Review

Heat Shock Proteins Mediate Intercellular Communications within the Tumor Microenvironment through Extracellular Vesicles

Renata F. Saito 1,2, Camila Maria Longo Machado 1,2, Ana Luiza Oliveira Lomba 1,2, Andréia Hanada Otake 1,2,*,# and Maria Cristina Rangel 1,2,*

1 Center for Translational Research in Oncology (LIM/24), Instituto do Câncer do Estado de São Paulo, Hospital das Clínicas HCFMUSP, Faculdade de Medicina, Universidade de São Paulo, São Paulo CEP 01246-000, Brazil; renata.saito@hc.fm.usp.br (R.F.S.); camilalongomachado@gmail.com (C.M.L.M.); analuizalomba@gmail.com (A.L.O.L.); mcrangel@gmail.com (M.C.R.)
2 Comprehensive Center for Precision Oncology (C2PO), Universidade de São Paulo, São Paulo CEP 01246-000, Brazil
* Correspondence: andreia.otake@hc.fm.usp.br
# These authors contributed equally to this work.

Abstract: From an evolutive perspective, tumor cells endure successive turnover upon stress conditions and pressure to adapt to new environments. These cells use exceptional communication skills to share biological information to “survive upon every metabolic cost”. The tumor microenvironment (TME) is a miscellaneous collection of cells, factors, and extracellular vesicles (EVs). EVs are small lipid bilayer-delimited particles derived from cells with sizes ranging from 100 to 1000 nm. Exosomes (<160 nm) are the minor subtype of EVs, originating from the endosomal pathways. The TME also contains “giant” vesicles, microvesicles (100–1000 nm, MV), originated from membrane blebbing. EVs can act as intercellular communication mediators, contributing to many biological processes, by carrying different biomolecules, such as proteins, lipids, nucleic acids, and metabolites. EV secretion can promote either tumor cell survival or manage their stress to death. Tumor-derived EVs transfer adaptive stress signaling to recipient cells, reprogramming these cells. Heat shock proteins (HSP) are prominent stress response regulators, specifically carried by exosomes. HSP-loaded EVs reprogram tumor and TME cells to acquire mechanisms contributing to tumor progression and therapy resistance. The intercellular communication mediated by HSP-loaded EVs favors the escape of tumor cells from the endoplasmic reticulum stress, hypoxia, apoptosis, and anticancer therapies. Extracellular HSPs activate and deactivate the immune response, induce cell differentiation, change vascular homeostasis, and help to augment the pre-metastatic niche formation. Here we explore EVs’ mechanisms of HSP transmission among TME cells and the relevance of these intercellular communications in resistance to therapy.

Keywords: extracellular vesicles; tumor-resistance; chaperones; heat shock proteins; CSC-derived exosomes; cancer

1. Introduction

Cancer cell survival is challenged by internal and external stress factors, which exert selective pressure on cancer cells, driving the emergence of genetic and phenotypic diversity that allows cell survival. Harsh tumor microenvironment (TME) conditions increase intracellular misfolded and damaged proteins; thus, from an evolutionary perspective, cancer adaptability involves proteotoxic stress adaptation. The proteostasis network preserves the proteome functionality by coordinating processes such as protein synthesis and folding, translocation, or degradation, to promptly respond to stress conditions [1,2]. However, severe or persistent stress stimulus can disturb the protein-folding equilibrium and surpass
the proteostasis “buffering” capacity, culminating in the induction of cell death. Heat shock proteins (HSPs) are a group of conserved protein families that play crucial roles in cellular protection against stress and the maintenance of cellular proteostasis. HSPs were originally named proteins induced by thermal stress; however, this nomenclature may also be used to describe proteins induced by other stressors, such as hypoxia, inflammation, and infection. The numerous members in the various human HSP families contributed to the inconsistencies in their nomenclature. In 2009, Kampinga et al. provided a guideline for a more consistent HSP nomenclature. Here, we reproduce the HSP nomenclature used in the original papers, and provide in parenthesis the information of the HSP family [3]. They mainly function as molecular chaperones, acting as protectors of misfolding protein accumulation by facilitating protein folding, trafficking, assembly, and degradation (reviewed in [4]). The interface between environmental stress and protein homeostasis impacts the evolution and selection of more adaptive phenotypes. In a model of Drosophila cell lines, it was evidenced that the direct folding of mutated proteins provided new functions that eventually conferred selective advantages to cells to survive in hostile environments [5]. In addition to their chaperone activity, HSPs play important roles in the cell cycle, apoptosis regulation, and cell signaling transduction [6]. Here, we exploit the idea that increased HSP expression and activity in cancer cells [7–9] can be an evolutionary advantage to respond environmental stress signals that result in cancer progression and resistance to therapy.

