Chapter 12
Hsp90 and Its Role in Heme-Maturation of Client Proteins: Implications for Human Diseases

Arnab Ghosh and Dennis J. Stuehr

Abstract Hemeproteins are essential for life and heme insertion is an essential step in their maturation. Maturation of hemeprotein requires that they incorporate heme and become active, but knowledge of this essential cellular process remains incomplete. However recent studies on chaperon Hsp90 has revealed that it drives functional heme insertion in vital hemeproteins like inducible nitric oxide synthase (iNOS), soluble guanylate cyclase (sGC) and hemoglobin (Hb). In all three cases Hsp90 interacts with the heme-free or apo-protein and then drives the heme insertion by an ATP dependent process before dissociating from the heme-replete proteins. Given the diverse role of chaperon Hsp90, and in particular to it being a major therapeutic target in drug discovery programs these findings add up to Hsp90's repertoire of being a druggable target and opens up more avenues in regulating growth of diseased cells in those pathologic conditions where these hemeproteins are dysfunctional.

Keywords Angiogenesis · Bronchodilation · Erythropoiesis · Heme · Hemeprotein · Metastasis

Abbreviations

AHSP	Alpha hemoglobin stabilizing protein
AIF	Apoptosis-inducing factor
Apaf-1	Apoptotic protease activating factor 1
Ask-1	Apoptosis signal-regulating kinase 1
ATP	Adenosine triphosphate
cGMP	Cyclic guanosine monophosphate

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ECM	Extracellular matrix
FLVCRb	Feline leukemia virus subgroup c receptor b
Hb	Hemoglobin
HDM	House dust mite
Her-2	Human epidermal growth factor receptor 2
Hip	hsp70-interacting protein
H-NOX	Heme nitric oxide/oxygen
Hop	hsp70/hsp90 organizing protein
Hsp90	Heat shock protein 90
iNOS	Inducible nitric oxide synthase
IPF	Idiopathic pulmonary fibrosis
MMPs	Matrix metalloproteinases
NO	Nitric oxide
NOS	Nitric oxide synthase
NOX	NADPH oxidase
per2	Period circadian regulator 2
ROS	Reactive oxygen species
sGC	Soluble guanylate cyclase
TGF-β1	Transforming growth factor-β1
VEGF	Vascular endothelial growth factor

12.1 Introduction

Hsp90 is a ubiquitously-expressed, ATP-dependent chaperone that helps to fold, stabilize, or modify the functions of select client proteins (Li and Buchner 2013; Makhnevych and Houry 2012). Hsp90 functions through its subdomain molecular motions and its inherent ATPase activity to help control client protein maturation, trafficking, and lifetime in cells (Faou and Hoogenraad 2012; Prodromou 2012; Taipale et al. 2010). The molecular-level impacts of hsp90 on various client proteins are just beginning to be elucidated (Krukenberg et al. 2011; Park et al. 2011; Ratzke et al. 2012; Southworth and Agard 2011). Hsp90 primarily exist as homodimers whose activity is regulated by ATP. It belongs to the HSP90 family of proteins which represents a well-conserved group of proteins with an average molecular mass of 90 kDa. There are two major Hsp90 isoforms, the Hsp90α1 (HspC1) and the Hsp90β (HspC3), commonly known as Hsp90 (Csermely et al. 1998; Chen et al. 2005). These two proteins in humans are expressed by genes *HSP90AA1 and HSP90AB1* respectively. While Hsp90α1 (HspC1) represents the stress-inducible isoform, Hsp90β (HspC3) is constitutively expressed.

Like many other chaperones, Hsp90 is a hydrophobic protein whose hydrophobicity further increases after heat shock (Yamamoto et al. 1991; Iwasaki et al. 1989). Hsp90 is the major soluble protein of the cell and most commonly located in the cytoplasm. It is also reported to be present in the extracellular milieu where they act as potent stimulators of immune responses (Pockley and Multhoff 2008; Pockley

et al. 2008). Hsp90 activity is regulated by post-translational modifications and its association with numerous co-chaperones and client proteins involved in signal transduction and transcriptional regulation. Hsp90 is known to associate with several heme proteins, and the ascribed outcomes include assisting in protein heme-maturation, stabilization, function, or activity, and in shaping enzyme product distribution (McClellan et al. 2007). Herein we present the current knowledge of Hsp90 function with regard to heme-maturation of client proteins, role of hsp90 in tumorigenesis and pulmonary diseases and how Hsp90 function in client hemeprotein maturation may relate to human diseases where Hsp90 is a therapeutic target.

