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REVIEW

# HSPA6 and its role in cancers and other diseases

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**Abstract**
Heat Shock Protein Family A (Hsp70) Member 6 (HSPA6) (Online Mendelian Inheritance in Man: 140555) belongs to the HSP70 family and is a partially conserved inducible protein in mammals. The *HSPA6* gene locates on the human chromosome 1q23.3 and encodes a protein containing two important structural domains: The N-terminal nucleotide-binding domain and the C-terminal substrate-binding domain. Currently, studies have found that HSPA6 not only plays a role in the tumorigenesis and tumor progresses but also causes non-tumor-related diseases. Furthermore, HSPA6 exhibits to inhibit tumorigenesis and tumor progression in some types of cancers but promotes in others. Even though HSPA6 research has increased, its exact roles and mechanisms are still unclear. This article reviews the structure, expression, function, research progress, possible mechanism, and perspective of HSPA6 in cancers and other diseases, highlighting its potential role as a targeted therapeutic and prognostic marker.

**Keywords** Heat shock protein (HSP) · HSPA6 · Cancer · Antitumor activity · Mechanism · Biomarker

## Introduction

Heat Shock Protein Family A (Hsp70) Member 6 (HSPA6) has the aliases Heat Shock 70 kDa Protein 6, and Heat Shock 70 kDa Protein B′ (HSP70B′). As a family member of the HSP70, HSPA6 was first identified in 1990 by Leung et al. [1]. Besides HSPA6, this HSP70 family includes HSPA1A, HSPA1B, HSPA1L, HSPA2, HSPA5, HSPA7, HSPA8, HSPA9, HSPA12A, HSPA12B, HSPA13 and HSPA14 [2]. *HSPA6* is located on human chromosome 1q23.3 with a length of 1929 nucleotides and encodes 643 amino acids. The molecular weight of HSPA6 protein is approximately 70-kDa, and its cellular localization is mainly in the cytosol and extracellular exosomes [3]. In addition, other locations of HSPA6, such as centriole, nucleus protein-containing complex co-localizing with COP9 signalosome and blood microparticle have been reported [4]. Interestingly, it note that the HSPA6 isoform is a heat-shock-inducible protein of HSP70 [5]. Homologous genes of *HSPA6* have been found in cotton-headed tamarin, pigs, cattle, and humans, but not in rodents [2]. The information for HSPA6 can be retrieved on Online Mendelian Inheritance in Man (OMIM, entry 140555) [6].

Currently, studies have found that HSPA6 not only plays a role in the tumorigenesis and tumor progression but also causes non-tumor-related diseases. Furthermore, HSPA6 inhibits the tumorigenesis and tumor progression in some type of cancers, but promotes it in others. Overall, research for HSPA6 expanded in the previous years, but its exact roles and mechanisms are unclear. This article reviews the structure, expression, function, research progress, possible mechanism and future prospects of HSPA6 in malignant tumors and other diseases.

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## The structure, physiological function, and expression for HSPA6

### The structure of HSPA6

HSPA6 protein contains two important and separate domain structures, namely a more conserved approximately 44-kDa NBD (N-terminal nucleotide-binding domain), which known as the ATPase domain, that binds and hydrolyzes ATP, and a variable approximately 28-kDa SBD (C-terminal substrate-binding domain), which known as the peptide-binding domain, that can bind with polypeptide substrates (Fig. 1A). In addition, there is a flexible so-called interdomain linker-containing protease-sensitive sites between NBD and SBD, which plays an important role in allosteric communication between them [7, 8]. The HSPA6 presents the motive EEVD, is responsible for the interaction between HSP70 and their cochaperones and other proteins of the HSPs family [8]. With exception of *HSPA12A* and *HSPA12B* genes encoding a divergent ATPase domain, all members of the HSP70 family have a highly conserved NBD. In addition, the NBD consists of four subdomains, IA, IB, IIA, and IIB, presenting a surface patch on the upper-right, front portion of the C terminus [7]. Wisniewska et al. [9] presented the first

