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MINI REVIEW

The human HSP70 family of chaperones: where do we stand?

Jürgen Radons[1]

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Abstract The 70-kDa heat shock protein (HSP70) family of molecular chaperones represents one of the most ubiquitous classes of chaperones and is highly conserved in all organisms. Members of the HSP70 family control all aspects of cellular proteostasis such as nascent protein chain folding, protein import into organelles, recovering of proteins from aggregation, and assembly of multi-protein complexes. These chaperones augment organismal survival and longevity in the face of proteotoxic stress by enhancing cell viability and facilitating protein damage repair. Extracellular HSP70s have a number of cytoprotective and immunomodulatory functions, the latter either in the context of facilitating the cross-presentation of immunogenic peptides via major histocompatibility complex (MHC) antigens or in the context of acting as "chaperokines" or stimulators of innate immune responses. Studies have linked the expression of HSP70s to several types of carcinoma, with Hsp70 expression being associated with therapeutic resistance, metastasis, and poor clinical outcome. In malignantly transformed cells, HSP70s protect cells from the proteotoxic stress associated with abnormally rapid proliferation, suppress cellular senescence, and confer resistance to stress-induced apoptosis including protection against cytostatic drugs and radiation therapy. All of the cellular activities of HSP70s depend on their adenosine-5′-triphosphate (ATP)-regulated ability to interact with exposed hydrophobic surfaces of proteins. ATP hydrolysis and adenosine diphosphate (ADP)/ATP exchange are key events for substrate binding and Hsp70 release during folding of nascent polypeptides. Several proteins that bind to distinct subdomains of Hsp70 and consequently modulate the activity of the chaperone have been identified as HSP70 co-chaperones. This review focuses on the regulation, function, and relevance of the molecular Hsp70 chaperone machinery to disease and its potential as a therapeutic target.

Keywords Hsp70 · Structure · Regulation · Disease relevance · Inhibitors · Function · Therapeutic implications

✉ Jürgen Radons
j.radons@mail.de

[1] Scientific Consulting International, Mühldorfer Str. 64, 84503 Altötting, Germany

Abbreviations

17-AAG	17-Allylamino-17-demethoxy-geldanamycin
ADD70	AIF-derived decoy for HSP70
AIF	Apoptosis-inducing factor
APC	Antigen-presenting cell
BAG	Bcl-2-associated athanogene
CHIP	C-terminal Hsp70-interacting protein
CRLM	Colorectal liver metastasis
CSC	Cancer stem cell
DC	Dendritic cell
DSG	15-Deoxyspergualin
EGCG	(−)-Epigallocatechin-3-gallate
ERK	Extracellular signal-regulated kinase
FOLFOX	5-Fluorouracil, leucovorin, and oxaliplatin
GGA	Geranylgeranyl acetone
HCC	Hepatocellular carcinoma
HIF	Hypoxia-inducible factor
Hip	Hsp70 interacting protein
Hop	Hsp70/Hsp90 organizing protein
HSE	Heat shock element
HSF	Heat shock factor
HSP/Hsp	Heat shock protein
HSR	Heat shock response
JDP	J-domain protein

JNK	c-Jun N-terminal kinase
MB	Methylene blue
mHsp	Membrane-bound Hsp
MAPK	Mitogen-activated protein kinase
MDSC	Myeloid-derived suppressor cell
MMP	Matrix metalloproteinase
mTOR	Mammalian target of rapamycin
NBD	Nucleotide binding domain
NEF	Nucleotide exchange factor
NF-κB	Nuclear factor kappa B
PES	2-Phenylethynesulfonamide
NK	Natural killer
NSCLC	Nonsmall cell lung carcinoma
PI3K	Phosphatidylinositol-3 kinase
SBD	Substrate binding domain
SGC	Sulfogalactosylceramide
SIGLEC/ Siglec	Sialic acid-binding immunoglobulin-like lectin
STAT	Signal transducer and activator of transcription
TGF	Transforming growth factor
TNF	Tumor necrosis factor

Introduction

The 70-kDa heat shock protein (HSP70) family constitutes one of the most conserved protein families in evolution. HSP70s are monomeric proteins that reside in any adenosine-5′-triphosphate (ATP)-containing eukaryotic intracellular compartment and can also be found in cell membranes (Gehrmann et al. 2005; Hantschel et al. 2000; Multhoff et al. 1995; Multhoff and Hightower 1996) and the extracellular milieu (Pockley et al. 2014) as well as in bacteria and certain Archaea (Lindquist and Craig 1988). Although heat shock proteins (HSPs) were accidentally discovered as a set of genes whose expression is elevated by heat shock in *Drosophila melanogaster* in 1962 by the epoch-making work of Ritossa, the relevant gene products were only identified a decade later (Tissieres et al. 1974). The expression of HSPs can be induced by insults other than thermal stress, including ischemia, heavy metals, nutrient deprivation, irradiation, infections, inflammation, and exposure to organics and oxidants (Lindquist and Craig 1988). Stress-induced upregulation of HSP promotes cell survival in the face of endogenous or exogenous challenges that have the potential to induce cell damage and death. Table 1 provides information on the most common members of the human HSP70 family. HSP70-encoding genes leading to the expression of compartment-specific isoforms that fulfill organelle-specific functions are present in all eukaryotes, and multiple Hsp70 isoforms encoded by distinct genes are frequently co-expressed in the same cytosol. HSP70s are assisted by a broad panel of co-chaperones that constitute up to 3 % of the total protein mass of unstressed cells (Finka et al. 2015b).

Table 1 The human HSP70 family of chaperones

Protein	UniProt ID	Alternative names	Cellular localization	Length (aa)	Gene	Gene ID	Chromosome	Stress-inducible
HspA1A	P0DMV8	Hsp70-1, Hsp72, HspA1, Hsp70-1A, Hsp70i	Cytosol, nucleus, cell membrane, extracellular exosomes	641	*HSPA1A*	3303	6p21.3	Yes
HspA1B	P0DMV9	Hsp70-2, Hsp70-1B	Cytosol, nucleus, extracellular exosomes	641	*HSPA1B*	3304	6p21.3	Yes
HspA1L	P34931	Hsp70-1L, Hsp70-hom, Hsp70-1t, Hum70t	Cytosol, nucleus	641	*HSPA1L*	3305	6p21.3	No
HspA2	P54652	Heat shock 70kD protein 2, Hsp70.2	Cytosol, nucleus, cell membrane, extracellular exosomes	639	*HSPA2*	3306	14q24.1	No
HspA5	P11021	Hsp70-5, BiP, Grp78, Mif-2	ER, extracellular exosomes	654	*HSPA5*	3309	9q33.3	No
HspA6	P17066	Hsp70-6, Hsp70B'	Cytosol, extracellular exosomes	643	*HSPA6*	3310	1q23	Yes
HspA7	P48741	Hsp70-7, Hsp70B	Blood microparticles, extracellular exosomes	367	*HSPA7*	3311	1q23.3	Yes
HspA8	P11142	Hsp70-8, Hsc70, Hsc71, Hsp71, Hsp73	Cytosol, nucleus, cell membrane, extracellular exosomes	646	*HSPA8*	3312	11q24.1	No
HspA9	P38646	Hsp70-9, Grp75, HspA9B, MOT, MOT2, PBP74, mot-2, mtHsp70, mortalin	Mitochondria, nucleus	679	*HSPA9*	3313	5q31.1	No
HspA12A	O43301	Hsp70-12A, FLJ13874, KIAA0417	Intracellular, extracellular exosomes	675	*HSPA12A*	259217	10q26.12	No
HspA12B	B7ZLP2	Hsp70-12B, RP23-32L15.1, 2700081N06Rik	Endothelial cells, intracellular, blood plasma	685	*HSPA12B*	116835	20p13	No
HspA13	P48723	Hsp70-13, Stch	ER, extracellular exosomes, microsomes	471	*HSPA13*	6782	21q11	No
HspA14	Q0VDF9	Hsp70-14, Hsp70L1	Cytosol, membrane	509	*HSPA14*	51182	10p13	Yes

These co-chaperones are crucial, as they trigger the binding of clients to HSP70s by affecting the interplay between HSP70s and nucleotides. Hsp70-1 is among the first and most prominent molecule found in stressed cells. Compared to normal cells, tumors frequently express elevated basal Hsp70-1 levels that are further enhanced in response to a number of pathological and environmental stresses. To fulfill the various cellular housekeeping and stress-induced functions, HSP70s function as ATP-driven unfolding machines that are capable of shifting substrate polypeptides between various folding states. Upon exposure to different stressors, HSP70s are able to bind to misfolded proteins and prevent their aggregation. Consistent with these properties, several investigations have implicated HSP70s in a wide spectrum of disease and nondisease conditions (Ciocca et al. 2013; Gunther et al. 2015; Hom et al. 2012; Molvarec et al. 2010; Pockley et al. 2014; Qu et al. 2015). Cancer cells rely especially heavily on the Hsp70-based buffering system for survival. Recently, increasing attention has been devoted to the role of HSP70s in immune events. The expressions of a number of putative HSP70 receptors including scavenger receptors (e.g., SREC-1, LOX-1, FEEL-1), the collagen/thrombospondin receptor CD35, and the C-type lectin receptor CD91 (α_2-macroglobulin receptor) have been identified on a range of cell types, some of which (e.g., CD14, CD40, and Toll-like receptor (TLR)-2/TLR-4) have been suggested to facilitate the uptake of exogenous HSP70s and modulate the phenotype and function of antigen-presenting cells (APCs) and T cell sub-populations, as well as the nature and potency of innate and adaptive immune responses (reviewed by Binder et al. 2004; Theriault et al. 2006). However, the function of CD14, CD40, and TLRs as HSP70 receptors should be perceived with great care because of reports suggesting that the pro-inflammatory actions of HSP70s might rely on the binding of LPS which also interacts with these receptors (Wallin et al. 2002), even though much of the evidence argues against this concept (Henderson et al. 2010; Henderson and Pockley 2010; Vabulas and Wagner 2005). Given the importance of these proteins in the maintenance of cellular homeostasis and, more broadly, in the coordination and orchestration of a range of biological and immunological events, insights into the physiological and pathophysiological role of HSP70s are essential, as they will facilitate the development of novel approaches for combating cancer and other human diseases. In this respect, the therapeutic potential of targeting HSP70 and modulating HSP70 expression is attracting much interest.

