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Review

The Challenging Riddle about the Janus-Type Role of Hsp60 and Related Extracellular Vesicles and miRNAs in Carcinogenesis and the Promises of Its Solution

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Abstract: Hsp60 is one of the most ancient and evolutionarily conserved members of the chaperoning system. It typically resides within mitochondria, in which it contributes to maintaining the organelle's proteome integrity and homeostasis. In the last few years, it has been shown that Hsp60 also occurs in other locations, intracellularly and extracellularly, including cytosol, plasma-cell membrane, and extracellular vesicles (EVs). Consequently, non-canonical functions and interacting partners of Hsp60 have been identified and it has been realized that it is a hub molecule in diverse networks and pathways and that it is implicated, directly or indirectly, in the development of various pathological conditions, the Hsp60 chaperonopathies. In this review, we will focus on the multi-faceted role of this chaperonin in human cancers, showing the contribution of intra- and extracellular Hsp60 in cancer development and progression, as well as the impact of miRNA-mediated regulation of Hsp60 in carcinogenesis. There are still various aspects of this intricate biological scenario that are poorly understood but ongoing research is steadily providing new insights and we will direct attention to them. For instance, we will highlight the possible applications of the Hsp60 involvement in carcinogenesis not only in diagnosis, but also in the development of specific anti-cancer therapies centered on the use of the chaperonin as therapeutic target or agent and depending on its role, pro- or anti-tumor.

Keywords: Hsp60; chaperonopathies; carcinogenesis; extracellular vesicle (EV); miRNA; chaperonotherapy

1. Stress Responses and the Chaperoning System

Since their first appearance on Earth, living organisms have been oppressed by physical and chemical stressors, such as radiations, extreme temperatures and pH, and hypoxia. These challenges required adaptation, including the development of anti-stress mechanisms. Today, it is possible to observe the success of these protection strategies, since living beings are observable in a wide range of ecosystems, even in those seemingly incompatible with life, such as sulfurous lakes, deep depth of the oceans, and permafrost [1–8].

One of the most important cellular anti-stress machineries is the chaperoning (or chaperone) system, which is highly conserved in the three phylogenetic domains, Bacteria,

Archaea, and Eucarya [9]. It is composed of molecular chaperones and co-chaperones, and their co-factors, interactors, and receptors, which form functional networks working together to ensure protein homeostasis, under normal and stressful conditions [10,11]. The genes of many molecular chaperones are constitutively expressed, but others, named Heat shock protein (Hsp), are transcriptionally upregulated by exposure to stressors (e.g., heat, hyperthermia, hypoxia, heavy metals, ethanol, infections, radiation, and UV) and, thereby, protect against protein misfolding and aggregation and maintain proteins in their functional native state [12–15]. Hsps are commonly classified according to their molecular weight, and, even if not all the chaperones can be considered Hsps, the two terms are commonly used as synonymous [16–18].

Hsp60, One of the Most Ancient Anti-Stress Molecules

Hsp60 belongs to one of the oldest and evolutionarily most conserved protein families of the chaperoning system [19–21]. These proteins are present in all living species, including plants, where they were first discovered [22–24], and, considering their unique molecular characteristics, they were named "chaperonins" to distinguish them from other chaperones [25].

The canonical classification divides chaperonins into two main groups. Group I chaperonins are found in bacteria, as well as inside eukaryotic organelles of endosymbiotic origin (mitochondria in animal cells, and chloroplasts in plant cells), and work together with a co-chaperonin which helps the closing of the folding cage. Group II chaperonins are found in the eukaryotic cytosol and in Archaea, and do not require a co-chaperonin since they have a built-in lid [26]. More recently, a third group (Group III chaperonins) has been discovered and it is now under characterization [27,28].

In humans, the Group I chaperonin is Hsp60 (or Cpn60, or HSPD1), and the Group II chaperonin is CCT (chaperonin-containing TCP-1) or TRiC (T-complex protein Ring Complex). The former typically resides inside mitochondria but also occurs in various other locations intra- and extracellularly, while CCT is in the cytosol.

Hsp60 and CCT form macromolecular double-ring complexes with a central internal cavity in which polypeptides in need of assistance for folding or refolding are encapsulated and assisted to achieve their functional final conformation (native state) via an ATP-dependent mechanism [29–31].

A considerable part of our knowledge about how human chaperonins assemble and work derives from the study of their bacterial homologues. The bacterial counterpart of human Hsp60 is the chaperonin GroEL [32], which, together with the co-chaperonin GroES (the bacterial counterpart of human the Hsp10 or Cpn10), forms a tubular, double-ring complex with a central cavity similar to that described above for the eukaryotic Hsp60 and CCT complexes, inside which protein folding occurs [33,34]. Human Hsp60 has been found in various conformations (monomer, single heptameric ring, double-ring tetradecamer), depending on their concentration, levels of ATP, and presence of Hsp10 and substrate [21,35–37]. Early studies showed that human Hsp60 can assist in productive protein folding without forming a macro-double ring-complex [38,39]. However, more recent studies, based on transmission electron microscopy and X-ray crystallographic investigations, have provided strong evidence that it likely uses both double- and single-ring intermediates during its ATPase cycle [40–42].