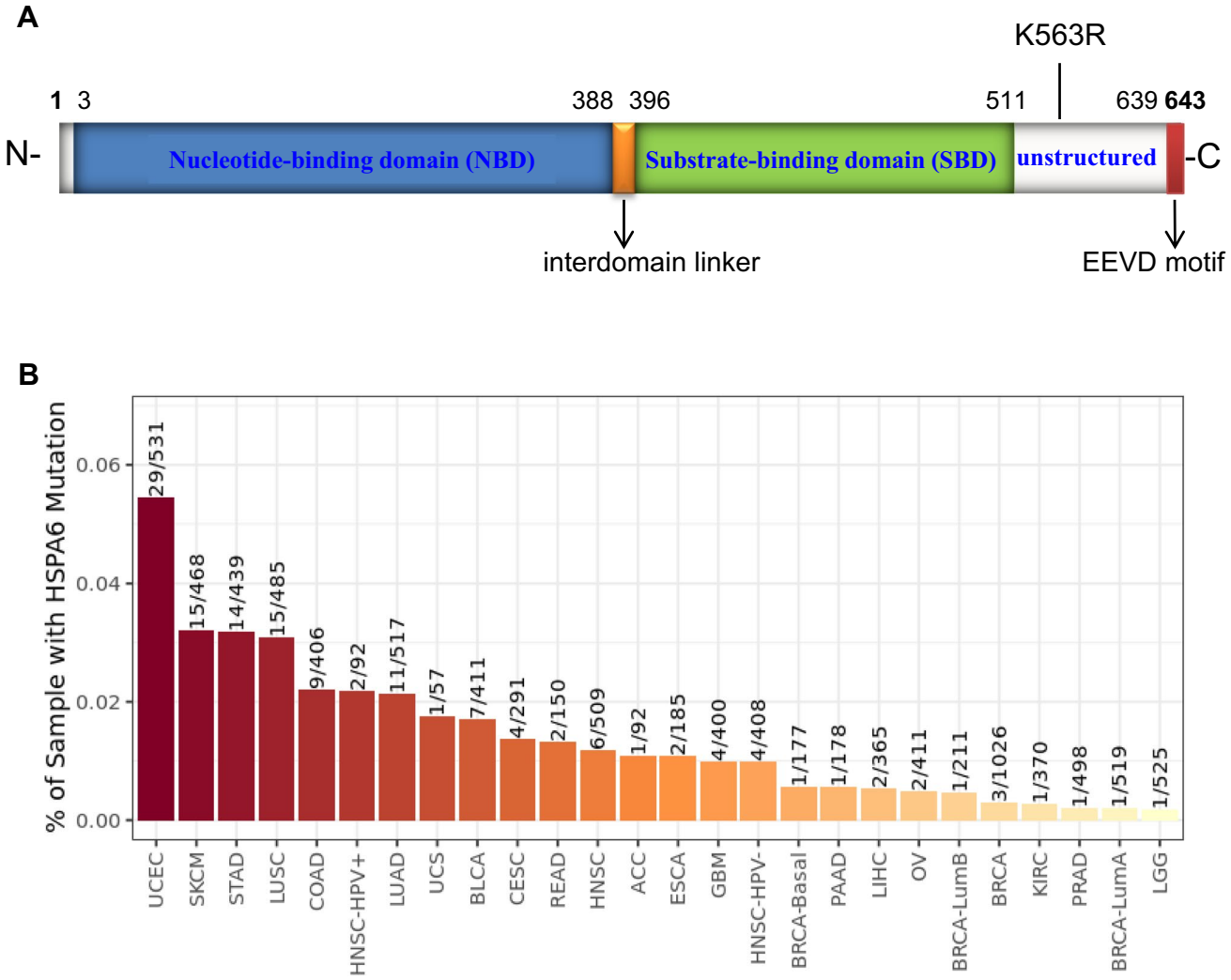


**Fig. 1** The structure and mutations of HSPA6. **A** The structure of HSPA6. The blue rectangle indicates the N-terminal nucleotide-binding domain (NBD) (aa 3-388), whereas the green rectangle indicates the C-terminal substrate-binding domain (SBD) (aa 396-511). The orange rectangle indicates a flexible so-called interdomain linker between NBD and SBD. In addition, the white rectangle indicates unstructured C-terminal region (aa 512-639) and the red rectangle indicates EEVD motif (aa 639-643). The C-terminal K563R shows one site of amino acid mutation. K, Lysine. R, Arginine. **B** Mutations of HSPA6 in cancerous tissues. Mutations of HSPA6 in cancerous tissues were performed by TIMER2.0 in TCGA (http://timer.comp-genomics.org/). The abbreviations and corresponding terms are presented in Fig. 3. (Color figure online)

crystal structures of the NBDs of HSPA6, demonstrating that the human HSP70s NBD acts through a conserved mechanism and contributes little to isoform specificity. The SBD is composed of an N-terminal β-sandwich subdomain (SBDβ) that is in contact with the peptide directly and a C-terminal α-helical domain (SBDα) that serves as a lid encapsulating the client proteins [10]. SBDβ and SBDα, respectively, are more rigid and more flexible. SBDβ is made up of β-strands arranged in β-sheets and contains a binding site to the client protein with a central hydrophobic cleft, which is responsible for the interaction with motifs composed of hydrophobic amino acid residues in the client protein. On the other hand, SBDα is a fully α-helical subdomain that covers the client protein binding site [11].

The allosteric mechanism of HSPA6 enables the conformational changes of NBD when binding with ATP and ADP. And the conformational changes could be transmitted to SBD. The SBD has a poor affinity for its substrates when it is ATP-bound state. When ADP binds to the NBD, the SBD has higher affinity to substrates through a conformational change. Interestingly, reverse direction conformational changes are caused by substrate binding to SBD. This bidirectional heterotrophic allosteric mechanism has been revealed in the Hsp70 family members [12, 13].

## The physiological function of HSPA6

Through ATP binding, ATP hydrolysis, and ADP release, HSPA6 likely goes through the cycle of ATP hydrolysis and nucleotide exchange that allows substrate binding and release, thereby enabling HSPA6 undergo a series of physiological functions [14]. Under physiological conditions, HSPA6 acts as a molecular chaperone that promotes protein folding and mediates the correct assembly of client proteins [3]. In addition, Hageman et al. [15] explored the substrate specificity and/or possible functional differences of HSP70 members, finding that HSPA6 own unusual activity in comparison to other canonical HSP70s and may have evolved to sustain specific critical functions under extreme stress conditions.

HSPA6 is required for protein translocation processes and induces the disassembly of specific protein complexes. Under stress conditions, HSPA6 is not only closely linked to the degradation machinery of misfolded polypeptides and prevents or reverses protein aggregation and molecular crowding, but also protects cells against unnatural damage [16]. HSPA6 stabilizes newly synthesized polypeptides and plays a critical role in their folding and transport. Moreover, the HSPA6 can counteract protein misfolding and aggregation, convert stable misfolded proteins into native proteins, or control the subsequent degradation of client proteins [5, 17, 18].

## The methylation modification and mutations of HSPA6

There is increasing evidence that HSP70s are regulated by a huge number of post-translational modifications (PTMs), also known as the chaperone code, including acetylation, methylation, phosphorylation, ubiquitination, AMPylation, and ADP-ribosylation [19, 20]. HSPA6 protein might subject to all kinds of post-translational modifications, such as methylation. HSPA6 can be post-translationally trimethylated at K563 residue (Lys-563) that is catalyzed by the methyltransferase METTL21A (recombinant human methyltransferase like 21a) both in vitro and in vivo [21] (Fig. 1A). This modification is stimulated by ATP. Surprisingly, Jakobsson et al. [21] found that the trimethylation of HSP70s alter the affinity of a molecular chaperone for certain disease-associated proteins. For example, the trimethylation of HSPA8 reduced the affinity for both monomeric and fibrillar forms in the Parkinson's-related protein α-synuclein, a major compound in its pathogenesis [21]. When lysine was mutated to arginine (K → R) at the Lys-563 residue of HSPA6, METTL21A completely lost the ability to methylate HSPA6 in vitro. However, its resulting pathological changes remain unclear.

