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Hsp70 in Cancer: Partner or Traitor to Immune System

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ABSTRACT

Heat shock protein 70.1 (Hsp70.1), also known as Hsp70, is a highly conserved member of the heat shock protein family that exists in all living organisms and determines the protein fate as molecular chaperones.

Hsp70 basal expression is undetectable or low in most unstressed normal cells, however, its abundant presence in several types of human cancer cells is reported. Several studies support upregulated Hsp70 involved in tumor progression and drug resistance through modulation of cell death pathways and suppresses anticancer immune responses. However, numerous studies have confirmed that Hsp70 can also induce anticancer immune responses through the activation of immune cells in particular antigen-presenting cells (APCs).

Regarding the significant and the promising role of vaccines in cancer immunotherapy, identification and characterization of the overexpressed Hsp70 as a potential immune stimulatory factor can pave the path for development of highly effective anticancer vaccines.

In this review, we will discuss the interactions of Hsp70 with components of the immune system in cancers as well as possible strategies to harness Hsp70 for eliciting anticancer immune responses.

Keywords: Apoptosis; Cancer vaccines; Heat shock protein 70 (Hsp70); Single-chain variable fragment antibody; Tumor microenvironment

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INTRODUCTION

All living organisms are exposed frequently to different environmental stresses such as chemicals and

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physical variations which threaten their survival. Heat shock proteins (HSPs), also called "molecular chaperones", play crucial roles in countering the effects of stresses through facilitating the peptide folding, translocation to subcellular organelle, and proteolysis activation of misfolded proteins. It is well known that HSPs not only are induced in thermal shock but also are expressed in different physical and chemical stressors such as ultraviolet radiation, hypoxia, oxidative stress, and heavy metals.[1] Based on molecular weight, HSPs are classified into several families, including Hsp110, Hsp90, Hsp70, Hsp60, and small HSPs. There are thirteen members of the human Hsp70 family with different properties such as subcellular localization, tissue expression, and regulation of gene expression. In this family, the Hsp70.1, called Hsp70 in this review, is one of the most important members which has diverse and sometimes opposing functions. The expression pattern of Hsp70 depends on age, tissue, and different physiological and pathological conditions. While our knowledge of the Hsp70 function is limited to its chaperone activity, the effects of its function in various conditions, such as pregnancy[2] and particularly in cancers,[3] have led it to conclude that it has more complex roles than only a chaperone function. Surprisingly, many *in vitro* and *in vivo* studies demonstrated that upregulated cytoplasmic Hsp70 in cancer cells suppresses apoptotic pathways,[4] autophagy, and lysosomal cell death (LCD)[5-7] leading to tumor progression and establishment of chemo/radio-resistance.[8] Furthermore, the plasma membrane and cancer cell-secreted isoforms of Hsp70 can involve in immunosuppression leading to tumor progression. However, other pieces of evidence showed another side of the coin: Hsp70 can provoke anticancer immune responses. Given the contradictory roles of Hsp70 in interaction with the immune system, there are two different approaches for cancer immunotherapy, including targeting Hsp70 by monoclonal antibodies and/or CAR-T cells or its exacerbated presence by Hsp70-based vaccines. Which of these methods could provide a promising approach in cancer immunotherapy? In this review, we will discuss the interaction of various isoforms of Hsp70 within the cells and its interaction with components of the immune system as well as possible strategies to harness of Hsp70 for eliciting anticancer immune responses.

MATERIALS AND METHODS

We searched PubMed, Elsevier, UniProtKB, IEDB database, Human Protein Atlas knowledgebase, ExPASy web server, and Clinicaltrials.gov for articles that were published from 2000 to 2019 as well as bibliographies of articles to include additional relevant studies; using the following combinations of MeSH terms with a manual search: Hsp70, tumor biomarker, metastasis, angiogenesis, cancer immunotherapy, and clinical trials. Citations from all databases were imported into a single database (Endnote library, version X8, Thomson Reuters, USA) and duplicate articles were removed. Full texts of articles were carefully read, and data were extracted for data extraction in Microsoft Word and Excel sheets (version 2016, Microsoft Corporation, USA) and to display them by Visual Molecular Dynamics software (VMD version 1.9.1, the University of Illinois at Urbana-Champaign).