Cancer cells survivability and proliferation require continuous communication among tumor and TME cells [10]. The systemic release of extracellular vesicles (EVs) has been reported as a particular mechanism of cancer cells to propagate stress tolerance to other cells. EVs are small lipid bilayer-delimited particles derived from cells with sizes ranging from 100 to 1000 nm. Exosomes (<160 nm) are the minor subtype of EVs, originating from the endosomal pathways, and the “giant” EVs microvesicles (100–1000 nm, MVs) originate from membrane blebbing [11]. EVs can act as intercellular communication mediators, contributing to many cells’ biological processes, by carrying different biomolecules, such as proteins, lipids, nucleic acids, and metabolites [12,13]. The release of EVs from cancer cells as an adaptative stress response to harsh conditions has been well documented. We have previously shown that acquisition of resistance to treatment by melanoma cells could be mediated by EVs derived from treated tumor cells [14]. EVs are shed by tumors as unique forms of processing or reshaping of cell content in a way that they can share, with other cells, information received by a particular population of cells. Similarly, cancer cells can receive foreign information from EVs that signal them to survive, grow, migrate, or even die [15,16].

Considering the primary function of HSPs to maintain cellular homeostasis, it is important to explore the potential role of EVs loaded with HSPs (HSP-EVs) in activating the proteostasis network within the TME with prominent modulators to undergo internal proteotoxic stress [17]. Although the concept of EVs’ preconditioning mechanism remains under construction, it is mainly attributed to the EV’s internal cargo. In addition, some knowledge needs to be explored; in contrast to external uncovered HSPs, EVs can deliver HSPs to distinct cell types and at distant sites outside the body’s compartments in saliva or other fluids. In this review, we exploit the idea that HSP-EVs are shed by cancer cells upon stress stimulus and focus our discussion on HSP-mediated stress response adaptation within the TME, what contributes to tumor progression.

2. Extracellular Vesicle (EV)-Mediated Stress Propagation during Tumor Development and Progression

Cells release EVs because of their physiology and pathophysiology. EVs can be classified into three main subtypes [18] according to their biogenesis and size. Apoptotic bodies are generated by membrane disintegration after injuries and cell death activation, producing vesicles having a diameter ranging from 1 to 5 µM. Microvesicles are particles generated by the direct outward budding of the plasma membrane of viable cells, having vesicles in a size range of around 50 nm to 1000 nm in diameter. Finally, exosomes are generated by the
endosomal sorting complex required for the transport (ESCRT) system composed of different proteins able to interact with other proteins and promote the formation of intraluminal vesicles. Exosome formation starts after the invagination of the plasma membrane and the formation of an early-sorting endosome (ESE). The ESE contains cell-surface and soluble proteins associated with the extracellular milieu in addition to content from the trans-Golgi network and endoplasmic reticulum. Late-sorting endosomes (LSEs) are matured ESEs that generate multivesicular bodies (MVBs) containing intraluminal vesicles. MVBs form by double invagination of the plasma membrane and can later fuse with lysosomes or autophagosomes to be degraded. Release of exosomes occurs after the fusion of MVBs to the plasma membrane; these exosomes have a size of around 40 to 160 nm in diameter [19].