12.1.1 Heme Proteins in Biology

Heme proteins are the most ubiquitously distributed metalloprotein groups in nature. These proteins usually contain heme or an iron (Fe) protoporphyin IX (or a derivative of this macrocycle) in the active site. Depending on the nature of the side chains present in the macrocycle and the type of bond involved in the interaction with the protein, the hemes are classified in three major classes; a, b and c (Severance and Hamza 2009). Hemes a and c are synthesized from heme b, which is the most abundant heme found in hemeproteins, with chemical modification occurring in the side chains as shown in Fig. 12.1. This metallated protoporphyrin is corordinated to the protein through histidine, methionine, tyrosine or cysteine axial ligands (Paoli et al. 2002). The representative classes of heme proteins is summarized in Fig. 12.2.

Heme proteins are involved in a significant array of biological functions including cell energetics (cytochromes), oxygen transport and storage (hemoglobin and myoglobin), oxygenation (monooxygenases), hydrogen peroxide degradation (peroxidases, catalases), oxygen reduction (heme-copper oxidases), heme or small molecule sensing (FixL, HemeAT sensors), transcription regulation (Per2, RevErb

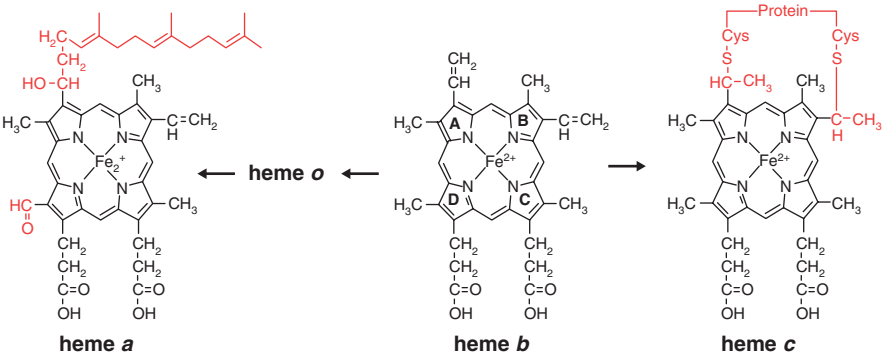

Fig. 12.1 Structures for heme a, b and c. Heme a and c are synthesized from heme b via side chain modifications shown in red. Pyrrole rings nomenclature (A, B, C and D) are depicted using the Hans Fischer system. (Adapted from Severance and Hamza 2009)

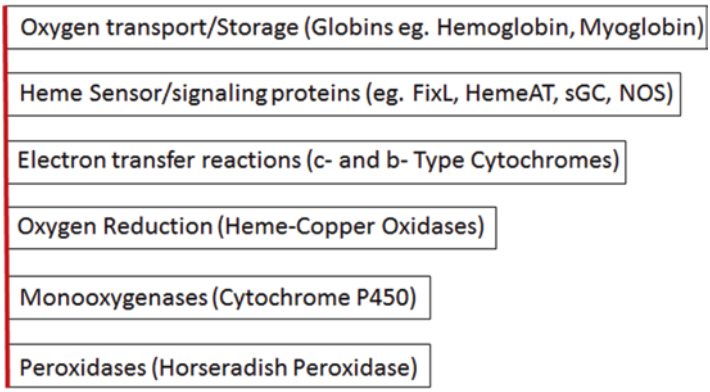

Fig. 12.2 Representative classes of heme proteins

αβ, Bach1), cell signaling (sGC, NOSs), numerous enzymatic transformations and host defense (Ponka 1999; Smith and Veitch 1998; Taylor and Zhulin 1999). It is remarkable that heme proteins use the same heme cofactor, Fe-protoporphyrin IX, to confer such diverse functions. This is attributed to the variety of interactions between the heme cofactor and the protein polypeptide chain, such as the coordination of heme iron by various axial ligands (His, Met, Cys, water, etc.) and the hydrogen-bonding interactions in the heme distal pocket (Lu et al. 2009; Spiro et al. 2013; Poulos 2014). Thus the reactive versatility of the heme iron is exploited via the structural nature of the protein matrix. In addition to these interactions, post-translational modifications (such as nitration, glycosylation, amino acid cross links such as Tyr-His cross links in heme-copper oxidase etc.) also play key roles in tuning the structure and function of heme proteins (Yamakura and Ikeda 2006; Liu et al. 2012; Yoshikawa et al. 1998).