Genetic and Structural Biochemistry of Hsp70

Three intron-less HSP70 genes, including HSPA1A, HSPA1B, and HSPA1L, are mapped between the human lymphotoxin β (LTB) and complement system genes embedded in the major histocompatibility complex class III (MHC III) region on the human chromosome (6p21.310,[9] Figure 1-A. Although the genes of HSPA1A and HSPA1B represent similar sequences (only differ in 8 bp) with different mechanisms in the regulation of expression. The HSPA1A and HSPA1B genes, are usually considered as Hsp70-1, and encode a similar protein with 99% identity but have a completely divergent 3' untranslated regions (3'-UTR). The Hsp70-1L shares 90% homology to HspA1A and HspA1B but is not inducible by heat shock.[10,11]

The human Hsp70 contains 641 amino acids with 70,052 Da in molecular weight and is consisted of two major conserved functional domains[12] including (I) A nucleotide-binding domain (NBD) or ATP-binding domain (ABD) at N-terminal which binds and hydrolyzes ATP. (II) A substrate-binding domain (SBD) at C-terminal (Figure 1-B). This domain forms a pocket to interact with extended polypeptides as a substrate or client protein. Besides, a~10 kDa subdomain of SBD acts as a flexible "lid" over the substrate-binding pocket. The NBD and SBD are connected by a highly conserved leucine-rich motif

(LRR) also termed as a flexible linker.

Expression Pattern of Hsp70 in Various Conditions

Under normal conditions, Hsp70 is expressed and accumulated during the mid-G1 and early S phase of the cell cycle in a cell type and cell cycle manner.[14,15] *In silico* analysis of expressed sequence tag (EST) data suggest that Hsp70s have a different expression pattern at 44 normal human tissues.[16]. HspA1A and HspA1B have high expression in the spleen and the esophagus, respectively. The expression profiles for Hsp70 (HspA1A and HspA1B) in major organs and tissues in the human body have prepared by The Human Protein Atlas (HPA) knowledgebase (www.proteinatlas.org/); using the integration of different omics technologies including transcriptomics and proteomics.[17] The expression levels of both HspA1A and HspA1B are measured by EST analysis and HPA are not similar and may be contradictory. Nevertheless, the results of both methods show that these two proteins do not have identical expression patterns in various normal tissues (Table 1). These data reveal that HspA1A and HspA1B differ only in two amino acid residues, they could be expressed and become active in tissue-specific manners. Furthermore, the EST analysis indicated that Hsp70 is preferentially expressed at specific stage development[16] so that both HspA1A and HspA1B are expressed at their highest levels in juvenile tissues (Figure 2), therefore these two members of Hsp70s play an important role in mammalian development. This interpretation is greatly supported by the outcomes of male mammalian models[18-20] as well as interesting findings obtained from various conditions of nondisease and disease (Table 2).

Hsp70 in Cancer

A great number of studies indicate that Hsp70 is expressed at undetectable or low levels in most unstressed normal cells while it is overexpressed in different types of cancers.[4] Although a few studies have determined that single nucleotide polymorphisms (SNPs) of the HspA1A gene be associated with several cancers, no mutation or amplifications have been found. Based on these findings, the expression of Hsp70 in many tumors should be regulated at transcriptional and translational levels. Elevated level of Hsp70 associated with overexpressed Heat shock factor 1 (HSF1) as the major transcription