Studies have been conducted to investigate the role of EVs in the transfer of the stress tolerance phenotype in different cancer models. Hypoxia leads to EV release and/or a higher cargo loading per vesicle, and transfers a hypoxic tolerance phenotype to TME, as well as promoting pro-tumoral effects, including induced proliferation, migration, angiogenesis, and immunomodulation [20]. The main cellular hypoxia effect is protein-folding instability with damaged and misfolded protein accumulation. Proteostasis instability affects the endoplasmic reticulum (ER), triggering a specific cellular state known as ER stress and the unfolded protein response (UPR) to restore homeostasis. Mahadevan et al. demonstrated that ER stress can be transmitted from cancer cells to bone marrow-derived myeloid cells, a phenomenon known as transmissible ER stress [21]. This communication was confirmed to be organized by EVs, which was firstly attributed to cancer cell soluble factors [22], and compelling evidence from cancer cells submitted to ER stress inducers demonstrates that this insult increases EV secretion [23,24].

EV-mediated remote stress preconditioning has identified several EV cargos, including mRNA, microRNA, and proteins that impact HIF-1α, UPR-, angiogenesis-, and autophagy signaling in recipient cells (reviewed in [20]). Given the critical role of HSPs in driving the stress response, their activity is one of the main cellular pro-survival mechanisms, and it would be logical to expect the presence of these proteins in EVs. Five major mammalian HSP families have been classified according to the guideline proposed by Kampinga et al.: HSP70 superfamily (HSP70 (HSPA) and HSP110 (HSPH)), DNAJ (HSP40), small heat shock proteins (HSPB), HSPC (HSP90), and chaperonins (HSPD/E) [3]. EVs containing diverse HSP members are passively or actively released by damaged, stressed, or dead cells. The expression of HSP90 (HSPC family) in exosomes derived from diverse normal cells was previously reported [25–28]. Later, B cell-derived exosomes were reported to have increased levels of chaperones under heat stress conditions [29]. Oral squamous cell carcinoma secretes HSP90-enriched EVs and promotes expression of HSP90, TRAP1, and HSP105 (HSP70 superfamily), which were correlated with poor prognosis in head and neck carcinoma patients [30]. A mitochondrial chaperonin, HSP60 (chaperonin family), is secreted into exosomes as a regular process independent of cell death induction [31]. A mitochondrial chaperone, GRP75/mt-HSP70 (HSP70 superfamily), is involved in EV secretion by breast cancer cells and its blockage decreases tumoral EV secretion [32]. The release of EV-HSP70 (HSP70 superfamily) can also be enhanced immediately in plasma after cardio-exercising, which follows the return to the baseline quantitate amount of HSPs in EVs extracted from patients’ serum [33].

3. Heat Shock Protein (HSP) Secretion by EVs Triggered by Chemo- or Radiotherapy

Several reports have underlined that stress induced by anticancer therapies, such as chemotherapy and radiotherapy, induce EV secretion from TME cells, resulting in drug resistance transfer to recipient cells (reviewed in [34]). Importantly, high levels of tumoral HSPs have been reported to be associated with poor prognosis and resistance to therapy (reviewed in [35]). Some authors revealed that diverse HSPs, including HSP27 (HSPB family), HSP60, HSP70, and HSP90, have a cytoprotective activity in reducing the sensitivity of tumor cells to anticancer drugs [36–41]. It has become apparent that HSPs
are released and are able to induce cellular responses in the extracellular milieu, including therapy resistance [42].

For many years, findings of extracellular HSPs were considered artifacts caused by cell necrosis, due to the absence of a peptide secretion signal in their sequence [43]. However, this concept was revised, showing HSPs to be released even in the absence of cell death [44], and further studies described diverse unconventional pathways of HSP secretion, including within EVs [42]. Importantly, HSPs are present in exosomes released by various cancer cells and different TME cells (Table 1). The exact mechanism by which HSPs are incorporated within EVs is still controversial. However, the promotion of malignant features by HSP-EVs and drug resistance are extensively reported [30,45–50].

Table 1. HSP-EVs secreted by cancer cells or different TME cells.

