmj.org

HDAC Inhibitors and Heat Shock Proteins (Hsps)

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(Received 12 April 2017, Accepted 30 October 2017)

ABSTRACT

Epigenetic alterations, including DNA acetylation, hypermethylation and hypomethylation, and the associated transcriptional changes of the affected genes are central to the evolution and progression of various human cancers, including pancreatic cancer. Cancer-associated epigenetic alterations are attractive therapeutic targets because such epigenetic alterations, unlike genetic changes, are potentially reversible. Several drugs that target epigenetic alterations, including inhibitors of histone deacetylase (HDAC) and DNA methyltransferase (DNMT), are currently approved for treatment of hematological malignancies and are available for clinical investigation in solid tumors. Histone deacetylases (HDACs) is well known to be associated with tumorigenesis through epigenetic regulation. HDACs comprise an ancient family of enzymes that play crucial roles in numerous biological processes and HDACs are found to be over expressed in many tumor types. Its inhibitors (HDACIs) induce differentiation and apoptosis of tumor cells. In addition, the activity of heat shock proteins (Hsps) can be regulated by HDACs. Hsps exist in many types of cells and these proteins can prevent aggregation and formation of toxic inclusion. Hsps are major molecular chaperones in prokaryotic and eukaryotic cells. This review summarizes mechanisms of histone deacetylase inhibitors action on Hsps and will describe the regulation of major cellular chaperones and heat shock factors by HDAC-mediated deacetylation.

Keywords: HDAC, HDAC inhibitor, Chaperon, Hsp, Acetylation

INTRODUCTION

Reversible acetylation is generally accepted as a posttranslational modification that regulates diverse protein activity. The best established protein targets for reversible acetylation are core histones, which have been known for almost 30 years [1]. A correlation between acetylation and transcriptional activity has been well established and the recent discovery that certain enzymes control the acetylation status of core histones showed that acetylation of histones plays a critical role in the regulation of chromatin structure and transcriptional activity [2,3]. The acetylation and deacetylation of histones are catalyzed by histone acetyltransferase (HAT) and histone deacetylases (HDACs) respectively. HDACs also deacetylate and affect the activity of other proteins. At least 50 non-histone proteins of known biological function have been identified, which may be acetylated and are substrates of HDACs, including chaperones and Heat shock proteins (Hsps) [4]. The acetylation and deacetylation of histones are catalyzed by HAT and HDACs respectively. HDACs also deacetylate and affect the activity of other proteins [5,6].

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HDACs

HDACs comprise an ancient family of enzymes that play crucial roles in numerous biological processes and HDACs are found to be over-expressed in many tumor types. HDACs contribute to cancer initiation and progression through their regulatory activities on cell cycle progression, epithelial differentiation, angiogenesis, metastasis and apoptosis [7]. HDACs deacetylate the ε-amino group of lysines located at the N-terminal tail of histones, which leads to a repressive chromatin formation (heterochromatin) and the suppression of gene expression [8]. In addition to the condensation of chromatin, HDACs

deacetylate various proteins to regulate their function. Non-histone protein targets of HDACs include transcription factors, transcription regulators, signal transduction mediators, DNA repair enzymes, nuclear import regulators, structural proteins, inflammation mediators and chaperone proteins [9].

Chaperones and their cofactors form molecular chaperone complexes that facilitate the structural maturation of client proteins [10]. Molecular chaperones are playing a critical role in maintaining nascent or refolding denatured polypeptides in a functionally mature conformation [11]. Inhibition and RNA knockdown of HDACs have been very useful in identifying reversible acetylation as a potential regulator of chaperone activity, and there is increasing support for the view that molecular chaperones play an important role in the development, maintenance and progression of cancers [12].

The state of acetylation can either increase or decrease the function or stability of the proteins or protein-protein interactions. Data from some research articles suggest that HDAC inhibitors could have multiple mechanisms of inducing cell growth arrest and cell death [13].

Four classes of HDACs have been identified: I, II, III and IV [14]. Class I HDACs (HDACs 1, 2, 3 and 8) are associated with reduced potassium dependency 3 (RPD3) deacetylase [15].

Class II HDACs are divided into two subclasses, class II a (HDACs 4, 5, 7 and 9) and class IIb [16] (HDACs 6 and 10) and are homologous to the yeast Hda1 deacetylase [17]. HDAC6 is highly expressed in mammary tumors [18].

Class III HDACs consists of seven HDACs (SIRT1 to SIRT7) members that share homologies with the yeast silent information regulator 2 (Sir2) families. The class IV family of HDACs has only one member, HDAC11. Classes II and IV require Zn^{2+} for activity. Sirtuins do not contain Zn^{2+} in the active site and they have a different mechanism of action that uses NAD^{+} as cofactor [19,20]. HDAC enzymes differ in their subcellular localization (Table 1), catalytic activity and susceptibility to different inhibitors [21].