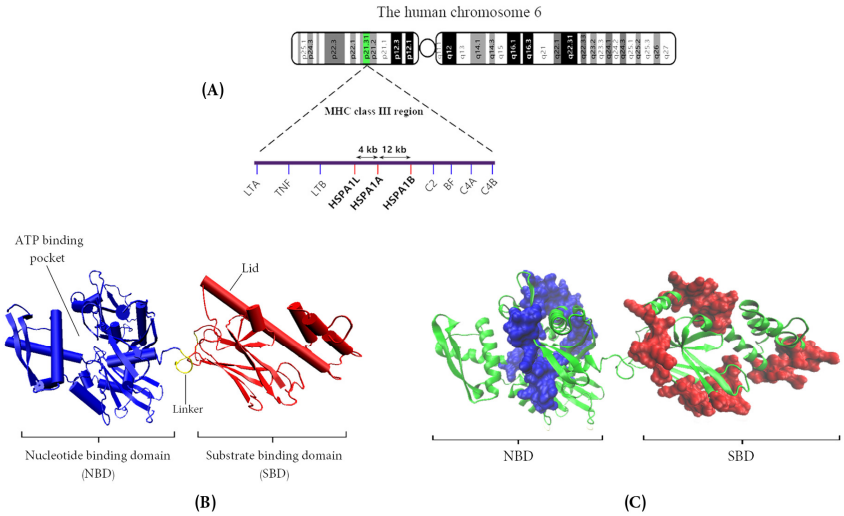

Figure 1. The human Hsp70. (A) three HSP70 gene loci including HSPA1A, HSPA1B, and HSPA1L are all located on the chromosome 6p21.31 within the major histocompatibility class III (MHC III) region. (B) The NBD (residues 1 to 383) and SBD (residue 397 to 507) are functional domains of Hsp70 that coupled together by a flexible linker (residues 384 to 396). (C) The Hsp70 provides ten specialized B-cell epitopes with more than 10 amino acid residues in both NBD and SBD (the epitopes at NBD shown in blue in and the epitopes at SBD shown in red). These epitopes predict by the IEDB database (http://www.iedb.org).[13] The secondary structures of Hsp70 (UniProtKB identifier: P0DMV8) visualized using VMD 1.9.1 bioinformatics software.[13]

Table 1. Expression pattern of Hsp70 (HSPA1A and HSPA1B) in various human normal tissues

Organ	Tissue/Cell type	HSPA1A		HSPA1B	
		HPA	EST	HPA	EST
Brain	Cerebral cortex	L	805 for Brain	M	235 for Brain
	Hippocampus	L		M	
	Caudate	L		H	
	Cerebellum	L		L	
Endocrine tissues	Pituitary gland	No data	167	No data	0
	Thyroid gland	H	776	M	271
	Parathyroid gland	M	0	L	0
	Adrenal gland	M	1805	M	411
Bone marrow and immune system	Appendix	M	No data	L	No data
	Bone marrow	L	61	ND	81
	Lymph node	L	10	L	10
	Lymph	No data	0	No data	0
	Thymus	No data	1982	No data	399
	Tonsil	M	115	M	0
	Spleen	M	7292	ND	1416
Muscle	Heart muscle	L	1692	H	347
	Skeletal muscle	M	262 for muscle	M	52for muscle
	Smooth muscle	M		M	
Lung	Lung	M	1467	L	153
	Nasopharynx	M	No data	H	No data
	Trachea	No data	2595	No data	797
	Bronchus	H	No data	H	No data
Live and gallbladder	Liver	M	397	M	62
	Gallbladder	H	No data	M	No data

Organ	Tissue/Cell type	HSPA1A		HSPA1B	
		HPA	EST	HPA	EST
Pancreas	Islets of Langerhans	L	480 for Pancreas	L	155 for Pancreas
	Exocrine glandular cells	M		ND	
Gastrointestinal tract	Salivary gland	M	0	M	0
	Oral mucosa	M	No data	M	No data
	Esophagus	H	2732	H	1839
	Stomach	M	641	M	145
	Duodenum	M	3914 for small intestine	L	380 for small intestine
	Small intestine	M		L	
	Colon- Endothelial and Glandular cells	M	320 for Colon	L	103 for Colon
	Colon- Peripheral nerve/ganglion	L		L	
	Rectum	M	No data	L	No data
Kidney and Urinary bladder	Cells in glomeruli	L	931 for Kidney	M	214 for Kidney
	Cells in tubules	M		ND	
	Urinary bladder	H	782	H	1108
Male organs	Testis	M	197	M	44
	Prostate	H	1410	H	237
	Epididymis	H	No data	H	No data
	Seminal vesicle	H	No data	H	No data

