Immunostimulatory functions of membrane-bound and exported heat shock protein 70

Running Title: Immunostimulatory functions of Hsp70

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Abstract

In the search for tumor-specific antigens, microbial and eukaryotic heat shock proteins (HSP) have been identified. Intracellularly, HSPs function as molecular chaperones supporting folding and transport of a great variety of polypeptides and proteins under normal physiological conditions and following stress stimuli. Furthermore, interferon-γ and elevated body temperature induced by exercise have been found to increase serum levels of HSPs in humans. Extracellularly localized or plasma membrane-bound HSPs elicit a potent anti-cancer immune response mediated either by the adaptive or innate immune system. Following uptake of HSP (HSP70 and gp96)-peptide complexes by antigen presenting cells (APCs) and "cross-presentation" of HSP-chaperoned peptides on MHC class I molecules, a CD8-specific T cell response is induced. Apart from chaperoning tumor-specific peptides, HSPs per se provide activatory signals for the innate immune system. Binding of peptide-free HSP70 to APCs via Toll-like receptors (TLRs) initiates the secretion of pro-inflammatory cytokines and thus results in a broad non-specific immunostimulation. An unusual membrane localization of Hsp70, the major heat-inducible member of the HSP70 family, on tumor cells but not on corresponding normal tissues was found to act as a tumor-specific recognition structure for natural killer (NK) cells. Soluble as well as cell membrane-bound HSP70 can directly activate the cytolytic and migratory capacity of NK cells. APCs and tumor cells actively release HSP70s in lipid vesicles with biophysical properties of exosomes. These HSP70-presenting exosomes are thought to stimulate the adaptive and innate immune system in vivo. Taken together, depending on their intra/extracellular localization, peptide loading status, origin and route of application, HSPs either exert immune activation as danger signals in cancer immunity or protect cells from lethal damage induced by exogenous stress stimuli.

Key Words: Heat shock proteins, exosomes, NK cells, exercise, protein transport, cancer, immunostimulation

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Introduction

Heat shock proteins (HSPs) are highly conserved proteins that inhabit nearly all subcellular compartments. Following physical as well as chemical stress the synthesis of HSPs is strongly induced in both, prokaryotic and eukaryotic cells in order to protect the cells from lethal damage. Members of the HSP70 family are involved in intracellular transport processes, support folding of nascent polypeptides and play key roles in antigen processing. Apart from their chaperoning functions (1-3), HSPs have been found to play key roles in tumor immunity mediated by antigen presenting cells (APC)s, T cells and natural killer (NK) cells (4). Most immunotherapeutical approaches exploit the carrier function of HSPs for tumor-specific antigenic peptides (5-7). Tumor-derived HSPs with a $MW_{[r]}$ of ~70 kDa (Hsc70, Hsp70) and 96 kDa (gp96) have been shown to chaperone immunogeneic peptides (6,8) into the MHC class I antigen presenting pathway (7,9). Several receptors including CD40 (10), the α-2 macroglobulin receptor CD91 (11-13) and members of the Toll-like receptor family (TLR-2, TLR-4) either alone or in combination with CD14, the LPS receptor (14-17), are thought to mediate binding and uptake of HSP70- and HSP90-peptide complexes. More recently, human APCs have been found to interact with HSPs via scavenger receptors LOX-1 and SRA-1 (18,19). The role of the collagen and thrombospondin receptor CD36 as an HSP70-selective receptor needs further investigation (20,21). After receptor-mediated uptake and re-presentation of HSP-chaperoned peptides on MHC class I molecules, a tumor-specific $CD8^+$ T cell response is induced (7,22). The process of an MHC class I-specific presentation of exogenous peptides is generally termed cross-presentation. Even in the absence of tumor-derived peptides, HSP70s have the capacity to stimulate the secretion of pro-inflammatory cytokines including interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α via a TLR-2/ TLR-4 and CD14-associated pathway by APCs (14). This "chaperokine" function of members of the HSP70 family results in a non-specific stimulation of the innate immune system (23).