<table>
<thead>
<tr>
<th>HSP</th>
<th>Cell Type and Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSP20</td>
<td>Gynecologic cancer cells [51]</td>
</tr>
<tr>
<td>HSP27</td>
<td></td>
</tr>
<tr>
<td>HSC70</td>
<td></td>
</tr>
<tr>
<td>HSP70</td>
<td></td>
</tr>
<tr>
<td>HSP90</td>
<td></td>
</tr>
<tr>
<td>HSP60</td>
<td>Human lung carcinoma cells [52]</td>
</tr>
<tr>
<td>HSP60</td>
<td></td>
</tr>
<tr>
<td>HSP70</td>
<td></td>
</tr>
<tr>
<td>HSP70</td>
<td>Hepatocellular carcinoma cells [53]</td>
</tr>
<tr>
<td>HSP70</td>
<td>Human peripheral blood mononuclear cells [54]</td>
</tr>
<tr>
<td>HSP70</td>
<td>Natural killer cells [46]</td>
</tr>
<tr>
<td>HSP70</td>
<td>Choriocarcinoma cells [23]</td>
</tr>
<tr>
<td>HSP72</td>
<td>Breast adenocarcinoma cells</td>
</tr>
<tr>
<td></td>
<td>Erythroleukemic cells [55]</td>
</tr>
<tr>
<td>HSC73</td>
<td>Dendritic cells [25]</td>
</tr>
<tr>
<td>HSP70</td>
<td>Prostate cancer cell [56]</td>
</tr>
<tr>
<td>HSP90</td>
<td></td>
</tr>
<tr>
<td>mt-HSP70</td>
<td>Breast cancer cells [32]</td>
</tr>
<tr>
<td>GRP78</td>
<td>Colon cancer cells [57]</td>
</tr>
<tr>
<td>HSP90</td>
<td>Cancer stem cell-like [58]</td>
</tr>
</tbody>
</table>

HSP (heat shock protein), HSC (heat shock cognate protein), mt-HSP (mitochondrial HSP), GRP (glucose-related protein).

4. Transfer of Therapy Resistance Mediated by HSP-EVs’ Release by Tumor Cells

Besides the advances in targeted therapies, some patients relapse even after an initial positive response to a therapy schedule and become unresponsive after a few cycles. Therapy resistance is documented for all cancer and therapy types. To date, the focus of drug resistance research has been on identifying genetic and epigenetic changes in cancer cells and/or cells from the TME. They usually aim for pro-survival signaling, apoptotic pathway inhibition, and controlled drug alteration. It was recently revealed that EVs also alter cancer cell plasticity to modify them to become chemotherapeutic-resistant [59–61]. EVs mediate drug resistance by reducing the concentration of the drug by exporting it from cancer cells or by dividing their cargo among TME cells [62]. These altered pathways evolve cells to diminish drug efficacy [61]. The role of intercellular transfer of HSPs mediated by EVs in the horizontal transmission of drug resistance in multiple cancer types is explored here.
Chemo- and radiotherapy stimulate secretion of EVs by tumor cells with pro-survival and pro-metastatic capacity [63–70]. Lv et al. showed that paclitaxel, irinotecan, and carboplatin promote the release of HSP-exosomes from HepG2 hepatocellular carcinoma cells [53]. Campanella et al. observed a decrease in HSP60 intracellular levels and an increase in nitrosative HSP60 expression via exosomes in a human lung-derived carcinoma cell line (H292) after treatment with the histone deacetylase inhibitor, suberoylanilide hydroxamic acid (SAHA) [71]. Similarly, evidence shows that radiotherapy also promotes the release of exosomes containing HSP72 (HSP70 superfamily) from PC-3 and DU-145 prostate cancer cells [64]. Shao et al. showed that glioblastoma cells treated with temozolomide (TMZ) secreted more microvesicles with HSP90 as cargo. In a combined TMZ plus an HSP90 inhibitor (geldanamycin) treatment, there were more glioblastoma cells undergoing apoptosis and diminished vesiculation [72]. Kıyga et al. observed increased expression of HSP70, HSP60, HSP90, and HSP27 (HSPB family) on EVs derived from glioblastoma cells after treatment and this increased expression was related to therapeutic resistance [73]. Lv et al. showed that chemotherapeutic treatment of hepatocarcinoma cells, HepG2 and PLC/PRF/5, increases secretion of exosomes expressing HSP60, HSP70, and HSP90 on their membranes. Interestingly, HepG2 cell-derived exosome secretion and the highest HSP levels in exosomes were observed in response to chemotherapy, showing that HepG2 cells exhibit resistance [53].