Organ	Tissue/Cell type	HSPA1A		HSPA1B	
		HPA	EST	HPA	EST
Female organs	Fallopian tube	M	No data	M	No data
	Breast- Adipocytes	M	647 for mammary gland	ND	104 for mammary gland
	Breast- Glandular cells	H		L	
	Breast- Myoepithelial cells	M		ND	
	Vagina	H	No data	M	
	Cervix, uterine- Squamous epithelial cells	H	123 for Cervix 721 for Uterus	H	103 for Cervix 266 for Uterus
	Cervix, uterine- Glandular cells	M		H	
	Endometrium	M		M	
	Ovary	M	74	M	83
	Placenta	M	169	L	13
	Umbilical cord	No data	0	No data	0
Adipose and soft tissue	Chondrocytes	H	2885 for Adipose	No data	591 for Adipose
	Fibroblasts	M		ND	
	Peripheral nerve	M		ND	
	Adipocytes	M		ND	
Skin	Fibroblasts	M	358 for Skin	ND	42 for Skin
	Keratinocytes	M		H	
	Langerhans	M		H	
	Melanocytes	M		H	

EST: results extracted from expressed sequence tag analysis.[16] HPA: results extracted from the Human Protein Atlas knowledge base (www.proteinatlas.org/). L: Low, M: Medium, H: High.

Table 2. Circulating level of Hsp70 in various conditions[22-25]

Conditions	Hsp70 level	Example
Normal	Increases	Different types of exercise, excessive use of cell phones
	Decreases	Human pregnancy and Aging process
Disease	Increases	Diabetes mellitus, Carotid intima-media thickness, Pulmonary diseases, Active chronic glomerulonephritis, Sepsis, Inflammation, and Cancers
	Decreases	*Helicobacter pylori* infection, Fatty liver diseases, Hepatic steatosis, Arteriosclerosis, Atrial fibrillation following coronary artery bypass surgery and Obstructive sleep apnea

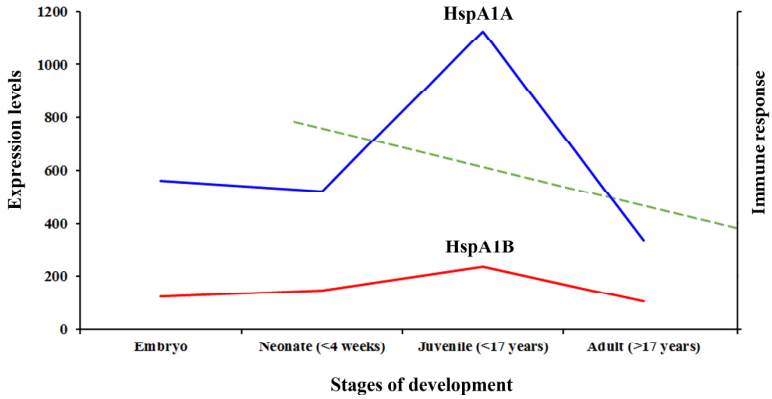

Figure 2. Expression of HspA1A and HspA1B in a lifetime. The peak of both Hsp70 levels is in the juvenile stage. The results extracted from expressed sequence tag analysis.[16] Synchronically, when age advances immune responses also will be decline.[21]

