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Review

Heat shock proteins in cancer stem cell maintenance: a potential therapeutic target?

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Summary. Cancer stem cells (CSCs) are a subpopulation of tumor cells with unlimited self-renewal capability, multilineage differentiation potential and long-term tumor repopulation capacity. CSCs reside in anatomically distinct regions within the tumor microenvironment, called niches, and this favors the maintenance of CSC properties and preserves their phenotypic plasticity. Indeed, CSCs are characterized by a flexible state based on their capacity to interconvert between a differentiated and a stem-like phenotype, and this depends on the activation of adaptive mechanisms in response to different environmental conditions. Heat Shock Proteins (HSPs) are molecular chaperones, upregulated upon cell exposure to several stress conditions and are responsible for normal maturation, localization and activity of intra and extracellular proteins. Noteworthy, HSPs play a central role in several cellular processes involved in tumor initiation and progression (i.e. cell viability, resistance to apoptosis, stress conditions and drug therapy, Epithelial-Mesenchymal Transition (EMT), bioenergetics, invasiveness, metastasis formation) and, thus, are widely considered potential molecular targets. Furthermore, much evidence suggests a key regulatory function for HSPs in CSC maintenance and their upregulation has been proposed as a mechanism used by CSCs to adapt to unfavorable environmental conditions, such as nutrient deprivation, hypoxia, inflammation. This review discusses the relevance of HSPs in CSC biology, highlighting their role as novel potential molecular targets to develop anticancer strategies aimed at CSC targeting.

Key words: Heat shock proteins, Cancer stem cell, Therapy, Chaperones

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Cancer stem cells

The Cancer Stem Cell (CSC) model suggests that tumors are hierarchically organized with a small subpopulation of cells at their apex, which are the sole drivers of tumor formation and propagation (Medema, 2013). CSCs are characterized by unlimited self-renewal ability, multilineage differentiation potential and long-term tumor repopulation capacity (Rich, 2016) and are responsible for tumor heterogeneity, since they can differentiate into non-tumorigenic intermediate progenitors and terminally differentiated progenies that constitute the complex structure of solid tumors (Clarke et al., 2006; Lettini et al., 2016). There are two possible ways through which CSCs can arise: i) oncogenic mutations and epigenetic modifications occurring within normal stem cells, ii) de-differentiation of fully differentiated cancer cells that acquire stem cell properties (Dean et al., 2005; Zhao et al., 2018). This

theory is opposed to the Stochastic Model, which suggests that tumor growth is a random process to which all cells can contribute. In this alternative view, cancer cells, after accumulating oncogenic mutations, acquire the same potential to form and maintain the tumor mass (Snyder et al., 2018).

However, recent studies suggest that the two models are not mutually exclusive; indeed, CSCs should not be considered as a "fixed state", but as a "flexible entity". This can be explained through the CSC plasticity concept, wherein there is a balance between CSCs and more differentiated cells, which are flexible and interconvertible. Therefore, CSCs differentiate into non-CSCs under some circumstances, and daughter, progenitor, transient and fully differentiated cells could de-differentiate into CSCs (Fig. 1). This dynamic equilibrium could be influenced by processes intrinsic to tumor cells and microenvironmental factors, which further enhance cancer complexity (Batlle and Clevers, 2017; Hermann and Sainz, 2018).

Of note, the interconnection between cancer stem cell features and tumor microenvironment (TME) plays a relevant role in remodeling cancer cell phenotype. Indeed, CSCs are located in a subcellular compartment, called "niche", which includes immune, fibroblastic, endothelial and epithelial cells, as well as extracellular matrix components. The tumorigenic potential and other CSCs features, such as self-renewal, cell differentiation and plasticity, are greatly influenced by the niche complex milieu. CSC niche also plays a pivotal role in cancer metastasis, since it is responsible for EMT, thus increasing the potential of tumor cells to migrate and disseminate in secondary sites (Zhao et al., 2018). Furthermore, stimuli from TME and genetic/epigenetic events play a fundamental part in adaptive drug resistance, a peculiar characteristic of CSCs (Kreso and Dick, 2014). Indeed, CSCs are resistant to apoptosis and anticancer agents and this is likely due to the activation of adaptive mechanisms and survival pathways induced by the unfavorable hypoxic environment of the niche. In such a context, a major role is played by the overexpression of antiapoptotic molecules, like Heat Shock Proteins (HSPs), the activation of DNA repair mechanisms, the upregulation of drug-efflux pumps and the increased protection against reactive oxygen species (ROS) (Sreepadmanabh and Toley, 2018). Thus, traditional therapies successfully target rapidly dividing cells that constitute the bulk of the tumor, but are ineffective against slow-cycling CSCs, which are not eradicated and are therefore responsible for tumor relapse after initial response to therapy (Clarke et al., 2006). Consequently, a major goal of translational research in cancer is to characterize druggable molecular targets to develop selective anticancer agents able to eradicate CSCs. In recent years, several studies focused the attention on HSPs based on the evidence that they are frequently overexpressed in CSCs and play a pivotal role in regulating their functions. This review will focus on the key aspects about HSP function in CSC maintenance and their potential role as molecular targets.

Heat shock proteins

Heat Shock Proteins are molecular chaperones

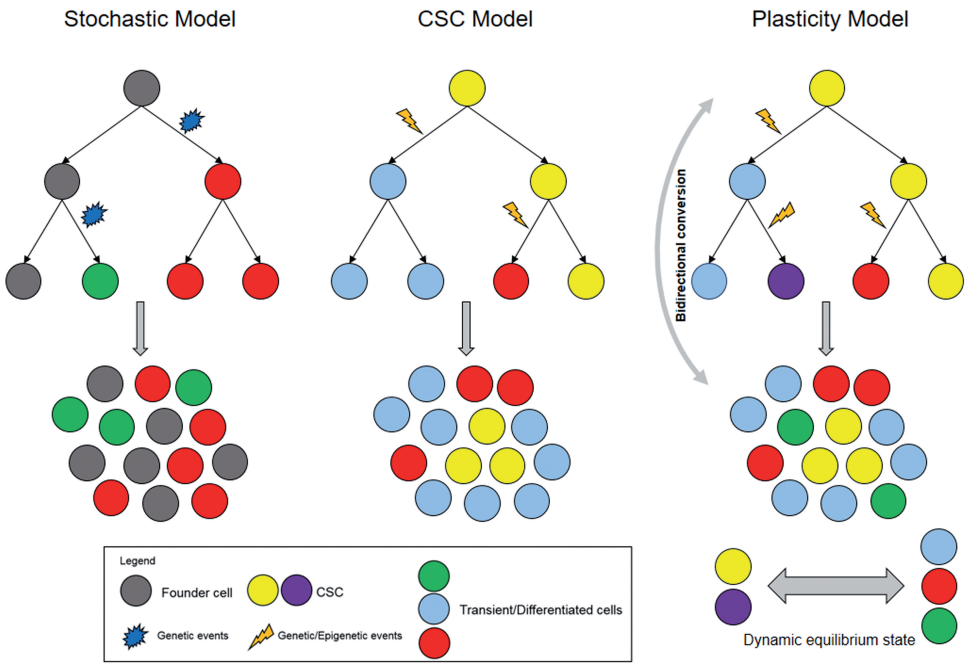

Fig. 1. Representative scheme of Stochastic, Cancer Stem Cells and Plasticity models. The Stochastic Model suggests that all tumor cells are characterized by tumor initiating capacity, thus contributing to tumor growth in a random manner, upon accumulation of oncogenic mutations. By contrast, the Cancer Stem Cell model suggests that tumor cells are hierarchically organized with a small subpopulation at their apex, which are the sole drivers of tumor formation. Cancer Stem Cells can differentiate into transient/differentiated cancer cells, which definitively lose the capacity to self-renew and drive tumor growth. The Plasticity Model is a combined model, in which Cancer Stem Cells undergo clonal evolution and therefore multiple Cancer Stem Cell clones coexist within the tumor. According to this hypothesis, a balance between Cancer Stem Cells and more differentiated cells characterizes heterogeneous tumor populations, with flexible differentiation of Cancer Stem Cells in differentiated cells and viceversa.

involved in many biological processes ensuring normal maturation, localization and activity of intra and extracellular proteins. HSPs were identified in 1962 by F. Ritorsa in *Drosophila melanogaster* (Ritossa, 1963) and later they were characterized as proteins whose expression is strongly induced after exposure to stress conditions, such as heat shock, heavy metals, hypoxia, nutrient deprivation, irradiation, oxidative and toxic stress, infections and inflammatory cytokines (Pirkkala and Sistonen, 2006; Wieten et al., 2007; Khalil et al., 2011).

HSPs in mammals were classified into six families according to molecular weight, sequence, structural and functional homology: HSP100, HSP90, HSP70, HSP60, HSP40 and small HSPs (sHSPs) (Kampinga et al., 2009). High molecular weight HSPs are ATP-dependent molecular chaperones, while sHSPs act in an ATP-independent manner (Kumar et al., 2016). The main functions of HSPs involved in CSCs regulation are schematically reported in Table 1.

Under physiological conditions, HSPs are responsible for protein homeostasis, because they are involved in protein folding and assembly and in preventing the aggregation of damaged proteins (Parcellier et al., 2003; Mayer and Bukau, 2005). Furthermore, HSPs regulate other biological processes including RNA stabilization/translation, protein synthesis and signaling pathways (Mathew and Morimoto, 1998; Schmitt et al., 2007; Izawa et al., 2008). In light of their relevance in cell physiology, an increase in HSP levels and/or activity has been related to the onset of several pathological conditions, such as neurodegenerative and cardiovascular disorders and cancer (Ciocca and Calderwood, 2005; Rehana, 2014). Indeed, HSP upregulation has been described in several malignancies, such as prostate, cervical, ovarian, renal, brain, lung, colorectal, hepatocellular, and breast carcinomas and myeloid leukemia (Ciocca and Calderwood, 2005). Overexpression of HSPs has also been observed in CSCs, likely in order to respond to stress conditions from tumor microenvironment, including hypoxia and pharmacological therapies (Torigoe et al., 2013). In the following paragraphs, the physiological functions of the main HSPs and their role in CSC maintenance are discussed.