Mutations of HSPA6 in cancerous tissues were identified by TIMER 2.0 database in TCGA, showing the highest mutation rate in uterine corpus endometrial carcinoma (UCEC) (25/531) and the lowest in brain lower grade glioma (LGG) in 26 different types of cancers (Fig. 1B). A cursory search on www.cbioportal.org for HSPA6 using a selection of "curated set of non-redundant studies" reveals 234 mutations detected in different cancers. The mutation frequency in those cancers including breast cancer would be actually pretty high, which depends on what databases we used [22]. Different mutation statuses might affect the other gene expressions, the disease progresses, or prognosis. Mechanistically, HSPA6 mutations may also alter the co-chaperone interactions, we guess.

## The gene expressions of *HSPA6*

HSPA6 is a member of the strictly heat-inducible HSP70 family that is very identical to HSPA1A (~ 85%) and expressed at low levels in most cells and tissues [1]. As for HSP70, its low basal levels in tissues may related with the rate of aging and longevity in mice, daphnia, and *Drosophila melanogaster* [23–25].

By analyzing the database of Human Protein Atlas (HPA), and Function Annotation of The Mammalian Genome (FANTOM5), Genotype-Tissue Expression (GTEx) [26], we found that the mRNA expression levels of *HSPA6* were different in various normal human tissues and cells. The *HSPA6* mRNA expression was highest in the cerebral cortex (511.3 nTPM), followed by lung (95.3 nTPM), hippocampal

formation (86.3 nTPM), spleen (64.1 nTPM), but lowest in the thymus (0.7 nTPM) (Fig. 2A). At the protein levels, high expression in four tissues (cerebral cortex, caudate, testis and smooth muscle); no detectable in two tissues (parathyroid gland and bone marrow); other 39 tissues show medium or low HSPA6 protein expression (Fig. 2B). High expression in the brain cerebral cortex, demonstrating its role in neurology.

Then to know HSPA6 expression levels in tumor tissues and the corresponding adjacent normal tissues, we conducted analysis by the Gene Expression Profiling Interactive Analysis 2 (GEPIA2) [27, 28] in 31 different tumors and their adjacent tissues, we found that, in six different types of tumor tissues (cervical cancer, cholangiocarcinoma, glioblastoma, pancreatic adenocarcinoma, kidney renal clear cell carcinoma and skin cutaneous melanoma), the HSPA6 expression levels were considerably lower than that of the corresponding adjacent tissues ($P < 0.01$) (Fig. 3A). However, the expression levels of HSPA6 in the other four different types of tumor tissues (adrenocortical carcinoma, acute myeloid leukemia, diffuse large B-cell lymphoma and thymoma) were considerably higher than that of the corresponding adjacent normal tissues ($P < 0.01$) (Fig. 3B), implying that HSPA6 would have, at least in part, dual effects, namely a tumor-suppressive role or a pro-tumorigenic role.

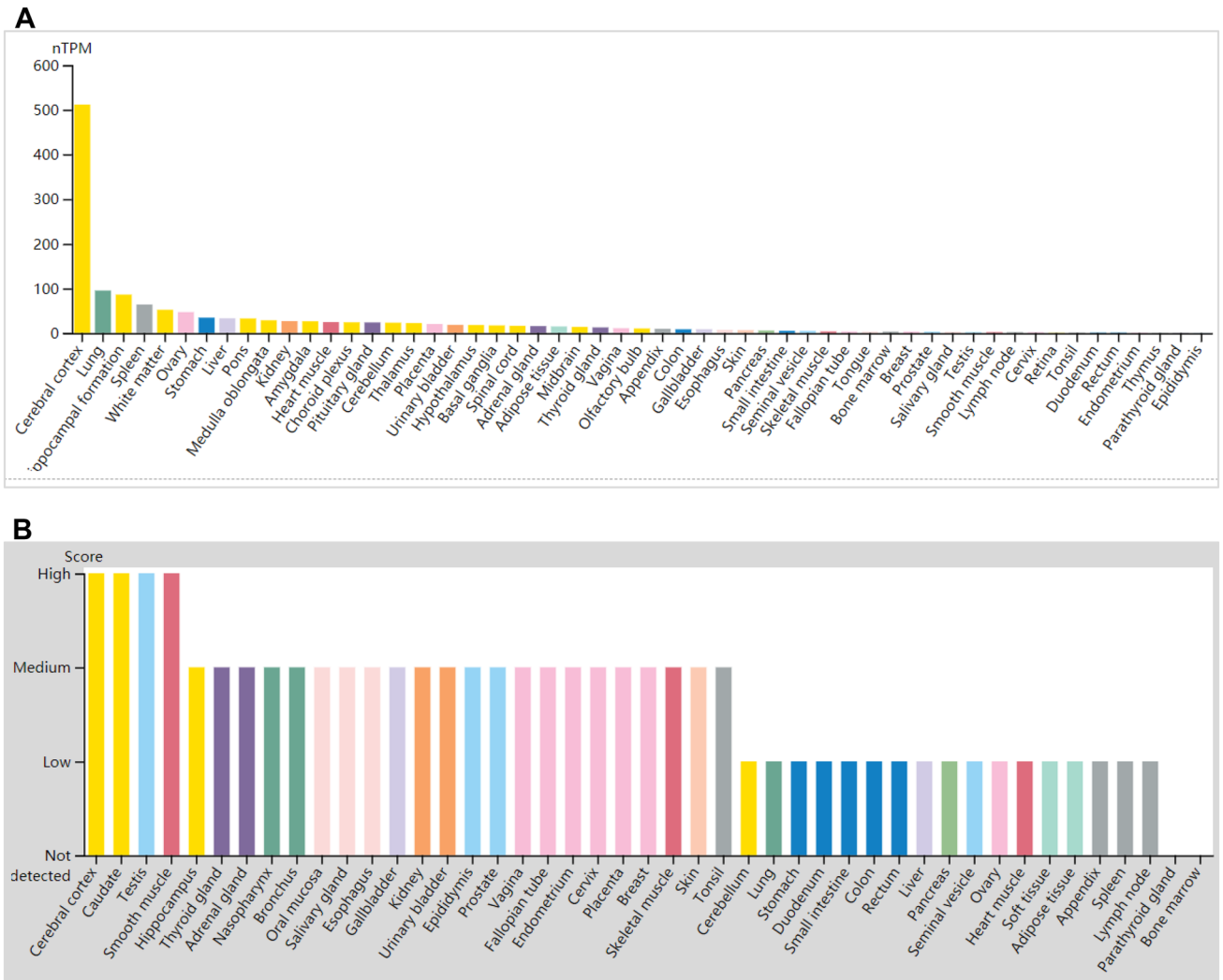


**Fig. 2** The expression of HSPA6 in various normal human tissues. **A** The mRNA expression of *HSPA6* in various normal human tissues. The same color represents the same type of organization, which is composed of organizations with common functional characteristics. **B** The protein expression of HSPA6 in various normal human tissues. The expression data are from HPA, GTEx, and FANTOM5 databases (https://www.proteinatlas.org/ENSG00000173110-HSPA6/tissue). (Color figure online)