An additional effect of peptide-free Hsp70 is its immunostimulatory activity upon NK cells (24-27). By flow cytometry, membrane-bound Hsp70 was selectively detected on the surface of tumors but not on corresponding normal tissues (28). Our group showed that membrane-bound Hsp70 provides a tumor-specific recognition structure for NK cells (24,29,30). The amount of plasma membrane-bound Hsp70 was correlated with the sensitivity towards lysis mediated by NK cells (31,32). Several clinically applied therapies including cytostatic drugs, γ-irradiation, insulin sensitizers and COX-1/COX-2 inhibitors have been found to enhance the Hsp70 membrane expression selectively on tumor cells (33-36). This therapy-induced, increased Hsp70 surface density correlates with an increased sensitivity of tumors towards NK cell-mediated lysis.

Cross-talk of NK cells with Hsp70 membrane-positive tumor cells appears to be mediated via the C-type lectin receptor CD94 (37,38). Thus, the understanding of physiological factors orchestrating NK cell activation might provide the basis for innovative approaches in cellular immunotherapy. The present review summarizes our current view on immunological effects of extracellular and membrane-bound HSPs on the innate and adaptive immune system and its relevance for exercise-initiated immunological effects.

Cross-talk of NK cells with Hsp70

As mentioned above, our group detected a tumor-selective plasma membrane localization of Hsp70 by selective cell surface iodination and by flow cytometry using an Hsp70-specific monoclonal antibody directed against the C-terminal substrate binding domain (24,28). Since this antibody also inhibits the cytolytic activity of Hsp70-expressing tumor cells (24), we assumed that its recognition epitope might be crucial for the interaction of NK cells with Hsp70. Peptide scanning of NK cells against the complete substrate binding domain identified an 8-mer sequence NLLGRFEL ($aa_{454-461}$) as the antibody epitope.

Based on these findings we were interested to identify the minimal Hsp70 peptide sequence with the capacity to bind to NK cells. Binding studies using different fluorochrome-labelled peptides all containing the core sequence NLLGRFEL revealed that the 14-mer Hsp70 peptide TKDNNLLGRFELSG ($aa_{450-463}$) termed TKD provides the minimal Hsp70 sequence interacting with NK cells. Furthermore, this peptide was shown to enhance the cytolytic activity of NK cells against Hsp70 membrane-positive tumors (39) identically to full-length Hsp70 or the C-terminal domain (25,40). These findings are in line with observations of the group of Colombo demonstrating that genetically engineered tumors secreting the inducible Hsp70 displayed an increased immunogenicity against cancer in a mouse model (41). The genetic manipulation of tumor cells did not affect the chaperone activity of Hsp70. Tumor rejection in these mice was mediated on the one hand via an increased amount of dendritic cells mediating a robust $CD8^+$ T cell response, and on the other hand by an enhanced susceptibility towards NK cells (42).

Since binding of Hsp70 protein as well as TKD peptide to NK cells was saturable and concentration-dependent (37,38), a receptor-mediated interaction was hypothesized. However, HSP receptors postulated for APCs were only weakly or not expressed on NK cells. In contrast, the cell surface density of the C-type lectin receptor CD94 was significantly up-regulated after co-incubation of NK cells either with Hsp70 protein or TKD plus cytokines (43). Moreover, a CD94-specific antibody did not only block Hsp70 binding to NK cells but also the cytolytic activity towards Hsp70 membrane-positive tumor cells (38). These data strongly suggested an involvement of CD94 in the interaction of NK cells with Hsp70.

Novel mode of NK cell-mediated tumor cell killing

The mechanism of lysis of Hsp70 membrane-positive tumor cells was characterized as a perforin-independent, granzyme B-mediated apoptosis (43). By affinity chromatography a 32 kDa protein representing granzyme B could be eluted from NK cell lysates after incubation with columns coupled either with full length Hsp70 or Hsp70 peptide TKD. These data strongly suggest that granzyme B has a high affinity not only for full length Hsp70 protein but also for TKD peptide which is exposed to the extracellular milieu by tumor cells. Our findings were supported by data of Judy Liebermann`s group who precipitated Hsp70 and Hsp27 from granzyme B affinity columns incubated with cell lysates (44).