The human HSP70 family

Intracellular HSP70s

The human HSP70 family comprises 13 gene products that differ from each other by expression level, subcellular location, and amino acid constitution (Table 1 and Fig. 1). They are encoded by a multigene family encompassing up to 17 genes and 30 pseudogenes (Brocchieri et al. 2008). Functional genes encoding human HSP70 proteins map to several chromosomes, as summarized in Table 1 (Harrison et al. 1987). The major stress-inducible HSP70s comprise Hsp70-1 (HspA1A) and Hsp70-2 (HspA1B), collectively termed Hsp70 or Hsp70-1, and these only differ from each other by two amino acids. The basal mRNA expression of *HSPA1A/B* varies in most tissues and exceeds the expression levels of the other Hsp70 isoforms in humans (Daugaard et al. 2007b). Hsp70-1t (HspA1L) and Hsp70.2 (HspA2) represent two cytosolic family members with high abundance in testis. Hsp70-1t is a constitutively expressed, noninducible chaperone that exhibits an ~90 % identity to Hsp70-1. Hsp70.2 is highly expressed not only in testis but also in brain and exhibits ~85 % homology to Hsp70-1. Hsp70.2 has been suggested to be crucially involved in spermatogenesis and meiosis (Zhu et al. 1997). Hsp70-5 (HspA5, BiP, Grp78) is constitutively expressed in the endoplasmic reticulum (ER) facilitating transport and folding of nascent polypeptides into the ER lumen. Hsp70-6 (HspA6, Hsp70B′) is an additional stress-inducible HSP70 family member that is highly homologous to Hsp70-1 and has no detectable basal expression level in most cells (Leung et al. 1990). *HSPA7* has long been considered as being a pseudogene that is transcribed in response to stress, with recent observations suggesting a high homology to *HSPA6* (Brocchieri et al. 2008). The human *HSPA7* promoter is an efficient and tightly regulated promoter that can be induced by cellular nutrient stresses (Siddiqui et al. 2008). Hsp70-8 (HspA8, Hsc70, Hsp73) is the cognate HSP70 family member that exhibits essential housekeeping functions such as folding and transport of polypeptides across intracellular membranes and ~86 % identity to Hsp70-1 (Dworniczak and Mirault 1987). Hsp70-9 (HspA9) is located in mitochondria and shows a 52 % identity to stress-inducible Hsp70-1 but is not induced upon stress. Hsp70-9 (HspA9) bears a 46 amino acid target signal that is responsible for delivering Hsp70-9 to the mitochondrial lumen (Mizzen et al. 1989). Less information is available for Hsp70-12A (HspA12A) and Hsp70-12B (HspA12B) that were originally found in atherosclerotic lesions of mice (Han et al. 2003). They represent distant members of the Hsp70 family due to the presence of an atypical ATP-binding domain (Han et al. 2003). Human Hsp70-12A is widely expressed, with the highest levels being found in brain, kidney, and muscle. In contrast, high levels of human Hsp70-12B expression have been reported in muscle, heart, and liver, with lower levels being expressed in kidney (Han et al. 2003). Hsp70-12B is specifically expressed in endothelial cells, thereby suggesting its contribution to the pathogenesis of endothelium-associated processes (Hu et al. 2006). Hsp70-13 (HspA13, Stch) represents the microsome-associated member of the HSP70 family and is constitutively expressed

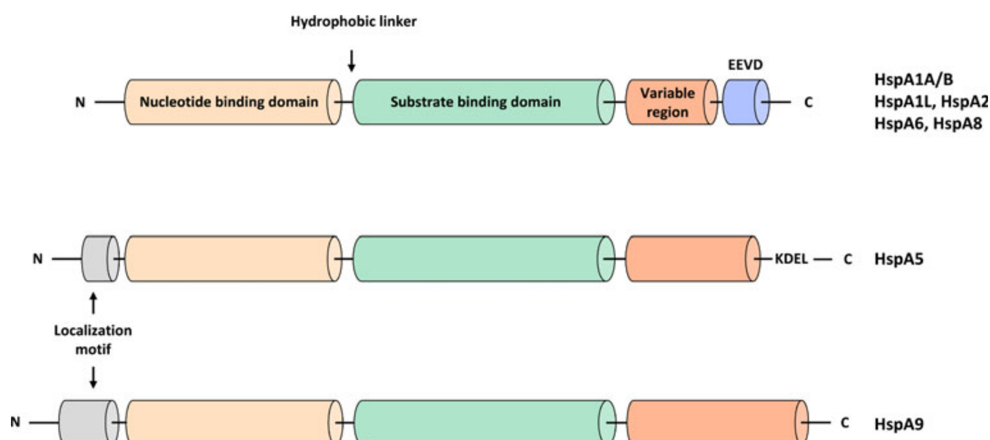

Fig. 1 The human HSP70 family. *Top*: Schematic representation of the common domain structure of selected HSP70 chaperones. *Bottom*: Amino acid sequence alignment of the human HSP70 family using Clustal Omega 1.2.1 (https://www.ebi.ac.uk/Tools/msa/clustalo/). Each protein is identified by its UniProtKB ID. *Asterisks* indicate identical amino acids; *dots* indicate similar amino acids. Conserved residues are marked by an *arrow*. The conserved nucleotide binding domain (NBD) is highlighted in *gray* with the exception of HspA7 where the predicted NBD (aa 8-341) obviously spans most of the polypeptide chain. The *black box* in HspA5 (aa 1-18) indicates the presumed N-terminal ER localization signal, in HspA9 (aa 1-46) it indicates the N-terminal transit peptide. The C-terminal ER retention signal (KDEL) in HspA5 is also marked accordingly. The C-terminal EEVD motif involved in binding of co-chaperones and other HSPs is highlighted in *gray*. The hydrophobic linker (DL/VLLLD) connecting the NBD and SBD is found in most of the HSP70 family members. *M* methylation site

in all human cell types (Otterson et al. 1994). Hsp70-14 (HspA14, Hsp70L1) is derived from human dendritic cells (DCs), with potent adjuvant effects that polarize responses towards Th1 (Wan et al. 2004). Hsp70-14 expression can be induced by overexpression of the DNA repair protein nibrin (Nbs-1, p95), thereby contributing to nibrin-mediated metastatic and transformation activity (Wu et al. 2011). An upregulated expression of Hsp70-14 has recently been reported in tumor tissues of patients with HBV-related early-stage hepatocellular carcinoma (HCC, Yang et al. 2015).

Cell surface and extracellular HSP70s

Various HSP70s are selectively expressed on the cell surface of virally or bacterially infected cells or on tumor cells and can be found in the extracellular milieu of normal and diseased individuals (Hantschel et al. 2000; Multhoff et al. 1995; Multhoff and Hightower 1996; Pockley et al. 1998, 2014). Extracellular HSP70s exist in a free soluble form, complexed to antigenic peptides, or associated with exosomes (Bausero et al. 2005; Gastpar et al. 2005; Lancaster and Febbraio 2005; Vega et al. 2008; see also Table 1). Although the first report of an ER/Golgi-independent release of Hsp70-1 from viable cells with intact cell membranes was made in the late 1980s by Hightower and Guidon (1989), the molecular mechanisms underlying HSP release continue to be a matter of debate given that cytosolic HSPs lack a consensus signal for secretion. Different mechanisms have been proposed, including exosomal release (Bausero et al. 2004; Gastpar et al. 2005; Lancaster and Febbraio 2005), export via cholesterol-rich microdomains (Broquet et al. 2003), secretory endolysosomes (Mambula and Calderwood 2006; Nylandsted et al. 2004), and secretory-like granules (Evdonin et al. 2006), as well as ubiquitination-triggered transports (Evdonin et al. 2004) and passive release after cell death by necrosis (Basu et al. 2000). In this context, Hsp70-1 although lacking a transmembrane domain has been found to locate to the cell surface of tumor cells but not on normal cells using the monoclonal antibody cmHsp70.1 (Gehrmann et al. 2005; Hantschel et al. 2000; Multhoff et al. 1995; Multhoff and Hightower 1996). Quantitative analysis revealed that approximately 15–20 % of the total Hsp70-1 is present in the plasma membrane of Hsp70-1-positive tumor cells (Gehrmann et al. 2008a). Membrane Hsp70-1 (mHsp70-1) might help to maintain stability of tumor cells and thus protect tumors from lethal damage induced by environmental stressors (Horvath et al. 2008). The demonstration that HSP70 family members interact with artificial membranes containing phosphatidylserine (PS; Arispe and De Maio 2000) and that Hsp70-1 is an integral membrane protein associated with raft (glycosphingolipid Gb3) and nonraft (PS) lipid components of tumor cells (Gehrmann et al. 2008a; Vega et al. 2008) suggest that the selective expression of this unique membrane form of HSP70 by tumor cells might be facilitated by changes in membrane lipid composition. Another interesting and potentially important finding has been that Hsp70-1 associates predominantly with PS outside of lipid rafts in the plasma membrane of tumor cells following irradiation or hypoxia-induced stress (Schilling et al. 2009). These data indicate the potency of environmental stressors in re-organizing the lipid bilayer and modulating the interplay between Hsp70-1 and lipid components. It should also be noted that radio(chemo)therapy enhances cell surface expression of Hsp70-1 on tumor cells (Gehrmann et al. 2005; Kleinjung et al. 2003), thus rendering

Fig. 1 (continued)

integral mHsp70-1 a tumor-specific recognition structure for therapeutic interventions. However, care should be taken when describing the presence of this membrane form of Hsp70 on viable tumor cells using commercially available monoclonal antibodies (the presence of membrane Hsp70 is detectable using the cmHsp70.1 monoclonal antibody), as these do not recognize the membrane form and identify extracellular Hsp70 bound to the plasma membrane surface (Stangl et al. 2011).

Structure and function

HSP70s are highly conserved molecules and display a common domain structure (Fig. 1) composed of (i) a 44-kDa N-terminal nucleotide binding domain (NBD) that binds and hydrolyzes ATP, (ii) a middle domain with protease sensitive sites, and (iii) a 28-kDa C-terminal substrate binding domain (SBD) that binds extended polypeptides (Flaherty et al. 1990). The NBD is conserved in all of the HSP70 family members, with the exception of the two *HSPA12* genes encoding a more divergent NBD. The NBD is composed of four subdomains (IA, IB, IIA, IIB) surrounding the ATP-binding pocket (Flaherty et al. 1990). Herein, Asp10 and Glu175 within subdomain IA, Lys71 of subdomain IB as well as Asp199 and Thr204 within subdomain IIA are highly conserved, acting as interaction sites for adenosine diphosphate (ADP; Arakawa et al. 2011). The SBD is subdivided into an N-terminal β-sheet (SBDβ) and a C-terminal α-helical subdomain (SBDα) acting as a flexible lid (Zhu et al. 1996). Eukaryotic cytosolic HSP70s also contain a G/P-rich C-terminal region harboring an EEVD motif involved in binding of co-chaperones and other HSPs (Hartl 1996). The EEVD motif is absent from specialized HSP70s such as the ER-resident Hsp70-5 or mitochondrial Hsp70-9, both bearing an N-terminal localization signal. Additionally, Hsp70-5 contains the C-terminal ER retention signal KDEL (Munro and Pelham 1987).

The functions of the different HSP70 family members depend on their cellular localization. Under physiological conditions, HSP70 family members act as molecular chaperones. Intracellular residing HSP70s protect cells against lethal damage induced by stress and support folding and transport of newly synthesized polypeptides and aberrant proteins as well as the assembly of multi-protein complexes (Hartl 1996). HSP70s exhibit an "unfoldase" activity that enables the recognition of stable misfolded or aggregated proteins followed by their unfolding and spontaneous refolding to natively refoldable species (Sharma et al. 2010). Stable misfolded polypeptides are recognized by Hsp70 in conjunction with dimeric Hsp40 and nucleotide exchange factors (NEFs) that convert the misfolded proteins into native proteins by repeated cycles of binding, ATP-dependent unfolding, and spontaneous refolding. Failure in unfolding/refolding might lead to binding of the misfolded substrate to "holdases," including Hsp90 and small HSPs, that keep the substrate in a nonaggregated folding-competent state and pass it to the Hsp70 unfoldase machinery for refolding. Alternatively, aggregated proteins can be converted to natively unfolded substrates by heterodimeric Hsp70/Hsp110 (reviewed by Finka et al. 2015a; Mattoo and Goloubinoff 2014). Hsp70/Hsp110 heterodimers form NEFs by acting reciprocally on each other and, cooperatively, they efficiently disassemble stable protein aggregates (Mattoo et al. 2013; Schuermann et al. 2008).