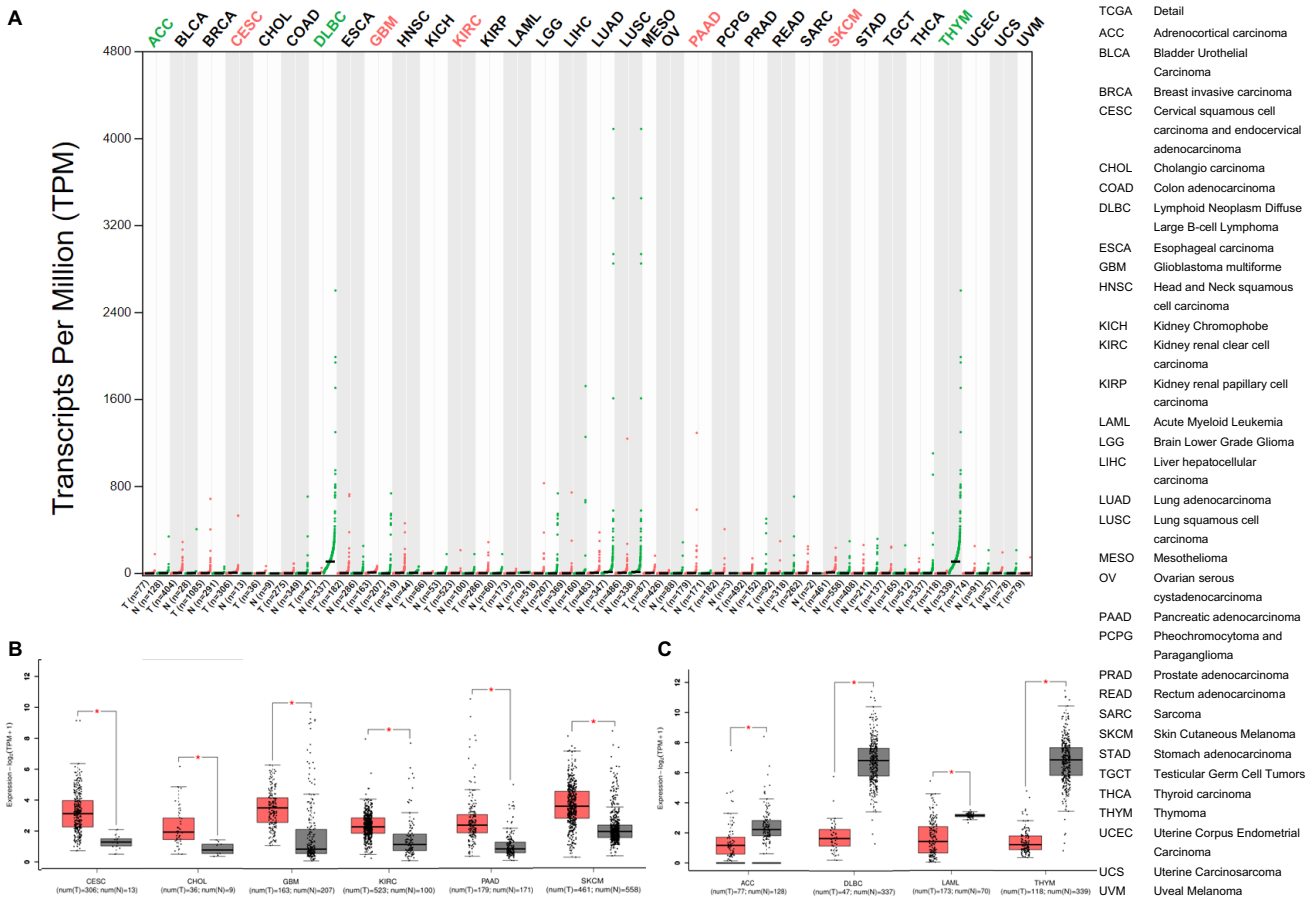


**Fig. 3** The expression profiles of HSPA6 in different types of cancerous tissues and corresponding adjacent tissues. **A** The expression profiles of HSPA6 in 33 different types of cancerous tissues and corresponding adjacent tissues. Names in red represents the significantly upregulated cancers while names green represents the significantly downregulated cancers. **B** HSPA6 was significantly upregulated in tumor tissues. **C** HSPA6 was significantly downregulated in tumor tissues. Red represents the cancerous tissue and gray represents the adjacent normal tissue. These data are from the GEPIA 2 database (http://gepia2.cancer-pku.cn/#analysis). (Color figure online)

## The tumor-suppressive role for HSPA6

The treatment of tumor patients is a difficult and long process, and drug resistance is bound to occur. Researchers have yet to clarify the mechanism of resistance and develop new drugs, in the hope of discovering drugs that have better curative effects, or have synergistic effects with existing drugs, shorten the treatment cycle, and benefit patients' health. Recent studies in the field of cancer biology demonstrated that the expression of HSPA6 was increased after drug treatment of tumor cells and verified the anti-tumor effect of HSPA6 [29, 30].