HSP90

HSP90 chaperones are essential to maintain cellular proteostasis favoring the correct folding of neo-synthesized proteins and preventing their aggregation (Hoter et al., 2018) in collaboration with other co-chaperones, especially HSP70 (Taipale et al., 2010; Johnson, 2012; Stankiewicz and Mayer, 2012). The HSP90 family consists of four members: i) two HSP90 isoforms called HSP90α (inducible/major form) and HSP90β (constitutive/minor form), localized in cytosol and nucleus (Li et al., 2012); ii) Glucose Regulated Protein 94 (GRP94), localized in Endoplasmic Reticulum (ER) (Marzec et al., 2012); iii) TNF Receptor-Associated Protein 1 (HSP75 or TRAP1), mainly localized in mitochondria, but also in ER (Amoroso et al., 2014) (Table 1). In addition, HSP90α is secreted as a C-terminal truncated form, called extracellular HSP90 (eHSP90), which is located on the external cell surface (Tsutsumi and Neckers, 2007).

HSP90 is a flexible dimer and each monomer consists of three domains: i) the N-terminal ATP-binding domain responsible for the ATPase activity, required to provide energy for chaperone conformational change and interaction with substrate proteins and co-chaperones, ii) the central domain responsible for client protein binding and structural flexibility, and iii) the C-terminal domain containing a conserved pentapeptide sequence (MEEVD) responsible for regulating ATPase activity and presenting the subcellular localization sequence (Trepel et al., 2010).

HSP90 has several hundreds of client proteins, whereby it is a key modulator of important cellular processes. HSP90 chaperone regulates several core cancer hallmarks by performing folding control of specific client proteins and, thus, regulates crucial functions in human carcinogenesis, such as cell proliferation, angiogenesis, metastasis formation, and apoptosis. (Fulda et al., 2010; Taipale et al. 2010). Particularly relevant is the chaperoning activity of HSP90 during malignant transformation and tumor progression, since highly proliferating tumor cells are enriched of many mutated and/or aberrant proteins and molecular chaperones play a crucial role in controlling the folding of these proteins, thus favoring cell

Table 1. Functions and cellular localization of main HSPs involved in CSCs regulation (Source GeneCards).

Family	Members	Localization	Functions
HSP90	HSP90α (HSP86) HSP90β (HSP84)	Cytosol	Maturation, structural maintenance and quality control of client proteins involved in cell cycle and signal transduction
	GRP94 (Endoplasmin)	Endoplasmic Reticulum	Processing and transport of secreted proteins; Ca^{2+} homeostasis
	TRAP1 (HSP75)	Mitochondria/ Endoplasmic Reticulum	Protection of mitochondrial integrity; folding control of client proteins
HSP70	GRP78 (BiP)	Endoplasmic Reticulum	Protein folding and quality control of newly synthesized proteins in Endoplasmic Reticulum
HSP40	DNAJA1	Cytosol, mitochondria	Protein transport into mitochondria
	DNAJB8	Cytosol, nucleus	Suppression of aggregation and toxicity of disease-associated polyglutamine proteins
Small HSP	HSP27 (HSPB1)	Cytosol, nucleus	Stress resistance and prevention of apoptosis

homeostasis (Miyata et al., 2013). Thus, overexpression of HSP90 has been observed in many human cancers (Neckers and Workman, 2012; Kolosenko et al., 2014; Patel et al., 2014; Shi et al., 2014), being frequently associated to poor prognosis (Wang et al., 2013; Su et al., 2016).

GRP94 is characterized by its ER exclusive localization, where it guarantees protein quality control, promoting the folding and assembly of a small number of substrate proteins, such as secreted and membrane proteins. Moreover, GRP94 is involved in Ca^{2+} homeostasis, being able to bind intracellular Ca^{2+}. Taken together, these features make GRP94 extremely important for cell survival (Marzec et al., 2012).

TRAP1 was initially discovered by Song et al. (1995) and characterized as the mitochondrial HSP90 chaperone by Felts et al. (2000). Subsequent reports described TRAP1 as a component of a multiprotein mitochondrial complex containing also HSP90 and Cyclophilin D (CYPD), involved in protection of mitochondrial integrity (Kang et al., 2007) and aimed at protecting cells against apoptosis and evading the toxic effects of oxidants and anticancer drugs (Montesano Gesualdi et al., 2007; Costantino et al., 2009). More recently, some evidence suggests that TRAP1 is also localized in the ER where it interacts with the proteasome regulatory particle TBP7/Rpt3 and other translational factors performing quality control on nascent proteins (Amoroso et al., 2012; Matassa et al., 2013). Thus, it has been proposed that TRAP1 controls the folding of its client proteins by direct protein interaction, mainly in mitochondria (Landriscina et al., 2010; Chae et al., 2013; Sciacovelli et al., 2013), or by a co-translational quality control in the ER, preventing their proteasomal degradation (Amoroso et al., 2012). In such a perspective, TRAP1 plays a crucial role in carcinogenesis, contributing to cell adaptation to the extracellular environment. In fact, the TRAP1 network is involved in drug resistance (Maddalena et al., 2013; Sisinni et al., 2014), regulation of the balance between proliferation and quiescence (Palladino et al., 2016; Sisinni et al., 2017), cell motility (Agliarulo et al., 2015), maintenance of stemness through the modulation of Wnt/β-Catenin pathway (Lettini et al., 2016), and modeling of tumor metabolism (Chae et al., 2013; Sciacovelli et al., 2013; Yoshida et al., 2013; Matassa et al., 2016).

HSP70

The HSP70 family is widely expressed in all organisms from archaebacteria to humans. The human HSP70 family includes at least eight members, differing from each other in subcellular location, amino acid constitution, level and modality of expression in specific tissues. HSP70s are monomeric proteins located in cytosol, nuclei, ER, mitochondria (Lancaster and Febbraio, 2005; Vega et al., 2008), on the surface of exosomes (Vicencio et al., 2015) and outside cells as free soluble forms (Pockley et al., 2014). HSP70 protein presents two domains: a peptide-binding domain (PBD) and an amino-terminal ATPase domain (ABD). The PBD can be further subdivided into a substrate-binding subdomain responsible for protein refolding and a C-terminal subdomain that acts as a 'lid' for the substrate binding pocket. The ABD, in turn, facilitates the release of the client protein after ATP hydrolysis (Saibil, 2013). Mainly, HSP70 proteins support the folding of newly synthesized polypeptides, the transport of proteins over cellular membranes and the building of multiprotein complexes (Jego et al., 2013). HSP70s are upregulated in different pathological conditions, as many human cancer types (Lianos et al., 2015), being able to bind misfolded proteins, prevent their aggregation and promote cancer cell survival, tumorigenicity and antiapoptotic activity (Radons, 2016).

One of the best-characterized HSP70 family chaperones is the 78-kDa Glucose-Regulated Protein (GRP78), which is also called BiP or HSPA5. GRP78 is located in the lumen of ER where it binds newly synthesized proteins, facilitating their assembly or targeting misfolded proteins for ER-associated degradation (Table 1). GRP78 plays a pivotal role in calcium homeostasis and ER stress control; moreover, it presents, together with GRP94, peculiar antiapoptotic properties, due to its ability to prevent ER stress and regulate the activation of URP signaling. (Wang et al., 2009).

HSP60

Mammalian HSP60 is mainly located in mitochondria where it interacts with HSP10 and mitochondrial HSP70 (Mortalin). However, HSP60 is also placed in the cytosol, on the cell surface and in the extracellular space (Cappello et al., 2008). HSP60 monomer consists of a 60-kDa protein subdivided into three domains: apical, intermediate and equatorial domains. Inside the mitochondria, it forms a ring-shaped heptamer. Two rings overlap each other and form a cylinder with a central cavity, where the bending of the synthetizing peptide occurs. HSP10 binds to the apical domain of the double HSP60 ring, creating a closed chamber for polypeptide folding (Cappello et al., 2011). Okamoto et al. showed that mammalian HSP60 mainly exists as a single heptameric ring and, in presence of ATP, it is converted into a tetradecameric double-ring structure (Okamoto et al., 2017). In normal conditions, HSP60 controls the transport and the folding of mitochondrial proteins, but it is reported that HSP60 mutation or abnormal expression are associated with various pathological conditions, such as inflammation, immune reactions, autoimmune diseases (Hjelholt et al., 2013; Tonello et al., 2015; Bross and Fernandez-Guerra, 2016) and different tumor types, including lung, breast, colon, cervix carcinomas and acute myeloid leukemia (Hanahan and Weinberg, 2011; Meng et al., 2018). The role of HSP60 in tumor cells is related to prevention of

apoptosis, by facilitating the stabilization of Survivin and the restraining of p53 function (Ghosh et al., 2008), and the promotion of metastasis by interaction with β-Catenin (Tsai et al., 2009).

HSP40

HSP40 family, also known as DnaJ chaperones, is a group of co-chaperones characterized by the presence of the conserved J-domain, which plays a fundamental role in regulating the ATPase activity of HSP70. HSP40 function depends on different mechanisms: i) they act, together with HSP70, as co-receptors of HSP90, thus, cooperating in the folding of HSP90 client proteins; ii) they directly bind their client proteins; iii) they induce the HSP70 folding function (Kampinga and Craig, 2010). The protein consists of three domains: the N-terminal domain is the J domain that interacts with HSP70, the central domain is implicated in substrate binding and the C-terminal domain has the real function of chaperone. Considering their architecture, HSP40s are classified in three main subfamilies: DNAJAs, DNAJBs, and DNAJCs based on the presence or absence of conserved domains defined by the canonical *Escherichia coli* DNAJ (Cheetham and Caplan, 1998). HSP40s are localized in cytosol, nuclei, endosomes, exosomes, mitochondria, ribosomes, and ER and are implicated in various human diseases, including microbial infections, autoimmune and neurodegenerative disorders, and cancer (Mitra et al., 2009; Lianos et al., 2015). DNAJB4, DNAJB6, and DNAJC15 are overexpressed in, respectively, lung, breast and ovary cancer (De Bessa et al., 2006; Tsai et al., 2006). DNAJC6 is also up-regulated in human hepatocellular carcinoma where it promotes tumor progression by induction of EMT (Yang et al., 2014).