HSP70s are powerful anti-apoptotic proteins and block apoptosis at a number of different levels. On the one hand, Hsp70 blocks mitochondrial translocation and activation of Bax, thereby preventing mitochondrial membrane permeabilization and release of pro-apoptotic factors (Stankiewicz et al. 2005; Yang et al. 2012), and on the other inhibits assembly of the death-inducing signaling complex (DISC; Guo et al. 2005b). Hsp70-1 also binds directly to apoptosis protease-activating factor 1 (Apaf-1) and blocks the recruitment of pro-caspase-9 to the mitochondrial apoptosome (Beere et al. 2000). Hsp70-1 also interacts with the mitochondrial intermembranal flavoprotein apoptosis-inducing factor (AIF), thereby avoiding caspase-independent chromatin condensation and apoptosis (Ravagnan et al. 2001). Hsp70-1 also inhibits caspase-3 and regulates c-Jun N-terminal kinase (JNK), p38 mitogen-activated protein kinase (MAPK), and extracellular signal-regulated kinase (ERK) in the apoptotic pathway (Gabai et al. 1997; Lee et al. 2005). In conjunction with the E3 ubiquitin ligase C-terminal Hsp70-interacting protein (CHIP), Hsp70-1 promotes proteasomal degradation of the mammalian apoptosis signal-regulated kinase 1 (Ask-1) and prevents apoptosis by forming a ternary complex with CHIP and Ask-1 (Gao et al. 2010). Interestingly, CHIP can downregulate the receptor tyrosine kinase c-Met that is critically involved in epithelial to mesenchymal transition (EMT), a crucial process in tumor invasiveness and metastasis (Jang et al. 2011). Hsp70-1 itself plays a central role in EMT by attenuating phosphorylation of the intracellular transforming growth factor (TGF)-β mediator Smad-2 (Li et al. 2011). In the nucleus, Hsp70-1 facilitates the ssDNA breaks repairing machines by binding to poly(ADP-ribose) polymerase 1 (PARP-1), thus mediating their assembly and stimulating their function (Kotoglou et al. 2009).

In the context of malignantly transformed cells, HSPs enhance cell growth, suppress senescence, and confer resistance to stress-induced apoptosis, cytostatic drugs, and radiation therapy (Gehrmann et al. 2008b). Senescence is a state in which cells stop dividing and has been considered a weapon for cancer treatment. Downregulation of Hsp70-1 triggers cell senescence via p53-dependent and p53-independent pathways (Yaglom et al. 2007). HSP70s have the capacity to stabilize lysosomal membranes and influence autophagy, thereby

promoting cancer cell survival (Daugaard et al. 2007a; Leu et al. 2009; Nylandsted et al. 2004). Evidence is accumulating for the crucial contribution of Hsp70 to anoikis and amorphosis, apoptotic processes that are triggered when cells lose contact with their extracellular matrix during cancer metastasis. In this regard, Hsp70-1 affects activity of focal adhesion kinase (Fak; Mao et al. 2003), c-Met (Jang et al. 2011), and the major survival kinase AKT (Koren et al. 2010). Hsp70 stabilizes the metastasis-promoting protein Wiskott-Aldrich syndrome protein family member 3 (Wasf-3) that is upregulated in high-grade tumors (Teng et al. 2012). Hsp70 is also involved in the modulation of the extracellular matrix, since the Hsp70 co-chaperone Bag-3 has been identified to interact with matrix metalloproteinase (MMP)-2 (Suzuki et al. 2011). MMP-2 can be activated directly by Hsp70 complexed to Hsp90 and Hsp70/Hsp90 organizing protein (Hop; Walsh et al. 2011). The Hsp70/Bag-3 complex modulates the activity of the transcription factors nuclear factor kappa B (NF-κB), FoxM1, HIF-1α, the translation regulator HuR, and the cell cycle regulators p21 and survivin (Colvin et al. 2014). Importantly, extracellular Hsp70-1 increases proMMP-9 secretion and activates MMP-9 expression via NF-κB and AP-1 (Lee et al. 2006), highlighting the crucial role of Hsp70 in tumor invasion and metastasis.

Immunomodulatory role of Hsp70

While intracellular HSP70s play a key role in proteomic homeostasis, extracellular HSP70s are considered as molecules with immunomodulatory functions (Henderson and Pockley 2010; Multhoff et al. 2012, 2015; Pockley et al. 2008), either as cross-presenters of immunogenic peptides via MHC antigens (Asea et al. 2000), as chaperokines that can stimulate innate and adaptive immunity (Asea et al. 2002), or as stimulators and targets for innate immune responses mediated by natural killer (NK) cells (Henderson and Pockley 2010; Kronenberg 2005; Moretta et al. 2005; Multhoff et al. 1999, 2012, 2015; Pockley et al. 2008; Smyth et al. 2001; Specht et al. 2015). Much work has been done in recent years to analyze the dual immunoregulatory role of endogenous and exogenous HSP family members (Borges et al. 2012; Galazka et al. 2014; Multhoff et al. 2012, 2015; Multhoff and Radons 2012; Pockley et al. 2008; Stocki et al. 2012). Briefly, HSP70s can act as stimulators of the adaptive immune response through their ability to bind antigenic peptides during intracellular antigen processing (Srivastava 2005). After their release from tumor cells into the extracellular compartment, HSP/peptide complexes bind to surface receptors on APCs followed by cross-presentation to $CD8^+$ cytotoxic T lymphocytes on MHC class I molecules eliciting specific tumor cell killing (Arnold-Schild et al. 1999; Singh-Jasuja et al. 2000). Cross-presented antigens can also be recognized by $CD4^+$ T lymphocytes that play a crucial role in modulating immune responses. HSPs carrying tumor antigen can further bind and activate a second receptor that induces an innate immune response that facilitates APC maturation, pro-inflammatory cytokine release, and expression of costimulatory molecules such as CD80 and CD86 interacting with their corresponding receptors (e.g., CD28) on T lymphocytes (Pockley et al. 2008). Due to their capacity to stimulate pro-inflammatory cytokine production, extracellular residing Hsp70 may act as a danger signal to the innate immune system and may also be relevant for the establishment of cancerous and autoimmune diseases (Asea et al. 2000; Paduch et al. 2009; Tsan and Gao 2004). HSP/peptide complexes from normal cells are internalized by either nonspecific or receptor-mediated endocytosis followed by lysosomal degradation and subsequent presentation to $CD4^+$ T cells through MHC class II molecules irrespective of residing costimulatory molecules. As a consequence, a T cell-mediated immune response occurs accompanied by the release of immunosuppressive Th2 cytokines. HSPs from pathogens harbor nonself epitopes that are presented to pro-inflammatory $CD4^+$ Th1 cells by APCs culminating in the release of pro-inflammatory cytokines such as IFN-γ and the induction of an inflammatory T cell response. Like HSP-chaperoned tumor antigens, APC maturation via a second receptor precedes antigen presentation.

Apart from its capacity to induce pro-inflammatory signaling, soluble HSP70s also exert anti-inflammatory properties (reviewed by Borges et al. 2012). In this regard, HSP70s are able to modulate cytokine production of DCs thereby providing a link between innate and adaptive immune responses (Heath and Carbone 2009). Remarkably, soluble inducible Hsp70-1A has been noted to induce a tolerogenic phenotype in monocyte-derived DCs (Stocki et al. 2012), underlining an important implication for a regulatory role of soluble forms of Hsp70. Tolerogenic DCs contribute to the formation of a "suppressive environment" mediating the peripheral generation of regulatory T (Treg) cells. Treg cells are crucially involved in suppressing the effector immune response that is detrimental to the host (Sakaguchi et al. 2008) and express a variety of effector molecules mediating the suppression of target cells, including anti-inflammatory cytokines like TGF-β, IL-10, and IL-35 (Collison et al. 2007). Hsp70-1A enhances the immunosuppressive activity of $CD4^+CD25^+FoxP3^+$ Treg cells via the PI3K/AKT, JNK, and p38 MAPK pathways, thereby highlighting its role in maintaining immune homeostasis (Wachstein et al. 2012). More intriguingly, extracellular Hsp70 has been identified as a ligand of the paired immunoreceptors sialic acid-binding immunoglobulin-like lectin (Siglec)-5 and Siglec-14 (Fong et al. 2015). SIGLECs block inflammatory processes by interacting directly with TLRs (Chen et al. 2014). In this context, a highly original series of experiments performed by Fong et al. have revealed an inflammatory balancing process mediated by

extracellular HSP70 and the identification of the Janus-like properties of these SIGLEC family members. Although HSP70 binding to Siglec-14 was shown to transmit a pro-inflammatory signal by boosting TLR signaling, stimulation via Siglec-5 exerts anti-inflammatory effects by blocking inflammatory TLR signaling. The discovery of Siglec-5 and Siglec-14 as HSP70 receptors is thus highly meaningful and may clarify previous contradictory findings on the immunomodulatory function of HSP70s. These intriguing novel findings are likely to provide an important insight into the apparently conflicting influences of HSP70s in immunity.

The Hsp70 chaperone cycle

Based on current knowledge, the following reaction cycle of HSP70 family members is proposed (Fig. 2). Central to the chaperone function of HSP70s is the transition between open and closed conformations of the SBD. In the ATP-bound open conformation, the SBDα and SBDβ are detached from each other and docked to different faces of the NBD, giving a low affinity and fast exchange rates for the substrate (Kityk et al. 2012). Sequential docking of the SBD subdomains to the NBD is influenced by various client molecules. In the ADP/peptide state, SBD plus linker collides with and samples the surface areas of subdomains IA and IIA of the NBD so that the IA/IIA cleft is closed (Bertelsen et al. 2009). ATP binding to the NBD modifies both substrate affinity and substrate binding to the SBD and stimulates ATP hydrolysis (Zhuravleva et al. 2012). Substrate-induced ATPase stimulation then leads to SBD release, enabling closure of the lid subdomain and embedding of the substrate (Bertelsen et al. 2009). ATP hydrolysis is facilitated by J-domain proteins (JDPs) of the HSP40 family (Craig et al. 2006) and ATP/ADP exchange by NEFs including Bcl-2-associated athanogene (BAG) domain proteins and Hsp70 binding protein 1 (HspBP1) interacting with the NBD of HSP70s. Bag-1 is the best studied member of the BAG family of proteins, having six homologs in humans that are characterized by the presence of the C-terminal Hsp70-binding BAG domain (Takayama et al. 1999). HspBP1 promotes nucleotide exchange in a manner quite different from Bag-1 and is required for efficient HSP70-mediated protein folding in the cytosol (Shomura et al. 2005). These co-chaperones function as both positive and negative regulators of HSP70 chaperone activity (Tzankov et al. 2008).