Phenazine methosulfate (PMS) has been confirmed to induce apoptosis of human malignant melanoma cells, and Hua et al. [31] first reported that PMS may be used as a new experimental chemotherapy drug. Transcriptional upregulation of HSPA6 was observed in malignant human melanoma cells treated with PMS. Cold atmospheric plasma (CAP) is used in tumor therapy because of its good anti-proliferative effect on cancer cells. The non-reactive oxygen species (non-ROS) component of CAP targets HSPA6, which exhibits pro-apoptotic activity and anti-proliferative in cancer cells [32]. In vitro and in vivo, 6-formylindolo [3,2-b] carbazole (FICZ) is useful as a nanomolar photosensitizer for photodynamic eradication in both melanoma and nonmelanoma skin cancer cells. Justiniano et al. [33] demonstrated that *HSPA6* was a stress response target gene with pronounced transcriptional upregulation after Ultraviolet A (UVA)/FICZ photodynamic treatment.

Studies [34, 35] have shown that garlic extract (GE) has these anti-tumor effects. Shin et al. [29] also demonstrated that HSPA6 enhanced the GE-mediated inhibitory effects of bladder cancer EJ cells proliferation, migration, and invasion, providing a potentially, new approach for the treatment of malignant tumors. Through the arrest of the G2/M-phase cell cycle, the proliferation of EJ cells was significantly inhibited by administration with GE. In addition, GE inhibited migration and invasion by suppressing matrix

metalloproteinases 9 (MMP-9) expression, thereby inhibiting the binding activities of AP-1, NF-κB, and Sp-1, the transcription factors at the promoter region of MMP-9 [36, 37]. Interestingly, as an outcome of GE treatment, the most highly up-regulated gene was *HSPA6*. Overall, the GE-mediated inhibitory effects were reinforced by HSPA6, which was confirmed by the inhibition of MMP-9 regulation associated with NF-κB, AP-1, and Sp-1, phosphorylation of AKT signaling, and mitogen-activated protein kinase (MAPK), and the induction of ATM-CHK2-Cdc25C-p21WAF1-Cdc2 cascade mediated by G2/M-phase [29].

Although histone deacetylase inhibitors (HDACi) remain largely ineffective in solid tumors [38], Duncan et al. [30] found that the anti-tumor activity of HDACi was significantly and synergistically enhanced by a new class of protein disulfide isomerase (PDI) inhibitors in the preclinical models of glioblastoma and pancreatic cancer. More importantly, Duncan et al. [30] identified ATF3 (activating transcription factor 3) as a driving factor of this synergistic anti-tumor effect, and the members of HSP40/HSP70 family genes *DNAJB1* and *HSPA6* were ATF3-dependent that triggered the anti-tumor effect of PDI and HDAC inhibitors. HSPA6 played a pro-apoptotic role in the downstream expression of ATF3 after PDI and HDAC inhibition. These studies hence fully showed that HSPA6 can mediate anti-tumor effects.

Colorectal cancer (CRC) is reported to be the third most common malignancy both in males and females [39], and chemoresistance remains a barrier to therapeutic effectiveness [40, 41]. The ubiquitin–proteasome system (UPS) plays a critical role in cellular homeostasis and functions [42], so the inhibition of proteasome activity may result in tumor cells death, especially in malignant cells that are very sensitive to proteasome inhibition [43, 44]. Fan et al. [45] found that ixazomib, a novel proteasome inhibitor, exerted the anti-tumor effects by upregulating the *HSPA6* gene, and affecting cell apoptosis and cell cycle pathways in CRC SW620 cells, suggesting HSPA6 might play a tumor-suppressive role under the effect of proteasome inhibitors.

Although the usage of Everolimus monotherapy in the treatment of metastatic renal cell carcinoma (mRCC) was reduced, Yang et al. [46] investigated the relationship between efficacy for Everolimus and *HSPA6* expression. By comparing Everolimus monotherapy alone or combined with a vascular disrupting agent BNC105P in the mRCC treatment, HSPA6 was found to be closely related to clinical benefit (CB), and gene expression signatures, thus might provide a theoretical basis for patients to choose appropriate treatment methods, such as Everolimus monotherapy alone or combination with BNC105P.

Currently, breast cancer has not only the highest incidence among malignant tumors worldwide but also the tumor with the second-highest death rate among women [47]. Shen et al. [48] identified that the *HSPA6* gene was highly upregulated and a target for thymoquinone (TQ), a natural small molecule compound, in the triple-negative breast cancer (TNBC) cells. Further analysis demonstrated that the overexpression of HSPA6 inhibited breast cancer cell growth, migration and invasion. Meanwhile, knockdown of HSPA6 in TNBC cell line BT-549 promoted growth, migration, and invasion. Moreover, a positive correlation between high expression of HSPA6 and long overall survival (OS) in both TNBC and all subtypes of breast cancer was revealed. Interestingly, TQ can induce the expression of HSPA6, thereby tapping into its tumor-inhibiting effect. As we are known, TQ is the main bioactive constituent extracted from the seed of *Nigella sativa* plant [49] and numerous studies have proved its potent anti-tumor effects in vivo and in vitro [50–52].

## HSPA6 upregulation promotes tumorigenesis and tumor progression

HSPA6 is not only a potential target for tumor suppression but also has a pro-tumor effect that plays a key role in the tumorigenesis, tumor progression, and tumor prognosis, namely in its oncogenic role [48, 53].

Wang et al. [54] probed a role of Rho guanine nucleotide exchange factor 10-like protein (ARHGEF10L) in the tumor progression of gastric cancer (GC) and found that the expression of ARHGEF10L was increased in GC tissues compared to the surrounding normal tissues. The cell proliferation, cell migration, and tube-like structure formation ability of the GC cell line SGC7901 were elevated by overexpression of ARHGEF10L. The expression of HSPA6 was increased in SGC7901 cells when ARHGEF10L was overexpressed. But the precise mechanism for ARHGEF10L in enhancing the expression of HSPA6 in tumorigenesis remains to be clarified. Nevertheless, the expression of ARHGEF10L can stimulate the GC occurrence by increasing the expression of HSPA6 [54].