12.1.2 Maturation of Heme Proteins

In a majority of cases, the heme cofactor of hemeproteins is essential for function, but because free heme is reactive its production is tightly regulated (Taketani 2005). The specific steps of heme biosynthesis are well-documented and take place in both the cytosolic and mitochondrial compartments of a cell (Dumont et al. 1991; Steiner et al. 1996; Wang et al. 1996), with the final three steps occurring in the mitochondria. However, with the exception of cytochrome c biogenesis (Richard-Fogal et al. 2009) and some aspects of heme acquisition and catabolism (West and Oates 2008; Yi et al. 2009), relatively little is known about how heme is transported out of mitochondria in eukaryotic cells and how it becomes inserted into soluble proteins in the cytosol, or how these processes might be regulated (Fleming and Hamza 2012; Severance and Hamza 2009). These steps are critical, given that free heme is

potentially cytotoxic (Tsiftsoglou et al. 2006) and is normally kept at low intracellular levels (Wijayanti et al. 2004). New studies have identified a mitochondrial heme exporter FLVCRb, which is essential for erythropoiesis (Byon et al. 2013). How this heme exporter pertains to heme transport into soluble hemeproteins in the cytosol remains to be investigated. Heme insertion into catalase was shown to possibly occur within the peroxisome (Lazarow and de Duve 1973), and myeloperoxidase trafficking in cells was found to depend on its heme content (Nauseef et al. 1992). More importantly recent studies from our group (Ghosh et al. 2011; Ghosh and Stuehr 2012) as well as previous studies from Osawa's group (Billecke et al. 2004) have shown specific involvement of chaperon Hsp90 in cytosolic heme insertion into soluble proteins. Inducible nitric oxide synthase (iNOS), soluble guanylate cyclase (sGC) and hemoglobin (Hb) are our new finds of hsp90 client proteins which show remarkable hsp90 dependence on their heme maturation.

12.1.3 HSP90 and Its Role in Client Hemeprotein Maturation

Osawa and colleagues showed for the first time that hsp90 is required for cellular heme insertion into neuronal nitric oxide synthase (nNOS) (Billecke et al. 2004). Later we uncovered a role for Hsp90 in iNOS heme insertion (Ghosh et al. 2011). In the latter case, Hsp90 was shown to primarily associate with an apo-iNOS monomer in cells, and then found to drive heme insertion into the apo-enzyme by an ATP-dependent process, after which the Hsp90 interaction with the heme-replete, mature iNOS fell apart. We then found that heme insertion into the β subunit of soluble guanylate cyclase (sGC) is also Hsp90-dependent (Ghosh and Stuehr 2012). Given that sGC structural homologs (H-NOX) and NOS have markedly different protein structures and heme environments (Crane et al. 1998; Martin et al. 2010), these findings hint that Hsp90 may play a broader role in heme protein maturation than was previously realized. In the sGC studies, Hsp90 associated primarily with the heme-free form of sGC-β1 in cells, but the association fell apart once heme became inserted. Although the association did not require hsp90 to have an intact ATPase activity, as judged from results using Hsp90 inhibitors radicicol or novobiocin, or by using an ATPase defective Hsp90 mutant (D88N), an intact ATPase activity was essential to actually drive heme insertion into the apo-sGCβ1. Thus, the model suggested for Hsp90 function in sGC maturation mimics the model proposed for driving heme insertion into apo-iNOS (Fig. 12.3). These similarities imply Hsp90 may operate through a common mechanism to target and stabilize heme-free forms of client heme proteins, and then enable their maturation by driving heme insertion in an ATP-dependent process. Other studies on transmembrane heme proteins like NADPH oxidases (NOX) have shown preliminary evidence of a Hsp90 regulation (Chen et al. 2011). NOX enzymes utilize NADPH to synthesize superoxide. This NOX functionality is dependent on its heme and is a major source of cellular reactive oxygen species (ROS) (Cai and Harrison 2000; Griendling et al. 2000). Because of the intrinsic toxicity of ROS, the regulation of

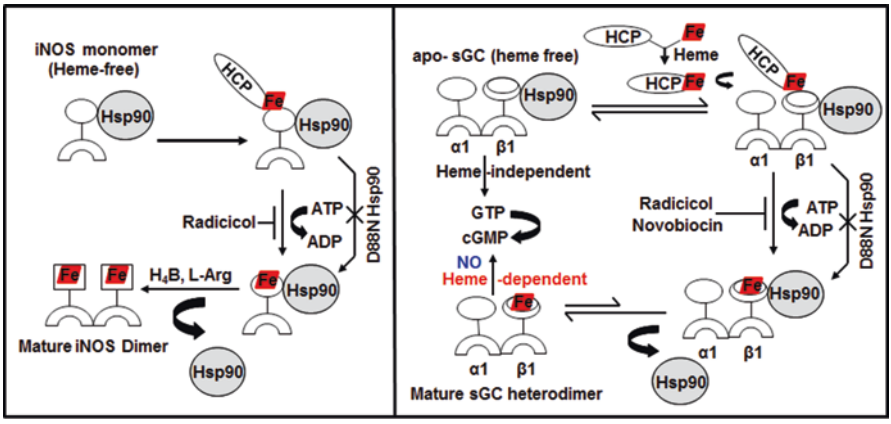

Fig. 12.3 Similarities in Hsp90-apo-protein interactions with respect to heme deplete/replete states of sGC and iNOS. HCP indicates an unknown heme carrier protein which may eventually be Hsp90

NOX enzymes has developed a significant degree of complexity. Hsp90 has been shown to bind to the C-terminal domain of NOX5 and regulate its superoxide production, suggesting that Hsp90 may regulate certain parameters of cellular redox. However a more in depth study or direct study relating Hsp90 to heme-maturation of NOX-5 is lacking and warrants further studies.