HSP40s/JDPs represent a heterogeneous group of co-chaperones bearing the remarkably conserved J-domain and are responsible for the stimulation of the ATPase activity of HSP70s and locking the client into the closed SBD (Ahmad et al. 2011; Fig. 2). HSP40s are homodimeric proteins that bind to unfolded or nonnative polypeptides via their C-terminal SBD in order to prevent aggregation. HSP40s represent intermediate molecules that complex with Hsp70 interacting

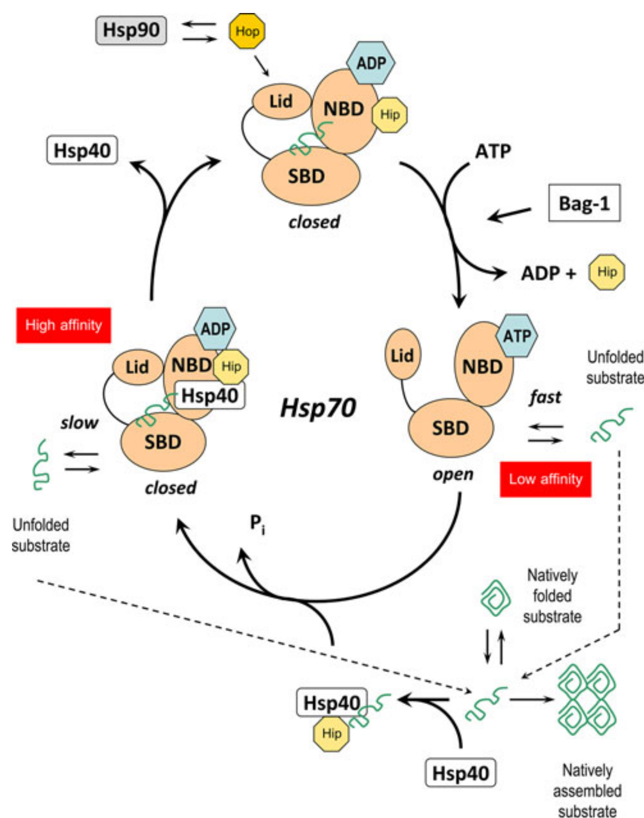

Fig. 2 Proposed model of the HSP70 chaperone reaction cycle. In the ATP-bound state, the nucleotide binding domain (NBD) and substrate binding domain (SBD) are docked, the substrate binding groove is open, and Hsp70 exhibits low affinity and fast exchange rates for its substrate. ATP hydrolysis is facilitated by J-domain proteins (JDPs) of the HSP40/DNAJ family and nucleotide exchange by nucleotide exchange factors (NEFs) such as Bag-1. Homodimeric HSP40s bind to unfolded or non-native polypeptides via their C-terminal SBD in order to prevent aggregation. After delivery of the Hsp40-bound substrate to Hsp70, Hsp40 interacts with the N-terminal ATPase domain of Hsp70 thereby inducing a conformational change which stimulates ATP hydrolysis and mediates closing of the substrate binding groove. In the ADP-bound state, the docking between the two domains of Hsp70 is interrupted and the chaperone exhibits high affinity and slow exchange rates for its substrate. Coupling to other chaperones is mediated by Hop (Sti-1) which binds to the C-terminal domain of Hsp70 and Hsp90 thereby passing clients to Hsp90. ADP release is mediated by the specific interaction of NEFs with the Hsp70 ATPase domain followed by a conformational change culminating in a low-affinity state and release of the substrate. The released substrate can be either folded into the native protein, rebound to Hsp70, or natively assembled into oligomers

protein (Hip) and transfer the unfolded protein to HSP70s via the interaction of Hsp40 with the N-terminal ATPase domain of Hsp70 (Greene et al. 1998). Hip binds to the NBD of Hsp70 and prevents ADP dissociation from Hsp70 (Höhfeld et al. 1995). Hip contributes to the interaction of Hsp70 with various target proteins via its own chaperone activity (Höhfeld et al. 1995). Substrate binding in synergy with the actions of JDPs stimulates ATP hydrolysis and concomitant closure of the substrate binding groove. Recruitment and transfer of substrate protein to Hsp70 also occur through interactions with

the SBD of Hsp70. In this respect, HSP40s associate with the C-terminal octapeptide GPTIEEVD of human Hsp70-1 via a C-terminal peptide-binding domain (Suzuki et al. 2010). Interestingly, some HSP40s target HSP70 activity to clients at precise locations in cells, and others bind client proteins directly, thereby delivering specific clients to HSP70 and directly determining their fate (Kampinga and Craig 2010).

In the ADP-bound state, the docking between the two domains of Hsp70 is interrupted and the chaperone exhibits high affinity and low exchange rates for its substrate stabilized by Hip. ADP release is mediated by the specific interaction of NEFs with the Hsp70-ATPase domain followed by a conformational change resulting in a low-affinity state and release of the substrate. The NEF-mediated ADP/ATP exchange is a prerequisite for re-opening of the lid and release of the substrate (Harrison et al. 1997). The released substrate can be either folded into the native protein, rebound to Hsp70, or natively assembled into oligomers. Cycling between ATP-bound and ADP/substrate-bound states requires HSP70s to visit a state with high ATPase activity and fast on/off kinetics of substrate binding. In the ATP state, the NBD surface groove opens up, thereby enabling productive NBD-SBD collisions that place the linker in the groove and dock the SBD on the IA area (Bertelsen et al. 2009).

The activity of the Hsp70 chaperone is regulated by various co-chaperones of the tetratricopeptide repeat (TPR) protein family, including Hip. Although Hip binds to the NBD of HSP70s, other members such as CHIP and Hop interact with the C-terminal domain of HSP70s and HSP90s (Connell et al. 2001; Hernandez et al. 2002). CHIP inhibits the ATPase cycle of cytosolic Hsp70 by blocking HSP70-mediated protein folding and facilitating ubiquitination and clearance of other client proteins (Ballinger et al. 1999; Connell et al. 2001). Hop, also known as stress-inducible protein 1 (Sti-1), stabilizes client proteins and mediates their transfer to Hsp90 in a coordinated folding process (Hernandez et al. 2002).

Regulation

Cells respond to environmental stress by enhancing their expression of HSPs. The rapid induction of HSPs in response to environmental stress is based on a variety of genetic and biochemical processes that are referred to as the heat shock response (HSR; Shamovsky and Nudler 2008). The HSR is an ancient and sophisticated cytoprotective mechanism to augment organismal survival and longevity in the face of proteotoxic stress from without and within (Craig 1985). The HSR is highly significant in human pathology, as HSP levels increase in cancer and promote tumorigenesis, and are seen to decline in protein aggregation disorders such as Alzheimer's disease (Calderwood and Gong 2012; Hayashida et al. 2010). The expression of five members of the HSP70 family is induced by stress (Table 1). HSR is regulated mainly at the transcription level by heat shock factors (HSF). Among them, HSF-1 is considered as being the key transcription factor of stress-inducible HSPs (Pirkkala et al. 2001). Under nonstress conditions, HSF-1 exists as an inactive monomer in the cytoplasm in association with Hsp70 and Hsp90 (Akerfelt et al. 2010). In response to stress, Hsp70 and Hsp90 proteins are released followed by the formation of HSF-1 homotrimers that are capable of binding to heat shock elements (HSEs) upstream of *HSP* promoters, thereby triggering *HSPA* transcription (Mason and Lis 1997; Westwood et al. 1991). The mammalian target of rapamycin kinase (mTOR) plays a key role in response to proteotoxic stress due to its capacity to directly phosphorylate, and thus activate, HSF-1 (Chou et al. 2012). Proteotoxic stress often results from the mTOR-mediated overproduction of cellular components contributing to several processes that might become pathological such as cellular senescence, adipogenesis, and glucose homeostasis. Hence, the mTOR signaling pathway is implicated in an increasing number of pathological conditions, including cancer, obesity, type 2 diabetes, and neurodegeneration (Laplante and Sabatini 2012).

As already outlined, constitutively high levels of Hsp70-1 are frequently observed in cancer cells, in which the chaperone confers resistance to stress-induced apoptosis, serves in suppressing default senescence, and is associated with the development of metastasis and drug resistance. In tumors, Hsp70-1 may also be expressed irrespective of HSF-1 transcriptional activity (Wigmore et al. 2007). Possible candidates for Hsp70 synthesis in the absence of stress include the transcription factors signal transducer and activator of transcription (STAT)-1/3, NF-IL6, and C/EBP. STAT-3 binds to *HSPA1* promoter sequences that are different to those that are used by HSEs and competes with HSF-1 for Hsp70-1 expression on stress (Stephanou and Latchman 2005). In contrast, STAT-1 recognizes interferon-γ-activated sequences without competing with HSF-1 for transcription (Stephanou et al. 1999). STAT-responsive elements are also recognized by NF-IL6 and C/EBP that cooperate with HSF-1 for inducing Hsp70-1 (Stephanou and Latchman 2005). These molecules can also bind to *HSPA1* proximal promoter regions together with STATs, thus upregulating *HSPA1* transcription regardless of stress (Akira et al. 1992; Kinoshita et al. 1992). The p53 family member ΔNp63α (Wu et al. 2005), the deacetylase and longevity factor sirtuin-1 (Westerheide et al. 2009), and the mTOR complex 1 (mTORC-1; Chou et al. 2012) have been identified as putative cancer-related HSF-1 regulators in tumors, contributing to Hsp70-1 overexpression by stimulating HSF-1 expression and/or activity. Several inflammatory mediators and signaling molecules such as NF-κB and TNF are strictly linked to HSP gene expression and protein functions. In this context, the NF-κB subunit p65/RelA functions as a transcription factor for numerous HSPs, including Hsp70-1

that in turn may have anti-apoptotic functions in cancer cells (Guzhova et al. 1997; Rappa et al. 2012).

Apart from its transcriptional regulation, HSP70 protein levels have also been found to be regulated at the post-transcriptional level by micro-RNAs (miRNAs) that are critically involved in transformation, differentiation, and proliferation. Although global alterations in miRNAs can be observed in a number of disease states including cancer (Tu et al. 2015), little information on the role of miRNAs in the regulation of the *HSPA* expression is available. In mouse cardiac tissues, miR-1, miR-21, and miR-24 have been found to upregulate HSF-1 and Hsp70-1 after exposure to ischemia (Yin et al. 2009). In contrast, miR-1432-3p has been identified as being a negative regulator of *HSPA1B*, irrespective of HSF-1 in pancreatic ductal adenocarcinoma cells (MacKenzie et al. 2013). Brain-enriched miR-181a specifically regulates *HSPA5* expression and outcome from cerebral ischemia, whereas miR-181 potentially targets *HSPA1A*, *HSPA5*, and *HSPA9* (Ouyang and Giffard 2013). Ji et al. (2014) have reported altered levels of miR-15a and *HSPA1B* in the spermatozoa of varicocele patients and described miR-15a-mediated stress regulation of *HSPA1B* in sperm. These findings contribute to understanding the molecular mechanisms involved in varicocele-related sperm damage. Chi et al. (2014) have validated *HSPA12B* as being a target of miR-134 and provided evidence for the neuroprotective potential of downregulated miR-134 by modulating Hsp70-12B protein expression. More recently, miR-16-1 (Zhang and Cheng 2014), miR-133b-5p (Xia et al. 2015), and miR-215 (Fesler et al. 2015) have been characterized as regulators of HSP70 expression. Of note, cells overexpressing Hsp70-1 had higher levels of miR-23a and its loss was less severe in heat-stressed cells, thereby implying that upregulation of miR-23a in Hsp70-1-expressing cells might result from changes in the turnover of miRNA at different levels (Roufayel et al. 2014). However, future approaches analyzing the regulatory potential of miRNAs in *HSPA* expression will shed light on the post-transcriptional regulation of these chaperones.