*Actinidia chinensis* Planch. Root extracts (acRoots), a traditional Chinese medicine (TCM), is used for anti-tumor treatment and its mechanisms in the lung cancer cell lines were explored [55]. HSPA6 has been found to be expressed significantly higher in some lung cancer cells sensitive to acRoots treatment, while decreased in other insensitive cells. After knocking out HSPA6, the proliferation of the sensitivity cells was significantly increased, but there was no obvious variation of cell proliferation in the less-sensitive cells. In addition, the cell sensitivities in sensitive and less-sensitive cells to acRoots were improved in these knockout cells. Mechanically, the interaction of HSPA6 and HSPA6-dominated molecular networks could alter the sensitivity of lung cancer cells to drugs. The expression for HSPA6 and HSPA12b, another member of the heat shock protein 70 families, had obviously upregulated in both sensitive

and less-sensitive cells. Moreover, HSPA1a and HSPA6 exhibited significant coordination functions in physiological and pathological conditions to control cell survival [56]. Meanwhile, Wang et al. [55] found that lung cancer cells without p53 expression were considerably more sensitive to acRoots than that of p53 expression cells, demonstrating that the upregulation of HSPA6 treated by acRoots might interact and suppress with p53, a tumor suppression gene, and its correlative pathways.

Heat shock proteins (HSPs) were overexpressed not only in human hepatocellular carcinoma (HCC) tissues but also related to the invasiveness and prognosis of human HCC patients. As an important marker for HCC invasion and deterioration, an increase of HSPs intracellular concentrations was strongly correlated with the tumorigenesis and tumor progression of HCC [57, 58]. Upregulation of HSPA6 expression was also associated with early unfavorable prognosis of hepatitis B virus (HBV)-related HCC. Yang et al. [53] found that most of the HSPs in tumor tissues were expressed higher than non-tumor tissues, while HSPA6 was increased in non-tumor tissues. Thus, HSPA6 was identified as a risk factor for the earlier recurrence of HCC [53]. Coto-Llerena et al. [59] demonstrated that transcriptional enhancer factor domain family member 4 (TEAD4) was shown to be overexpressed in HCC and was closely related to aggressive HCC characteristics and poor outcomes. The proliferation and migration rates of HCC cells and tumor growth were increased by overexpression of TEAD4, and HSPA6 was the most up-regulated gene after overexpression of TEAD4. This study disclosed that the oncogenic effect for TEAD4 was partly mediated by regulation of HSPA6.

Esophageal cancer (EC) is a seriously aggressive malignancy with a high incidence and mortality, and its tumor development is closely connected with immune-related genes (IRGs). Guo et al. [60] recently identified nine optimal immune-related prognostic genes, including *HSPA6*, *ANGPTL3*, *CACYBP*, *DKK1*, *EGF*, *FGF19*, *GAST*, *OSM* and *NR2F2*, all of which were high-risk, showing that *HSPA6* was an up-regulated gene. Wang et al. [61] established a 6 immune-related prognostic signal model whereas Chen et al. [62] constructed a 7-immune-related gene predictive risk model, both included *HSPA6* gene. They claimed that those models can provide an accurate evaluation of prognosis for EC patients, as well as the diagnosis and individual treatment, specifically for immuno-therapeutics. Furthermore, Zhu et al. [63] recently established prognostic biomarkers as an immune risk score signature (IRSS) including 6 independent immune genes, CCL25, S100A3, STC2, HSPA6, GPER1 and OSM, which might effectively predict the prognosis for EC, head and neck squamous cell carcinoma (HNSC) and GC.

As an abundant protein chaperone, HSP90 is overexpressed in a variety of tumor tissues and is a target for cancer therapeutics [64]. Mutations of HSP90 cochaperones could resulted in a wide range of human disorders [65]. Geldanamycin is a natural, small molecule that inhibits the function of HSP90 family proteins by the close integration of an amino-terminal pocket in HSP90 [66]. As an inhibitor of HSP90, 17-demethoxygeldanamycin (17-AAG) is not only the derivative of geldanamycin but also a potential antitumor agent [67]. However, HSP90 inhibitors will upregulate other HSPs, especially members of the HSP70 family including HSPA6 [68]. But some cell lines showed no HSPA6 induction after the administration of HSP90 inhibitor [69], probably due to other different mechanisms including heat shock and proteasome inhibition. Nevertheless, HSPA6 is expected to be a sensitive marker for HSP90 suppression [70].

Manumycin A (MA) is a natural antibiotic isolated from a marine microorganism. MA has been demonstrated an anti-tumor effect in vitro and in vivo preclinical models. But MA may act as a stressor to induce adaptive stress responses of cancer cells to minimize its toxicity. With potent cytoprotective factors, HSPs, including HSPA6, are deemed as effective cancer-related pro-survival factors and can counteract the harmful effects of various stressful stimulations [71]. As expected, Sojka et al. [72] found that the anti-tumor effect of MA could be offset by the upregulation of the HSPA6 protein. Overall, the anti-tumor activity of natural products, such as MA, was inhibited by the cytoprotective mechanisms including upregulation of the inducible HSPA6 protein level [73]. Recently study also showed MA functions for inhibiting proteasome through ubiquitin–proteasome system (UPS), thereby increasing HSPA6 protein [74].

Magnetic fluid hyperthermia (MFH) was regarded as a potential therapeutic approach for cancer. Court et al. [75] found that the expression of the *HSPA6* gene was upregulated following MFH treatment. More importantly, HSPA6 was identified as a potential molecular target and its inhibition might exert a synergistic effect to enhance the therapeutic effect of MFH in ovarian cancer [75]. Meanwhile, HSPA6 was also a prognostic marker of human brain glioma and its high expression led to a shorter survival time [76].

## Roles for HSPA6 in non-tumor-related diseases

HSPA6 has been gradually explored for its new roles in clinical multidisciplinary research other than tumor-related research. HSPA6 not only influences tumorigenesis and tumor progression but also plays an important role in other diseases at pathological and pathophysiological conditions.

## The digestive system diseases

Both Behçet's disease (BD) and Crohn's disease (CD) are not only chronic and recurrent inflammatory diseases but also have similar colonoscopy characteristics. Feng et al. [77] found that, except for the clinical features, medical imageology, and pathological features, HSPA6 was helpful to distinguish intestinal BD and CD. Thus, HSPA6 might be a valuable, diagnostic biomarker.