factor for heat shock proteins, in many cancers can prove this interpretation.[26] Subsequently, increased level of Hsp70 has emerged as a candidate biomarker for poor prognosis as its expression level was found to significantly correlate with clinical staging and overall survival rate in various types of human malignancies including breast, lung, prostate, liver, esophagus colon and cervix cancers.[2-6] The upregulation of Hsp70 can guarantee cancer cell survival via its chaperone function. It has been shown that under stress condition (i.e. cancer), Hsp70 levels not only increase in the cytoplasm but also it appears at the plasma membrane and it is even secreted into the extracellular milieu. Since 1995, several studies showed that a large variety of human cancer cells such as pancreatic carcinomas, glioblastoma, breast, ovarian, head and neck, colorectal, non-small cell lung cancer (NSCLC), prostate, and acute lymphoblastic leukemia are Hsp70 plasma membrane positive, however, normal cells are negative for Hsp70.[8,27-29] A little later, it was proved that the density of the membrane Hsp70 can be enhanced on tumor cells by various drugs[30-32] and standard cancer therapeutic methods.[3,33,34] Interestingly, it was also found that the circulating tumor cells (CTCs) that are responsible for metastasis[35] also represent Hsp70 in their plasma membranes.[36] Surprisingly, Hsp70 releases into the extracellular milieu of tumor cells.[37,38] Indeed, it lacks a consensual signal peptide thus it cannot be export via the classical endoplasmic reticulum-Golgi protein transport mechanisms.[39] The exact mechanism of Hsp70 release from cancer cells is not clear, six possible mechanisms are suggested including; (I) fusion of endolysosomes with the plasma membrane,[39] (II) secretion from dying cells,[40] (III) by secretory-like granules,[41] (IV) specific interaction with membrane phospholipids,[42] (V) refuge in tumor-derived exosomes (TDEs) that leave the cells through the plasma membrane blebbing,[43,44] and (VI) formation of pores and stable multi-conductance ion channels.[45,46] Regardless of the possible mechanism, both membrane and extracellular Hsp70s become available for the components of the immune system as three different forms including free soluble, complexed with tumor antigenic peptides and TDEs. Not surprisingly, B-cell specialized epitopes in Hsp70 can be predicted by the IEDB database (http://www.iedb.org)[47] (Figure 1) which can involve in the immune responses that will be discussed in the following section.

Hsp70 and Immune System

As previously mentioned, it is estimated that after birth both HspA1A and HspA1B levels gradually increase after birth and reach the maximum level in juvenility and then decrease with age. Interestingly, there is a similar pattern for the immune system: from childhood, the immune system starts to mature but as age advances, the function of both the innate and adaptive immune systems decline,[21,48] (Figure 2). Moreover, the dendritic cells (DCs) are a shred of evidence that can demonstrate the alignment between Hsp70 and the immune system. Firstly, many compelling pieces of evidence have shown that both free Hsp70 and Hsp70- tumor antigenic peptide complex can bind to their receptors on the DCs such as CD14 and Toll-like receptor 2 and 4 (TLR2/4), leading

to the maturation and activation of these cells.[49-53] Then, the activated and matured DCs interact with $CD8^+$ cytotoxic T lymphocyte (CTL) to initiate an adaptive immune response.[54,55] Therefore, it is concluded that Hsp70 may also play a The upregulation role in the activation of the immune system in normal conditions. Secondly, a recent study found that the expression of antigen presentation genes in DCs is reduced in healthy aged compared to young individuals.[56] Although the interaction between Hsp70 and immune system components is not completely understood under normal conditions, it has been widely studied in cancer settings.

Part I: Hsp70, A Partner to Immune System

Hsp70 is found to activate natural killer (NK) cells in cancer. In the presence of IL-2 as a pro-inflammatory cytokine, the membrane Hsp70 on tumor cells can activate $CD57^+/CD94^+$ NK cells.[57,58] leading to the secretion of granzyme B. Moreover, membrane Hsp70 enhances uptake of granzyme B by cancer cells in a perforin-independent fashion. This function of Hsp70 is due to a 14-mer sequence at its SBD, also known as TKD peptide (Residues: 450 to 463: TKDNNLLGRFELSG, Figure 1) which is appeared to the extracellular side of tumor cells.[59,60] In addition, the Hsp70 positive TDEs can also activate NK cells.[61]

Another well-known mechanism for immune-stimulatory effects of Hsp70 is through activation of the antigen-presenting cells (APCs), precisely DCs. lines of evidence support extracellular Hsp70 role a danger signal for APCs and induce their functional maturation. It has been shown that Hsp70 interacts with CD14 and TLR2/4 on APCs resulting in the release of nitric oxide (NO) as well as proinflammatory cytokines such as TNF-α, IFN-γ, and IL-1β.[45,62,63] Moreover, extracellular Hsp70 induces the release of high mobility group protein B 1 (HMGB1) that is a proinflammatory cytokine and decisively implicated in cancer.[64] Lastly, it was determined that the free extracellular Hsp70 can also act as a damage-associated molecular pattern (DAMP) and induces proinflammatory cytokines in the human lung cancer cells through RAGE signaling.[65]