From a functional point of view we demonstrated that membrane-bound Hsp70 not only facilitates binding and uptake of granzyme B but also initiates

apoptosis in Hsp70 membrane-positive tumors in a perforin-independent manner. Classically, NK cell-mediated tumor cell killing involves the exocytosis of cytotoxic granules containing perforin and serine proteases (45). After internalization procaspases are cleaved into their activated form by granzyme B promoting DNA fragmentation followed by programmed cell death (46-49). The mannose 6-phosphate receptor was discussed to be involved in the process of endocytosis/pinocytosis of both, granzyme B and perforin (50-52). Our data provide evidence for a novel granzyme B-mediated but perforin-independent induction of apoptosis selectively in Hsp70 membrane-positive tumor cells (Figure 1). Normal cells lacking an Hsp70 membrane expression do not provide targets for granzyme B. As shown in Figure 1, contact of NK cells with membrane-bound Hsp70 or TKD results in an up-regulated expression of the C-type lectin receptor CD94 and thus initiates the production and secretion of high amounts of the apoptosis-inducing enzyme granzyme B, but not of perforin. Released granzyme B interacts with Hsp70 (53) which forms ion channels in artificial lipid bilayers (54). The cell surface of Hsp70 membrane-positive tumor cells facilitates internalization and initiates apoptosis in a perforin-independent manner. This hypothetical mechanism is supported by the finding that enhanced cytolytic activity of NK cells against Hsp70 membrane-positive tumor cells could be blocked by the Hsp70-specific monoclonal antibody. This antibody interacts with TKD and thus prevents binding and internalization of granzyme B into tumor cells.

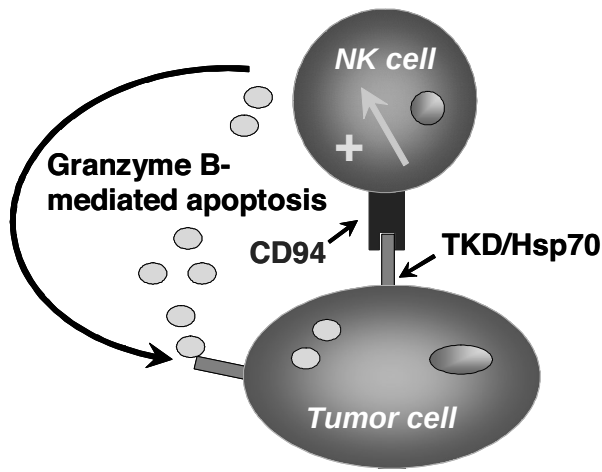

Figure 1: Granzyme B-induced apoptosis of Hsp70 membrane-positive tumor cells. Membrane-bound Hsp70 on tumor cells interacts with the C-type lectin receptor CD94 on the surface of NK cells and mediates a perforin-independent release of granzyme B initiating apoptosis in tumor cells.

Anchorage and exosomal export of Hsp70

Although the immunological role of membrane-bound Hsp70 appears apparent, the mechanisms of transport, membrane anchorage and export remained elusive. Cytosolic HSPs do not contain leader peptides enabling membrane localization; however, transport of other proteins across membranes is one of the major tasks of members of the HSP70 family. In the cytosol HSP70s frequently co-operate

with DnaJ molecules and members of the BAG family. Cross-linking experiments revealed a co-localization of Hsp70 with Hsp40 and the silencer of death domain Bag-4 on the plasma membrane (35). Regarding these results it was conceivable that Hsp70 in concert with co-chaperones might fulfill shuttle functions from inside the cell to the plasma membrane. Functionally, Hsp70/Hsp40 and Hsp70/Bag-4 complexes on the cell surface perform dual tasks. On the one hand they confer protection against radiation-induced cell cycle arrest, on the other hand they provide recognition structures for the cytolytic attack mediated by NK cells via granzyme B.