Little is known about the post-translational processing of HSP70s. Although some of the amino acid residues are phosphorylated, the effect of these on Hsp70 function remains unclear. Phosphorylation of Ser400 in Hsp70-1 is critical for the nuclear location of the chaperone (Frisan et al. 2012). A constitutively phosphorylated Hsp70-1 mutant is able to restore the cytosolic-nuclear shuttling capacity of HSP70s, thereby protecting differentiating erythroblasts from apoptosis in patients with myelodysplastic syndromes in which the nucleocytosolic trafficking of Hsp70-1 is blocked (Frisan et al. 2012). Further post-translational modifications of HSP70s involve acetylation (Hsp70-1/2, Hsp70-8, Hsp70-9, Hsp70-12A), malonylation (Hsp70-9), methylation (Hsp70-1/2, Hsp70.2, Hsp70-5, Hsp70-6, Hsp70-8), and conjugation with the ubiquitin-like interferon-inducible protein ISG15 (Hsp70-8), respectively. Since proteins that can be ISGylated play important roles in translation, glycolysis, stress responses, and cell motility, it can be speculated that ISGylation of HSP70s targets these chaperones in several fundamentally important cellular pathways, thereby providing insight into the physiological role of interferon-induced ISG15 and ISG15 conjugation (Giannakopoulos et al. 2005). These data, together with previous studies revealing an anti-viral function of ISG15 (Lenschow et al. 2007), imply a putative role of ISGylated HSP70s in limiting virus replication. Methylation represents a critical step in the post-translational modification of HSP70s. The only known Hsp70 methyltransferase identified so far is METTL21A (Jakobsson et al. 2013), and this enzyme is responsible for trimethylation of a conserved lysine residue found in several human HSP70s (see Fig. 1). In the case of Hsp70-8, trimethylation alters the affinity of the chaperone for both the monomeric and fibrillar forms of the Parkinson disease-associated protein α-synuclein (Jakobsson et al. 2013). These data unveil a novel role for protein methylation as a regulatory mechanism for molecular chaperones, particularly HSP70s.

Role of HSP70s in disease and nondisease

Although the original reports on the presence of Hsp70 and Hsp60 in the peripheral circulation of normal individuals by Pockley et al. (1999) were controversial, there is now a wealth of literature indicating that HSPs are present in the peripheral circulation in health and a range of diseased conditions (Ciocca et al. 2013; Gunther et al. 2015; Hom et al. 2012; Molvarec et al. 2010; Pockley et al. 2014; Qu et al. 2015). Furthermore, the level of HSP70 increases in the peripheral circulation during different kinds of exercise (Heck et al. 2011; Horn et al. 2007; Sandström et al. 2008), and excessive use of mobile phones may lead to enhanced *HSPA* gene expression and serum HSP70 levels compared to moderate use (Balakrishnan et al. 2014). Concordantly, serum levels of C-reactive protein also increase upon mobile phone-induced radiation, highlighting the role of serum HSP70 and C-reactive protein as potential biomarkers in systemic inflammation. There are a number of particularly interesting findings relating to changes in the serum concentration of HSP70 during the aging process (Rea et al. 2001). There is evidence for an age-dependent decrease in the serum concentration of Hsp70 in a normal population, with higher Hsp70 levels correlating with inflammation and frailty in the elderly (Njemini et al. 2011), with low levels of circulating serum Hsp70 being proposed to serve as an indicator of a healthy state and a biomarker for longevity (Terry et al. 2004). The fact that the concentration of inducible Hsp70-1 in the peripheral circulation inversely correlates with pro-inflammatory markers highlights the crucial role of pro-inflammatory profiles in reducing Hsp70-1

(Marotta et al. 2007). These and other findings (Gehrmann et al. 2014a) suggest that circulating serum Hsp70 might also serve as a biomarker of inflammation in healthy individuals.

Hsp70-1 is also present in the peripheral circulation of healthy nonpregnant and pregnant individuals. In normal human pregnancy, circulating Hsp70-1 levels are decreased, showing a positive correlation with gestational age and a negative correlation with maternal age (Molvarec et al. 2007b). The lower serum Hsp70 levels might be implicative of an unknown regulatory mechanism aimed at maintaining immune tolerance in pregnancy. Contrarily, elevated circulating Hsp70-1 levels correlate with an increased risk of several pregnancy complications such as transient hypertension, preeclampsia, and superimposed preeclampsia, reflecting systemic inflammation, oxidative stress, and hepatocellular injury in preeclampsia (Molvarec et al. 2006). Serum Hsp70-1 levels are significantly higher in patients with the syndrome of hemolysis, elevated liver enzymes, and low platelet count (HELLP syndrome) than in severely preeclamptic patients without HELLP syndrome (Molvarec et al. 2007a). In the latter, enhanced serum Hsp70-1 levels correlate strongly with markers of tissue damage and disease severity (Madach et al. 2008). Notwithstanding these issues, additional investigations are needed in order to establish the detection of circulating Hsp70 levels as a useful tool in discriminating healthy and diseased individuals.

HSP70s and cancer

Hsp70 plays a pivotal role in carcinogenesis acting as a potential tumor biomarker. In a broad range of human cancers, Hsp70-1 is frequently constitutively overexpressed to promote cancer cell survival, tumorigenicity, and anti-apoptotic activities. Hsp70-1 overexpression correlates with elevated tumor cell proliferation, clinical stage or increased grade, and poor prognosis in patients with nonsmall cell lung carcinoma (NSCLC; Malusecka et al. 2001), breast, endometrial, and uterine cervical carcinoma (Ciocca and Calderwood 2005), as well as in patients with acute myeloid leukemia (Thomas et al. 2005), colorectal carcinoma (Hwang et al. 2003; Sun et al. 1997), and prostate and hepatocellular carcinoma (Abe et al. 2004; Chuma et al. 2003). In osteosarcomas, Hsp70-1 overexpression functions as a predictive immunohistochemical marker, correlating with responses to neoadjuvant chemotherapy (Trieb et al. 1998). Recent studies on patients with brain tumors revealed substantial expression of Hsp70 in gliomas and meningiomas (Alexiou et al. 2014), as well as in large-cell medulloblastoma (Alexiou et al. 2013). However, additional investigations are required in order to better assess their relationship with tumor aggressiveness and patient prognosis. Moreover, Hsp70-1 expression was significantly associated with malignant rather than benign ovarian tumors (Athanassiadou et al. 1998) and correlated to the clinical stage and survival of malignant cutaneous melanoma (Lazaris et al. 1995), oral cancer (Kaur et al. 1998), and bladder cancer (Syrigos et al. 2003). With respect to breast cancer, Hsp70-1 upregulation obviously occurs through activation of the Her-2/neu pathway during mammary tumorigenesis (Khaleque et al. 2005). Primary and metastatic breast cancers also show an overexpression of Hsp70.2 (HspA2), acting as a critical regulatory growth element in conjunction with Hsp70-1 (Daugaard et al. 2007a). Hsp70-1 and Hsp70.2 display specific and nonoverlapping functions in regulating tumor cell growth and survival, since concomitant depletion of both chaperones led to wide differences in cancer cell morphology, gene expression profiles, and cell cycle distributions (Rohde et al. 2005). In patients with renal cancer, Hsp70-1 upregulation was associated with better prognosis irrespective of stage and histological grade (Santarosa et al. 1997). Tumor tissues of patients with HBV-related early-stage HCC overexpress a great variety of HSP70s including Hsp70-1 and Hsp70-12A, the latter correlating with poor survival (Yang et al. 2015). Upregulated levels of Hsp70-4/-5/-6 were associated with earlier recurrence of HCC. These exciting findings warrant further analyses regarding a possible preventive or therapeutic impact of these HSPs in HCC. Interestingly, Hsp70-1 expression is frequently reduced in patients with esophageal squamous cell carcinoma and can be considered as an independent negative prognostic factor of this disease (Kawanishi et al. 1999; Shiozaki et al. 2000).

Hsp70-1 is expressed in the plasma membrane of a large proportion of different tumor entities but not in the plasma membrane of corresponding normal cells/tissues (Gehrmann et al. 2008a, 2012, 2015; Multhoff et al. 1995; Multhoff 2007; Pfister et al. 2007; Schilling et al. 2009; Vega et al. 2008). Tumor cells expressing mHsp70-1 are in general highly resistant to radiochemotherapy most likely due to blockage of the NF-κB, JNK, and ERK signaling pathways (Gabai et al. 2005; Pocaly et al. 2007). An ongoing screening program of more than 1000 patients with diverse solid tumors by the group of Gabriele Multhoff clearly demonstrates that more than 50 % of the patients harbor an Hsp70-1 membrane-positive tumor phenotype. The membrane density of Hsp70-1 on cancer cells is selectively increased in response to treatments such as radiochemotherapy but not on normal cells (Gehrmann et al. 2002). In metastatic disease, the membrane density of Hsp70-1 is considerably higher on metastases compared to primary and relapse tumors (Gehrmann et al. 2012), suggesting that mHsp70-1 might facilitate metastases, support adherence of tumor cells to endothelial cells and organs, or might confer resistance to an unfavorable milieu during metastasis. In line with these findings mHsp70-1 expression has been associated with an unfavorable prognosis and a decreased overall survival in patients with lower rectal carcinoma and squamous cell carcinoma of the lung (Pfister et al. 2007). Based on these data, mHsp70-1 positivity can be considered

as a ubiquitous selective tumor-specific marker of "aggressive" disease. Remarkably, the better survival of patients with mHsp70-1-positive tumors metastasizing to the liver obviously results from the capacity of hepatic NK cells to recognize and eliminate mHsp70-1-positive tumor cells (Pfister et al. 2007).

There have been numerous interesting and eminent observations relating to the presence of circulating HSP70s in cancer as well as a putative relationship between HSP70 levels with treatment response and tumor volume (Bayer et al. 2014; Breuninger et al. 2014; Gehrmann et al. 2014b; Gunther et al. 2015). In patients with HCC, total HSP70 serum levels are significantly enhanced compared to controls and individuals with chronic hepatitis (Gehrmann et al. 2014a). The same study revealed that patients with liver cirrhosis who evolved HCC had elevated HSP70 levels compared to patients who did not develop HCC. Consistent with these findings, patients with pancreatic cancer have higher serum Hsp70 levels than healthy controls and individuals with chronic pancreatitis (Dutta et al. 2012). A significant correlation of serum Hsp70 levels with the gross tumor volume has been shown for adeno and squamous cell NSCLC (Bayer et al. 2014; Breuninger et al. 2014; Gehrmann et al. 2014b; Gunther et al. 2015). From these data, it can be hypothesized that circulating Hsp70 has the capacity to discriminate between inflammation/disease and cancer, rendering circulating Hsp70 a potential biomarker for cancer (Pockley et al. 2014).

Although several studies have investigated the association between *HSPA* polymorphisms and risk of cancer, the results remain controversial. A meta-analysis evaluating the association between the *HSPA* polymorphisms and cancer susceptibility suggested that the *HSPA1B* polymorphism rather than *HSPA1L* and *HSPA1A* polymorphisms was associated with the risk of cancer (He et al. 2014). Since this meta-analysis had several limitations, large and well-designed studies taking into consideration gene-gene and gene-environment interactions are warranted in order to validate these findings.