Class I HDACs 1, 2 and 3 are ubiquitously expressed and are almost exclusively found in the nuclei of normal cells [22]. Smooth muscle cells of either organ or vessel walls express the proteins as well. In addition, endothelial cells were positive to a variable degree. Inflammatory cells, especially lymphocytes and macrophages, occasionally expressed HDACs 1, 2 and 3. Expression of class I HDAC8 was found to be restricted to cells with smooth muscle/ myoepithelial differentiation and consequently has been suggested as a diagnostic marker for uterine tumors with smooth muscle differentiation [23,24].

Expression of class II HDACs including 4, 5, 6, 7 has been localized to the both nucleus and cytoplasm [25]. Expression of class II HDAC6 was not observed in lymphocytes, stromal cells or vascular endothelial cells (Table 1) [26]. Members of class III HDACs have been located in mitochondria, cytoplasm and nucleus (Table 2) [27].

HDAC INHIBITORS

HDAC inhibitors applies to compounds that target the HDACs and are currently being evaluated in clinical trials [25]. HDAC inhibitors represent a new class of chemotherapeutic agents that target both histone and non-histone proteins (Table 3). Since the discovery of the anti-tumor effect of Trichostatine A(TSA) in 1990 [28], many other HDAC inhibitors have been identified. Usually HDAC inhibitors are classified into six groups, including short-chain fatty acids (such as butyrate, phenylbutyrate, and valproic acid), hydroxamic acids [1], cyclic peptides (such as depsipeptide (FK228) and apicidin), benzamides (such as MS-275 and CI-994), electrophilic ketones (trapoxin and 2-amino-8-oxo-9), and hybrid molecules (CHAP31 and CHAP50). Two HDAC inhibitors, vorinostat [29] and romidepsin [30], are now approved by the US Food and Drug Administration (FDA) for the treatment of CTCL. HDAC inhibitors mediate a wide range of biological effects including induction of apoptosis and autophagy and inhibition of angiogenesis (Table 4) [31].

HEAT-SHOCK PROTEINS

Heat-shock proteins (Hsps) are a family of highly conserved proteins that are induced by various stimuli, such as infection, high temperature, free radicals and mechanical stress [32]. Hsps were first discovered in 1962. Hsps play essential roles as molecular chaperones in protein folding, protein traffics and cell signaling (Fig. 1). Molecular

Table 1. Expression of Different HDAC Isoforms in Variety of Human Tumors, Localization and Number of Amino Acids [23,31]

Classes	HDACs	Localization	Amino acids	Tumor
Class I	1	Nucleus	482	Colon, Prostate, CTCL
	2	Nucleus	488	Colon, Prostate, CTCL, gastric, Endometrial
	3	Nucleus	428	Colon, Prostate
	8	Nucleus/Cytoplasm	377	Colon
Class II	4	Nucleus/Cytoplasm	1084	Colon, Prostate, Breast
	5	Nucleus/Cytoplasm	1122	Colon, Acute Promyelocytic Leukemia (AML)
	7	Nucleus/Cytoplasm	855	Colon
	9	Nucleus/Cytoplasm	1069	Astrocytomas, Medulloblastomas
	6	Cytoplasm	1215	Breast, AML, CTCL
	10	Cytoplasm	669	Heptocellular carcinoma
Class IV	11	Nucleus/Cytoplasm	347	Breast

Table 2. Localization of Sirtuin Members and Number of Aminoacids [27]

		Amino acids	Localization
Class III	SIRT1	747	Nucleus, cytoplasm
	SIRT2	389	Cytoplasm
	SIRT3	399	Nucleus, mitochondria
	SIRT4	314	Mitochondria
	SIRT5	310	Mitochondria
	SIRT6	355	Nucleus
	SIRT7	400	Nucleolus

chaperones have multiple functions, and the potential of using chaperones in treatment is a new frontier of recent therapies against cancer, cardiovascular disease and neurodegeneration [11,33].

Chaperones are usually classified according to their molecular weight (Hsp40, Hsp60, Hsp70, Hsp90, Hsp100 and the small Hsps) [32]. The chaperones that participate broadly in *de novo* protein folding and refolding are Hsp70s and Hsp90s [34].

Various molecular chaperones exist in the cells, and they include tyrosine kinases and serine/threonine kinases as well, so they are very important for proper regulation of cell cycle and cell growth [35].