These findings indicate that increased HSP expression on EVs may confer advantages to cancer cells to resist and survive anticancer therapies. Notably, a comparative proteomic analysis of EVs derived from breast cancer patients who relapsed or not, showed a differential expression of HSP70 amongst both groups [74]. Likewise, Rothammer et al. observed higher HSP70 serum levels in breast cancer patients who exhibited contralateral recurrence or metastases after radiotherapy treatment [75]. This is of particular interest, as tumor recurrence results from therapy resistance and suggests the involvement of HSPs in this phenomenon.

5. Cancer Cell Intrinsic Mechanism Modulated by HSP-EVs That Impact Therapy Response

In addition to the transfer of HSP cargo from EVs to cancer cells, HSPs present on the external surface of EVs can interact with surface receptors of target cancer cells and contribute to resistance phenotype propagation. McCready et al. described that invasive cancer cells secrete HSP90α-EVs and also identified the pro-migratory protein plasminogen as a potential client protein of these extracellular chaperones [48]. Tsen et al. revealed another mechanism by which HSP90α, the inducible cytosolic isoform of HSP90, can modulate cancer cell migration. In this study, they provided evidence that extracellular HSP90α binds to the subdomain II of the extracellular part of low-density lipoprotein receptor-related protein 1 (LRP-1), which signals to Akt kinases, Akt1 and Akt2, to promote cell motility [76]. Similarly, Ono et al. showed that HSP90-EVs derived from metastatic oral cancer cells initiate epithelial-mesenchymal transition (EMT) in normal epithelial cells and promote migration and invasion of tumor cells. Moreover, these EV-driven migratory events were reversed by HSP90 depletion [77]. Tang et al. also demonstrated that breast-cancer-derived exosomes present HSP90α on their external surface and stimulate the migration of both normal stromal cells and tumor cells in a paracrine and autocrine mechanisms [78]. The intercellular transfer of chemoresistance mediated by HSPs-EVs was shown by Wang et al. [79]. In their study, they demonstrated that the transfer of DNAJB8 (HSP40 family) by EVs derived from oxaliplatin-resistant cells could transfer the resistance phenotype to recipient colon cancer cells. HSP-EVs can also mediate the communication of cancer cells with other stromal cells, such as endothelial cells, and promote angiogenesis. Yukawa et al. investigated the influence of exosomes secreted from hepatocellular carcinoma cells on angiogenesis and found that HepG2-derived exosomes expressing HSP70 are incorporated by HUVEC cells and induce lumen formation [80]. Notably, several reports have suggested a key role of HSP90 in regulating tumor angiogenesis, as multiple arms
of angiogenic signaling have been described as clients of this chaperone [81]. Feng et al. reported that HSP90 is directly associated with a smaller (165 amino acid) VEGF isofrom found in the outer surface of microvesicles (MVs) isolated from MDAMB231 and SKBR3 breast cancer cells. Interestingly, this association results in a sustained activation of VEGFRs and a consequent resistance to Bevacizumab. However, HSP90 inhibitors disrupt this client–protein interaction and the release of VEGF90K from the MVs restores Bevacizumab sensitivity [82]. HSP-EVs can also promote the activation of fibroblasts in the pre-metastatic niche (PNM). Sun et al. demonstrated that HSP60 present on the surface of EVs derived from tumor cells was crucial for EV-induced lung PMN formation. HSP60-EVs in circulation could mediate immunomodulatory effects and immune response. Colon carcinoma patients present HSP60 expression in macrophages and NK cells; at the same time, HSP-EVs are present in the blood of the patients. High levels of EVs in circulation are dependent on tumor presence because, after tumor removal, HSP60-EVs in circulation decrease [83]. The same scenario is found in other tumor types: in thyroid papillary carcinomas, HSP27-, HSP60-, and HSP90-EV levels in plasma decreased in number after surgical resection of the tumor [84]. These data support the idea that HSP60-EVs in circulation may be useful to follow up in patients’ recurrence after surgical treatments, for instance.