Small HSPs

Small HSPs are ATP-independent molecular chaperones, ubiquitously distributed in numerous species, from bacteria to humans and are characterized by a low molecular mass (<43 kDa) and are located in different cell compartments. The human sHSP family contains ten members: HSP27 (HSPB1, HSP25), myotonic dystrophy protein kinase-binding protein (MKBP, HSPB2), HSPB3, alphaA-crystallin (HSPB4), alphaB-crystallin (HSPB5), HSP20 (p20, HSPB6), cardiovascular heat shock protein (cvHSP, HSPB7), HSP22 (HSPB8), HSPB9 and HSPB10. Some of them are ubiquitously expressed, while others are restricted to specific tissues (Zeng et al., 2013). The protein presents three domains: a flexible N-terminal region (NTR) of variable length and sequence, a structured a-crystallin domain (ACD) that is conserved among all family members and a short C-terminal region (CTR) that is involved in stabilizing the oligomer (Kriehuber et al., 2010; Bar-Lavan et al., 2016). Many sHSPs form, through the interaction with themselves or other sHSPs, homo or hetero-oligomers containing up to 50 subunits. (McDonald et al., 2012; Mainz et al., 2015). The most important function of sHSP is to prevent the aggregation of proteins and polypeptides in response to specific stress conditions (Bakthisaran et al., 2015). Moreover, sHSP phosphorylation modulates a variety of cellular functions, such as apoptosis, cell cycle and differentiation (Garrido et al., 2012; Bakthisaran et al., 2015). The expression of various sHSP is upregulated in several pathological disorders and/or mutant sHSP have been associated to specific pathological conditions including cancer, neurological diseases, myopathies, and multiple sclerosis (Haslbeck et al., 2019).

HSP and cancer stem cell

The scientific community focused its attention on the correlation between CSCs and HSPs in cancer initiation and progression based on the assumption that HSPs are central in CSC biology and potentially relevant for more effective therapeutic interventions. Indeed, a strong rationale points out that HSPs play a central role in the regulation of several processes in cancer cells and are likely involved in CSC maintenance. Of note, different tumor types are characterized by increased HSP expression with parallel upregulation of their network of client/related proteins (Calderwood and Gong, 2016). The increased activity of these molecular networks allows the maintenance of cancer cell viability, this representing the prerequisite to consider HSPs an excellent target for cancer therapy (Ciocca and Calderwood, 2005). This concept is particularly relevant in the perspective of CSC biology, since these cell populations are resistant to conventional anticancer agents and maintain their stem phenotype under stressful conditions. In such a view, several studies reported the overexpression of different HSP family proteins in CSCs as a mechanism to upregulate the apoptotic threshold, survive in a hostile environment and escape from anticancer therapies (Hsu et al., 2011; Chiu et al., 2013; Lee et al., 2017) (Table 2).

Furthermore, HSPs play a pivotal role in regulating the plasticity of CSCs in response to different environmental conditions (Torigoe et al., 2013). Indeed, several reports demonstrated a key regulatory function for HSPs in CSC metabolic remodeling and suggested that HSP upregulation may provide a mechanism to adapt to unfavorable microenvironments, such as nutrient deprivation, hypoxia, and inflammatory stimuli (Liu et al., 2019). In particular, recent evidence suggests that cancer cells are able to remodel the balance between glycolytic and oxidative metabolism depending on the cell type and the extracellular environment. While breast CSCs are characterized by a prevalent glycolytic metabolism (Dong et al., 2013; Ciavardelli et al, 2014), other types of tumors, including pancreatic cancer and leukemia, rely mostly on oxidative phosphorylation for cancer progression (Lagadinou et al., 2013; Sancho et al., 2015). This is particularly relevant for CSCs that are able

to shift from one metabolic pathway to another in response to environmental stimuli (Deshmukh et al., 2016; Sancho et al., 2016). Thus, HSP upregulation could be an effective strategy to favor CSCs plasticity and metabolic remodeling, self-renewal, tumor-initiating ability, EMT, chemoresistance and, based on this rationale, HSP pharmacological inhibition may represent a strategy to target these specific CSCs features (Table 2).

GRP78 and GRP94

GRP78 and GRP94 are ER chaperones implicated in cytoprotective functions (Luo and Lee, 2013) and protection from ER stress (Zhu and Lee, 2015). GRPs are involved in tumor proliferation, invasion and resistance to anticancer therapy, indeed these molecules are overexpressed in many types of cancers such as breast, colorectal, gastric, lung, and pancreatic carcinomas (Lee, 2014).

Interestingly, recent studies indicated that GRPs play a pivotal role in stem cell biology, since they are involved in mammary gland development (Zhu et al., 2014) and are important regulators of hematopoietic stem cell homeostasis (Wey et al., 2012; Luo et al., 2013). In particular, GRP78 is fundamental for embryonic cell growth and pluripotent cell survival in a murine model (Luo et al., 2006).

The correlation between GRPs and CSC has been highlighted by several studies in different cancer models (Table 2). GRP78 and GRP94 are essential for increased chemo/radioresistance, an intrinsic character of CSCs. Indeed, Bartkowiak et al. demonstrated that GRP78 and GRP94 are upregulated in breast cancer disseminated cells (Bartkowiak et al., 2010), whereas GRP78 mediates resistance to radiation in a breast cancer stem cell-like subpopulation (Li et al., 2013). Furthermore, the same authors reported that GRP78 is significantly increased in breast CSCs and its silencing enhances the sensitivity to radiation treatment (Li et al., 2013). Consistently, other reports suggest that breast cancer cells with high CD44 and low CD24 expression ($CD44^+/CD24^-$) possess tumor-initiating capacity with stem cell-like characters (Ricardo et al., 2011) and that GRP78 and GRP94 are overexpressed in this stem cell breast cancer subpopulation compared to their original cell lines (Nami et al., 2016).

GRP78 plays a crucial role in head and neck cancer stem cell biology. It is overexpressed at the plasma membrane of head and neck cancer initiating cells (CIC) (Wu et al., 2010) and in $CD44^+/CD24^-$-sorted cell lines for which GRP78 is relevant for stemness properties (Chiu et al., 2013). Indeed, $GRP78^+/CD44^+/CD24^-$ cells show increased chemo-radioresistance and invasiveness compared to parental cells and this cell subpopulation exhibits a pronounced EMT phenotype and highest tumorigenic potential, as demonstrated by sphere formation and xenograft tumor growth. The mechanistic relevance of GRP78 in the regulation of CSC phenotype

Table 2. Role of HSPs in CSC regulation.

HSP	Tumor Type	Role in CSCs	References
GRP78	Breast	Overexpression in CSCs	Bartkoviak et al. 2010; Nami et al. 2016
		Radioresistance	Li et al. 2013
	Head and Neck	Self-renewal, radioresistance, tumorigenicity	Wu et al. 2010
		Stemness conversion, chemoradioresistance, invasiveness, EMT, tumorigenicity	Chiu et al. 2013
	Ovary	Tumorigenicity	Mo et al. 2015
GRP94	Breast	Overexpression in CSC	Bartkoviak et al. 2010; Nami et al. 2016
HSP27	Lung	Chemoresistance, stemness marker expression	Hsu et al. 2011
	Prostate	Clonogenic ability, cell viability, invasiveness	Tang et al. 2010
	Colon	Apoptosis protection	Lin et al. 2012
	Breast	Stemness properties	Wei et al. 2011
	Ovary	Stemness marker expression, tumorigenicity	Yasuda et al. 2017
HSP40 (DNAJB8)	Colon	Stemness marker expression, tumor-initiating ability	Morita et al. 2014
	Renal, Colon, Breast, Lung	Stemness marker expression, tumorigenicity	Nishizawa et al. 2012
HSP90	Lymphoma	HIF1α stability	Newman et al. 2012
	Leukemia	Cell viability	Peng et al. 2007
	Thyroid	Stemness marker expression, clonogenic ability	White et al. 2016
	Head and Neck	Stemness marker expression, EMT	Subramanian et al. 2017
	Breast	Self-renewal	Lee et al. 2017
		Stemness marker expression, clonogenic ability	Stivarou et al. 2016
	Lung	Cell viability	Le et al. 2018
	Prostate	Self-renewal, stemness marker expression, tumor-initiating ability	Nolan et al. 2017
HSP75 (TRAP1)	Glioblastoma	Drug resistance, clonogenic ability	Wu et al. 2016
	Colon	Stemness marker expression, clonogenic ability, Wnt/β-Catenin pathway regulation	Lettini et al. 2016

was proven by knockdown experiments, in which GRP78 silencing restored the parental phenotype (Chiu et al., 2013). Finally, Mo et al. demonstrated that murine ovarian cancer stem-like cells exhibit higher levels of plasma membrane GRP78 (^{mem}GRP78) than control cells, showing increased tumorigenic ability. Intriguingly, pharmacological treatment with antibodies directed against the C-terminal domain of GRP78 suppresses sphere formation and tumor growth *in vitro* and *in vivo* (Mo et al., 2015). Altogether, this evidence suggests that GRP targeting deserves to be evaluated as a potential therapeutic strategy for eradication of refractory CSCs.

HSP27

HSP27 is a molecular chaperone belonging to the sHSP family, overexpressed in a broad range of cancer types and involved in tumorigenicity, chemoresistance (Concannon et al., 2003; Straume et al., 2012), cell invasiveness and poor patient survival (Bauer et al., 2012; Ciocca et al., 2013). The cytoprotective function of HSP27 is primarily related to its role in apoptosis regulation (Table 1). Indeed, HSP27 prevents apoptosis through sequestration of cytochrome c and inhibition of caspase pathway (Bruey et al., 2000; Pandey et al., 2000).