More recently, the group of Arispe and DeMaio demonstrated a direct interaction of HSP70s with the lipid phosphatidylserine in plasma membranes of PC12 tumor cells (55). Earlier studies of the same group showed that Hsc70 has the capacity of inducing ion conductance channels in artificial bilayers that were regulated by the ATP/ADP content (54). Other laboratories detected HSP70 in detergent-soluble microdomains which were found to be enriched in sphingolipids (54,56). By fluorescence resonance energy transfer (FRET) imaging an association of HSP70 with TLR-4 clusters in lipid rafts could be observed upon stimulation with lipopolysaccharides (LPS) (54,57,58). The group of Triantafilou demonstrated that TLR-4 is targeted to the Golgi apparatus along with HSPs (58). These results indicate that, on the one hand, HSPs support binding and transfer of LPS via the TLR-4 complex to the cell surface. On the other hand, HSPs also assist trafficking and targeting of LPS into the Golgi compartment.

As well as HSP70, gp96, an ER residing HSP90 member harboring the KDEL retention sequence, was recently found to be localized in the plasma membrane of tumor cells (59). It was speculated that gp96 might reach the plasma membrane through the ER-Golgi compartment by masking or suppressing the ER-retention sequence.

Several groups have reported on an active release of Hsp70 from viable tumor cells that could be further increased by exogeneous stress (56,60,61). An alternative vesicular pathway bypassing the ER-Golgi compartment was hypothesized for this Hsp70 export (56). Members of the Rab GTPase family are key regulators of vesicular transport pathways (for a review see refs. 62-64). Figure 2 summarizes the current understanding of the regulatory role of Rab GTPases. Early endocytic events are primarily regulated by Rab5 and Rab15. The former facilitates segregation of cargo into clathrin-coated vesicles and promotes cytoskeletal motility and homotypic early endosome fusion in collaboration with other effector molecules (65). In contrast, Rab15 blocks the vesicular transport to early endosomes thus opposing the Rab5 activity (66,67). Molecules reaching the early endosomes are recycled or undergo lysosomal degradation. Recycled molecules are either sorted into Rab4-containing microdomains within early endosomes permitting fast transfer to the plasma membrane (68) or become transported to perinuclear recycling endosomes where Rab11 regulates transport to the plasma membrane (69). Rab11 has also been found to participate in exocytosis from the trans-Golgi network to the plasma membrane (70). Molecules destined for degradation are delivered in a Rab7-dependent transport step from early to late endosomes (71). Downstream of late endosomes, Rab7 facilitates transport to lysosomes (72). Recycling of certain molecules such as the mannose 6-phosphate receptor from late endosomes to the trans-Golgi network is mediated by Rab9 as an additional branch of the endocytic pathway (73).

In a recent study we adressed the question whether differences in the Hsp70/Bag-4 and Hsp70/Hsp40 membrane expression pattern were associated with a different capacity of export. Moreover, we wanted to analyse whether these molecules were released from plasma membrane-positive tumor cells in soluble form or within membrane-coated vesicles (74). Our studies led to the observation that spontaneous release of soluble Hsp70 by tumor sublines differentially expressing Hsp70 on their cell surface was rather low. In contrast, detergent-soluble vesicles actively released by tumors contained high amounts of Hsp70/Bag-4 and Hsp70/Hsp40. Biochemical and biophysical characterization identified these vesicles as exosomes corresponding to internal vesicles produced by inward budding of endosomal membranes in a process sequestering particular lipids and proteins (75). Comparative Western blot analyses of whole cell lysates and exosomal fractions derived from Hsp70 membrane-positive ($Hsp70^+$) or -negative (Hsp70-) tumor lines revealed that cytosolic proteins including tubulin, Bag-4, Hsp70, and Hsc70 were present in