Hsp90

Hsp90 is a non-fibrous protein that gained its name on the basis of its molecular weight, 90kDa. Hsp90 is the most abundant globular protein (as much as 2% of total cellular protein) present in the eukaryotic cytoplasm that plays a significant role in the cells by acting as a molecular chaperone for various proteins associated with some important signaling pathways in the cells. Hsp90 is involved

Table 3. HDAC Substrates Include Histones and Nonhistone Proteins [79]

Function	*Proteins*
DNA binding transcriptional factors	p53, c-Myc, AML1, BCL-6, E2F1, E2F2, E2F3, GATA-1, GATA-2, GATA-3, GATA-4, Ying Yang 1 (YY1), NF-κB (RalA/p65), MEF2, CREB, HIF-1α, BETA2, POP-1, IRF-2, IRF-7, SRY, EKLF
Steroid receptors	Androgen receptor, estrogen receptor α, glucocorticoid receptor
Transcription coregulators	Rb, DEK, MSL-3, HMGI(Y)/HMGA1, CtBP2, PGC-1α
Signaling mediators	STAT3, Smad7, β-catenin, IRS-1
DNA repair enzymes	Ku70, WRN, TDG, NEIL2, FEN1
Nuclear import	Rch1, importin-α7
Chaperone protein	HSP90
Structural protein	α-Tubulin
Inflammation mediator	HMGB1
Viral proteins	E1A, L-HDAg, S-HDAg, T antigen, HIV Tat

in the posttranslational folding and stabilization of more than 200 client proteins which are required for the activity of key regulators of cell signaling that promote tumor cell growth and radioresistance [34,37]. The chaperone-assisted maturation of client proteins often leads to an enhanced activity and stability [38]. It is also responsible for stabilization of multiple mutated, chimeric and/or over-expressed signaling proteins that promote cancer cell growth and/or survival and the expression of Hsp90 is 2- to 10-fold higher in tumor cells than in normal cells [39]. Although initially recognized as a stress-induced protein, the realization that many Hsp90 targets are critical for normal and oncogenic signaling has identified Hsp90 as an important modulator in cell signaling and a promising target in cancer therapy [40]. Recent studies showed that Hsp90 is also involved in the assembly of small nucleolar ribonucleoproteins (snoRNPs) and RNA polymerase [41].

Structurally, Hsp90 is a flexible homodimeric protein composed of three different domains, an N-terminal ATP-binding domain (N-domain), a middle domain (M-domain), and a C-terminal dimerization domain (C-domain), which adopts structurally distinct conformations. ATP binding triggers directionality in these conformational changes and leads to a more compact state. To achieve its function, Hsp90 works together with a large group of cofactors, termed co-chaperones [34]. Hsp90 itself is regulated by various posttranslational modifications such as phosphorylation, acetylation, nitrosylation and methylation which tightly control the function of Hsp90 and thus influence the maturation of client proteins [42].

Regulation of HSP90 Functions by HDAC Inhibitors

Post-translational modifications such as hyper-phosphorylation, S-nitrosylation and reversible hyper-acetylation have been shown to regulate the chaperone function of Hsp90. Hsp90 acetylation and its influence on the chaperone machinery has been extensively investigated in recent years. Hsp90 acetylation dramatically affects its function, but the identity and importance of individual acetylated residues have not been determined [43].

Lysine acetylation is a reversible modification mediated by opposing actions of HATs and HDACs in which an acetyl group is covalently linked to lysine residues of target proteins. Deacetylation of Hsp90 drives the formation of Hsp90 client complexes and promotes the maturation of the client proteins. Hsp90 can be acetylated at different sites [44]. HDAC6 is the major deacetylase that is responsible for