The implication of HSP27 in CSCs regulation is still under evaluation (Table 2). Previous reports suggest that HSP27 is upregulated in lung CSCs, where it is involved in drug resistance. Its pharmacological inhibition by Quercetin enhanced chemotherapy activity, as well as promoting the downregulation of stemness genes, such as *Oct4, Nanog* and *Sox2* (Hsu et al., 2011). Moreover, HSP27 inhibition by Quercetin impaired spheroid formation, cell viability and invasion in prostate CSCs (Tang et al., 2010). Consistently, Lin et al. demonstrated that colon CSCs ($CD133^+$) exhibit increased levels of HSP27 and the constitutive activation of HSP27 network induces resistance to apoptosis in this cell population. Of note, HSP27 inhibition, by siRNA or chemical agents, favors the sensitization of CSCs to hypoxia and serum depletion (Lin et al., 2012). HSP27 increased levels were confirmed in breast CSCs, selected by ALDH positive expression, where the chaperone is involved in regulation of NF-κB pathway. Intriguingly, HSP27 inhibition impaired stemness properties, including mammosphere formation and EMT features in $ALDH^+$ population (Wei et al., 2011). Finally, Yasuda et al. showed that HSP27 is overexpressed in gynecological CSCs and HSP27 knockdown results in reduction of $ALDH^+$ cells and sphere formation (Yasuda et al., 2017). In the perspective to use HSP inhibitors in clinic, it has been suggested that HSP27 inactivation could be useful in combination with HSP90 inhibitors, in order to lower doses of HSP90 targeting agents and reduce their typical toxicity (Lee et al., 2012).

HSP40

The role of HSP40 in CSC regulation is still challenged, even though several members of this family are overexpressed in breast CSCs (Table 2), such as DNAJA1, DNAJB1, DNAJC9, DNAJC10, DNAJC12 (Sterrenberg et al., 2011). In addition, DNAJA1 expression was associated with malignancy and radio-resistance in glioblastoma (Wang et al., 2006).

Subsequent reports focused their attention on the peculiarity of DNAJB8, member 8 of DNAJ subfamily B, which is expressed predominantly in colorectal CSCs (Table 2). Indeed, Morita et al. showed that DNAJB8 expression was greater in side population (SP) cells derived from SW480, HCT15 and HT29 cells with respect to parental cells. Moreover, DNAJB8-overexpressing HT29 cells exhibited higher percentage of SP cells and higher expression levels of stem cell markers, such as SOX2, LGR5 and POU5F1 compared to control HT29 cells. Finally, this cell subpopulation displayed greater sphere forming and tumor-initiating ability in *in vitro* and *in vivo* models than parental cells (Morita et al., 2014). Consistently, Nishizawa et al. reported that DNAJB8 is preferentially expressed in SP cells derived from renal, colon, breast and lung carcinoma. Indeed, this study demonstrated the central role of DNAJB8 in CSC regulation: DNAJB8-transduced cells exhibited higher percentage of SP cells and increased tumor-initiating ability compared to parental cells. Of note, DNAJB8 silencing significantly reduced SP cells and tumorigenicity (Nishizawa et al., 2012).

HSP90

Increasing evidence indicates that HSP90 plays a key role in stemness maintenance in cancer (Table 2). In fact, Newman et al. showed that the HSP90 inhibitor 17-AAG eradicates lymphoma CSCs *in vitro* and *in vivo* by disrupting the transcriptional function of HIF1α, a client protein of HSP90 (Newman et al., 2012). Moreover, HSP90 inhibition by IPI-504 treatment significantly decreased the number of leukemia stem cells (Peng et al., 2007). White et al. demonstrated that novel HSP90 inhibitors, such as WGA-TA and KU711, promote reduction of CD44 and β-Catenin expression, decrease ALDH activity and impair thyrosphere formation in thyroid cancer cell lines (White et al, 2016). Consistently, another research group reported that KU711 and KU757 treatment inhibits orosphere formation, paralleled by downregulation of CD44 and BMI1 expression, ALDH activity and EMT features (Subramanian et al., 2017).

In addition, Lee et al. suggested that HSP90α could positively regulate self-renewal in breast CSCs by facilitating the nuclear translocation of c-Myc and EZH2 to maintain BMI1 expression. Of note, the authors demonstrated that 17-DMAG, an HSP90 inhibitor, suppresses self-renewal of breast CSCs through BMI1 downregulation (Lee et al., 2017). A recent paper reported that Panaxynol, a natural active principle derived from Panax Ginseng, disrupts HSP90 function

by binding to N-terminal and C-terminal ATP-binding pockets without increasing HSP70. Low concentrations of Panaxynol selectively inhibit CSC population of NSCLC cells by inducing apoptosis (Le et al., 2018).

Finally, Nolan et al. reported a novel function for extracellular HSP90 (eHSP90) as a mediator of stemness maintenance in prostate cancer models, based on its ability to upregulate stem-like markers, promote self-renewal, and enhance prostasphere growth (Nolan et al., 2017). In such a scenario, $CD44^+/CD24^-$ mammosphere showed increased levels of eHSP90 in parallel to stemness marker expression including CD49f and SOX2. Furthermore, selective inhibition of HSP90 by monoclonal antibodies decreased mammosphere formation *in vitro* and mammosphere-derived tumors *in vivo* (Stivarou et al., 2016). Taken as a whole, these reports indicated that HSP90 is a key regulator of CSC features, and therapeutic strategies based on its inhibition could be relevant in clinical approach.

TRAP1

Some evidence suggests that HSP75 protein (TRAP1) is involved in the maintenance or regulation of stemness in cancer (Im, 2016). First, many reports demonstrated that TRAP1 is upregulated in human malignancies (Amoroso et al., 2014) and its levels correlate with a drug-resistant phenotype (Costantino et al., 2009; Maddalena et al., 2013; Sisinni et al., 2014; Wu et al., 2016) and poor clinical outcome (Gao et al., 2012, Maddalena et al., 2017). Moreover, TRAP1 plays a role in regulation of migration/invasion of cancer cells (Matassa et al., 2014; Agliarulo et al., 2015) and TRAP1 inhibition impaired glioblastoma (GBM) cells proliferation/migration and decreased neurosphere formation (Wu et al., 2016). Furthermore, since most tumors preferentially use aerobic glycolysis to support cellular growth, downregulation of TRAP1 sensitized GBM cells to temozolomide chemotherapy through metabolic reprogramming (Wu et al., 2016) (Table 2).

TRAP1 contributes to mitochondrial bioenergetics, favoring a context-dependent reprogramming of cancer cell metabolism with suppression of oxidative metabolism and enhancement of glycolysis (Chae et al., 2013; Sciacovelli et al., 2013; Yoshida et al., 2013). Intriguingly, Kadye et al. suggested that TRAP1-mediated modulation of mitochondrial activity might be crucial in stem cell maintenance, survival and differentiation (Kadye et al. 2014). In such a context, our research group demonstrated, for the first time, that TRAP1 regulates CSC phenotype by modulating Wnt/β-Catenin pathway in human colorectal carcinomas (CRCs). Indeed, TRAP1 is preferentially expressed by intestine stem cells and CSCs isolated from CRC cells, being co-expressed with CSC markers (CD44, CD133, CD166), and it is responsible for the clonogenic ability of CRC cells. Moreover, TRAP1 modulates the expression of components of Wnt/β-Catenin pathway, and its silencing results in attenuation of Wnt/β-Catenin signaling and in loss of the stem-like signature with gain of a more differentiated phenotype. Mechanistically, TRAP1 maintains the stem phenotype through the regulation of β-Catenin ubiquitination/phosphorylation, being able to interact with β-Catenin and prevent its phosphorylation and degradation (Lettini et al., 2016). Altogether, this evidence suggests that TRAP1 plays a pivotal role in CSC maintenance and supports the concept that TRAP1 inhibition could be a strategy to target CSCs in human malignancies.

Conclusions

The identification of molecular pathways that control CSC biology is essential for developing novel and effective therapeutic anticancer strategies. In such a view, the notion that HSPs play a pivotal role in CSC regulation/maintenance is an emerging concept that might provide novel targets for innovative therapeutic approaches. Indeed, many HSPs are overexpressed in CSCs of a variety of human tumors and their upregulation is likely to be involved in several functions of CSCs, such as protection from apoptosis and drug resistance, cell plasticity, bioenergetics, invasiveness, EMT, metastasis formation (Tang et al., 2010; Sterrenberg et al., 2011; Li et al., 2013; White et al., 2016) (Table 2). Based on this rationale, HSP inhibition has been proposed, among others, as a strategy to design and deliver anticancer therapies focused on CSC targeting. In a clinical perspective, this issue is extremely relevant since CSCs are resistant to apoptosis and are not eradicated by current treatments that target mostly differentiated cancer cells, which constitute the bulk of the tumor (Nguyen et al., 2012). Thus, drug-resistant CSCs are likely responsible for tumor recurrence after initial response to therapy (Reya et al., 2001).

In this contest, we above described several preclinical studies, in which HSP inhibitors have been used to target CSCs and to induce loss of peculiar CSC features, such as self-renewal, stem marker expression, tumor-initiating ability, chemoresistance and EMT (Table 2). These reports provide the molecular rationale that CSCs might be sensitive to HSP inhibition, thus offering a window of vulnerability in terms of effective and innovative tumor treatments. Intriguingly, targeting HSP proteins could achieve the goal to eradicate CSCs, avoiding tumor relapse and safeguarding healthy normal tissue.