Recently we found that Hsp90 chaperones hemoglobin (Hb) maturation in erythroid and non-erythroid cells (RAW, A549 cells) following a similar mechanism and further reinforcing this concept (Ghosh et al. 2018) (Fig. 12.4). While in erythroid cells, Hb-α and Hb-β/γ (-$\beta_{adult}/\gamma_{fetal}$ Hb) are independently chaperoned by AHSP (alpha Hb stabilizing protein) and Hsp90 respectively, Hsp90 promotes heme-maturation of both Hb-αβ subunits in non-erythroid cells which lack AHSP. Although a role for AHSP in Hb-α maturation had been established (Kihm et al. 2002; Feng et al. 2005; Yu et al. 2007), what chaperones might aid partner globin maturation (Hb-γ and Hb-β) was unclear. Our study revealed that Hsp90 enabled maturation of both Hb-γ and Hb-β by associating with and stabilizing their immature, heme-free forms and by driving their heme insertion reactions in an ATP-dependent manner. This was demonstrated in the erythroid-like K562 cell line and in two human erythroid progenitor cell types (HiDEP-1 and HUDEP-2) during their in vitro differentiation to mature erythroid cells, implying that Hsp90's role is relevant for globin maturation during erythropoiesis. Thus, Hsp90 appeared to act as a counterpart to AHSP by chaperoning Hb-γ and Hb-β for their heme insertion reactions, as would be required to form functional fetal (α2γ2) and adult (α2β2) tetramers during erythropoiesis. Under all circumstances, the globin associations of Hsp90 or AHSP remained specific. Together, our findings support the view that Hb-α and Hb-β maturation are independently chaperoned in erythroid cells (Weiss and dos Santos 2009) and confirm that Hb-α maturation in erythroid cells is independent of Hsp90. However, when Hb-α was coexpressed with Hb-β in nonerythroid cells either

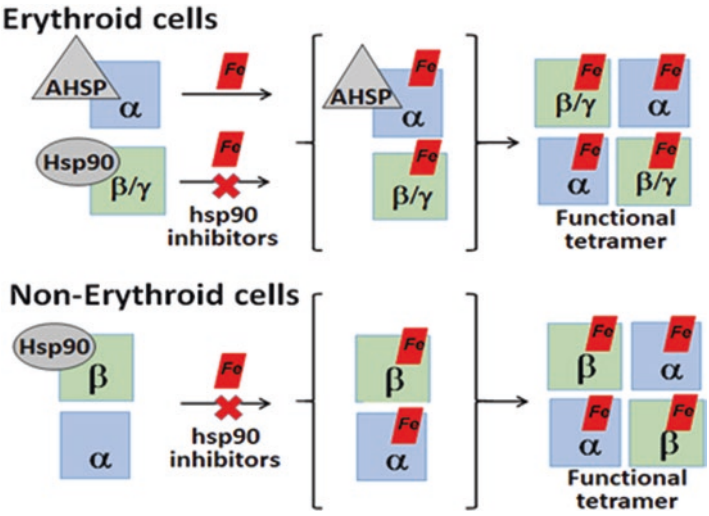

Fig. 12.4 Hsp90 chaperons Hb maturation

naturally or as a consequence of transfection, we saw that its heme insertion then became Hsp90-dependent. Thus, Hsp90 is needed for heme insertion into at least two (Hb-β and Hb-γ) and as many as three (Hb-α) globins, depending on the circumstances under which they are expressed (i.e., with or without AHSP), and therefore Hsp90 plays an unexpectedly broad role in globin maturation. This concept may help explain how Hb maturation can succeed in nonerythroid cells, which all lack AHSP expression. A model for Hb maturation that incorporates our current findings is presented in Fig. 12.4. Thus Hsp90-assisted heme insertion into Hb-β and Hb-γ appeared to follow the tenets established for maturation of other heme proteins like NOS and sGC (Ghosh et al. 2011; Ghosh and Stuehr 2012).

Our findings also revealed that low doses of nitric oxide (NO) can contribute to sGC maturation by triggering a rapid Hsp90-dependent heme insertion into the apo-sGCβ1 (heme-free) population, ultimately resulting in a mature sGC heterodimer (Ghosh et al. 2014). Our study showed that at resting or steady state the majority of apo-sGCβ1 is bound to Hsp90 and activation by NO caused a transient swapping of binding partners of sGC-β1, i.e. from Hsp90 to sGC-α1 subunit, which forms the active sGCα1β1 heterodimer. This partner protein swap resulting in elevated active sGC-α1β1 heterodimer showed for the first time that NO can trigger a change in protein interactions by inserting heme into apo-sGCβ1. This finding filled a void in an earlier work done by Ignarro and colleagues in the 80s which showed that NO-heme moiety could be transferred into heme-free or apo-sGCβ1, through an exchange reaction with NO-hemeproteins to activate the enzyme (Ignarro et al. 1986). This suggests that a NO-Hsp90 synergy may be essential for maturation and activation of certain hemeproteins.