HSP70s in nonmalignant pathologies

Despite its impact in carcinogenesis, HSP70s play critical roles in other pathological conditions. Overexpression of Hsp70-1 in patients with myelodysplastic syndromes correlates with poor prognosis (Duval et al. 2006). Enhanced plasma levels of Hsp70-1 are found in patients with idiopathic inflammatory myopathy, although these did not correlate with clinicopathological parameters (Svitalkova et al. 2014). Hsp70-1 upregulation in the peripheral lung tissues of patients with chronic obstructive pulmonary disease is closely related to disease severity and smoking status (Dong et al. 2013). In patients with heart failure, plasma concentrations of Hsp70-1 increased gradually with the progression of disease stages, thereby suggesting that Hsp70-1 could be used as a potential screening biomarker for the early diagnosis of heart failure (Li et al. 2013b) and as an independent prognostic marker of mortality (Jenei et al. 2013). Of note, Hsp70-2 protein expression in peripheral blood mononuclear cells is significantly lower in multiple sclerosis (MS) patients with GG genotype compared to AA genotype, thereby indicating an implication of the G allele of *HSPA1B* polymorphism in the development of MS (Boiocchi et al. 2014). Interestingly, allele A of the *HSPA1B* polymorphism correlates with the clinical course of Crohn's disease in the Chinese population (Chen et al. 2013).

A growing body of evidence now indicates that the level and localization of ER-resident Hsp70-5 is altered in different models of neurodegenerative disorders including Parkinson's disease, Alzheimer's disease, and progressive retinal degeneration (Holtz and O'Malley 2003; Kroeger et al. 2012; Lee et al. 2010). These disorders are characterized by activation of the unfolded protein response (UPR) as a result of ER stress and altered expression of Hsp70-5, a key UPR mediator (Hoozemans et al. 2012). Consequently, apoptosis pathways are activated through cross-talk between the ER and mitochondria (Rutkowski and Kaufman 2004). ER stress is therefore considered to be a common mediator of apoptosis in neurogenerative disorders. Recent investigations documented an age-dependent modification of the ER structure and concomitant reduction of the Hsp70-5 expression and activation in models of neurogenerative disorders (Erickson et al. 2006; Nuss et al. 2008). This raises the question of whether the loss of Hsp70-5 function might serve as a predisposing factor for many age-associated neurogenerative disorders including Parkinson's disease, Alzheimer's disease, and age-related macular degeneration. However, further studies to decipher the role of Hsp70-5 are needed.

The list of diseases with significant changes of HSP70 levels is steadily increasing and involves obesity, alcoholic and nonalcoholic fatty liver disease, hepatic steatosis, diabetes, chronic glomerulonephritis, stroke, and seizure-related pathological events as well as asthma, renal and cardiovascular disease, infections, and after surgery/trauma (for a review, see Pockley et al. 2014; Qu et al. 2015). In these cases, Hsp70 levels can be increased or decreased reflecting the ambivalent nature of HSP70s in pathology. For example, serum Hsp70 levels are increased in patients with both type 1 (Pagetta et al. 2003) and type 2 diabetes mellitus (Nakhjavani et al. 2010). Serum Hsp70 is also increased in women with gestational diabetes mellitus correlating well with HbA1c levels (Garamvolgyi et al. 2015). A randomized study in patients with atherosclerosis exhibited lower levels of circulating Hsp70-1 and anti-Hsp70-1 compared to controls, implying that these molecules play a putative role in arterial calcification and might function as biomarkers for the progression of atherosclerotic disease (Dulin et al. 2010). Notwithstanding this issue, further investigations are needed to gain insight into the pathophysiological role of Hsp70, particularly of Hsp70

levels in biological fluids that have been associated with a wealth of clinical conditions (see above).

Therapeutic implications

As already mentioned, HSP70s that are constitutively expressed at low levels in nonmalignant cells are abundantly expressed in a great variety of human cancers and often associated with metastasis and poor clinical outcome. Since particularly Hsp70-1 and Hsp70-2 have been validated to play a central role in tumorigenesis (Daugaard et al. 2005; Rohde et al. 2005), studies aiming to reduce their expression or activity could represent a valuable strategy in anti-cancer approaches. In this regard, silencing of Hsp70-1 and Hsp70-2 is cytotoxic selectively towards tumor cells (Nylandsted et al. 2000), and an efficient cancer therapeutic treatment is often associated with decreased HSP70 levels (Yiu et al. 2010). Although several RNA interference studies have confirmed the pivotal role of HSP70s in growth and survival of cancer cells (Matokanovic et al. 2013; Peng et al. 2013), no clinically available RNAi-derived inhibitors have been tested up to date in clinical trials.

Targeting inhibition of intracellular or membrane-bound HSP70s might represent a further promising approach in cancer therapy. The group of Gabriele Multhoff has convincingly demonstrated that mHsp70 acts as a target structure for the induction of antibody-dependent toxicity in tumor cells using the monoclonal antibody cmHsp70.1 (Stangl et al. 2011). Membrane HSP70s also serve as tumor-specific target structure for the recognition by activated NK cells through induction of granzyme B-mediated apoptosis (Gross et al. 2003; Multhoff et al. 1997, 2001). Notably, binding of recombinant granzyme B to mHsp70-1 followed by perforin-independent endocytosis induces selective tumor cell killing (Gehrmann et al. 2012). Notably, a mHsp70-1-positive tumor phenotype is associated with a significantly decreased overall survival in tumor patients and could therefore serve as a negative prognostic marker (Pfister et al. 2007).

Based on promising pre-clinical data using ex vivo-activated NK cells in different tumor mouse models, a phase I clinical trial in tumor patients was performed in order to test feasibility, safety, and tolerance of ex vivo-stimulated autologous NK cells in patients with metastasized colorectal carcinoma and NSCLC (Krause et al. 2004). Since this study showed promising clinical results regarding safety and tolerability, a proof-of-concept phase II randomized clinical trial was initiated to test the efficacy of ex vivo-stimulated autologous NK cells in patients with mHsp70-1-positive NSCLC following radiochemotherapy (Specht et al. 2015). The primary endpoint of this study will be the comparison of the progression-free survival of patients treated with ex vivo-activated NK cells compared to patients treated with radiochemotherapy alone.

In a different setting, the observation that mHsp70 functions as tumor-specific target structure encouraged the generation of autologous therapeutic anti-tumor vaccines for the treatment of cancer patients. A therapeutic vaccine, consisting of hydroxyapatite ceramic particles in combination with tumor cell membrane proteins including Hsp70, demonstrated tolerability and a positive response in certain cancer patients (Ciocca et al. 2007). However, due to the small number of patients enrolled, further studies are warranted to analyze the efficacy of anti-tumor vaccines. Several ongoing studies on the immunization of cancer patients with autologous HSP-based anti-tumor vaccines complexes reveal promising results (Ciocca et al. 2012). Currently, magnetic nanocarriers coated with Hsp70-1 have been validated to boost anti-tumor immune responses in experimental glioma, thereby rendering this technology a potentially effective platform for the development of innovative anti-cancer strategies (Shevtsov et al. 2015).

An important finding relates to the presence of Hsp70-1 on tumor-derived exosomes (TDEs) of mHsp70-1-positive tumors (Gastpar et al. 2005). TDE-associated Hsp70-1 can interact with myeloid-derived suppressor cells (MDSCs) capable of suppressing T cell activation and promoting cancer development. Of note, TDE-associated human Hsp70-1 activates human MDSCs and triggers their suppressive function in humans. These findings highlight the pivotal role of TDE surface-expressed Hsp70-1 in restraining anti-tumor immune surveillance by promoting the suppressive functions of MDSCs (Chalmin et al. 2010). An alternative approach in the preparation of HSP-based vaccines represents the tumor/DC fusion technology (Enomoto et al. 2006; Gong et al. 2010), because this technique has the capacity to target individual tumor cell populations such as cancer stem cells (CSCs; Murshid et al. 2011). T cells induced by DC-CSC fusion vaccines selectively kill ovarian CSCs and radiation-resistant cells enriched in CSCs (Weng et al. 2011). The efficaciousness of Hsp70/peptide complexes from tumor/DC fusion cells derived from DC-CSC fusion vaccines in targeting the CSC subpopulation is currently being investigated. Hsp70-peptide complexes (Hsp70.PC-F) extracted from fusions of DCs and radiation-enriched tumor cells have been used to treat mice with pre-existing lung metastases (Weng et al. 2013). Immunization of mice with the Hsp70.PC-F vaccine resulted in a T cell-mediated immune response against radioresistant tumor cells. The combination of chaperone vaccine with radiotherapy inhibited the growth of primary tumors and the number of tumor cells metastasizing to lung, implying that the Hsp70.PC-F vaccine induced specific immunity towards radioresistant populations of mammary tumor cells by complementing radiotherapy (Weng et al. 2013).

Chemical compounds modulating HSP70 functions

The modulation of Hsp70 chaperone activity has emerged as a promising anti-cancer strategy in pre-clinical and clinical trials, with intensive efforts having been made to develop HSP70 inhibitors. HSP70 inhibitors have the potential for use in single-agent or combinatorial therapies in order to supplement the hitherto existing conventional radiochemotherapeutical approaches and molecularly targeted drugs. Up to date, only a few studies identified chemical compounds that have the capacity to directly modulate the activity of HSP70s by interacting with different chaperone subdomains (Table 2). 15-Deoxyspergualin (DSG), a synthetic derivative of spergualin from *Bacillus* sp., was about the first compound known to stimulate the ATPase activity of Hsp70-8 (Brodsky 1999). DSG binds to the C-terminal EEVD motif, that is the same site of binding to the TPR-domain co-chaperones (Nadler et al. 1998). DSG showed significant anti-tumor activity in mice (Plowman et al. 1987), but in a phase II trial, it was inefficient against metastatic breast cancer resistant to frontline chemotherapy (Dhingra et al. 1994). Since DSG has a very low bioavailability and poor stability, several analogs with improved properties were generated. Among them, LF 15-0195 was found to be less toxic and more potent than DSG in a renal allograft rejection primate model, suggesting that further chemical optimization might improve the performance of these compounds (Lebreton et al. 1999; Yang et al. 2003). The dihydropyrimidine NSC 630668-R/1 was identified to inhibit yeast Hsp70-8 activity and to block Hsp70-8-mediated protein translocation in vitro (Fewell et al. 2001). MAL3-101 represents a second-generation derivative of dihydropyrimidines with blocking Hsp70-8 ATPase activity (Fewell et al. 2001). MAL3-101 exhibits anti-myeloma effects in vitro and in vivo in a xenograft plasmacytoma model, as well as on primary tumor cells and bone marrow endothelial cells from myeloma patients (Braunstein et al. 2011). A similar derivative, MAL2-11B, inhibits the ATPase activity of Hsp70 as well as the ATPase activity of a chaperone-like protein, T antigen, required for polyomavirus (PyV) replication (Wright et al. 2009). Infection by members of the PyV family contributes to AIDS-related dementias and renal transplant rejection (Jiang et al. 2009). This molecule class is implicated in altering the processing of the microtubule-associated protein, Tau. In this respect, SW02 was shown to stimulate the ATPase activity of Hsp70 in vitro and to promote Tau accumulation in Alzheimer's disease models (Jinwal et al. 2009).