Recently, in this regard, researchers developed several HSP inhibitors, such as natural/synthetic compounds, peptides, antibodies and vaccines (Wu et al., 2017). Despite the efficacy exhibited by some HSP inhibitors in pre-clinical and clinical studies, none of them was approved by FDA for routine clinical practice. The reasons for failure of HSP inhibitors use in patient treatment are various. First, HSP inhibitors cause side effects, like severe organ-specific toxicities (i.e., liver or ocular toxicity). Second, the current generation of HSP

inhibitors lacks specificity, causing contemporary impairment of multiple HSP proteins. This strategy affects the activity of a broad range of client proteins, indiscriminately causing several side effects in treated patients. Third, HSP inhibitions can severely affect normal cells due to the key role played by HSPs in cellular physiology. Finally, some HSP inhibitors promote a cellular compensatory effect that induce the upregulation of other HSP members. For example, HSP90 inhibition induces overexpression of HSP27, HSP70 and other HSPs (Wu et al., 2017). Thus, major challenges in this research field are i) to design novel HSP inhibitors to improve the specificity toward target proteins and ii) to increase the accuracy of drug delivery in specific subcellular compartments, making these anticancer agents more efficacious and less toxic. In this perspective, the recent design/development of novel paralog/isoform selective HSP inhibitors is likely to improve their anticancer activity and minimize their toxicity profile, thus enhancing the chance to obtain a novel class of anticancer agents (Olotu et al., 2018).

Finally, considering the complexity of the HSP biology and the cell- and context-dependent role played by different HSPs in human malignancies, it is crucial to improve our knowledge about molecular mechanisms and functions of HSPs in CSCs. In the era of personalized medicine, this issue is extremely relevant to develop novel strategies for tumor selection, based on novel biomarkers and/or gene signatures, as prerequisite for the development of CSC targeted strategies.

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References

Agliarulo I., Matassa D.S., Amoroso M.R., Maddalena F., Sisinni L., Sepe L., Ferrari M.C., Arzeni D., Avolio R., Paolella G., Landriscina M. and Esposito F. (2015). TRAP1 controls cell migration of cancer cells in metabolic stress conditions: Correlations with AKT/p70S6K pathways. Biochim. Biophys. Acta 1853, 2570-2579.

Amoroso M.R., Matassa D.S., Laudiero G., Egorova A.V., Polishchuk R.S., Maddalena F., Piscazzi A., Paladino S., Sarnataro D., Garbi C., Landriscina M. and Esposito F. (2012). TRAP1 and the proteasome regulatory particle TBP7/Rpt3 interact in the endoplasmic reticulum and control cellular ubiquitination of specific mitochondrial proteins. Cell Death Differ. 19, 592-604.

Amoroso M.R., Matassa D.S., Sisinni L., Lettini G., Landriscina M. and Esposito F. (2014). TRAP1 revisited: novel localizations and functions of a 'next-generation' biomarker (review). Int. J. Oncol. 45, 969-977.

Bakthisaran R., Tangirala R. and Rao Ch.M. (2015). Small heat shock proteins: Role in cellular functions and pathology. Biochim. Biophys. Acta 1854, 291-319.

Bar-Lavan Y., Shemesh N. and Ben-Zvi A. (2016). Chaperone families and interactions in metazoa. Essays Biochem. 60, 237-253.

Bartkowiak K., Effenberger K.E., Harder S., Andreas A., Buck F., Peter-Katalinic J., Pantel K. and Brandt B.H. (2010). Discovery of a novel unfolded protein response phenotype of cancer stem/progenitor cells from the bone marrow of breast cancer patients. J. Proteome Res. 9, 3158-3168.

Batlle E. and Clevers H. (2017). Cancer stem cells revisited. Nat. Med. 23, 1124-1134.

Bauer K., Nitsche U., Slotta-Huspenina J., Drecoll E., Von Weyhern C.H., Rosenberg R., Höfler H. and Langer R. (2012). High HSP27 and HSP70 expression levels are independent adverse prognostic factors in primary resected colon cancer. Cell Oncol. (Dordr). 35, 197-205.

Bross P. and Fernandez-Guerra P. (2016). Disease-associated mutations in the HSPD1 gene encoding the large subunit of the mitochondrial Hsp60/Hsp10 chaperonin complex. Front. Mol. Biosc. 3, 49.

Bruey J.M., Ducasse C., Bonniaud P., Ravagnan L., Susin S.A., Diaz-Latoud C., Gurbuxani S., Arrigo A.P., Kroemer G., Solary E. and Garrido C. (2000). HSP27 negatively regulates cell death by interacting with cytochrome C. Nat. Cell Biol. 2, 645-652.

Calderwood S.K. and Gong J. (2016). Heat shock proteins promote cancer: It's a protection racket. Trends Biochem. Sci. 41, 311-323.

Cappello F., Conway de Macario E., Marasà L., Zummo G. and Macario A.J. (2008). HSP60 expression, new locations, functions and perspectives for cancer diagnosis and therapy. Cancer Biol. Ther. 7, 801-809.

Cappello F., David S., Peri G., Farina F., Conway de Macario E.C., Macario A.J. and Zummo G. (2011). HSP60: molecular anatomy and role in colorectal cancer diagnosis and treatment. Front. Biosci. (Schol Ed). 3, 341-351.

Chae Y.C., Angelin A., Lisanti S., Kossenkov A.V., Speicher K.D., Wang H., Powers J.F., Tischler A.S., Pacak K., Fliedner S., Michalek R.D., Karoly E.D., Wallace D.C., Languino L.R., Speicher D.W. and Altieri D.C. (2013). Landscape of the mitochondrial Hsp90 metabolome in tumours. Nat. Commun. 4, 2139.

Cheetham M.E. and Caplan A.J. (1998). Structure, function and evolution of DNAJ: conservation and adaptation of chaperone function. Cell Stress Chaperones. 3, 28-36.

Chiu C.C., Lee L.Y., Li Y.C., Chen Y.J., Lu Y.C., Li Y.L., Wang H.M., Chang J.T. and Cheng A.J. (2013). GRP78 as a therapeutic target for refractory head-neck cancer with CD24(-)CD44(+) stemness phenotype. Cancer Gene Ther. 20, 606-615.

Ciavardelli D., Rossi C., Barcaroli D., Volpe S., Consalvo A., Zucchelli M., De Cola A., Scavo E., Carollo R., D'Agostino D., Forlì F., D'Aguanno S., Todaro M., Stassi G., Di Ilio C., De Laurenzi V. and Urbani A. (2014). Breast cancer stem cells rely on fermentative glycolysis and are sensitive to 2-deoxyglucose treatment. Cell Death Dis. 5, e1336.

Ciocca D.R. and Calderwood S.K. (2005). Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications. Cell Stress Chaperones 10, 86-103.

Ciocca D.R., Arrigo A.P. and Calderwood S.K. (2013). Heat shock proteins and heat shock factor 1 in carcinogenesis and tumor development: an update. Arch. Toxicol. 87, 19-48.

Clarke M.F., Dick J.E., Dirks P.B., Eaves C.J., Jamieson C.H., Jones D.L., Visvader J., Weissman I.L. and Wahl G.M. (2006). Cancer stem cells--perspectives on current status and future directions: AACR Workshop on cancer stem cells. Cancer Res. 66, 9339-9344.

Concannon C.G., Gorman A.M. and Samali A. (2003). On the role of Hsp27 in regulating apoptosis. Apoptosis. 8, 61-70.

Costantino E., Maddalena F., Calise S., Piscazzi A., Tirino V., Fersini A.,

Ambrosi A., Neri V., Esposito F. and Landriscina M. (2009). TRAP1, a novel mitochondrial chaperone responsible for multi-drug resistance and protection from apoptotis in human colorectal carcinoma cells. Cancer Lett. 279, 39-46.

De Bessa S.A., Salaorni S., Patrao D.F., Neto M.M., Brentani M.M. and Nagai M.A. (2006). JDP1 (DNAJC12/Hsp40) expression in breast cancer and its association with estrogen receptor status. Int. J. Mol. Med. 17, 363-367.

Dean M., Fojo T. and Bates S. (2005). Tumour stem cells and drug resistance. Nat. Rev. Cancer 5, 275-284.

Deshmukh A., Deshpande K., Arfuso F., Newsholme P. and Dharmarajan A. (2016). Cancer stem cell metabolism: a potential target for cancer therapy. Mol. Cancer 15, 69.

Dong C., Yuan T., Wu Y., Wang Y., Fan T.W., Miriyala S., Lin Y., Yao J., Shi J., Kang T., Lorkiewicz P., St Clair D., Hung M.C., Evers B.M. and Zhou B.P. (2013). Loss of FBP1 by Snail-mediated repression provides metabolic advantages in basal-like breast cancer. Cancer Cell 23, 316-331.

Felts S.J., Owen B.A., Nguyen P., Trepel J., Donner D.B. and Toft D.O. (2000). The hsp90-related protein TRAP1 is a mitochondrial protein with distinct functional properties. J. Biol. Chem. 275, 3305-3312.

Fulda S., Galluzzi L. and Kroemer G. (2010). Targeting mitochondria for cancer therapy. Nat. Rev. Drug Discov. 9, 447-464.

Gao J.Y., Song B.R., Peng J.J. and Lu Y.M. (2012). Correlation between mitochondrial TRAP-1 expression and lymph node metastasis in colorectal cancer. World J. Gastroenterol. 18, 5965-5971.

Garrido C., Paul C., Seigneuric R. and Kampinga H.H. (2012). The small heat shock proteins family: the long forgotten chaperones. Int J. Biochem. Cell Biol. 44, 1588-1592.

Ghosh J.C., Dohi T., Kang B.H. and Altieri D.C. (2008). Hsp60 regulation of tumor cell apoptosis J. Biol. Chem. 283, 5188-5194.

Hanahan D. and Weinberg R.A. (2011). Hallmarks of cancer: the next generation. Cell 144, 646-674.

Haslbeck M., Weinkauf S. and BuchnerJ. (2019). Small heat shock proteins: Simplicity meets complexity. J. Biol. Chem. 294, 2121-2132

Hermann P.C. and Sainz B. Jr (2018). Pancreatic cancer stem cells: A state or an entity? Semin. Cancer Biol. 53, 223-231.

Hjelholt A., Carlsen T., Deleuran B., Jurik A.G., Schiøttz-Christensen B., Christiansen G. and Birkelund S. (2013). Increased levels of IgG antibodies against human HSP60 in patients with spondyloarthritis. PLoS One 8, e56210.