Some studies also have shown a cross-talk between NK and DCs. Based on a scenario, there is a dialog between NK cells and DCs for the production of IFN-γ by NK cells. In the first curtain, Hsp70 induces the expression of MHC class I chain-related gene A (MICA) on DCs. In the last curtain, the interaction between MICA with its receptor (NKG2D) on NK cells leads to the production of IFN-γ by NK cells.[66] In another show, a 20-mer sequence at the SBD of Hsp70 (residues: 407 to 426: GGVM TALIKRNSTIPTKQTQ) induces upregulation of MHC class II and costimulatory molecules such as CD40 and CD86, leading to the maturation and cytokine production of DCs[49,50] which in return interact with CTL to initiate an anticancer adaptive immune response.

Interestingly, there are various receptors for the extracellular Hsp70- tumor antigenic peptide complex on the APCs and even on endothelial/epithelial cells such as TLR2/4, CD40, FEEL-1 and LOX-1.[51-53] Occupation of these surface receptors by the Hsp70-tumor antigenic peptide complex leads to a receptor-mediated endocytosis and antigen cross-presentation onto MHC class I [molecule67,68] which in turn can induce anticancer CTL responses.[69,70] Therefore, Hsp70 mediates the coupling of innate to adaptive immunity by activation of DCs.

Part II: Hsp70, a Traitor to Immune System

"Treason is greatest where trust is greatest". John Dryden (1631- 1700)

Despite the well-understood effects of Hsp70 in the induction of cell-mediated immune responses against cancer, a limited number of studies show that Hsp70 can also induce tolerance in some types of human malignancies. Generally, cancer-produced Hsp70 isoforms act through autocrine signaling on the tumor cells and through paracrine signaling on immune and endothelial/epithelial cells which can result in tumor progression and induction of cancer tolerance. The results from both human and mice models demonstrated that refuged Hsp70 inside and membrane of TDEs contributes to the immunosuppressive activity of myeloid-derived suppressor cells (MDSCs).[71] In addition, an *in vitro* model also indicated that enhanced immunosuppressive activity of T_{reg} by the free extracellular Hsp70 leads to increasing in TGF-β and IL-10 as suppressor cytokines but decreasing in TNF-α and IFN-γ as proinflammatory cytokines.[72] Furthermore, several studies are recently confirmed that Hsp70 is involved in angiogenesis and metastasis procedures.[73-75] However, an *in vitro* study on various human cancer cell lines is showed that Hsp70 plays a contradictory role in metastasis in which silencing of

Hsp70 gene expression enhances the migration ability of the cells.[76]

Hsp70 in Cancer Immunotherapy

As Hsp70 has a dual role in cancer immunity, it has been used in both activation and suppression of immunotherapy. The stimulation and suppression strategies based on Hsp70 are illustrated in Figure 3. In the stimulating strategy, tumor-derived or exogenous Hsp70 is used as a vaccine to evoke anticancer immune responses. Based on a wide range of studies, Hsp70 vaccine vehicles can be prepared from different approaches (Table 3). Up to now, all developed Hsp70-based anti-cancer vaccines were found to effectively induce anticancer immune responses and suppress tumor growth in different animal cancer models. However, none of the developed Hsp70-based anticancer vaccines have been approved for clinical practices. Among the reported Hsp70 vaccines, only two vaccines have been found to be promising in cancer: (i) Hsp70PC with Imatinib in patients with chronic; myeloid leukemia (in the phase I clinical trial) and in patients with high-risk breast cancer (at the phase II clinical trial)[77] and (ii) stimulated autologous NK cells by TKD peptide in patients with metastasized non-small cell lung cancer (at the phase II clinical).[78]