Compounds displacing ATP from HSP70 are expected to be powerful tools in modulating HSP70 chaperone function. The ATP analog Ver-155008 binds to the NBD of both Hsp70-8 and Hsp70-1 and thereby acts as an ATP-competitive inhibitor preventing allosteric control between NBD and SBD (Schlecht et al. 2013). VER-155008 inhibits the proliferation of human colorectal cancer cells with concomitant reduction in cellular levels of Raf-1 and Her-2 (Williamson et al. 2009). VER-155008 also induced Hsp90 client protein degradation and caspase-3/7-dependent apoptosis in human breast cancer cells (Massey et al. 2010). Unfortunately, the bioavailability of VER-155008 is low because it is rapidly degraded in vivo, and its level in tumor tissues never reaches the predicted pharmacologically relevant level. The NBD is also the target structure of apoptozole, a small apoptosis-inducing molecule. Apoptozole binds to the ATP-binding pocket of human Hsp70-1 and Hsp70-8 and inhibits the ATPase activity of both chaperones (Cho et al. 2011; Williams et al. 2008). Notably, the identity of the interaction site has come under scrutiny because of reports demonstrating that apoptozole failed to interact with human Hsp70-1 (Evans et al. 2015). Methylene blue (MB), azure C, and myricetin also abolish the ATPase activity of HSP70 (Jinwal et al. 2009). MB selectively inactivates Hsp70-1, but not Hsp70-8, by oxidizing Cys306 within the NBD that is not conserved in Hsp70-8 (Miyata et al. 2012). MB reduces the levels of several Hsp70-1 substrates, including Tau (Congdon et al. 2012). MB also improves cognitive functions in the rTg4510 tauopathy mouse model (O'Leary et al. 2010; Wang et al. 2010) and has been explored in clinical phase IIb trials in patients with Alzheimer's disease (Wischik et al. 2008). Due to its enviable safety record, MB is used clinically for multiple indications, even though there are some disadvantages of the drug including eye and urine discoloration as well as nausea induction. Similarly, the flavonol myricetin directly inhibits Hsp70 activity and facilitates chaperone-mediated Tau turnover in Tau-overexpressing cells (Jinwal et al. 2009). In *Escherichia coli*, myricetin binds to a noncanonical site on the NBD of DnaK and allosterically blocks binding of DnaK to the co-chaperone, DnaJ (Chang et al. 2011). These data clarify the role of endogenous Hsp70 in Tau processing and suggest an unexpected avenue for therapeutic intervention. Although these initial results were encouraging, the first-generation compounds often lacked sufficient selectivity.

The search for more potent and selective inhibitors yielded the rhodocyanine MKT-077 that preferentially binds and inhibits the ADP-bound form of Hsp70-9 and Hsp70-8 (Rousaki et al. 2011; Wadhwa et al. 2000). MKT-077 locates itself in a negatively charged pocket close to, but not identical to, the NBD of Hsp70-8 (Rousaki et al. 2011). This allosteric HSP70 inhibitor has originally been described to exhibit anti-tumor activity in vitro (Koya et al. 1996) and in vivo (Chiba et al. 1998). Unfortunately, a phase I trial revealed severe renal dysfunction in a panel of patients with solid tumors (Propper et al. 1999). Interestingly, combined inhibition of Hsp70-5 and Hsp90 with MKT-077 and 17-allylamino-17-demethoxygeldanamycin (17-AAG) in vivo conferred potent anti-tumor effects against HCC, providing an alternative therapeutic approach for liver cancer therapy (Guo et al. 2014). Due to the low stability of MKT-077, optimization scaffolds yielding novel compounds with improved drug-like properties were

Table 2 Modulators of HSP70 functions

Compound	Interaction site	References
ATP analogs		
Ver-155008	NBD	Schlecht et al. (2013)
Spergualin derivatives		
15-Deoxyspergualin	EEVD	Nadler et al. (1998)
LF 15-0195	EEVD[a]	Lebreton et al. (1999), Yang et al. (2003)
Dihydropyrimidines		
NSC 630668-R/1	n.d.	Fewell et al. (2001)
MAL3-101	n.d.	Fewell et al. (2001)
MAL2-11B	n.d.	Wright et al. (2009)
SW02	n.d.	Jinwal et al. (2009)
Flavonoids		
(−)-Epigallocatechin-3-gallate (EGCG)	NBD	Ermakova et al. (2006)
Myricetin	NBD	Chang et al. (2011)
Curcumin	NBD	Angelo et al. (2013)
Imidazoles		
Apoptozole	NBD?	Cho et al. (2011)
Rhodocyanines and analogs		
MKT-077	Near NBD	Rousaki et al. (2011)
JG-13	NBD[a]	Li et al. (2013a)
JG-48	NBD[a]	Li et al. (2013a)
JG-98	NBD	Li et al. (2013a)
YM-1	NBD[a]	Abisambra et al. (2013), Koren et al. (2012), Li et al. (2013a)
YM-8	NBD	Miyata et al. (2013)
Phenylethylsulfonamide		
2-Phenylethynesulfonamide (PES)	SBD	Balaburski et al. (2013)
2-(3-Chlorophenyl)ethynesulfonamide (PES-Cl)	SBD	Balaburski et al. (2013)
Benzothiazine		
Methylene blue	NBD	Jinwal et al. (2009), Miyata et al. (2012)
Azure C	NBD[a]	Jinwal et al. (2009)
Lipids		
Geranylgeranyl acetone	SBD	Otaka et al. (2007)
Fatty acyl benzamides	SBD	Liebscher et al. (2007)
Sulfoglycolipids		
3-Sulfogalactoglycerolipid	NBD	Mamelak and Lingwood (2001)
3-Sulfogalactosylceramide (SGC)	NBD	Mamelak and Lingwood (2001)
Adamantyl-SGC	NBD	Whetstone and Lingwood (2003)
Peptides		
A3-APO	C-terminal lid	Rozgonyi et al. (2009)
Peptide aptamer A8	SBD	Rerole et al. (2011)
Peptide aptamer A17	NBD	Rerole et al. (2011)
ADD70 (AIF-derived decoy for HSP70)	SBD	Gurbuxani et al. (2003)
Pyrrhocoricin	SBD	Kragol et al. (2002)

NBD nucleotide binding domain, *SBD* substrate binding domain, *n.d.* not determined

[a] Proposed interaction site based on structural analogy

developed. Among them, JG-98 was identified as an allosteric inhibitor of the Hsp70/Bag-3 interaction with more potent anti-proliferative properties in cancer cells than MKT-077 (Li et al. 2013a, 2015). Since the activity of JG-98 was also positively evaluated in xenograft mouse models (Li et al. 2015), it can be proposed that targeting the Hsp70/Bag-3 interaction might represent a promising, novel anti-cancer strategy. A further microsomal stable MKT-007 analog, JG-13, has been described as an active anti-cancer compound. JG-13 interferes with Hsp70-8/Bag-1 interactions and reduces Tau levels in vitro (Li et al. 2013a). Similarly, JG-48 and the stable and soluble MKT-077 analog YM-1 downregulate Tau levels in neuronal cells (Abisambra et al. 2013; Li et al. 2013a). YM-1 which shows promising anti-cancer properties in vitro (Koren et al. 2012), as well as JG-48, rescued long-term potentiation deficits in hippocampal slices from rTg4510 mice (Li et al. 2013a). YM-08 is a further refinement of YM-1 that binds to Hsp70-8 and has reduced potency but a dramatically increased blood–brain barrier permeability, thereby identifying YM-08 as a promising scaffold for the development of HSP70 inhibitors suitable for use in the CNS (Miyata et al. 2013). However, no clinical data regarding the efficacy of these interesting compounds in targeting Hsp70 in disease are available.

Notably, rationally designed decoy targets of HSP70 that are derived from AIF have been demonstrated to sensitize cancer cells to apoptosis by neutralizing HSP70 function (Schmitt et al. 2003, 2006). These inhibitors, called ADD70 (AIF-derived decoy for HSP70), bind to the SBD of Hsp70-1 and block Hsp70-1/AIF association and consequently the cytoprotective action of Hsp70-1 (Gurbuxani et al. 2003). In animal models of colon cancer and melanoma, ADD70 showed promising effects on tumor size and growth and sensitized cancer cells to chemotherapy (Schmitt et al. 2006).

Another approach is based on peptide aptamers that selectively bind and inhibit Hsp70. Two of them, A8 and A17, are specific for inducible Hsp70-1 and bind to its SBD or NBD, respectively (Rerole et al. 2011). Both aptamers specifically block Hsp70-1 chaperone function and enhance the sensitivity of cancer cells to apoptosis induced by anti-cancer drugs. The peptide aptamers are able to induce an in vivo immune response, as demonstrated by the strong infiltration of immune cells in the regressing tumors of aptamer-treated animals (Rerole et al. 2011). Since tumors selectively express HSP70s on the cell surface, it would be fascinating to couple peptide aptamers to nanoparticles containing HSP70 antibodies such as the membrane Hsp70-specific monoclonal antibody cmHsp70.1 in order to specifically guide the aptamers into the malignant cells. A superior uptake of cmHsp70.1 antibody-conjugated gold nanoparticles into mHsp70-1-positive tumor cells has been reported (Gehrmann et al. 2015). Ongoing studies are evaluating the suitability of cmHsp70.1 antibody-conjugated gold nanoparticles for the detection of mHsp70-1-positive tumors in vivo and whether these nanoparticles can be used for therapeutic approaches. This could open new perspectives towards better clinical diagnostics and personalized therapeutic interventions in HSP70-expressing human tumors.

The NBD is also the site of action of the flavonoids (−)-epigallocatechin-3-gallate (EGCG) and curcumin. EGCG, the most abundant and powerful catechin in cancer prevention and treatment (Yang et al. 2007), directly interacts with the NBD of Hsp70-5, thereby inhibiting its ATPase activity (Ermakova et al. 2006). EGCG also stimulates apoptosis induction in cancer cells by interfering with the anti-apoptotic Hsp70-5/caspase-7 complex and suppresses the transformed phenotype. These results clearly indicate the inhibitory effect of EGCG on the anti-apoptotic properties of Hsp70-5 that plays a critical role in the development of drug resistance. Curcumin, a potent anti-inflammatory and anti-tumorigenic agent, has shown efficacy and low toxicity in several types of cancer. Curcumin upregulates Hsp70 at the mRNA and protein level in human schwannoma cells (Angelo et al. 2011), and a biotinylated curcumin interacts with the Hsp70 NBD at the ATPase site and also at a second binding site on the back of the SBD (Angelo et al. 2013). Currently, the addition of curcumin to 5-fluorouracil, leucovorin, and oxaliplatin (FOLFOX)-based chemotherapy enhances killing in patient-derived colorectal liver metastasis (CRLM) cultures by targeting CSCs (James et al. 2015). A phase I dose escalation study combining curcumin with first line FOLFOX chemotherapy in patients with CRLM has confirmed the safety and tolerability of this approach (James et al. 2015). A randomized phase II study comparing participants receiving FOLFOX only with those receiving FOLFOX + curcumin is currently recruiting.