Hoter A., El-Sabban M.E. and Naim H.Y. (2018). The HSP90 family: Structure, regulation, function, and implications in health and disease. Int. J. Mol. Sci. 19, E2560.

Hsu H.S., Lin J.H., Huang W.C., Hsu T.W., Su K., Chiou S.H., Tsai Y.T. and Hung S.C. (2011). Chemoresistance of lung cancer stemlike cells depends on activation of Hsp27. Cancer 117, 1516-1528.

Im C.N. (2016). Past, present, and emerging roles of mitochondrial heat shock protein TRAP1 in the metabolism and regulation of cancer stem cells. Cell Stress Chaperones 21, 553-562.

Izawa S., Kita T., Ikeda K. and Inoue Y. (2008). Heat shock and ethanol stress provoke distinctly different responses in 3'-processing and nuclear export of HSP mRNA in *Saccharomyces cerevisiae*. Biochem J. 414, 111-119.

Jego G., Hazoumé A., Seigneuric R. and Garrido C. (2013). Targeting heat shock proteins in cancer. Cancer Lett. 332, 275-285.

Johnson J.L. (2012). Evolution and function of diverse Hsp90 homologs and cochaperone proteins. Biochim. Biophys. Acta 1823, 607-613.

Kadye R., Kramer A.H., Joos-Vandewalle J., Parsons M., Njengele Z., Hoppe H. and Prinsloo E. (2014). Guardian of the furnace: mitochondria, TRAP1, ROS and stem cell maintenance. IUBMB Life. 66, 42-45.

Kampinga H.H. and Craig E.A. (2010). The HSP70 chaperone machinery: J proteins as drivers of functional specificity. Nat. Rev. Mol. Cell Biol. 11, 579-592.

Kampinga H.H., Hageman J., Vos M.J., Kubota H., Tanguay R.M., Bruford E.A., Cheetham M.E., Chen B. and Hightower L.E. (2009). Guidelines for the nomenclature of the human heat shock proteins. Cell Stress Chaperones 14, 105-111.

Kang B.H., Plescia J., Dohi T., Rosa J., Doxsey S.J. and Altieri D.C. (2007). Regulation of tumor cell mitochondrial homeostasis by an organelle-specific Hsp90 chaperone network. Cell 131, 257-270.

Khalil A.A., Kabapy N.F., Deraz S.F. and Smith C. (2011). Heat shock proteins in oncology: diagnostic biomarkers or therapeutic targets? Biochim. Biophys. Acta 1816, 89-104.

Kolosenko I., Grander D. and Tamm K.P. (2014). IL-6 activated JAK/STAT3 pathway and sensitivity to Hsp90 inhibitors in multiple myeloma. Curr. Med. Chem. 21, 3042-3047.

Kreso A. and Dick J.E. (2014). Evolution of the cancer stem cell model. Cell Stem Cell 14, 275-291.

Kriehuber T., Rattei T., Weinmaier T., Bepperling A., Haslbeck M. and Buchner J. (2010). Independent evolution of the core domain and its flanking sequences in small heat shock proteins. FASEB J. 24, 3633-3642.

Kumar S., Stokes J. 3rd, Singh U.P., Scissum Gunn K., Acharya A., Manne U and Mishra M. (2016). Targeting Hsp70: A possible therapy for cancer. Cancer Lett. 374, 156-166.

Lagadinou E.D., Sach A., Callahan K., Rossi R.M., Neering S.J., Minhajuddin M., Ashton J.M., Pei S., Grose V., O'Dwyer K.M., Liesveld J.L., Brookes P.S., Becker M.W. and Jordan C.T. (2013). BCL-2 inhibition targets oxidative phosphorylation and selectively eradicates quiescent human leukemia stem cells. Cell Stem Cell 12, 329-341.

Lancaster G.I. and Febbraio M.A. (2005). Exosome-dependent trafficking of HSP70: a novel secretory pathway for cellular stress proteins. J. Biol. Chem. 280, 23349-23355.

Landriscina M., Laudiero G., Maddalena F., Amoroso M.R., Piscazzi A., Cozzolino F., Monti M., Garbi C., Fersini A., Pucci P. and Esposito F. (2010). Mitochondrial chaperone Trap1 and the calcium binding protein Sorcin interact and protect cells against apoptosis induced by antiblastic agents. Cancer Res. 70, 6577-6586.

Le H.T., Nguyen H.T., Min H.Y., Hyun S.Y., Kwon S., Lee Y., Le T.H.V, Lee J., Park J.H. and Lee H.Y. (2018). Panaxynol, a natural Hsp90 inhibitor, effectively targets both lung cancer stem and non-stem cells. Cancer Lett. 412, 297-307.

Lee A.S. (2014). Glucose-regulated proteins in cancer: molecular mechanisms and therapeutic potential. Nat. Rev. Cancer 14, 263-276.

Lee C.H., Hong H.M., Chang Y.Y. and Chang W.W. (2012). Inhibition of heat shock protein (Hsp) 27 potentiates the suppressive effect of Hsp90 inhibitors in targeting breast cancer stem-like cells. Biochimie 94, 1382-1389.

Lee Y.C., Chang W.W, Chen Y.Y., Tsai Y.H., Chou Y.H., Tseng H.C., Chen H.L., Wu C.C., Chang-Chien J., Lee H.T., Yang H.F. and Wang B.Y. (2017). Hsp90 mediates BMI1 expression in breast cancer stem/progenitor cells through facilitating nuclear translocation of c-Myc and EZH2. Int. J. Mol. Sci. 18, 9.

Lettini G., Sisinni L., Condelli V., Matassa D.S., Simeon V., Maddalena

F., Gemei M., Lopes E., Vita G., Del Vecchio L., Esposito F. and Landriscina M. (2016). TRAP1 regulates stemness through Wnt/β-catenin pathway in human colorectal carcinoma. Cell Death Differ. 23, 1792-1803.

Li J., Soroka J. and Buchner J. (2012). The Hsp90 chaperone machinery: conformational dynamics and regulation by co-chaperones. Biochim. Biophys. Acta 1823, 624-635.

Li B., Cheng X.L., Yang Y.P. and Li Z.Q. (2013). GRP78 mediates radiation resistance of a stem cell-like subpopulation within the MCF7 breast cancer cell line. Oncol. Rep. 30, 2119-2126.

Lianos G.D., Alexiou G.A., Mangano A., Mangano A., Rausei S., Boni L., Dionigi G. and Roukos D.H. (2015). The role of heat shock proteins in cancer. Cancer Lett. 360, 114-118.

Lin S.P., Lee Y.T., Wang J.Y., Miller S.A., Chiou S.H., Hung M.C. and Hung S.C. (2012). Survival of cancer stem cells under hypoxia and serum depletion via decrease in PP2A activity and activation of p38-MAPKAPK2-Hsp27. PLoS One 7, e49605.

Liu C.C., Chou K.T., Hsu J.W., Lin J.H., Hsu T.W., Yen D.H., Hung S.C. and Hsu H.S. (2019). High metabolic rate and stem cell characteristics of esophageal cancer stem-like cells depend on the Hsp27-AKT-HK2 pathway. Int. J. Cancer 145, 2144-2156.

Luo B. and Lee A.S. (2013). The critical roles of endoplasmic reticulum chaperones and unfolded protein response in tumorigenesis and anticancer therapies. Oncogene 32, 805-818.

Luo S., Mao C., Lee B., and Lee A.S. (2006). GRP78/BiP is required for cell proliferation and protecting the inner cell mass from apoptosis during early mouse embryonic development. Mol. Cell. Biol. 26, 5688-5697.

Luo B., Tseng C.C., Adams G.B. and Lee A.S. (2013). Deficiency of GRP94 in the hematopoietic system alters proliferation regulators in hematopoietic stem cells. Stem Cells Dev. 22, 3062-3073.

Maddalena F., Sisinni L., Lettini G., Condelli V., Matassa D.S., Piscazzi A., Amoroso M.R., La Torre G., Esposito F. and Landriscina M. (2013). Resistance to paclitxel in breast carcinoma cells requires a quality control of mitochondrial antiapoptotic proteins by TRAP1. Mol. Oncol. 7, 895-906.

Maddalena F., Simeon V., Vita G., Bochicchio A., Possidente L., Sisinni L., Lettini G., Condelli V., Matassa D.S., Li Bergolis V., Fersini A., Romito S., Aieta M., Ambrosi A., Esposito F. and Landriscina M. (2017). TRAP1 protein signature predicts outcome in human metastatic colorectal carcinoma. Oncotarget 8, 21229-21240.

Mainz A., Peschek J., Stavropoulou M., Back K.C., Bardiaux B., Asami S., Prade E., Peters C., Weinkauf S., Buchner J. and Reif B. (2015). The chaperone alphaB-crystallin uses different interfaces to capture an amorphous and an amyloid client. Nat. Struct. Mol. Biol. 22, 898-905.

Marzec M., Eletto D. and Argona Y. (2012). GRP94: an HSP90-like protein specialized for protein folding and quality control in the endoplasmic reticulum. Biochim. Biophys. Acta 1823, 774-787.

Matassa D.S., Amoroso M.R., Agliarulo I., Maddalena F., Sisinni L., Paladino S., Romano S., Romano M.F., Sagar V., Loreni F., Landriscina M. and Esposito F. (2013). Translational control in the stress adaptive response of cancer cells: a novel role for the heat shock protein TRAP1. Cell Death Dis. 4, e851.

Matassa D.S., Agliarulo I., Amoroso M.R., Maddalena F., Sepe L., Ferrari M.C., Sagar V., D'Amico S., Loreni F., Paolella G., Landriscina M. and Esposito F. (2014). TRAP1-dependent regulation of p70S6K is involved in the attenuation of protein synthesis and cell migration: relevance in human colorectal tumors. Mol. Oncol. 8, 1482-1494.