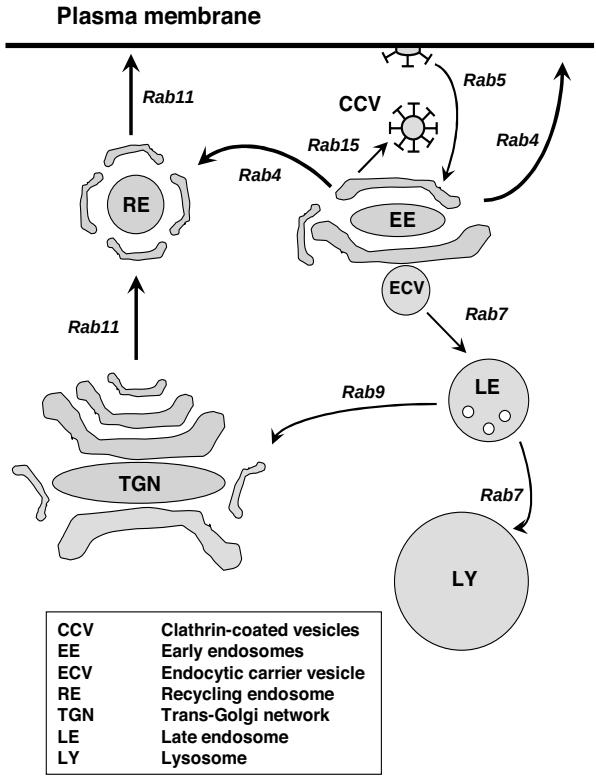

Figure 2: Schematic representation of the endocytic pathway regulated by Rab GTPases. Endocytosed molecules are rapidly transported to early endosomes characterized by Rab4 and Rab5. Retrograde transport to the plasma membrane occurs as a result of sorting into Rab4-containing microdomains within early endosomes. On the other hand, sorting into Rab5-containing microdomains results in transport to perinuclear localized, recycling compartments and to lysosomes for degradation, respectively. Subsequently, recycling transport from perinuclear recycling endosomes to the plasma membrane is mediated by Rab11 that also takes part in exocytosis from the trans-Golgi network to the plasma membrane. In this context, Rab7 facilitates transport from early to late endosomes, whereas Rab9 mediates cycling of molecules from late endosomes to the trans-Golgi network. Rab7 also takes part in the trafficking of molecules to lysosomes. Potential transport of Hsp70 from the cytosol to the plasma membrane involves EE, TGN, RE.

both, whole cell lysates and exosomes, whereas ER-residing proteins (i.e. Grp94, Calnexin) were absent in exosomes. A representative analysis of the protein composition in both celluar fractions is illustrated in Figure 3.

Since Rab GTPases are key regulators of membrane trafficking and localized to distinct membrane-bound compartments (65), we compared their distribution pattern in whole cell lysates and exosomes. As shown in Figure 3, the small GTPase Rab4 (early endosome to plasma membrane) was found to be highly enriched in exosomes, and small amounts of Rab11 (trans-Golgi to plasma membrane) were also detectable. In contrast, Rab7 (late endosome to lysosome) and Rab9 (late endosome to trans-Golgi network) were completely absent in exosomes. From these results we hypothesized that tumor-derived exosomes originate predominantly from early endosomes, minor amounts might originate from the trans-Golgi network. These findings document the intracellular transport route of HSP70-containing vesicles from the cytosol to the plasma membrane (Figure 2).