Taken together, all these reports clearly demonstrate that HSP-EVs can contribute to tumor heterogeneity response to anticancer therapies by inducing EMT, migration, and angiogenesis. It is interesting to stress that HSP-EVs can also propagate cancer drug resistance by interacting with and modulating crucial components of the immune response.

6. Immunological Roles of Cancer HSP-EVs That Impact Therapy Response

Many studies have been conducted to reveal the immunological consequences of tumoral HSP-EVs. All processes related to the immune system are tissue-context dependent on which the response is occurring. In the TME and in the presence of HSP-EVs, this idea is no different. Therefore, HSP-EVs can have an anti-tumor or pro-tumor role (Figure 1). Chalmin et al. and Diao et al. provided a mechanistic insight linking HSP72 and HSP70 present in tumor-derived exosomes (TDEs) and tumor-induced immunosuppression, respectively. They showed that both HSP70 and HSP72 are present in TDEs and they can bind to toll-like receptor 2 (TLR2) in myeloid-derived suppressor cells (MDSCs), triggering Stat3 activation and, promoting the suppressor activity of MDSCs [85,86]. Chalmin et al. also observed that dimethyl amiloride reduced exosome secretion and Stat3 phosphorylation in MDSCs, resulting in an enhanced cytotoxic effect on T cells under cyclophosphamide treatment [86]. In line with this, Gobbo et al. evaluated the blockage of HSP70 and TLR2 association by using the peptide aptamer A8, which targets the extracellular domain of membrane-bound HSP70 on exosomes. They observed that this peptide impaired MDSCs’ activity induced by cisplatin and 5-fluorouracil treatment, and potentiated the anti-tumor effect of these chemotherapeutic drugs [87]. Additionally, Ono et al. showed that HSP90-EVs derived from metastatic oral cancer cells are taken up by macrophages, resulting in M2 polarization [77].

On the contrary, there is also evidence that HSP-EVs can modulate innate immune responses, which leads to tumor control. Gastpar et al. demonstrated that high-HSP70/Bag-4 surface-positive exosomes act as natural killer (NK) cell attractants and elicit a strong NK lytic capacity to HSP70 membrane-positive tumors [46]. Elsner et al. also found that HSP70-EVs derived from human melanoma cells induce the activation of mouse NK cells and result in tumor growth and metastasis reduction [88]. Additionally, the encounter of myeloma-HSP-expressing exosomes and dendritic cells efficiently stimulates their maturation to promote T helper 1 (Th1) and cytotoxic T lymphocyte (CTL) anti-tumoral responses [89]. Similarly, HSP70-enriched exosomes derived from a tumor heat-treatment promote tumor regression in murine models mediated by a Th1 immune response [90]. Menay et al. showed the presence of HSP70 in the lumen and HSP90 on the surface of exosomes isolated from mice bearing a very aggressive T-cell lymphoma. The immunogenic properties of these HSP exosomes were found to induce Th1 response in naïve-syngeneic mice, resulting
in protection against secondary challenges [91]. Sen et al. demonstrated that the exposure of naïve murine macrophages is activated by HSP70-rich exosomes released from murine breast carcinoma cell lines post hyperthermia treatment. Moreover, other anti-tumoral responses were observed, such as increased macrophage migration and release of TNF-α and RANTES, which triggered a cytotoxic response against breast cancer cells [92]. Vega et al. also showed that exosomes enriched in HSP70 activate macrophages to increase TNF-α production [93].

Hurwitz MD et al. showed that the prostate cancer cell lines PC-3 and DU-145 secrete HSP72 exosomes after irradiation treatment. These exosomes also promote the increase in pro-inflammatory cytokines IL-6 and TNF-α and the expression of CD8+ T and NK cells [64]. HSP-EVs play a pivotal role in stimulating an anti-tumor immune response after anticancer therapies. Lv et al. showed that hepatocarcinoma HepG2 cells secrete HSP-rich exosomes in response to paclitaxel, irinotecan, and carboplatin. These secreted EVs elicit an NK-cell-mediated anti-tumor response after granzyme B production. Exosome treatment in NK cells decreased the expression of inhibitory receptor CD94 and increased the expression of activating receptors CD69, NKG2D, and NKp44 [53].