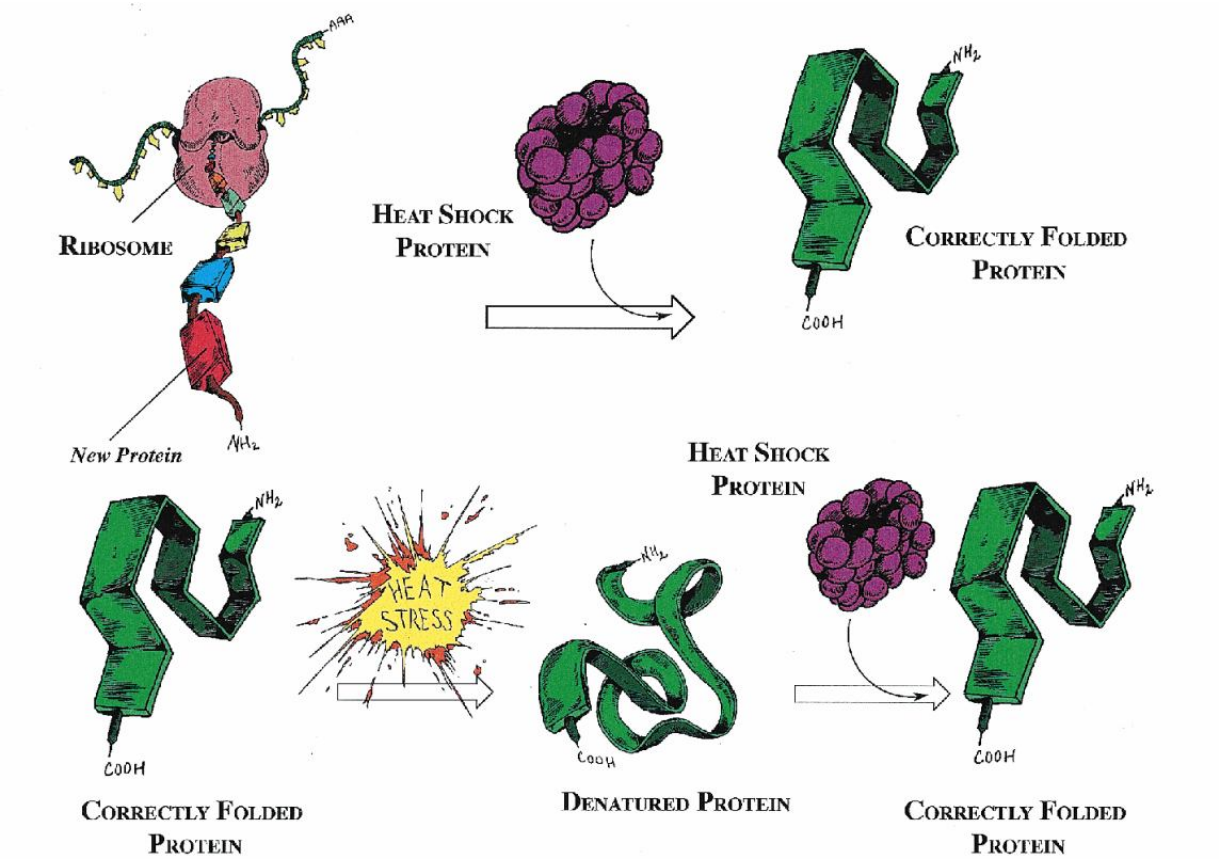

Fig. 1. Two functions of heat shock proteins. *Top:* As new proteins are being produced by ribosome, heat shock proteins assist in correct folding of polypeptide chain into functional protein. Presence of heat shock protein *(purple)* assures that the new protein will assume its functional three-dimensional configuration. *Bottom:* After stress event, heat shock proteins also assist in refolding or degradation of damaged or denatured proteins [36].

deacetylation of Hsp90 and α-tubulin to modulate microtubulin-dependent transportation, recruitment of mis-folded proteins. HDAC6 shuttles between the cytoplasm and nucleus to achieve its biological functions. Hsp90 has been shown to be overexpressed in several tumor types [45], and inhibition of Hsp90 by HDAC inhibitors has been reported to induce tumor cell-specific apoptosis. Following treatment with a variety of HDAC inhibitors or following siRNA mediated knockdown of HDAC6, reversible hyper-acetylation of Hsp90 has been documented.

HDAC inhibitor like LAQ824 [46], LBH589 [38], SAHA [47] have been shown to induce hyperacetylation and inhibition of chaperone function of Hsp90; this was shown to cause the misfolding, polyubiquitylation and degradation of HSP90-chaperoned client proteins. Necker's study pointed out that lysine 294, an acetylation site in the M-domain, strongly influences the binding between Hsp90 and its client proteins and co-chaperones. Hsp90 with mutations that mimic acetylated lysine 294 have reduced binding affinity to its client proteins including hypoxia inducible factor (HIF-1a) [48]. HDAC inhibitors produce a marked inhibition of HIF-1a expression and are currently in clinical trials partly based on their potent antiangiogenic effects. The HDAC inhibitors TSA and sodium butyrate can increase Hsp90 acetylation level, reduce the Hsp90-HIF-1a interaction, and promote the proteasomal degradation of HIF-1a. HIF-1a, a pro-angiogenic transcription factor, is hyperacetylated as a result of treatment by HDAC

inhibitors, resulting in its degradation [12,43].

In other hand, Kekatpure in 2009 has shown that acetylation of the Lys294 residue of Hsp90 was important for regulating the activation of aryl hydrocarbon receptor (AhR) signaling. The AhR is a client protein of Hsp90. In the absence of ligand, the AhR is present in the cytosol as a component of a complex with a dimer of the chaperone Hsp90 and the cochaperone p23. Treatment of KYSE450 (esophageal squamous cell carcinoma), HCA7 (colon adenocarcinoma), 1483 (head and neck squamous cell carcinoma), A549 (lung adenocarcinoma), and MSK-Leuk1 (oral leukoplakia) cells with TSA and SAHA causes acetylation of Hsp90 and depletion of several HSP90 client proteins. Inactivation of HDAC6 causes Hsp90 hyperacetylation and subsequent dissociation from the cochaperone, p23, with a loss of chaperone activity. Kekapture found that HDAC6 physically interacted with Hsp90. Inhibiting or silencing HDAC6 caused hyperacetylation of Hsp90, resulting in the loss of interaction between Hsp90 and HDAC6. Interestingly, neither treatment with TSA nor SAHA caused a significant increase in Hsp90 acetylation in HDAC6 knockdown cells [49].