The apoptotic cell death of inflammation-induced intestinal epithelial cells (DLD-1) is suppressed by cigarette smoke, which may have a protective effect on ulcerative colitis (UC). The expression of cytoprotective stress proteins including HSPs may be induced by cigarette smoke [78]. Regeling et al. [79] found that HSPA6 was a susceptibility factor of UC and strongly expressed in the human colonic epithelial cells. In addition, HSPA6 can stabilize a protein called anti-apoptotic B-cell lymphoma-extralarge (Bcl-XL) through physical interaction. Thus, HSPA6 can provide epithelial protection against intestinal damage (excessive apoptosis) caused by colitis [79].

The enterovirus A71 (EV-A71) infection normally causes hand, foot and mouth disease (HFMD), but it can also produce dangerous complications including aseptic meningitis, brain stem encephalitis and acute flaccid paralysis. Recently, Su et al. [80] found that HSPA6 was induced to support the replication cycle of EV-A71 and was a positive regulator to facilitate the EV-A71 life cycle. Interestingly, HSPA6 was necessary only for the internal ribosome entry site (IRES)-mediated translation, and it promoted the IRES activity of EV-A71 through cellular proteins rather than viral proteins. The knockdown of HSPA6 affected the IRES-mediated translation of encephalomyocarditis virus (EMCV), echovirus 9 virus (echo 9), coxsackievirus A16 (CV-A16), or hepatitis C virus (HCV), suggesting that HSPA6 might facilitate the function of cellular proteins for viral IRES activities [80].

## The cardiovascular diseases

Nitric oxide (NO) is primarily produced by the vascular endothelial cells and plays an important role in inhabiting smooth muscle cells (SMC) migration and proliferation [81, 82]. Endothelial nitric oxide synthase (eNOS) is the most active enzyme in endothelial cells for producing NO, and its lack is associated with some vascular diseases [83]. By microarray analysis, McCullagh et al. [84] found HSPA6 was a target of eNOS. More importantly, the overexpression of HSPA6 inhibited SMC proliferation through modification in gene therapy or pharmacological methods, thus we can prevent neointimal hyperplasia of vascular repair in human atherosclerosis [84]. In arteriovenous malformations (AVMs), Takagi et al. [85] found that *HSPA6* was a death-related differentially expressed gene, playing a role in neuron cell deaths, infiltrating cells, and vascular cells. HSPA6 also affected various stages and severities of the AVMs.

22q11.2 deletions are the second most common cause for congenital heart defects (CHDs), and its major pathogenic gene is *TBX1*. Fa et al. [86] reported canonical Wnt/β-catenin signalings which regulate *TBX1* is activated by the long noncoding RNA (lncRNA) *lnc-TSSK2-8*. The lncRNA lnc-TSSK2-8 is located in the 22q11.2 region and can protect β-catenin from degradation. The effects were mediated by HSPA6.

As a multifactorial injurious event, myocardial infarction (MI) has high morbidity and mortality. In the blood samples of MI patients, Wu et al. [87] identified the expression level of HSPA6 was increased. Thus, HSPA6 may be a diagnostic biomarker for early-stage and early recovery in MI patients [87].

## The immune system diseases

Immune thrombocytopenia (ITP) is an autoimmune-mediated hemorrhagic disease [88]. Parallel reaction monitoring (PRM) demonstrated that the expression levels of five targeted proteins including HSPA6, HSPA8, YWHAH, PRDX6, and ITGB3 were significantly decreased, which might be connected with the pathogenesis of ITP. Liu et al. [89] also found that the PI3K–Akt signaling pathway was strongly linked to the apoptosis-related proteins. Downregulation of HSPA6 can regulate apoptosis on ITP pathogenesis via the PI3K–Akt signaling [89].

Furthermore, HSPA6 can be used as a potential diagnostic biomarker for rheumatoid arthritis, which helps to predict the therapeutic efficacy of methotrexate (MTX) [90].

## The nervous system diseases

As a prospective target for protective methods in neurodegeneration, HSP can counter the protein misfolding and aggregation of neurodegenerative disorders through cellular repair and protective mechanisms [91]. The stress-sensitive sites of HSPA6 targeting have been explored in human neuronal cells, which disrupted the periphery of nuclear speckles and associated with HSPA1A dynamics of stress-sensitive cytoplasmic and nuclear structures [92, 93]. Through small interfering RNA (siRNA) knockdown, Deane et al. [94] demonstrated that both HSPA6 and HSPA1A helped to protect differentiated human neuronal cells from damage caused by cellular stress.

As a chronic autoimmune demyelinating disease, multiple sclerosis (MS) influences the central nervous system (CNS) and is also affected by HSPA6. Chiricosta et al. [95] demonstrated that HSPA6 was one of the upregulated genes