All these reports demonstrate that the same HSP-EVs can sometimes act as a danger signal, increasing tumor immunogenicity and inducing an active response. On the other hand, HSP-expressing EVs can induce immunosuppression and compromise anticancer therapy efficacy. Furthermore, there is growing evidence that HSPs inside or on the membrane of EVs contribute to tumor progression and resistance to therapy. However, once the timeline and order of these events are understood, physicians can use them to manage the patient’s treatment better and improve their follow-ups (Table 2).

**Figure 1.** The dual role of EV-HSPs inside the tumor microenvironment. (HSP: heat shock protein; MDSC: myeloid-derived suppressor cell; NK: natural killer). Created with BioRender.com (accessed on 10 May 2023).
Table 2. EV-HSPs dual role in cancer.

<table>
<thead>
<tr>
<th>Chaperone</th>
<th>Activity</th>
<th>Anti-Tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSP70</td>
<td>Promote cell-survival, protect against oxidative stress and others, promote protein folding and degradation, and promote cell migration and invasion.</td>
<td>Induce tumor cells’ apoptotic death and sensitize them to chemo- or radiotherapies.</td>
</tr>
<tr>
<td>HSP72</td>
<td>Promote angiogenesis, protect cancer cells from oxidative stress. Suppress apoptosis and promote cell invasion, and migration.</td>
<td>Favor arresting of tumor growth, promote apoptosis, sensitize to chemotherapy.</td>
</tr>
<tr>
<td>HSP90</td>
<td>Promote protein folding and stabilization of multiple proteins, promote cell survival, and suppress apoptosis.</td>
<td>Promote apoptosis, sensitize to chemo- and radiotherapy.</td>
</tr>
</tbody>
</table>

7. Future Perspectives

Research to date strongly supports that HSPs promote resistance to stress conditions by protecting cells against cell death [94]. An overview of molecular evidence of HSPs, such as HSP70, HSP60, HSP27, HSP40 (HSP40 family), and HSP90, on the regulation of apoptosis and necrosis, has been provided by Takayama et al. and Beere et al. [95,96]. HSPs exhibit antia apoptotic activity by interacting with key elements that regulate events occurring either upstream or downstream of caspase activation [94]. HSPs can also inhibit TNF-induced cell death [97] and favor the activity of survival factors, such as the Akt pathway [98]. The HSP’s ability to sustain cell survival following stress stimuli by coordinating multiple events within apoptotic pathways could be propagated by EVs. For example, Cesa et al. identified that inhibitors of apoptosis proteins (IAPs) are specific client substrates of HSP70 [99]. Considering that overexpression of IAPs has been associated with resistance to chemotheraphy [100], it is highly likely that the delivery of HSP70 by EVs could inhibit the turnover of IAPs in target cells, favoring their accumulation and resulting in tumoral apoptosis resistance. We speculate that anticancer therapy stress increases the intracellular expression of HSPs that are secreted by EVs, mainly exosomes, and promotes an antiapoptotic cytoprotective phenotype in the target cancer cells, conferring protection against a second therapy’s stressful stimulus, thereby favoring tumor repopulation (Figure 2). It is clear that HSPs are present in EVs derived from tumor cells or TME cells. There is evidence indicating that HSPs are either integrated on the EV’s membrane or in its lumen. However, the localization of HSPs in EVs is questionable, considering possible technical artifacts, the undetermined mechanism of HSP incorporation in EVs, and recent evidence of exosome secretion from other EVs leads to a raised discussion about EVs being uptaken by other EVs [101].