Table 12.1 Hsp90 chaperon hemeprotein interactions in eukaryotes

Protein	Function of heme	Associated Hsp90 chaperon, possible function
Eukaryotic initiation factor 2-alpha (eIF2) kinase HRI (heme regulated inhibitor)	Repress kinase activity, signal transduction	Amplify/enable function
NO synthases (NOSs)	Catalytic O_2 activation	Aid heme maturation, boost activity of heme replete enzyme, stabilize protein
Cytochrome P450 2B1	Catalytic O_2 activation	GRP94 (?), enable heme reconstitution
NADPH oxidase 1, 2, and 5	Catalytic O_2 reduction	Boost activity and influence enzyme products
Soluble guanylate cyclase (sGC)	Gas sensing signal transduction	Aid heme maturation, boost activity and increase enzyme lifetime
Hemoglobin (Hb)	O_2 transport	Aid heme maturation

Based on our recent finds of client hemeproteins of Hsp90 and previous literature on Hsp90 regulation of hemeproteins, we present a summary of currently known Hsp90 hemeprotein interactions in Table 12.1.

12.1.4 HSP90 and Human Diseases

Hsp90 is the epicenter to a broad spectrum of human diseases ranging from various form of cancers (Trepel et al. 2010; Miyata et al. 2013; Tóth et al. 2015), neurodegenerative diseases (Luo et al. 2010), asthma and pulmonary diseases (Ghosh et al. 2016; Bonniaud et al. 2017), autoimmune and endocrine disorders to many forms of retinal dysfunction (Ratajczak et al. 2015; Aguilà and Cheetham 2016). The ability of Hsp90 to protect unfolded proteins from aggregation, assist in proteosomal degradation and modulate several growth and signaling pathways simultaneously, makes it an attractive target for therapeutic intervention (Miyata et al. 2013). Elevated levels of chaperon Hsp90 has been found in a wide spectrum of cancers suggesting a central role in survival and growth of malignant cells (Calderwood et al. 2006; Whitesell and Lindquist 2005; Ciocca and Calderwood 2005). Such enhanced levels of Hsp90 is postulated as a protective effect from various stress parameters (e.g. hypoxia, ischemia, heavy metals etc.) which cells encounter under pathologic conditions. Since several oncoproteins are clients of Hsp90, targeting Hsp90 represents a useful anti-cancer approach. Over the years numerous oncoproteins have been identified as putative targets and Hsp90 inhibition is a promising approach in anti-cancer strategies (Xu and Neckers 2007; Kamal et al. 2003). These approaches are strengthened by the fact that inhibition of Hsp90 contributes to degradation of many oncoproteins thereby intensifying these strategies (Ali et al. 2006; Jackson et al. 2004; Chen et al. 2010).

12.1.5 Role of HSP90 in Tumorogenesis

Hsp90 and its chaperon proteins are involved in multiple cellular signaling pathways which regulate apoptosis and promote cell survival (Trepel et al. 2010). In this context, three acquired capabilities of cancer cells growth and sustenance are, evasion of apoptosis, sustained angiogenesis and tissue invasion and metastasis (Miyata et al. 2013). A great body of literature indicates critical inhibitory contribution of Hsp90 to apoptosis which is key to normal cell growth or adverse tumor progression. Hsp90 is known to inhibit apoptosis by directly binding to Apaf-1, blocking cytochrome c-mediated oligomerization of Apaf-1 and activation of pro-caspase 9 (Pandey et al. 2000). It also inhibits apoptosis by forming a ternary complex with pro-apoptotic kinase Ask-1 and Akt (Zhang et al. 2005). Moreover Hsp90 exerts anti-apoptotic activity by blocking the mitochondrial-cytosolic transition of apoptosis-inducing factor (AIF) and endonuclease G (Fulda et al. 2010). With regard to angiogenesis Hsp90 is known to promote angiogenesis and metastasis by chaperoning certain client proteins including VEGF, NOS, and MMP-2 (Whitesell and Lindquist 2005). Together these functions of chaperon Hsp90 make it clearly anti-apoptotic and pro-angiogenic (Miyata et al. 2013). Additionally Hsp90 also plays a role in the modulation of the extracellular matrix (ECM), since Hsp90β was identified as interaction partner of MMP-3 in the extracellular matrix accountable for mammary epithelial invasion and morphogenesis (Correia et al. 2013). In combination with the co-chaperones Hsp70, Hsp40, Hip, Hop, and p23, extracellular Hsp90α is known to interact with and promote the proteolytic activity of MMP-2 in an ATP-independent manner (Sims et al. 2011; Song et al. 2010). It is known that degradation of ECM is a signal for the beginning of invasion and metastasis, and MMPs are important molecules involved in this process (Nelson et al. 2000). Hsp90 and MMP-9 is known to constitute as a complex in anaplastic large cell lymphomas, and MMP-9 could be activated by Hsp90 to promote cell invasion (Lagarrigue et al. 2010). These functions of Hsp90 highlight its critical role in fostering tumour growth and metastasis.