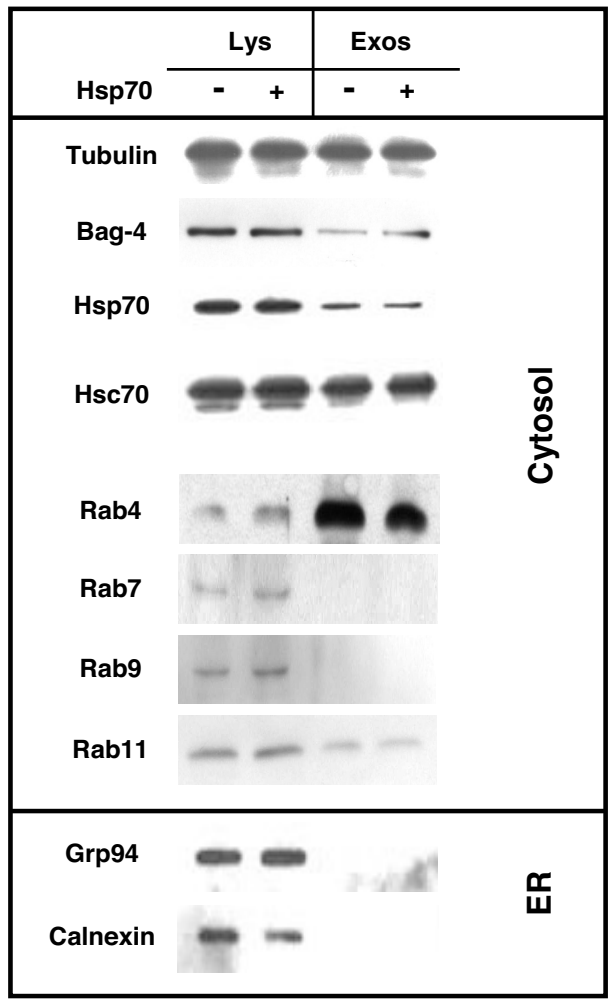

Figure 3: Comparative analysis of the protein composition in whole cell lysates (Lys) and exosomal (Exos) fractions derived from Hsp70 membrane-positive (Hsp70+) and membrane-negative (Hsp70-) tumor lines, as determined by Western blot analysis using specific antibodies directed against cytosolic tubulin, Bag-4, Hsp70, Hsc70, Rab4, Rab7, Rab9, Rab11, and ER-residing proteins, Grp94 and Calnexin.

The exact localization of Hsp70 was documented by immunogold electron-microscopy. Hsp70 was visualized by 5 nm gold particles on the plasma membrane of tumor cells (Figure 4, left). Although the

exosomal lumen of Hsp70 membrane-positive and -negative tumor cells contained comparable amounts of Hsp70, the exosomal surface revealed significant differences. As illustrated in Figure 4 (right), Hsp70 is predominantly found in duplicates on the surface of Hsp70-positive exosomes. In contrast, exosomes derived from Hsp70 membrane-negative tumor cells completely lack Hsp70 on their surface (Figure 4, right). Taken together, our results indicate that despite identical amounts of cytosolic proteins in the exosomal lumen, the surface of exosomes reflected that of the tumor cell membranes from which they originated.

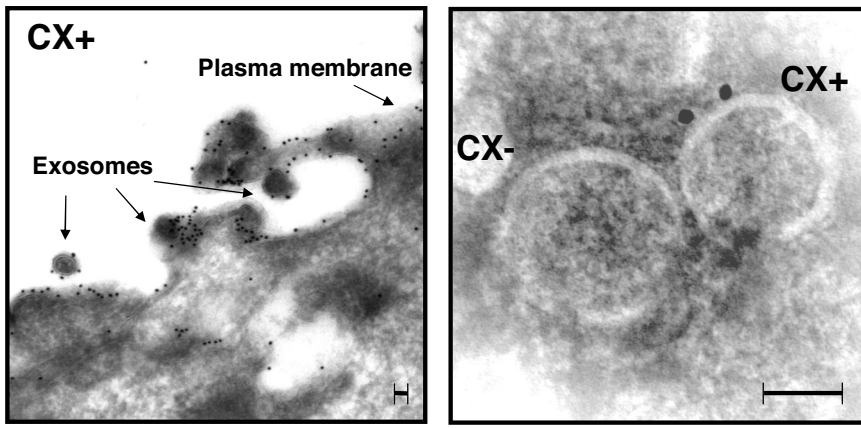

Figure 4: Immunoelectron microscopic view of the Hsp70 localization on the plasma membrane of Hsp70 membrane-positive tumor cells (left) and on the surface of exosomes derived from Hsp70+ and Hsp70- tumor cells (right; scale bar, 50 nm). Localization of Hsp70 was visualized by using an anti-Hsp70 monoclonal antibody coupled to 5 nm gold

Furthermore, Hsp70 surface-positive exosomes but not their surface-negative counterparts have the capacity to stimulate the cytolytic capacity of NK cells in a similar manner like soluble full length Hsp70 protein or TKD peptide. These data provide an explanation how Hsp70 reactivity in NK cells is induced by tumor-derived exosomes *in vivo*.