Gene deletion of HDAC6 or HDAC9 led to hyperacetylation of Hsp90 at Lys294 in regulatory T cells (Tregs) that contain the transcription factor Foxp3, in association with the nuclear translocation of HSF-1 and the induction of key heat shock response genes, such as those that encode Hsp27 and Hsp70. Although the exact mechanism remains unknown, a functioning heat shock response is considered important for the physiologic suppressive capabilities of Tregs. Consistent with this concept, Beier found that knockout of the gene encoding Hsp70 diminished the ability of wild-type Tregs to constrain T cell proliferation [50].

The location of K294 at the junction of the charged linker region and the middle domain places it in a region that is flexible, surface exposed and, based on recent structural data, potentially involved in both intra molecular contacts as well as dynamic domain-domain or protein-protein interactions. In yeast Hsp90, K274 is equivalent to K294 in the human protein. In yeast Hsp90 Thr273, Pro275, Try277, Phe292 and Try344 form a hydrophobic pocket for the N-terminal domain although hyperacetylation of Hsp90 following HDAC inhibitor impairs ATP binding, mutation of K294 did not affect ATP binding, suggesting that nucleotide binding is likely regulated by acetylation of other lysine residues within Hsp90. Acetylation of K294 decreases affinity for most clients and certain cochaperones while deacetylation increases these interactions. Through its impact on Hsp90 complex dynamics, reversible acetylation of K294, and potentially of other sites in the chaperone, likely provides an additional layer of physiologic control of Hsp90 function in response to environmental signals. Untangling the interplay of various post-translational modifications influencing this process is a challenging but necessary step toward understanding the regulation of this critical chaperone [12]. Activation of client proteins by the Hsp90-based chaperone machine involves an ordered association with several cochaperones p23 [51], cdc37 and Aha-1, linked to the ATPase cycle of Hsp90, which may also direct client protein specificity [12].

Bhalla reported that treatment with a HDAC inhibitor panobinostat (LBH589) increased the acetylation and inhibition of chaperone function of nuclear Hsp90, leading to proteasomal degradation and the depletion of ATR (Ataxia telangiectasia and rad3-related protein), Chk1 (Check point kinase 1), and BRCA1 (Breast cancer 1). This ultimately stopped the DNA repair process [52].

Overall, Rao *et al.'s* studies supported this finding, where hyper-acetylation of Hsp90 induced either by HDAC6 inhibition after LBH589 treatment, or by the depletion of HDAC6 levels by treatment with siRNA to HDAC6, inhibited the ATP, co-chaperone p23 and client protein binding to Hsp90, directing the client proteins to polyubiquitylation and proteasomal degradation. Hyperacetylation of Hsp90 increased the binding of Hsp90 to biotinylated-geldanamycin (GM) and its analogue 17-allyl-amino-demethoxy geldanamycin (17-AAG). Hypoacetylated, the chaperone protein HSP90 protects client proteins such as Bcr-Abl, Akt, c-Raf, glucocorticoid receptor (GR), epidermal growth factor receptor (EGFR) and ErbB2 from degradation in K562 leukemia cells and acute myeloid leukemia [53,54]. Hsp90 ATPase activity is regulated by acetylation of a specific lysine residue in the beginning of the middle domain [12]. The degradation of Bcr-Abl following HDACi-mediated hyperacetylation of the molecular chaperone Hsp90 has been proposed to be a

major effector mechanism of the HDAC inhibitor. In addition, MRLB-223, vorinostat and romidepsin, were all capable of killing the IL-3-dependent FDCP1 mouse myeloid cell line that was engineered to grow independently of IL-3 through forced expression of the Hsp90 client protein Bcr-Abl. All three inhibitors induced hyperacetylation of Hsp90 and degradation of Bcr-Abl [55].

The Yang, *et al.* study determined the identity and functional significance of the domain-specific seven lysine residues in Hsp90 that are hyper-acetylated, following treatment with pan-HDAC inhibitors that also inhibit HDAC6. Remarkably, hyperacetylated Hsp90α was extra-cellular and acted as a chaperone for matrix metalloproteinase (MMP-2), which promoted *in vitro* invasion by breast cancer cells. Treatment with anti-acetyl lysine-69 hsp90α antibody markedly inhibits the invasiveness of breast cancer cells. Extra-cellular HSP90α acts as a chaperone and assists in the maturation of the MMP-2 to its active form. Acetylation promotes not only the extra-cellular location of Hsp90α but also facilitates its chaperone association with MMP-2. Due to intra-tumoral stress in the primary breast cancers, increased expression, hyperacetylation and extracellular location of Hsp90α promotes MMP maturation, increased tumor invasion and metastasis. This may be responsible for the overall negative impact of high Hsp90α expression on the survival in breast cancer patients. Therefore, perhaps it is the level of acetylated HSP90α which is the important determinant of metastases and overall prognosis in breast cancer. As its corollary, it would be important to determine whether the combination of LBH589 and anti-AcK69 HSP90α antibody will inhibit *in vivo* invasion and metastasis by breast cancer cells [52].