Matassa D.S., Amoroso M.R., Lu H., Avolio R., Arzeni D., Procaccini C., Faicchia D., Maddalena F., Simeon V., Agliarulo I., Zanini E., Mazzoccoli C., Recchi C., Stronach E., Marone G., Gabra H., Matarese G., Landriscina M. and Esposito F. (2016). Oxidative metabolism drives inflammation-induced platinum resistance in human ovarian cancer. Cell Death Diff. 23, 1542-1554.

Mathew A. and Morimoto R.I. (1998). Role of the heat-shock response in the life and death of proteins. Ann. NY Acad. Sci. 851, 99-111.

Mayer M.P. and Bukau B. (2005). Hsp70 chaperones: cellular functions and molecular mechanism. Cell. Mol. Life Sci. 62, 670-684.

McDonald E.T., Bortolus M., Koteiche H.A. and Mchaourab H.S. (2012). Sequence, structure, and dynamic determinants of Hsp27 (HspB1) equilibrium dissociation are encoded by the N-terminal domain. Biochemistry 51, 1257-1268.

Medema J.P. (2013). Cancer stem cells: the challenges ahead. Nat. Cell. Biol. 15, 338-344.

Meng Q., Li B.X. and Xiao X. (2018). Toward developing chemical modulators of Hsp60 as potential therapeutics. Front. Mol. Biosci. 5, 35.

Mitra A., Shevde L.A. and Samant R.S. (2009). Multi-faceted role of HSP40 in cancer. Clin. Exp. Metastasis 26, 559-567.

Miyata Y., Nakamoto H. and Neckers L. (2013). The therapeutic target Hsp90 and cancer hallmarks. Curr. Pharm. Des. 19, 347-365.

Mo L., Bachelder R.E., Kennedy M., Chen P.H., Chi J.T., Berchuck A., Cianciolo G. and Pizzo S.V. (2015). Syngeneic murine ovarian cancer model reveals that ascites enriches for ovarian cancer stem-like cells expressing membrane GRP78. Mol. Cancer Ther. 14, 747-756.

Montesano Gesualdi N., Chirico G., Pirozzi G., Costantino E., Landriscina M. and Esposito F. (2007). Tumor necrosis factor-associated protein 1 (TRAP-1) protects cells from oxidative stress and apoptosis. Stress 10, 342-350.

Morita R., Nishizawa S., Torigoe T., Takahashi A., Tamura Y., Tsukahara T., Kanaseki T., Sokolovskaya A., Kochin V., Kondo T., Hashino S., Asaka M., Hara I., Hirohashi Y. and Sato N. (2014). Heat shock protein DNAJB8 is a novel target for immunotherapy of colon cancer-initiating cells. Cancer Sci. 105, 389-395.

Nami B., Ghasemi-Dizgah A. and Vaseghi A. (2016). Overexpression of molecular chaperons GRP78 and GRP94 in CD44(hi)/CD24(lo) breast cancer stem cells. Bioimpacts 6, 105-110.

Neckers L. and Workman P. (2012). Hsp90 molecular chaperone inhibitors. Are we there yet? Clin. Cancer Res. 18, 64-76.

Newman B., Liu Y., Lee H.F., Sun D. and Wang Y. (2012). HSP90 inhibitor 17-AAG selectively eradicates lymphoma stem cells. Cancer Res. 72, 4551-4561.

Nguyen L.V., Vanner R., Dirks P. and Eaves C.J. (2012). Cancer stem cells: an evolving concept. Nat. Rev. Cancer 12, 133-143.

Nishizawa S., Hirohashi Y., Torigoe T., Takahashi A., Tamura Y., Mori T., Kanaseki T., Kamiguchi K., Asanuma H., Morita R., Sokolovskaya A., Matsuzaki J., Yamada R., Fujii R., Kampinga H.H., Kondo T., Hasegawa T., Hara I. and Sato N. (2012). HSP DNAJB8 controls tumor-initiating ability in renal cancer stem-like cells. Cancer Res. 72, 2844-2854.

Nolan K.D., Kaur J. and Isaacs J.S. (2017). Secreted heat shock protein 90 promotes prostate cancer stem cell heterogeneity. Oncotarget 8, 19323-19341.

Okamoto T., Yamamoto H., Kudo I., Matsumoto K., Odaka M., Grave E. and Itoh H. (2017). HSP60 possesses a GTPase activity and

mediates protein folding with HSP10. Sci. Rep. 7, 16931.

Olotu F., Adeniji E., Agoni C., Bjij I., Khan S., Elrashedy A. and Soliman M. (2018). An update on the discovery and development of selective heat shock protein inhibitors as anti-cancer therapy. Expert Opin. Drug Discov. 13, 903-918.

Palladino G., Notarangelo T., Pannone G., Piscazzi A., Lamacchia O., Sisinni L., Spagnoletti G., Toti P., Santoro A., Storto G., Bufo P., Cignarelli M., Esposito F. and Landriscina M. (2016). TRAP1 regulates cell cycle and apoptosis in thyroid carcinoma cells. Endocr. Related Cancer 23, 699-709.

Pandey P., Farber R., Nakazawa A., Kumar S., Bharti A., Nalin C., Weichselbaum R., Kufe D. and Kharbanda S. (2000). Hsp27 functions as a negative regulator of cytochrome c-dependent activation of procaspase-3. Oncogene 19, 1975-1981.

Parcellier A., Gurbuxani S., Schmitt E., Solary E. and Garrido C. (2003). Heat shock proteins, cellular chaperones that modulate mitochondrial cell death pathways. Biochem. Biophys. Res. Commun. 304, 505-512.

Patel K., Wen J., Magliocca K., Muller S., Liu Y., Chen Z.G., Saba N. and Diaz R. (2014). Heat shock protein 90 (HSP90) is overexpressed in p16-negative oropharyngeal squamous cell carcinoma, and its inhibition *in vitro* potentiates the effects of chemoradiation. Cancer Chemother. Pharmacol. 74, 1015-1022.

Peng C., Brain J., Hu Y., Goodrich A., Kong L., Grayzel D., Pak R., Read M. and Li S. (2007). Inhibition of heat shock protein 90 prolongs survival of mice with BCR-ABL-T315I–induced leukemia and suppresses leukemic stem cells. Blood 110, 678-685.

Pirkkala L. and Sistonen L. (2006). Heat shock proteins (HSPs): Structure, function and genetics. Encycl. Life Sci. 1-7.

Pockley A.G., Henderson B. and Multhoff G. (2014). Extracellular cell stress proteins as biomarkers of human disease. Biochem. Soc. Trans. 42, 1744-1751.

Radons J. (2016). The human HSP70 family of chaperones: where do we stand? Cell Stress Chaperones 21, 379-404.

Rehana K. (2014). Heat shock proteins in neurodegenerative disorders and aging. J. Cell. Commun. Signal. 8, 293-310.

Reya T., Morrison S.J., Clarke M.F. and Weissman I.L. (2001). Stem cells, cancer, and cancer stem cells. Nature 414, 105-111.

Ricardo S., Vieira A.F., Gerhard R., Leitão D., Pinto R., Cameselle-Teijeiro J.F., Milanezi F., Schmitt F. and Paredes J. (2011). Breast cancer stem cell markers CD44, CD24 and ALDH1: expression distribution within intrinsic molecular subtype. J. Clin. Pathol. 64, 937-946.

Rich J.N. (2016). Cancer stem cells: understanding tumor hierarchy and heterogeneity. Medicine (Baltimore). 95, S2-7.

Ritossa F. (1963). New puffs induced by temperature shock, DNP and salicylate in salivary chromosomes of *D. melanogaster*. Drosophila Information Service. 37, 122-123.

Saibil H. (2013). Chaperone machines for protein folding, unfolding and disaggregation. Nat. Rev. Mol. Cell. Biol. 14, 630-642.

Sancho P., Burgos-Ramos E., Tavera A., Bou Kheir T., Jagust P., Schoenhals M., Barneda D., Sellers K., Campos-Olivas R., Graña O., Viera C.R., Yuneva M., Sainz B. Jr. and Heeschen C. (2015). MYC/PGC-1α balance determines the metabolic phenotype and plasticity of pancreatic cancer stem cells. Cell Metab. 22, 590-605.

Sancho P., Barneda D. and Heeschen C. (2016). Hallmarks of cancer stem cell metabolism. Br. J. Cancer 114, 1305-1312.

Schmitt E., Gehrmann M., Brunet M., Multhoff G. and Garrido C. (2007). Intracellular and extracellular functions of heat shock proteins: repercussions in cancer therapy. J. Leukoc. Biol. 81, 15-27.

Sciacovelli M., Guzzo G., Morello V., Frezza C., Zheng L., Nannini N., Calabrese F., Laudiero G., Esposito F., Landriscina M., Defilippi P., Bernardi P. and Rasola A. (2013). The mitochondrial chaperone TRAP1 promotes neoplastic growth by inhibiting succinate dehydrogenase. Cell Metab. 17, 988-999.

Shi Y., Liu X., Lou J., Han X., Zhang L., Wang Q., Li B., Dong M. and Zhang Y. (2014). Plasma levels of heat shock protein 90 alpha associated with lung cancer development and treatment responses. Clin. Cancer Res. 20, 6016-6022.

Sisinni L., Maddalena F., Lettini G., Condelli V., Matassa D.S., Esposito F. and Landriscina M. (2014). TRAP1 role in endoplasmic reticulum stress protection favors resistance to anthracyclins in breast carcinoma cells. Int. J. Oncol. 44, 573-582.

Sisinni L., Maddalena F., Condelli V., Pannone G., Simeon V., Li Bergolis V., Lopes E., Piscazzi A., Matassa D.S., Mazzoccoli C., Nozza F., Lettini G., Amoroso M.R., Bufo P., Esposito F. and Landriscina M. (2017). TRAP1 controls cell cycle G2-M transition through the regulation of CDK1 and MAD2 expression/ubiquitination. J. Pathol. 243, 123-134.

Snyder V., Reed-Newman T.C., Arnold L., Thomas S.M. and Anant S. (2018). Cancer stem cell metabolism and potential therapeutic targets. Front. Oncol. 8, 203.