Role of HSPs in tumor immunity

Homologous members of distinct HSP families are present in nearly all cellular compartments including cytosol, nucleus, mitochondria, lysosomes, endosomes, endoplasmic reticulum, and on intracellular and plasma membranes (1,28,76-79). Moreover, an association of membrane-bound HSP70 family members in detergent-resistant caveoli (80) and in lipid rafts has been described (56). HSPs have been detected in body fluids of cancer patients and in supernatants of tissue cultures (60,81). Also exercise has been found to induce the release of Hsp70 into the serum of humans (82). The extracellular localization of HSPs with molecular weights of 60, 70, and 90 kDa is frequently associated with the appearance of HSP-specific antibodies in the serum. Apart from the induction of a humoral immune response, HSPs also have the capacity to elicit a cellular immune

response. Depending on their localization and peptide-loading status a variety of different immunological functions have been established (83). On the one hand, elevated cytosolic and membrane-bound HSP levels were associated with protection against a second lethal stress stimulus as a result of non-lethal stress (35,84). On the other hand, membrane-bound and extracellularly localized HSPs also provide danger signals for the immune system (84). It was hypothesized that soluble HSPs might either originate from necrotic or apoptotic cell death, or be recruited from viable cells with the capacity to actively release HSPs in lipid vesicles from the endosomal compartment (60,85). In any case tumor-derived HSPs either unloaded or peptide-loaded have attracted significant attention from an immunological point of view. The group of Srivastava was among the first who reported their roles as adjuvant-free tumor vaccines (9,86). Injection of mice with HSP70 (the stress-inducible Hsp70 and the cognate Hsc70) or HSP90 (the glucose-related protein gp96)-peptide complexes purified from the cytosol or endoplasmic reticulum (ER) of tumors generated protective immunity against subsequent tumor challenge in mice. Apart from chaperoning tumor-derived peptides, the ER-residing glycoprotein gp96 is known to interact with cholesterol esterase, fibrillin, thyroglobin, MHC class II peptides and mediates proper folding of the immunoglobulin (Ig) light chain (79,87). The cytosolic stress proteins, Hsc70 (73kDa cognate Hsc70) and Hsp70 (72kDa inducible Hsp70), preferentially bind early folding products including nascent chains and support transport of other proteins across membranes (1,2).

Adoptive transfer experiments of different effector cell populations convincingly demonstrated an involvement of $CD8^+$ cytotoxic T lymphocytes (CTL) and of professional APCs in protecting mice from tumors from which the HSP preparations were derived (22,88,89). HSP90 and HSP70 peptide preparations of corresponding normal tissues failed to protect mice against subsequent tumor challenge. In general, "cross-presentation" describes the transfer of exogenous HSP-chaperoned peptides into the MHC class I pathway through an endosomal pathway stimulating a $CD8^+$ T cell response (9,90,91). With the identification of the molecular nature of HSP-specific receptors the mechanism of HSP-peptide complex uptake by APCs became clearer (20). Binding studies revealed that receptor-mediated uptake of HSP-peptide complexes into APCs was specific, saturable, and concentration-dependent (92-94). These findings provide an explanation of why small amounts of HSP-peptide complexes were highly efficient in immunizing against tumors.

In an effort to improve the efficacy of HSP peptide vaccines several laboratories designed HSP fusion constructs with bacterial antigens. The group of Huang took advantage of superantigens (SAg SEA) assisting HSPs in eliciting a potent anti-tumor immune response (95). HSP70-transduced tumor cells bearing SEA transmembrane fusion proteins were used successfully as a modified vaccine prolonging survival of B6 melanoma bearing mice. The immune response against malignant melanoma was mediated through CTLs and NK cells as demonstrated by an augmented cell proliferation of both effector cell types *in vivo*.