Considering the above facts it is not surprising that Hsp90 is central to tumorigenesis and the evidences are evergrowing. A study showed that Hsp90 derived from tumor cells (breast and colon tumors) displayed high ATPase activity relative to normal cells (Kamal et al. 2003). The tumor Hsp90 displayed very high affinity for Hsp90 inhibitor 17-AAG even at nanomolar levels. On this basis it was inferred that Hsp90 chaperons mutant proteins within these tumors resulting in chaperoning and stabilization of such oncoproteins, saving them from degradation and explaining Hsp90-dependent malignant progression which was unresolved before. Likewise Hsp90 was found to stabilize the conformation of mutant proteins that arise during transformation including v-Src, Bcr-Abl, and p53 (Nimmanapalli et al. 2001; Neckers 2002), replicating multiple effect of these mutants on the cancer cell phenotypes. An increased activity of Hsp90 has been described in lung cancer expressing a mutated form of the epidermal growth factor receptor which belongs to the group of Hsp90 client proteins (Shimamura and Shapiro 2008). In melanoma,

Hsp90 showed high expression in the tumors than nevi and was associated with disease progression, suggesting it to be a valuable drug target in melanoma as well as a useful diagnostic marker (McCarthy et al. 2008). Moreover in bladder and epithelial ovarian carcinomas a high expression of Hsp90 was observed at advanced stages, suggesting that it maybe an indicator of aggressiveness (Lebret et al. 2003; Elpek et al. 2003). Studies by Wang et al., showed that the level of plasma Hsp90α was significantly enhanced in patients with malignant tumours of breast, lung, pancreas and liver in comparison with normal people, and patients with benign tumours (Wang et al. 2009), highlighting the importance of cancer cell invasions.

The tumor selectivity of Hsp90 inhibitors makes Hsp90 a unique therapeutic target. What stands out here is the high ATPase of tumor Hsp90 which maybe the driving force as we see in our heme-maturation of client hemeproteins under normal conditions (Ghosh et al. 2011; Ghosh and Stuehr 2012). The ability of cancer cells within tumors to expand requires angiogenic ability and neovascularization. Since such angiogenic pathways are turned on during tumor progression (Hanahan and Folkman 1996) and the vascularization machinery becomes active enough to sprout new blood vessels and sustain neoplastic growth. As new blood vessel growth requires functional Hsp90 (Miyata et al. 2013), active heme-maturation of Hsp90 clients such as iNOS (Ghosh et al. 2011) Hb and sGC (Ghosh et al. 2018; Ghosh and Stuehr 2012) may also occur during angiogenesis within these malignant tumors to support these processes. These events can now be envisioned based on our recent finds.

12.1.6 Role of HSP90 in Asthma and Pulmonary Diseases

The ability of Hsp90 to modulate cell fate might have vital repercussions not only for cancer but also for other progressive human diseases, such as those of the pulmonary system e.g. asthma (Ghosh and Erzurum 2011) and pulmonary fibrosis (Mora et al. 2017). Asthma is defined by airway inflammation and hyper responsiveness, and contributes to morbidity and mortality world wide (Ghosh and Erzurum 2011). sGC is a key enzyme of the NO signaling pathway, and is activated by NO produced from NOS enzymes (Ghosh et al. 2014; Stasch et al. 2011), thereby activating synthesis of the second messenger cGMP, which produces vascular smooth muscle relaxation or vasodilation as a downstream effect (Murad 2006; Bryan et al. 2009). This constitutes the NO-sGC-cGMP signal pathway and is a well known dilation pathway in the vasculature. In a recent study (Ghosh et al. 2016) we found that the NO-sGC-cGMP pathway plays a significant role in lung bronchodilation, and that lung sGC becomes dysfunctional in asthma due to high levels of NO generated from iNOS induction during inflammation in the airway epithelium (Ghosh et al. 2016; Guo et al. 1995), which desensitizes the sGC resident in the airway smooth muscles below (Fig. 12.5). This dysfunctional sGC is heme-free, does not respond to its natural activator NO, but can be activated by sGC agonists like BAY 60-2770 (Pankey et al. 2011), which can activate the enzyme