**Table 1** Roles of HSPA6 in tumors and other diseases and its pathophysiological mechanisms

| Tumors or diseases | Mechanisms of action | References |
|---|---|---|
| Bladder cancer | HSPA6 acts on garlic extract in EJ cells to enhance its mediated inhibition of cell proliferation, migration and invasion by enhancing ATM-CHK2-Cdc25C-p21WAF1-Cdc2 cascade mediated by $G_2$/M phase, inducing phosphorylation of MAPK and AKT signals, and inhibiting transcription factor-related MMP-9 regulation | [29, 36, 37] |
| Glioblastoma and pancreatic cancer | HSPA6 is a key ATF3-dependent gene. ATF3 induces transcription of members of the HSP70 and HSP40 family and triggers anti-tumor responses after inhibition by protein disulfide isomerase and histone deacetylase inhibitors | [30] |
| Colorectal cancer | As a novel proteasome inhibitor, ixazomib exert the anti-tumor effects by upregulating the HSPA6 gene, and affecting cell apoptosis and cell cycle pathways in CRC SW620 cells, suggesting HSPA6 may play a tumor-suppressive role under the effect of proteasome inhibitors | [45] |
| Gastric cancer | The expression of Rho guanine nucleotide exchange factor 10-like protein can stimulate the occurrence of gastric cancer by promoting GTP-RhoA-Rock1-phosphorylation-ERM signal transduction, inducing EMT and increasing the expression of HSPA6 | [54] |
| Lung cancer | HSPA6 is defined as a key factor regulating cell sensitivity, which may be achieved through interaction with HSP70 family members, inter-HSPs family members, and other families. Since acRoots is sensitive to $p53^-$ cell lines, this suggests that HSPA6 upregulation treated by acRoots may interact with p53 or its related pathways | [55, 56] |
| Hepatocellular carcinoma | The upregulation of HSPA6 expression is associated with early poor prognosis of HBV-related hepatocellular carcinoma | [53, 57, 58] |
| Ulcerative colitis | HSPA6 can interact with stable anti-apoptotic Bcl-XL to provide epithelial protection against intestinal damage caused by colitis | [78, 79] |
| Enterovirus A71 | HSPA6 is required for internal ribosome entry site (IRES)—mediated translation, which promotes IRES activity of enterovirus A71 through cellular proteins | [80] |
| Cardiovascular disease | Overexpression of HSPA6 can inhibit the proliferation of smooth muscle cells (SMC) | [81, 82, 84] |
| Immune thrombocytopenia | The significant reduction of HSPA6 can regulate apoptosis in immune thrombocytopenia through the PI3K–Akt signaling pathway | [89] |
| Multiple sclerosis | The expression of HSPA6 is significantly up-regulated, leading to the deterioration and promotion of immune system response to the myelin autoantigen | [95] |
| Anorectal malformations | HSPA6 is an autosomal-recessive disease-causing gene. The biallelic recessive mutations of HSPA6 cause congenital anorectal malformations and limb malformations, a major phenotype for VATER/VACTERL association spectrum | [99] |

in six brain areas including optic chiasm, internal capsule, hippocampus, corpus callosum, frontal and parietal cortex. Thus, HSPA6 might have harmful effects on autoimmune diseases such as MS, which exacerbated and promoted the immune system response facing the myelin autoantigen.

## Other diseases

In low oxygen condition of human placentation, survival of trophoblast cells required metalloprotease modulated shedding of heparin-binding EGF-like growth factor (HB-EGF) and downstream signaling. Jain et al. [96] found that HSPA6 played a vital role in regulating the biosynthesis of matrix metallopeptidase 2 (MMP2) and was necessary for the shedding of HBEGF under hypoxia. HSPA6 not only helped trophoblast cells survive in the low oxygen environment encountered during the first trimester but also was crucial for a successful pregnancy outcome in women.

Anorectal malformations (ARMs) are rare diseases, occurring in about 1/10,000 to 1/40,000 live births, caused by poor differentiation of the primitive hindgut [97]. The VATER/VACTERL association requires at least three component features, including vertebral defects (V), ARMs (A), cardiovascular anomalies (C), tracheoesophageal fistula with or without esophageal atresia (TE), renal anomalies (R), and limb anomalies (L), based on the limb defect [98]. Kause et al. [99] found that HSPA6 was an autosomal-recessive disease-causing gene. The biallelic recessive mutations of HSPA6 cause congenital anorectal malformations and limb malformations, a major phenotype for VATER/VACTERL association spectrum.

Compared to the healthy periodontal ligament, Goodman et al. [100] found that HSP genes were significantly overexpressed in human periapical granulomas. Specifically, the expression level of HSPA6 was significantly increased in granulomas and lipopolysaccharide-treated (LPS-treated) macrophages, and more highly expressed inactive lesions.

Hence, HSPA6 was associated with the development of periapical lesions and might explain the different clinical outcomes.

Altogether, the roles of HSPA6 in tumors and other diseases and its pathophysiological mechanisms are summarized in Table 1.

## Conclusions and perspectives

In recent years, research of HSP in various diseases has been increasing. HSPA6, as a member of the HSP70 family, is attracting more attention in the scientific community. HSPA6 can play a beneficial role in both physiological and stress conditions. Studies have shown that the changes of HSPA6 can reflect the healthy or unhealthy state of the body, especially the translational modification of HSPA6 may result in pathological changes. Interestingly, HSPA6 is highly expressed in cerebral cortex, the reason of which still needs further investigation.

Study of HSPA6 modulators elucidate the anti-cancer effect of HSPA6 in tumors and avoid tumor occurrence and tumor progression, which imply excellent future clinical application. Moreover, HSPA6 has great research value in the treatment of radiation-resistant tumors, revealing its role in radiotherapy, chemotherapy, immunotherapy, and hyperthermia can provide more therapeutic strategies for clinicians.

The clinical manifestations of tumor diseases, including complicated pathogenesis, difficult control of risk factors, high recurrence and metastasis rate, and poor accuracy, have led to high tumor morbidity and mortality rate. New targets and mechanisms need to be developed and characterized before use. Currently, in the tumor biology fields, there are only a few studies on HSPA6 but there are insufficient experimental data. And the experimental results do not all show the inhibitory effect of HSPA6 on the tumors.

HSPA6 has a bidirectional effect on tumors, which not only inhibits tumors directly and indirectly but also acts as a risk factor for tumorigenesis and tumor progression under certain conditions. Thus it is ambivalent as a prognostic marker. Through analyzing the TCGA database by GEPIA 2, it does show low expression of HSPA6 was associated with longer overall survival (OS) for COAD and LGG, while high expression of HSPA6 was associated with longer OS for SKCM (Supplementary Fig. 1). We still do not know the conditions that induce its role in tumor prevention, proliferation and progression. Most studies substantiated that HSPA6 undergoes changes through action of some drugs or natural compounds, and exerts its anti-tumor effect. However, its mechanism of action still needs in-depth study, including the regulatory mechanism of HSPA6 at the RNA or protein level, its interaction with different proteins, and its differential expression in different organs and tissues.

HSPA6 has potential use as a diagnostic, prognostic biomarker of various physiological and pathological conditions, and is expected to be used as a drug target in various diseases. Although studies have shown that HSPA6 has antitumor effects after induction of many drugs or natural compounds, there are still hurdles in pharmaceutical applications of HSPA6. The signaling pathways and action mechanisms of HSPA6 are still unclear, thus more exploration is needed to fully understand HSPA6, clarify its molecular targeting effects, and provide guidance for the clinical diagnosis, treatment and prognosis of tumors and other diseases.

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## Declarations

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