The small molecule 2-phenylethynesulfonamide (PES) has been identified as being a novel Hsp70-1 inhibitor (Leu et al. 2009). PES alters the association of co-chaperones with Hsp70-1 and impairs the autophagal/lysosomal system, as well as the proteasomal pathway, and consequently leads to the functional inactivity of HSP70s and client proteins and, through this activity, impacts on the fate of substrate. PES binds to the SBD of Hsp70-1 in which the C-terminal helical lid of Hsp70-1 is required (Balaburski et al. 2013). PES, or the related compound 2-(3-chlorophenyl)ethynesulfonamide (PES-Cl), exhibit(s) potent anti-leukemic effects in vitro (Kaiser et al. 2011; Steele et al. 2009) and in vivo (Balaburski et al. 2013; Leu et al. 2009). PES reversibly disrupts the purinosome, a dynamic multi-protein complex composed of Hsp70, Hsp90, and several co-chaperones that are functionally involved in purine synthesis (French et al. 2013). The combinatorial treatment of cervical cancer cells with PES and the known anti-cancer drug methotrexate synergistically increases the cytotoxicity of these cells (French et al. 2013). Moreover, the direct inhibition of Hsp70-1 and Hsp70-8 by

PES and VER-155008 induces an early elongation pausing of ribosomes and a decrease in protein translation. PES and its derivatives can therefore be considered as an auspicious group of HSP70 inhibitors that hold promise as a basis for innovative anti-cancer strategies.

Sulfogalactoglycerolipid and sulfogalactosylceramide (SGC) are sulfoglycolipids that are capable of binding to the NBD of testis-specific Hsp70-1t and cognate Hsp70-8 from multiple organisms (Boulanger et al. 1995; Mamelak and Lingwood 2001), highlighting the potential problem of selectivity in this highly conserved family. A soluble SGC derivative, adamantyl-SGC (adaSGC), exhibits a heightened affinity for HSP70 (Mamelak and Lingwood 2001) and inhibits the ATPase activity of bovine brain Hsp70-8, a potential target in neurodegeneration (Whetstone and Lingwood 2003). In transfection experiments, adaSGC upregulated a mutant of the cystic fibrosis transmembrane receptor (CFTR) that is prone to misfolding and decomposition via ER-mediated degradation (Park et al. 2009), implying that blockage of the ATPase activity of HSP70s might repress ER-mediated degradation pathways that physiologically downregulate CFTR (Evans et al. 2010). These findings might have future clinical implications for the use of HSP70s as drug targets in cystic fibrosis and neurodegenerative misfolding disorders.

Targeting DnaK in infectious diseases

Anti-microbial resistance is an emerging worldwide concern in light of the widespread use of anti-microbial drugs in animals and humans. Unfortunately, the treatment of life-threatening infections is especially problematic because clinical strains rapidly acquire multiple-drug resistance. The development of novel anti-microbial strategies is thus urgently required. In this regard, DnaK is considered as being a potential target in anti-bacterial therapies. Pharmacological inhibitors including pyrrhocoricin bind to DnaK and selectively kill susceptible bacteria (Otvos et al. 2000). Pyrrhocoricin specifically binds to the SBD of *E. coli* DnaK, thereby blocking its ATPase activity (Kragol et al. 2002). The proline-rich peptide A3-APO represents a family of a new class of anti-bacterial peptides with improved stability and activity (Otvos et al. 2005). A3-APO emerged as a viable pre-clinical candidate by virtue of its ability to disintegrate the bacterial membrane, inhibit DnaK, lack of eukaryotic toxicity, and withstand proteolytic degradation in body fluids. A3-APO binds to the C-terminal helical lid of bacterial DnaK and inhibits chaperone-assisted protein folding in bacteria (Rozgonyi et al. 2009). In mouse models of *Staphylococcus aureus* and *Propionibacterium acnes* intradermal infections, A3-APO efficiently ameliorates resistance to aerobic and anaerobic intradermal infections (Ostorhazi et al. 2013). A3-APO diminishes the production of correctly folded *Bacillus cereus* diarrheal enterotoxin, restricts lethal toxin-induced *Bacillus anthracis* replication in mouse macrophages, and improves survival in systemic mouse challenge models (Otvos et al. 2014). From these data, one can speculate that the inhibition of bacterial protein folding together with other types of anti-microbial modes might represent a novel strategy in combating resistant or life-threatening infections (Otvos et al. 2014), even though these compounds have not entered clinical trials to date.

The SBD is also the interaction site of geranylgeranyl acetone (GGA; Otaka et al. 2007) and fatty acyl benzamides (Liebscher et al. 2007). GGA induces the expression of mammalian Hsp70-1 (Hirakawa et al. 1996) and acts in a cytoprotective capacity in a number of pathological lesions such as stress-induced cell damage in the gastrointestinal tract, heart ischemia, hepatectomy, cerebral infarction, and colitis. GGA also binds to *Helicobacter pylori* DnaK with 26 times higher affinity than to human Hsp70-1 (Grave et al. 2015). In human cells, GGA-induced Hsp70-1 protects gastric cells from injury induced by *H. pylori*. In contrast, binding of GGA to DnaK suppresses the activity of the *H. pylori* chaperone and is accompanied by morphological alterations (Grave et al. 2015). *E. coli* DnaK assists protein folding by catalyzing the *cis*/*trans* isomerization of secondary amide peptide bonds in unfolded or partially folded proteins. This activity of DnaK can be specifically targeted by fatty-acylated benzamides. One of these compounds blocks *cis*/*trans* isomerase activity of DnaK, probably through binding to its SBD and the chaperone-mediated refolding of aggregated proteins after heat shock (Liebscher et al. 2007). Additionally, this compound harbors anti-bacterial activity against *E. coli*, thereby identifying the isomerase activity of DnaK as a crucial survival parameter in heat-stressed bacteria. These data further imply that acyl benzamido inhibitors may be promising anti-bacterial molecules, even though their specificity for prokaryotic Hsp70 has not been demonstrated yet.

Concluding remarks

HSP70s are crucial players in the chaperone network with diverse functions in cell survival and proteostasis. Several studies have demonstrated that changes in HSP70s are associated with a profile of healthy and diseased conditions. In this respect, Hsp70 has potential utility as a biomarker for diverse physiological and pathological conditions and drug target in a remarkably wide range of diseases. Since HSP70s represent key mediators of the anti-stress defensive alliance that can promote tumor cell growth and survival, there is an intensive search for HSP70 modulators that will provide clinicians and researchers with novel innovative compounds suitable for anti-cancer therapies. However, the field of HSP70 modulators is currently in its infancy in many instances, and extensive work has yet to be done before it is clear how this chaperone can be best exploited. Consideration of the findings that are presented in this review raises the question of whether

targeting of a single HSP70 isoform is the optimal strategy. The most recent trend relates to targeting extracellular or membrane-bound HSP70s. In tumor sublines, mHsp70-1 alters radiation sensitivity of tumor cells and its decreased membrane presence augments apoptosis induction after X-ray irradiation (Murakami et al. 2015). These exciting observations should be kept in mind for the development of novel approaches in treating radiation-resistant tumors. The detection of Hsp70-1 in the serum of patients using a recently established immunoassay (Breuninger et al. 2014) has emerged as a useful tool to second-guess the radiation sensitivity of tumors.

Several lines of evidence indicate that inflammation plays a pivotal role in modulating radiation responsiveness of tumors. Sublethal doses of ionizing radiation induce a nuclear DNA damage response and trigger cellular damage in tumors by inducing pro-inflammatory pathways predominantly mediated via activation of NF-κB, the central linker between inflammation, carcinogenesis, and radioresistance. In recent years, inhibition of NF-κB by synthetic compounds and nutraceuticals of different sources has been approved for tumor radiosensitization. Among them, curcumin has emerged as one of the best studied plant-derived polyphenols. Curcumin confers radiosensitizing effects in cancer cells by suppressing radiation-induced NF-κB activation (Chendil et al. 2004). Curcumin also enhances chemotherapy efficacy in isolated patient-derived CRLM (James et al. 2015), as mentioned before. Curcumin and other plant-derived polyphenols including EGCG have potential chemopreventive properties and are pharmacologically safe. These phytochemicals sensitize tumor cells to radiation therapy and chemotherapeutic agents by inhibiting pathways responsible for treatment resistance (Garg et al. 2005; Nambiar et al. 2011). We and others have shown that EGCG inhibits NF-κB activation and production of tumorigenic factors in cancer cells (Aggarwal and Gehlot 2009; Härdtner et al. 2012; Hoffmann et al. 2011; Hönicke et al. 2012), thereby blocking chronic inflammation, one of the hallmarks of cancer. The 10-year prospective cohort study by the group of Kazue Imai revealed that consumption of green tea significantly delayed cancer onset in humans indicative of primary cancer prevention (Nakachi et al. 2000). In a randomized phase II clinical trial, the same amount of green tea prevented colorectal adenoma recurrence in polypectomy patients (Shimizu et al. 2008). Keeping in mind that targeting NF-κB should also reduce the expression of pro-tumorigenic HSPs and could thus be considered a form of therapeutic use of chaperones termed “chaperonotherapy,” the use of suitable HSP70 modulators in combination with radio-/chemotherapy is encouraged to improve the radiation sensitivity of tumors. This approach might be an attempt to supplement the hitherto existing conventional methods to disturb the concert between inflammation, malignant transformation, and radiochemoresistance of cancer cells. An intriguing novel approach aims to increase anti-tumor immune responses by targeting immune cells to tumors that specifically express Hsp70 on their cell surface or by blocking tumor-specific immune suppression mechanisms such as activation of myeloid suppressor cells (Goloudina et al. 2012). An ambitious HSP70 inhibition scenario relates to its combination with HSP90 inhibitors. HSP70 expression can be induced by HSP90 inhibitors in vitro and in vivo (Guo et al. 2005a; Lanneau et al. 2008). In this respect, the HSP70 inhibitor Ver-155008 that binds to the NBD of Hsp70-8 and Hsp70-1 boosts the apoptosis-inducing capacity of the HSP90 inhibitor 17-AAG (Massey et al. 2010). A similar anti-cancer effect has been observed after combinatorial treatment of cancer cells with ADD70 and 17-AAG (Schmitt et al. 2006). However, future and efficient chaperonotherapeutic molecular targeting of HSP70s by hyperspecific agents or techniques will shed light on their suitability as local radio-/chemosensitizer of HSP70-expressing tumors. The development of such agents will represent a huge challenge for the future to realize the potential of targeting Hsp70.

Recently, the group of Tangchun Wu explored an intriguing idea on the suitability of *HSPA* promoters for the use as genetic sensors for a broad panel of potentially deleterious substances to which cells are exposed. This group developed an elegant and multifunctional in vitro assessment for numerous toxicities (Xin et al. 2012) in which the *HSPA1A* promoter was positioned upstream of a luciferase reporter gene in HepG2 cells according to the method described by Peng et al. (2013). In this study, toxicogenic substances including the carcinogenic and mutagenic benzo[*a*]pyrene and formaldehyde triggered *HSPA1A* transcription enabling fast recognition of hazardous factors. The newly developed *HSPA1A* promoter-driven luciferase reporter is several times more sensitive than the expression of Hsp70-1A, thereby validating the relative luciferase activity in HepG2-luciferase cells as a sensitive and responsive indicator of the toxicity of organic compounds (Xin et al. 2012). Together with the observation that the HepG2-luciferase cells had higher sensitivity, better reproducibility, and lower costs in comparison to other in vitro toxicity tests examined in this study, one can speculate that HepG2-luciferase cells might have future clinical implications with respect to the development of novel approaches in toxicity assessment. However, this study had several limitations such as the low number of single compounds or mixtures of organic chemicals tested. Therefore, large studies taking into consideration many toxic agents and combinations thereof are warranted to validate these findings. However, this technique offers exciting opportunities to rapidly screen the toxicity of such organic pollutants and might hold potential to analyze the impact of environmental stressors in regulating Hsp70-mediated immune responses.

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