Hsp70

Hsp70 is expressed at low levels in normal, non-stressed cells; its expression is, however, induced by different cellular stresses, such as heat shock or oxidative stress. In addition, the expression level of Hsp70 is frequently higher in transformed cells. Histone acetylation modification plays an important role in both initiation of transcription and elongation of the Hsp70 gene [56]. Hsp70 consist of an N-terminal ATPase domain of 45 kDa, with a weak ATPase activity which can be stimulated by binding to unfolded proteins and synthetic peptides and a C-terminal substrate binding domain of 25 kDa which is further subdivided into a β-sandwich subdomain of 15 kDa and a C-terminal α-helical subdomain [57].

Under certain pathological conditions the protein quality control machinery is not sufficient to prevent the accumulation of misfolded proteins. Expression of the major Hsp70 protects cells from heat-induced apoptosis. Hsp70 has been reported to act in some situations upstream or downstream of caspase activation, and its protective effects have been said to be either dependent on or independent of its ability to inhibit c-Jun N-terminal kinases (JNK) activation. Purified Hsp70 has been shown to block procaspase processing *in vitro* but is unable to inhibit the activity of active caspase 3. Since some aspects of Hsp70 function can occur in the absence of its chaperone activity. Hsp70 can inhibit apoptotic processing which lead to cytochrome c-mediated procaspase 9 processing, but not with deleted ATPase Hsp70 [58].

Overexpression of Hsp70 protects cells, tissues and organs from harmful assaults such as lethal temperature. It has been reported that no additional Hsp70 synthesis occurs if the cells are re-exposed to the same or a different type of stimulation. One possible mechanism for this retardation of Hsp70 synthesis is that overexpression of Hsp70 down-regulates its gene transcription and expression [59,60]. Hsp70 gene transcription is initiated by a group of transcriptional factors named heat shock factors (HSFs). Among these HSFs, HSF1 is known to have a binding domain for the promoter region of the Hsp70 gene and to be responsible for the heat shock-induced increase in Hsp70 gene expression. Like other transcription factors, HSF1 needs to be activated before promoting Hsp70 gene expression. The activation of HSF1 involves a series of processes including phosphorylation, translocation from the cytosol to the nucleus, formation of a trimer, binding to heat shock elements (HSE) and initiating Hsp70 gene expression [61]. Hsp70 is now known to regulate apoptotic cell death both directly by interfering with the function of several proteins that induce apoptotic cell death as well as indirectly by increasing levels of the anti-death protein Bcl-2. Despite these new insights into the ways in which Hsp70 functions as an anti-death protein, further surprises are likely as we continue to gain insight into the functioning of this

multifaceted protein. The Hsp70 superfamily consists of multiple members, and each member seems to have distinct properties in terms of structure, cellular localization, function and response to stress. The functions of the Hsp70 superfamily proteins are regulated and/or modified by co-chaperones. Again, the pathways appear to be ancient and conserved across a variety of species and the interactions are complex and in several cases not well understood [61].

Hsp70 is constitutively expressed in skeletal muscle, its expression levels are increased rapidly, and several-fold, in response to cellular stress, which provides protection to the cell. While the function of Hsp70 as a molecular chaperone becomes especially important during cellular stress, the mechanisms by which increased levels of Hsp70 provides cytoprotection are increasingly being linked to the direct regulation of specific cell signaling pathways by Hsp70 [56] The substrate binding and release cycle is driven by the switching of Hsp70 between the low-affinity ATP bound state and the high-affinity ADP bound state. Thus, ATP binding and hydrolysis are essential in vitro and in vivo for the chaperone activity of Hsp70 proteins. This ATPase cycle is controlled by co-chaperones of the family of J-domain proteins, which target Hsp70s to their substrates, and by nucleotide exchange factors, which determine the lifetime of the Hsp70-substrate complex. Additional co-chaperones fine-tune this chaperone cycle. For specific tasks the Hsp70 cycle is coupled to the action of other chaperones, such as Hsp90 and Hsp100 [62].