Song H.Y., Dunbar J.D., Zhang Y.X., Guo D. and Donner D.B. (1995). Identification of a protein with homology to hsp90 that binds the type 1 tumor necrosis factor receptor. J. Biol. Chem. 270, 3574-3581.

Sreepadmanabh M. and Toley B.J. (2018). Investigations into the cancer stem cell niche using in-vitro 3-D tumor models and microfluidics. Biotechnol. Adv. 36, 1094-1110.

Stankiewicz M. and Mayer M.P. (2012). The universe of Hsp90. Biomol. Concepts 3, 79-97.

Sterrenberg J.N., Blatch G.L. and Edkins A.L. (2011). Human DNAJ in cancer and stem cells. Cancer Lett. 312, 129-142.

Stivarou T., Stellas D., Vartzi G., Thomaidou D. and Patsavoudi E. (2016). Targeting highly expressed extracellular HSP90 in breast cancer stem cells inhibits tumor growth *in vitro* and *in vivo*. Cancer Biol. Ther. 17, 799-812.

Straume O., Shimamura T., Lampa M.J., Carretero J., Øyan A.M., Jia D., Borgman C.L. and Soucheray M. (2012). Suppression of heat shock protein 27 induces long-term dormancy in human breast cancer. Proc. Natl. Acad. Sci. USA 109, 8699-8704.

Su J.M., Hsu Y.Y., Lin P. and Chang H. (2016). Nuclear accumulation of heat-shock protein 90 is associated with poor survival and metastasis in patients with non-small cell lung cancer. Anticancer Res. 36, 2197-2203.

Subramanian C., Kovatch K.J., Sim M.W., Wang G., Prince M.E., Carey T.E., Davis R., Blagg B.S.J. and Cohen M.S. (2017). Novel C-terminal heat shock protein 90 inhibitors (KU711 and Ku757) are effective in targeting head and neck squamous cell carcinoma cancer stem cells. Neoplasia 19, 1003-1011.

Taipale M., Jarosz D.F. and Lindquist S. (2010). HSP90 at the hub of protein homeostasis: emerging mechanistic insights. Nat. Rev. Mol. Cell. Biol. 11, 515-528.

Tang S.N., Singh C., Nall D., Meeker D., Shankar S. and Srivastava R.K. (2010). The dietary bioflavonoid quercetin synergizes with epigallocathechin gallate (EGCG) to inhibit prostate cancer stem cell characteristics, invasion, migration and epithelial-mesenchymal transition. J. Mol. Signal. 5, 14.

Tonello L., Conway de Macario E., Marino Gammazza A., Cocchi M.,

Gabrielli F., Zummo G., Cappello F. and Macario A.J. (2015). Data mining-based statistical analysis of biological data uncovers hidden significance: clustering Hashimoto's thyroiditis patients based on the response of their PBMC with IL-2 and IFN-gamma secretion to stimulation with Hsp60. Cell Stress Chaperones 20, 391-395.

Torigoe T., Hirohashi Y., Yasuda K. and Sato N. (2013). Constitutive expression and activation of stress response genes in cancer stem-like cells/tumour initiating cells: potent targets for cancer stem cell therapy. Int. J. Hyperthermia 29, 436-441.

Trepel J., Mollapour M, Giaccone G. and Neckers L. (2010). Targeting the dynamic HSP90 complex in cancer. Nat. Rev. Cancer 10, 537-549.

Tsai M.F., Wang C.C., Chang G.C., Chen C.Y., Chen H.Y., Cheng C.L., Yang Y.P., Wu C.Y., Shih F.Y., Liu C.C., Lin H.P., Jou Y.S., Lin S.C., Lin C.W., Chen W.J., Chan W.K., Chen J.J. and Yang P.C. (2006). A new tumor suppressor DnaJ-like heat shock protein, HLJ1, and survival of patients with non-small-cell lung carcinoma. J. Natl. Cancer Inst. 98, 825-838.

Tsai Y.P., Yang M.H., Huang C.H., Chang S.Y., Chen P.M., Liu C.J., Teng S.C. and Wu K.J. (2009). Interaction between HSP60 and beta-catenin promotes metastasis. Carcinogenesis 30, 1049-1057.

Tsutsumi S. and Neckers L. (2007). Extracellular heat shock protein 90: a role for a molecular chaperone in cell motility and cancer metastasis. Cancer Sci. 98, 1536-1539.

Vega V.L., Rodríguez-Silva M., Frey T., Gehrmann M., Diaz J.C., Steinem C., Multhoff G., Arispe N. and De Maio A. (2008). Hsp70 translocates into the plasma membrane after stress and is released into the extracellular environment in a membrane-associated form that activates macrophages. J. Immunol. 180, 4299-4307.

Vicencio J.M., Yellon D.M., Sivaraman V., Das D., Boi-Doku C., Arjun S., Zheng Y., Riquelme J.A., Kearney J., Sharma V., Multhoff G., Hall A.R. and Davidson S.M. (2015). Plasma exosomes protect the myocardium from ischemia-reperfusion injury. J. Am. Coll. Cardiol. 65, 1525-1536.

Wang C.C., Liao Y.P., Mischel P.S., Iwamoto K.S., Cacalano N.A. and McBride W.H. (2006). HDJ-2 as a target for radiosensitization of glioblastoma multiforme cells by the farnesyltransferase inhibitor R115777 and the role of the p53/p21 pathway. Cancer Res. 66, 6756-6762.

Wang M., Wey S., Zhang Y., Ye R. and Lee AS. (2009). Role of the unfolded protein response regulator GRP78/BiP in development, cancer, and neurological disorders. Antioxid. Redox Signal. 11, 2307-2316.

Wang J., Cui S., Zhang X., Wu Y. and Tang H. (2013). High expression of heat shock protein 90 is associated with tumor aggressiveness and poor prognosis in patients with advanced gastric cancer. PLoS One 8, e62876.

Wei L., Liu T.T., Wang H.H., Hong H.M., Yu A.L., Feng H.P. and Chang W.W. (2011). Hsp27 participates in the maintenance of breast cancer stem cells through regulation of epithelial-mesenchymal transition and nuclear factor-κB. Breast Cancer Res. 13, R101.

Wey S., Luo B. and Lee A.S. (2012). Acute inducible ablation of GRP78 reveals its role in hematopoietic stem cell survival, lymphogenesis and regulation of stress signaling. PLoS One 7, e39047.

White P.T., Subramanian C., Zhu Q., Zhang H., Gallagher R., Timmermann B.N., Blagg B.S.J. and Cohen M.S. (2016). Novel HSP90 inhibitors effectively target functions of thyroid cancer stem cell preventing migration and invasion. Surgery 159, 142-151.

Wieten L., Broere F., van der Zee R., Koerkamp E. K., Wagenaar J. and van Eden W. (2007). Cell stress induced HSP are targets of regulatory T cells: a role for HSP inducing compounds as anti-inflammatory immuno-modulators? FEBS Lett. 581, 3716-3722.

Wu M.J., Jan C.I., Tsay Y.G., Yu Y.H., Huang C.Y., Lin S.C., Liu C.J., Chen Y.S., Lo J.F. and Yu C.C. (2010). Elimination of head and neck cancer initiating cells through targeting glucose regulated protein 78 signaling. Mol. Cancer 9, 283.

Wu J., Liu Y., Cho K., Dong X., Teng L., Han D., Liu H., Chen X., Chen X., Hou X., Peng F., Bi Y., Shen C. and Zhao S. (2016). Downregulation of TRAP1 sensitizes glioblastoma cells to temozolomide chemotherapy through regulating metabolic reprogramming. Neuroreport 27, 136-144.

Wu J., Liu T., Rios Z., Mei Q., Lin X. and Cao S. (2017). Heat shock proteins and cancer. Trends Pharmacol. Sci. 38, 226-256.

Yang T., Li X.N., Li X.G., Li M. and Gao P.Z. (2014). DNAJC6 promotes hepatocellular carcinoma progression through induction of epithelial–mesenchymal transition. Biochem. Biophys. Res. Commun. 455, 298-304.

Yasuda K., Hirohashi Y., Mariya T., Murai A., Tabuchi Y., Kuroda T., Kusumoto H., Takaya A., Yamamoto E., Kubo T., Nakatsugawa M., Kanaseki T., Tsukahara T., Tamura Y., Hirano H., Hasegawa T., Saito T., Sato N. and Torigoe T. (2017). Phosphorylation of HSF1 at serine 326 residue is related to the maintenance of gynecologic cancer stem cells through expression of HSP27. Oncotarget 8, 31540-31553.

Yoshida S., Tsutsumi S., Muhlebach G., Sourbier C., Lee M.J., Lee S., Vartholomaiou E., Tatokoro M., Beebe K., Miyajima N., Mohney R.P., Chen Y., Hasumi H., Xu W., Fukushima H., Nakamura K., Koga F., Kihara K., Trepel J., Picard D. and Neckers L. (2013). Molecular chaperone TRAP1 regulates a metabolic switch between mitochondrial respiration and aerobic glycolysis. Proc. Natl. Acad. Sci. USA 110, E1604-1612.

Zeng L., Tan J., Lu W., Lu T. and Hu Z. (2013). The potential role of small heat shock proteins in mitochondria. Cellular Signal. 25, 2312-2319.

Zhao Y., Dong Q., Li J., Zhang K., Qin J., Zhao J., Sun Q., Wang Z., Wartmann T., Jauch K.W., Nelson P.J., Qin L. and Bruns C. (2018). Targeting cancer stem cells and their niche: perspectives for future therapeutic targets and strategies. Semin. Cancer Biol. 53, 139-155.

Zhu G., Wang M., Spike B., Gray P.C., Shen J., Lee S.H., Chen S.Y., and Lee A.S. (2014). Differential requirement of GRP94 and GRP78 in mammary gland development. Sci. Rep. 4, 5390.

Zhu G. and Lee A.S. (2015). Role of the unfolded protein response, GRP78 and GRP94 in organ homeostasis. J. Cell. Physiol. 230, 1413-1420.

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