Another approach exploits HSP fusion constructs consisting of the viral E7 protein of human papilloma virus type 16 and Bacillus Calmette Guerin (BCG) mycobacterial Hsp65 as a vaccine (96). Mice immunized with these constructs developed a strong type 1 immune response mediating tumor regression and con-

ferring resistance to tumor challenge with the cervical cancer cell line TC-1. Again, an important role for $CD8^+$ T lymphocytes was determined (97). Previous studies using mycobacterial Hsp65 also resulted in loss of tumorigenicity and conferred protection against murine reticulum cell sarcoma mediated through both, cytotoxic $CD4^+$ and $CD8^+$ T cells (98).

A variety of HSPs were found on the plasma membrane of tumor cell lines as determined by selective cell surface protein profiling (77). These findings were confirmed by a broad screening programme of human tumor biopsies in our laboratory. Phenotypic analyses revealed that Hsp70, the major stress-inducible member of the HSP70 group, is frequently found on the plasma membrane of colon, lung, pancreas, mammary, head and neck and metastases derived thereof (24,28,99). Also bone marrow samples of patients suffering from acute and chronic myeloid leukemia are frequently Hsp70 membrane-positive (100). Interestingly, the corresponding normal tissues were always found to be Hsp70 membrane-negative. These Hsp70 membrane-positive tumors were efficiently eliminated by NK cells that had been pre-stimulated with low dose IL-2 plus Hsp70 peptide TKD (25). Adoptive transfer of these TKD-stimulated NK cells in tumor-bearing mice revealed identical results *in vivo* (42,101,102). It is known that IL-2-activated NK cells are able to induce regression of established lung and liver tumors (103-106). Our group identified a specific migratory capacity of NK cells towards Hsp70-positive tumor cells and supernatants derived thereof. The same effect could be observed for the Hsp70 peptide TKD (107). From these results we speculated that killing of Hsp70-positive tumors *in vivo* might be related to an enhanced migratory capacity of pre-activated NK cells.

Therefore, our findings have further clinical implications with respect to the development of an NK cell-based cellular immunotherapy. In a recently published clinical phase I trial the use of *ex vivo* TKD/low-dose IL-2-activated autologous NK cells has been tested with regard to tolerability, feasibility, and safety in patients with multiple metastasized colon and lung carcinomas (108). As demonstrated in this study, re-infusion of TKD-activated autologous NK cells is feasible and safe. Repeated injection cycles revealed clinical responses even in these heavily pre-treated, therapy-refractory tumor diseases.

Potential immunological role of Hsp70 in exercise

It is very well known that endurance exercise results in an up-regulated Hsp70 expression in healthy humans (82,109-112). Apart from the intracellular up-regulation, soluble Hsp70 was significantly increased in the plasma/serum (109) and in the brain (113) of endurance athletes. However, the biological significance of this release has not yet been identified. Previous studies indicate that hepatosplanchnic tissues (114) and glial cells (61,115) release Hsp70 by an exocytotic mechanism which is independent of cell necrosis. As mentioned above, extracellular HSPs play key roles in initiating immunoregulatory functions. Therefore, one might speculate that elevated extracellular Hsp70 levels after exercise might act as beneficial danger signals protecting against bacterial inflammation via cytokine secretion (23,116). On the other hand it is not clear as to whether Hsp70 might be exposed on the cell surface of muscle tissues following exercise and thus might provide a target structure for NK cells (30). This hypothesis might be

supported by the finding that intensive exercise also results in an increase of pro- and anti-inflammatory cytokines (117). Future kinetic studies are required to determine as to whether exercise-induced Hsp70 release in association with cytokines positively or negatively affects the immune system of athletes.

Concluding remarks

In summary, recent observations imply a crucial role of extracellularly localized and membrane-bound HSPs in inducing an efficient cellular immune response against cancer. Apart from their cross-priming activity, even in the absence of chaperoned tumor-peptides members of the HSP70 family are considered as potent activators of the innate immune response. Tumor-derived exosomes as well as soluble Hsp70 proteins are involved in the activation of an anti-tumor immune response mediated by NK cells. These findings might have further implications to broaden the understanding of an exercise-induced Hsp70 release in athletes.

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