A major player in therapy resistance and tumor repopulation is the cancer stem cell (CSC). One of the distinguishing characteristics of CSCs is their high tolerance to oxidative stress, hypoxia, and nutritional shortage [102], and it has been shown that HSP70 and HSP90 are involved in the development and maintenance of the CSC phenotype, as well as the cytoprotective machinery that allows these cells to survive stress conditions [103,104]. These chaperones are constantly secreted by CSCs and have been widely reported as being involved in cancer-stemness-associated events, such as EMT, angiogenesis, treatment resistance, tumor immunosuppression, and metastasis [104]. Importantly, CSCs release EVs that perform a variety of biological roles in tumors, including transferring stem-like features to non-CSCs and mediating cell–cell communication in the TME [105,106]. The ability of CSCs to release EVs that carry specific proteins and transcription factors to surrounding cells has a stronger impact on tumor heterogeneity [107]. Consequently, the investigation of EVs transporting key molecular chaperones involved in establishing and sustaining the CSC phenotype may become very attractive. To our knowledge, the only EV chaperones secreted by CSCs reported to date are exosomal HSP90 and HSP70, which are both found in prostate cancer. Hypoxia-stressed prostate cancer cells secrete exosomes rich in HSP90 and HSP70 [56], which seem to play a role in the establishment of the CSC phenotype.
Prostate cancer cell organoids with CSC-like properties secrete abundant amounts of HSP90 and EPCAM-containing exosomes, as well as exhibiting expression of multiple stemness markers [58]. Furthermore, extracellular HSP90 (eHSP90) has been linked to the overexpression of a cohort of stemness-associated markers and the EMT marker Snail in prostate CSCs. Additionally, eHSP90 has been implicated in boosting self-renewal, tumoroid formation, and treatment resistance associated with metastatic propensity [108]. Further research looking for HSPs in CSC-EVs and investigating the mechanisms by which they contribute to the maintenance of CSCs is needed and their modulation may represent an important weapon in the elimination of these hard-to-treat cells.

![Figure 2](image-url) Roles of extracellular vesicles (EVs) derived from tumor microenvironment (TME) after injuries acting in tumor repopulation (ER: endoplasmic reticulum; HSP: heat shock protein; MDSC: myeloid-derived suppressor cell; NK: natural killer). Created with BioRender.com (accessed on 15 April 2023).

8. Concluding Remarks

The interplay between EV-mediated communication and HSP cargo has profound implications for tumor biology and therapeutic strategies. Thus, interfering in HSP-EVs has emerged as a new potential target therapy. However, interfering in HSP signaling is challenging due to the overlap among HSP family members, and because they can vary widely depending on the disease context. While evidence suggests that targeting EV-HSPs may be a promising strategy for cancer therapy, it is unlikely that analyzing HSPs inside EVs alone would be a reliable method for predicting bad or good therapy responses for different types of cancer. It is crucial to consider other primordial factors that can influence therapy response, such as tumor stage, mutation status, history of disease, age of patient, and overall health status. Although HSP inhibitors could eventually lead to improved cancer treatment outcomes for some patients, to anticipate drug resistance it is crucial to better understand the crosstalk between HSP networks and other molecular factors in the TME to influence treatment response.
32. Huang, M.-B.; Wu, J.Y.; Lillard, J.; Bond, V.C. SMR peptide antagonizes mortalin promoted release of extracellular vesicles and affects mortality protection from complement-dependent cytotoxicity in breast cancer cells and leukemia cells. *Oncotarget* 2019, 10, 5419–5438. [CrossRef]
34. O’neill, C.P.; Gilligan, K.E.; Dwyer, R.M. Role of Extracellular Vesicles (EVs) in Cell Stress Response and Resistance to Cancer Therapy. *Cancers* 2019, 11, 136. [CrossRef]
40. Gabai, V.L.; Budagova, K.R.; Sherman, M.Y. Increased expression of the major heat shock protein Hsp72 in human prostate cancer cells is dispensable for their viability but confers resistance to a variety of anticancer agents. *Oncogene* 2005, 24, 3328–3338. [CrossRef]


60. Kreger, B.T.; Johansen, E.R.; Cerione, R.A.; Antonyak, M.A. The Enrichment of Survivin in Exosomes from Breast Cancer Cells Treated with Paclitaxel Promotes Cell Survival and Chemoresistance. *Cancers* 2016, 8, 111. [CrossRef]


100. Hunter, A.M.; LaCasse, E.C.; Korneluk, R.G. The inhibitors of apoptosis (IAPs) as cancer targets. *Apoptosis* 2007, 12, 1543–1568. [CrossRef]


Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.