In suppression strategies, the membrane/extracellular Hsp70 can be a target for anticancer drugs to inhibit the immune suppressive function of Hsp70. Targeting of tumor markers such as Hsp70 by monoclonal antibodies or their fragments has been suggested to be an effective approach to cancer targeted therapies. For this, the cmHsp70.1 antibody was established by immunization of BALB/c mice through TKD peptide. This mouse antibody has a high affinity for membrane Hsp70 expressed in various tumor cells. It also is revealed that cmHsp70.1 is able to induce antibody-dependent cellular cytotoxicity (ADCC) of the membrane Hsp70 positive tumor cells in mice.[79] Additionally, imaging of the membrane Hsp70 positive CT26 mouse tumor cells by the cmHsp70.1-conjugated gold nanoparticles showed that this antibody can be used as a promising diagnostic and therapeutic tool.[80] Furthermore, it is revealed that the cmHsp70.1 can be used for the isolation and quantification of CTCs from peripheral blood of different tumor patients.[36] Recently, a novel anti-Hsp70 truncated single-chain fragment variable (scFv) has been isolated by Phage display technology (PDT),[81] known as G6A scFv. Although *in silico* analysis, surface plasmon resonance (SPR) and

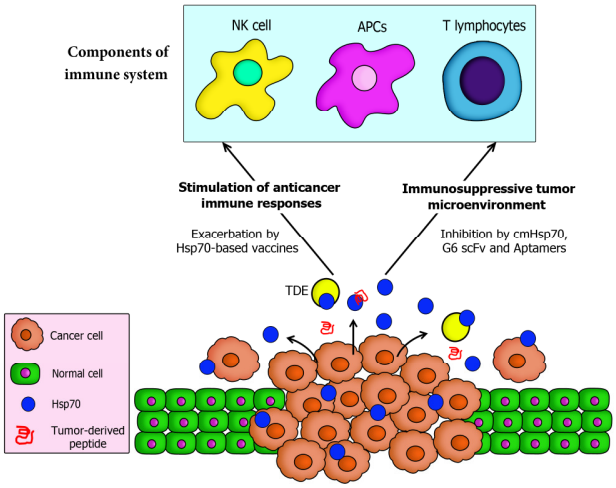

Figure 3. Hsp70 in cancer immunotherapy. The Hsp70 isoforms released from tumor cells have a dual function: On the one side, Hsp70 suppresses anticancer immune responses. On another side, Hsp70 stimulates anticancer immune responses. Based on the Hsp70 function, therapeutic approaches can be categorized as stimulation and suppression strategy, respectively. Both strategies are two sides of the one coin: in stimulation strategy, Hsp70-based vaccines stimulate the immune system but in suppression strategy, Hsp70 targeted by certain compounds to enhance immune system activities.

Table 3. Hsp70 based anticancer vaccines

Vaccine	Model of study	Immune response	Reference
Induction of Hsp70 on cancer cell surfaces and releasing into the extracellular milieu by physical and chemical stimuli	*in vitro*	Enhanced NK cell activity	(1)
	in vitro	Activation of Mast cells	(2)
	in vitro	DCs activation and maturation and T cell activation	(3)
Secretory Hsp70 from engineered tumor cells	*in vitro* and *in vivo*	CTL response	(4)
Tumor-derived Hsp70 (Hsp70PC)-Imatinib mesylate complex	Clinical	Activation of NK cell and T cells	(5)
Hsp70.PC-F obtained from the fusion of DC and tumor cells	*in vivo*	Increased $CD8^+$ and memory T cells	(6)
SC injection of Hsp70-melanoma peptide complex with IV delivery of the plasmid pPD-1A encoding sPD-1	*in vivo*	Increased tumor-infiltrating lymphocytes	(7)
Hsp70- HPV16 E7 fusion protein	*in vivo*	Enhanced CTL response	(8)
Repeated IV delivery of autologous Hsp70 isolated from murine Dalton's lymphoma and sarcoma (S-180)	*in vivo*	Enhanced CTL response	(9)
Hsp70- AFP fusion protein	*in vivo*	Increased $CD8^+$ T cell responses	(10)
IT delivery of pure soluble rhHsp70	*in vivo*	Enhanced CTL response and production of IFN-γ	(11)
Local injection of pure recombinant human Hsp70 (rhHsp70) by ALZET osmotic pump	*in vivo*	Increase both innate and adaptive immune responses	(12)
Hsp70- anti-mesothelin scFv fusion protein	*in vivo*	DC maturation and CTL response	(13)
The IV infusion of ex vivo stimulated autologous NK cells by TKD peptide	Clinical	Enhanced NK cell activity and T cell activation	(14)
Human DKK1 and human Hsp70 fusion DNA	*in vivo*	Increased $CD4^+$ and $CD8^+$ T cells, and decreased T_{reg} cells in the spleen	(15)