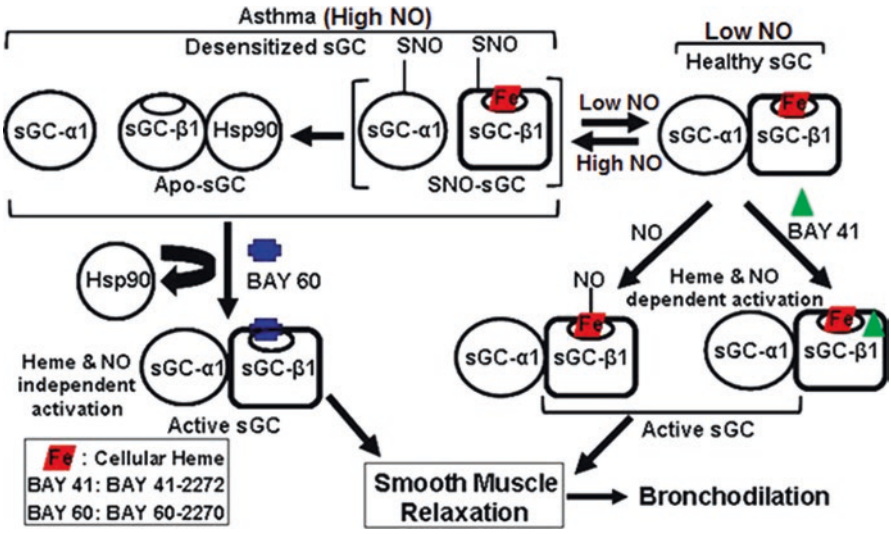

Fig. 12.5 Schematic representation depicting ways by which different populations of sGC can be activated in healthy and diseased conditions which leads to smooth muscle relaxation and ultimate bronchodilation

independent of NO to produce bronchodilation and such agonists are future drugs for asthma. In this study Hsp90 was associated with heme-free sGC, and seemed to stabilize it rather than priming it for degradation, which makes this pathological sGC more drug receptive. In other studies on asthma, relating to airway epithelium and inflammation, Hsp90 is more directly implicated. Extracellular Hsp90α (eHSp90α) is shown to mediate HDM-induced human bronchial epithelial dysfunction, suggesting that eHsp90α is a potential therapeutic target for treatment of asthma (Dong et al. 2017). Another report (Pezzulo et al. 2018) suggests that Hsp90 inhibition reverts IL-13, IL-17-induced goblet cell metaplasia in the human airway epithelia. Hsp90 inhibitors have been also been shown to reduce airway inflammation in mouse models of allergic asthma (Dimitropolou et al. 2010), and another study showed that Hsp90 inhibitors hampered airway relaxation (Intapad et al. 2012). Given our current understanding of iNOS heme-maturation most of these processes of Hsp90 inhibition may involve inhibition of iNOS heme-insertion, suppressing iNOS induction in the airway epithelium and reducing inflammation (Ghosh et al. 2016).

Idiopathic pulmonary fibrosis (IPF) is a progressive disease of the lung parenchyma, causing significant morbidity and mortality (Hopkins et al. 2016; Overgaard et al. 2016). Transforming growth factor (TGF)-β1 is a key cytokine involved in the process of fibrogenesis (Froese et al. 2016). It causes myofibroblast proliferation and differentiation and increases the synthesis of collagen, fibronection and other extracellular matrix components (ECM). A recent study (Sibinska et al. 2017) demonstrated that Hsp90 has a direct role in TGF-β1 signaling pathway and Hsp90 inhibition reduced lung fibrogenesis and fibrosis progression in mice. The study

also showed that Hsp90 is overexpressed in IPF lungs and fibrosis can be inhibited using a water soluble hsp90 inhibitor, 17-DMAG, which targets the Hsp90 ATPase similar to 17-AAG (Sibinska et al. 2017; Kamal et al. 2003). Another study (Sontake et al. 2017) found that Hsp90 was elevated in expression and in its ATPase activity in lung biopsies of patients with IPF. These findings bear great semblance with regard to Hsp90 expression and ATPase to what we see in a majority of cancers (Miyata et al. 2013) and suggests that targeting Hsp90 is an effective strategy for treating fibrotic lung disease. More recently sGC agonists (Sander et al. 2017) are also being tried to treat age-related fibrosis. Since Hsp90 and other chaperon machinery slows down with age (Verbeke et al. 2001), causing low NO levels which may reduce sGC activation thereby making such sGC agonists as the drugs of choice. Moreover a slow Hsp90 chaperon machinery may cause reduced heme-maturation causing buildup of heme-free sGC, which can be activated by BAY 60-2770 like drugs, further encouraging the use of these agonists to treat age-related fibrosis.