Regulation of HSP70 Functions by HDAC Inhibitors

The regulation of Hsp70 gene is a complex and precise mechanism. The Hsp70 gene may be expressed both at a low basal level under normal growth conditions and at a high induced level after heat shock [63]. Under non-heat-shock conditions, the promoter sequences of Hsp70 are occupied by at least two transcription factors, GAGA factor (GAF), TATA-binding protein, and RNA polymerase (pol) II. Upon heat shock, the inducible expression of Hsp70 is mediated by the interaction of the HSFs with HSEs, which are located in the 5′-upstream region of Hsp70 gene. Zhao et al showed that HDAC inhibitors caused the hyperacetylation of core histone H3, implicating the involvement of chromatin modulation in Hsp gene transcription. These data suggested a close correlation among histone acetylation, Hsp gene expression and longevity in *D. melanogaster* [64]. Moreover, HDAC inhibitor-induced H3 hyperacetylation increased the HSF binding to HSE, promoted the association of RNA pol II with the 5'-coding region, and the downstream region of Hsp70. These results suggested that histone acetylation modification plays an important role in both initiation of transcription and elongation of the Hsp70 gene. The results from the Zhao study indicated that upon transcriptional induction, a significant chromatin alteration occurred at the promoter of Hsp70 gene in *D. melanogaster*. The function of Hsp70 depends on the cellular location: intracellularly, it has cytoprotective and antiapoptotic functions, whereas extracellularly it exerts immunostimulatory functions. Secreted Hsp70 is, for example, involved in cross-presentation of cancer-derived antigenic peptides, a function that is explored currently in immunotherapeutic approaches against cancer additionally, membrane-bound Hsp70 can stimulate antigen presenting cells (APCs) to release proinflammatory cytokines and can provide a target structure for NK cell-mediated lysis. The Hsp70 gene may be expressed both at a low basal level under normal growth conditions and at a high induced level after heat shock. The Hsp70s are central players in protein folding and proteostasis control. Increasing Hsp70 levels has also proven effective in preventing toxic protein aggregation in disease models [65].

Studies by Marinova *et al.* have showed that valproic acid induced functional Hsp70 through class I HDAC inhibitors in rat cortical neurons and this type of induction may contribute to the neuroprotective and therapeutic effects of valproic acid. The data suggested that the phosphatidylinositol 3-kinase/Akt pathway and Sp1 are likely to be involved in this process [66]. In this situation, the functional role of HSP70 might be the protection of cells against HDAC inhibitor-induced apoptosis [67].

On the other hand Lv, *et al.* reported that injection of valproic acid markedly prevented the reduction of Ac-H3 and Ac-H4, upregulated the expressions of Hsp70 and Bcl-2, reduced apoptosis and finally promoted locomotion recovery [68].

The major properties of valproic acid that make it

interesting to today's researchers were not known to exist more than 10 to 30 years ago. They are that valproic acid:

a) Increases the activity of the neurotransmitter gamma amino butyric acid (GABA) through several mechanisms.

b) Is a histone deacetylase inhibitor.

c) Induces the mobilization of heat shock proteins, Hsp70 in particular.

d) Promotes the selective differentiation of certain stem and progenitor cells [69].

Recent studies have shown that inhibition of class I HDACs induced 78 kDa glucose regulated protein/binding immunoglobulin protein (GRP78/BiP) and Hsp70 protein chaperones [70].

A study by Park *et al*. has shown that a subset of HDAC inhibitors, valproic acid, TSA, SAHA and sodium butyrate targeting class I and class II HDACs induced early differentiation in embryonic stem cells and simultaneously induced Hsp70 expression in mouse and human cells but class III HDAC inhibitors, nicotinamide and splitomycin, failed to induce differentiation or Hsp70 expression. These results confirmed that class I and II HDACs help maintain embryonic stem cells properties [71].

Rao *et al*. has determined that the stress induced by nutrient withdrawal or treatment with pan-HDAC inhibitor panobinostat (LBH589) resulted in hyperacetylation of Hsp70, which induced autophagy in the cultured breast cancer MB-231 and MCF-7 cells [72].

Human cancer cells frequently express Hsp70 on their cell surface, whereas the corresponding normal tissues do not. In addition, several clinically applied reagents, such as alkyl-lysophospholipids, chemotherapeutic agents, and anti-inflammatory reagents, have been found to enhance Hsp70 surface expression on cancer cells. Jensen have found that inhibition of HDAC activity leads to surface expression of Hsp70 on various hematopoietic cancer cells, an occurrence that was not observed on native or activated peripheral blood cells. HDAC-inhibitor -mediated Hsp70 surface expression was confined to the apoptotic Annexin V positive cells and blocked by inhibition of apoptosis. Other chemotherapeutic inducers of apoptosis such as etoposide and camptothecin also led to a robust induction of Hsp70 surface expression. Hsp70 expression however was not caused by induction of apoptosis, since activated CD4 T cells remained Hsp 70 surface negative despite effective induction of apoptosis. Inhibition of endolysosomes or normal ER/Golgi transport did not affect Hsp70 surface expression. Intracellular calcium and the transcription factor Sp1, that has previously been shown to be important for the intracellular stress mediated by HDAC-inhibitors, were not involved in Hsp70 surface expression. Jensen *et al*. also found that HDAC-inhibitors decreased cellular Plasma Membrane Electron Transport (PMET) activity and that selective inhibition of PMET activity with extracellular NADH induced a robust Hsp70 surface expression. The data from Jensen *et al*. suggest that inhibition of HDAC activity selectively induces surface expression of Hsp70 on hematopoietic cancer cells and that this may increase immunorecognition of these cells [73].