IV: Intravenous, SC: Subcutaneous, IT: Intratumoral, scFv: single-chain antibody variable fragment, AFP: alpha-fetoprotein.

cell staining indicated that purified G6A scFv has good quality for binding,[82] more studies should be conducted to address its diagnostic and therapeutic functions at *in vivo* models. Noteworthy, a scFv has several advantages resulted from its minimized size in comparison to the full antibodies such as better penetration into the tumor, high blood clearance and also reduced immunogenicity.[83] Moreover, an anti-tumor marker scFv can be used to design chimeric antigen receptor T cells, also known as CAR T-cells, that are a promising cancer therapeutic approach.[84] Accordingly, it is claimed that an anti-Hsp70 specific CAR T-cell designed particularly for the treatment of particular leukemia.[85] Of note, there are also many chemical derivatives that inhibit Hsp70. Nevertheless, none of these Hsp70 inhibitory molecules have found their way to the clinic due to non-specificity and low bioavailability.[86]

CONCLUSION

It has been known for more than a decade that Hsp70 not only plays a key role in the development of human organs, but also it has important functions in various human diseases such as cancer. Nowadays, Hsp70 is suggested to be a potential biomarker of some disorders especially cancer.[99] However, the role of Hsp70 in cancers is dual and mysterious. On one hand, Hsp70 can protect tumor cells via suppression of apoptosis and induction of cancer tolerance, leading to

tumor progression and invasion. On the other hand, Hsp70 especially the membrane-bound and extracellular one can induce apoptosis and provoke potent antitumor immune responses thereby suppress tumor growth. This contradiction in the role of Hsp70 in cancer has led to the development of Hsp70-based cancer targeted therapy with two different approaches, including blocking Hsp70 by monoclonal antibodies and the use of Hsp70 protein as an immune potentiator in Hsp70-based vaccines. While neither one of these approaches has been translated to a clinical approach yet, they are believed to be promising therapeutic strategies for cancer targeted therapy.

Although the dual function of Hsp70 has not been addressed to date, the answer may lie in the amino acid residues in domains of Hsp70 or its context-dependent mode of function. In detailed, proteins hold structural domains that allow their interactions with specific sequences on other proteins, protein-protein interaction (PPI).[100] These interactions play key roles in cancer signaling.[101] However, the exact structure of the Hsp70 has been determined by methods such as crystallography, but the role of its domains and subdomains in the PPI network are still unclear. In a simple interpretation, the paradox in functions of the Hsp70 may be due to the difference in the interactions of Hsp70 with the other proteins in different contexts, which may have different and sometimes conflicting results. For example, a piece of evidence showed that the metastatic ability of various human cancer cells enhanced by downregulation of Hsp70.[76] Thus, the context-dependent PPIs of Hsp70 as a putative therapeutic target for the development of novel therapeutic approaches must be determined in different type of cancers, otherwise it is not possible to predict exactly how Hsp70 acts.

On the other hand, an important reason behind the poor therapeutic efficacy of Hsp-70 based vaccines can be tumor immunosuppressive microenvironment which is believed to suppress the anticancer immune responses elicited by cancer vaccines.[102] For example, the results from the studies on A431 squamous carcinoma cells and hepatocarcinoma cells are found that the extracellular Hsp70 promotes tumor progression through interaction with TLR2/4.[103,104] whereas it had previously been shown that Hsp70 can lead to activation and maturation of DCs via binding to TLR2/4.[49-53] Therefore, manipulation of tumor milieu has been suggested as an important strategy for enhancing the therapeutic efficacy of anticancer vaccines.[105] Of note, the effectiveness of immunotherapy is highly dependent on the cancer type, grade, and other criteria.[106] Therefore, to develop an effective Hsp70 immunotherapy strategy, the PPIs of Hsp70 and contexts of different cancers must be considered. In other words, stimulation and suppression strategies might warily elect to depend on the type of cancer which also means personal medicine.

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