12.1.7 Therapeutic Aspects of HSP90 Inhibition

Hsp90 has emerged as an important molecule in anti-tumour therapy, and several drug classes have been found to target its ATP-binding domain resulting in inactivation of the chaperone. The first hsp90 inhibitor, 17-AAG (tanespimycin), entered clinical trials in 1999, while a second inhibitor 17-DMAG entered the first-in-human study in 2004 (Trepel et al. 2010). From this period onwards, many other Hsp90 inhibitors are currently undergoing clinical evaluation in cancer patients, owing to extensive efforts in rational drug design and discovery (Eccles et al. 2008; Chiosis and Tao 2006). Various other newer class of hsp90 inhibitors are also on the rise (Neckers and Workman 2012; Fuhrmann-Stroissnigg et al. 2017).

A wealth of evidence now indicates that, after successive dosing of Hsp90 inhibitors to animals bearing human tumours, blocks tumour growth efficiently. However, tumours often start to re-grow after withdrawal of the inhibitor (Fadden et al. 2010). A similar observation is made in patients with solid tumours treated with several structurally unrelated Hsp90 inhibitors, thus rendering hsp90 inhibitors an unsuitable agent in monotherapy. However in certain tumour entities, single-agent inhibitor therapy was found to suppress tumour growth (Socinski et al. 2013). Likewise Hsp90 inhibitors in combination with cutting-edge targeted therapies is the pathway to move forward. It has been shown that a combination of the Hsp90 inhibitor SNX-5422 and trastuzumab (herceptin), a monoclonal antibody that blocks the Her-2 receptor, led to a synergistic regression of tumour growth in a xenograft model of human breast cancer (Fadden et al. 2010). As another example, a phase II trial, in which the combinatorial administration of 17-AAG (tanespimycin) plus trastuzumab displayed significant anti-cancer activity in patients with Her-2-positive metastatic breast cancer, which was previously seen to progress on trastuzumab (Modi et al. 2011). Thus the synergistic effects in tumour regression observed in

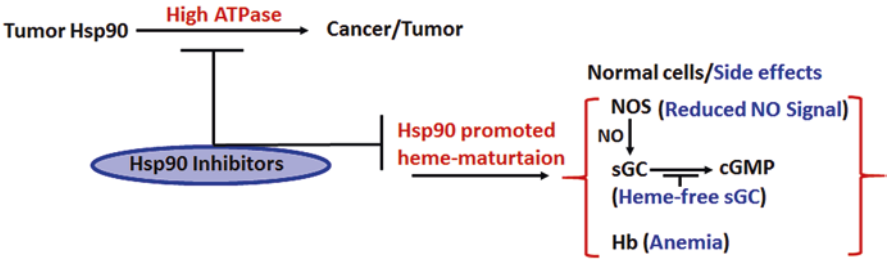

Fig. 12.6 Good effect of Hsp90 inhibitors on tumor cells while adverse effect on normal cells

animal studies after combinatorial administration of hsp90 inhibitors and potent anti-cancer drugs hold true in human trials, hence targeted therapies hold potential for future drug designing. Based on our current knowledge of hemeprotein maturation and the role played by Hsp90, use of Hsp90 inhibitors which target the ATPase function of the chaperon can also give rise to certain side effects (Fig. 12.6). These inhibitors can adversely effect normal cells by blocking the heme-maturation of iNOS, sGC or Hb (Fig. 12.6). For example, the Hsp90 inhibitors that are being developed for cancer treatment might unintentionally block Hb maturation in the recipient. Indeed, anemia has been commonly reported as a side effect during the clinical trials of Hsp90 inhibitor drug candidates (Pillai and Ramalingam 2012; Do et al. 2015). Likewise the Hsp90 inhibitors being used for asthma and IPF may also block heme-maturation of sGC, creating pathologic heme-free sGC and obstructing bronchodilation cascades. Hence widespread use of these Hsp90 inhibitors may require a more cautious approach or a combination therapy with sGC agonist like drugs may help in future therapeutics in such cases.

12.2 Conclusions

There are at least 13 Hsp90 inhibitors (Kim 2009) currently undergoing clincal trials from a broad range of tumors including breast, prostrate, gastrointestinal, melanoma, and hematological malignancies, and in vitro studies continue to identify newer small molecule compounds that selectively target Hsp90 chaperon in its various paralog forms (Taldone et al. 2014; Gomez-Monterrey et al. 2012). Thus this evergrowing progress of Hsp90 inhibitor drugs and their relevance to specific diseases holds great promise for further exciting developments in the future. Given the novel role of Hsp90 in hemeprotein maturation the effects of an overactive or a downregulated Hsp90 can both be deleterious to cellular homeostasis. This is now more evident from the fact that Hsp90 regulates heme maturation of three key hemeproteins (iNOS, sGC and Hb) and intertwines Hb maturation to the NO-sGC-cGMP dilation cascade. Together these realizations provide a platform to explore these concepts which may help in future drug designing.

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