Furthermore, administration of HDAC inhibitors, including valproic acid, sodium butyrate, TSA, and SAHA has been reported to induce increased expression of Hsp70 alongside neuroprotection. The effects of HDAC inhibitors on both Hsp70 and p-Akt were shown to be dose and agent dependent. Specifically, Faraco_demonstrated that the extent of the effect of SAHA on Hsp70 levels varies with different administered doses but did not observe any effect of this agent on p-Akt levels [74], as was later shown by Kim, when applying sodium butyrate or TSA [75].

The Zhao *et al*. results demonstrated that sodium butyrate and TSA were able to extend the lifespan and promote Hsp22 and Hsp70 expression in *D. Melanogaster*. However, the optimal concentrations of these inhibitors, and probably the mechanisms of their actions, vary with the genetic background [76]. In addition, results of experiments by Marinova *et al*. revealed that the histone acetyltransferase p300 was recruited to the Hsp70 promoter in rat astrocytes after valproic acid treatment, and that p300 formed a complex with the transcription factor NF-Y. NF-Y also appears to interact with the transcription factor Sp1 in astrocytes and neurons. NF-Y has been identified as important for acetylation responsiveness of the Hsp70 promoter in Xenopus. The Marinova *et al*. data implied that the recruited p300 interacts with NF-Y. The importance of the formation of a multiprotein complex between PCAF/p300 and Sp1/NF-Y for TSA induction of transforming growth factor beta II promoter activity in pancreatic cell lines has been demonstrated. A study from Marinova laboratory also observed that Sp1 plays a role in

Hsp70 induction by valproic acid in cortical neurons. Taken together, these results suggest that the formation of a complex between NF-Y and Sp1 and the recruitment of p300 may be necessary for the induction of Hsp70 by valproic acid [77]. The Marinova study demonstrated for the first time that Class I HDAC inhibition by valproic acid and other compounds increased levels of dimethylation and trimethylation of histone H3K4 in rat cortical neurons and astrocytes, suggesting the interplay between histone acetylation and histone methylation. Hsp70 protein levels were markedly increased under these experimental conditions and Histone 3 dimethyl lysine 4 (H3K4Me2) levels associated with the Hsp70 promoter in astrocytes were robustly increased after treatment with valproic acid or MS-275. These findings have profound implications for the use of HDAC inhibitors to induce neuroprotective proteins in neurodegenerative conditions [69].

Moreover the protein CoREST, which belongs to HDAC1 and 2 repressor complexes, has recently been implicated in the control of Hsp70 expression in non-heat shocked and heat shocked cells [78].

PERSPECTIVES AND CONCLUSIONS

As we come closer to understanding the molecular mechanisms inherently responsible for tumorigenesis, as well as the full range of HDAC inhibitors cellular actions, we will be able to target in a more appropriate way and to pair cancer therapies for clinical use. In order to establish rigorous patient selection criteria and optimal drug combinations to properly design further trials and maximize the clinical gain, the bridge between the biological function and the therapeutic benefit of these drugs needs to be further elucidated. Histone acetylation and deacetylation, as key factors in the regulation and dynamic changes of gene expression affect chromatin structure and its interaction with regulatory factors.

Overall, the emerging understanding of the impact of lysine acetylation of molecular chaperones and cochaperones is defining novel strategies which exploit this mechanism and can be harnessed for cancer therapy.

It is clear that Hsps play a crucial role in maintaining oncogenic protein homoeostasis. Hsps inhibition offers great promise in the treatment of a wide variety of solid and haematological malignancies.

A number of HDAC inhibitors are used in clinical trials for anticancer therapy. Today, we believe that histone deacetylation, similar to the induction of HSPs, is an essential event in the cellular defense to stress. Our review thus establishes a new link between two targets that are the subject of numerous studies in oncology: HSPs and HDACs. Strategies combining specific HDACs inhibitors with thermotherapy could pothole the effect of these inhibitors on tumor cells.

Novel combination therapies for each tumour type need to be developed based on preclinical data. Combination of HDAC inhibitors with conventional chemotherapy or targeted therapies, may lead to greater efficacy and improved clinical outcomes.

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