Hsp60 has been found in extramitochondrial sites, such as cytoplasm and plasma-cell membrane, as well as in extracellular sites, inside extracellular vesicles (EVs), in circulation, and in body fluids [43–49]. Consequently, in addition to its canonical chaperoning function, Hsp60 also performs various other non-canonical activities, "moonlighting functions" unrelated to protein quality control. (Figure 1) [21,49–51]. For instance, it has been observed that increased amounts of Hsp60 on the surface of cancer cells act as a signal to stimulate the immune system, leading to the activation and maturation of dendritic cells and the generation of an antitumor T-cell response [52–54]. A non-canonical function of human Hsp60 that is still under scrutiny is the regulation of cell apoptosis [55]. Some studies suggested a

pro-apoptotic role, involving pro-caspase 3 proteolytic activation [56,57], whereas other investigations support an anti-apoptotic role, involving various mechanisms: the sequestration of Bax-containing complexes [58,59], the maintenance of mitochondria integrity and ATP generation [60], and the triggering of the IKK/NF-κB survival pathway [61]. This dual role in cell apoptosis/survival regulation has been observed both in normal and tumor cells [59,62], thus it may affect cancer progression either positively or negatively.

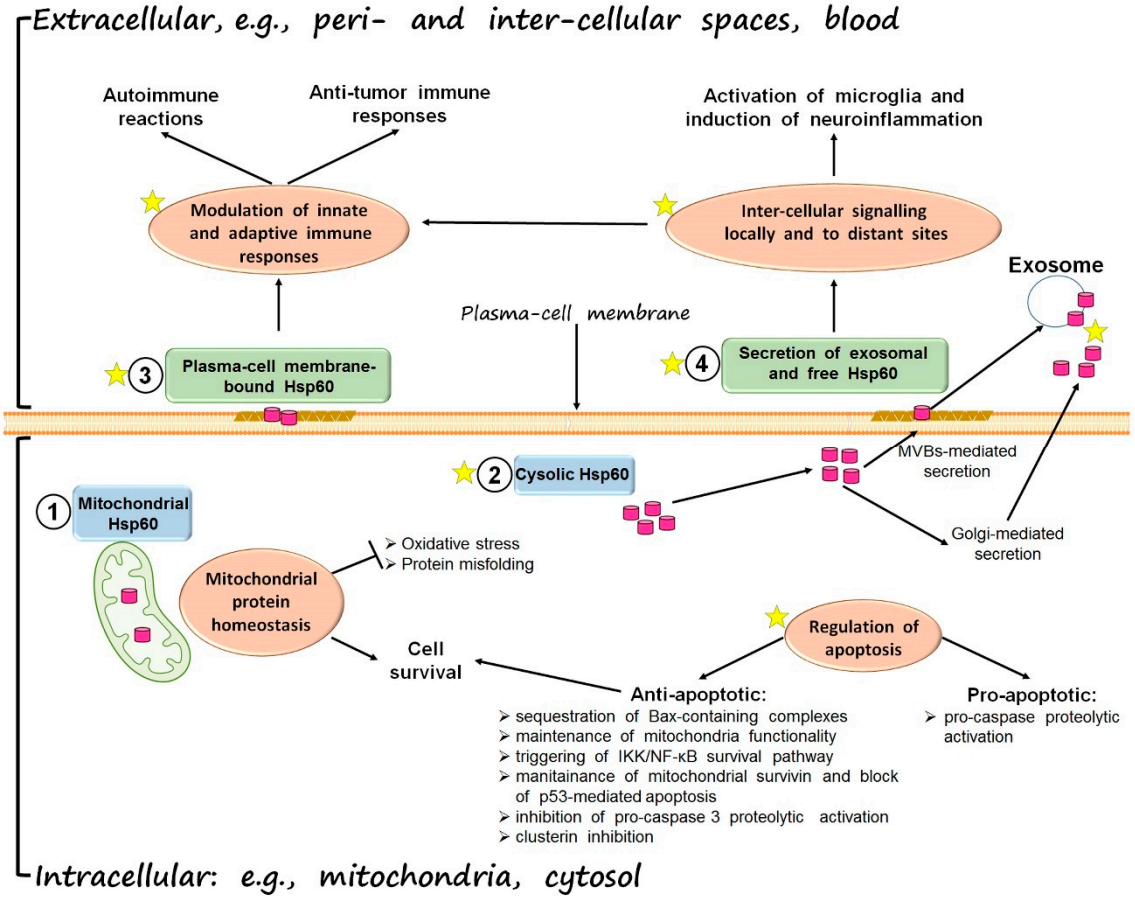

Figure 1. Hsp60 plays multiple roles intra- and extracellularly. Its canonical functions pertain to maintenance of protein homeostasis inside mitochondria, whereas its non-canonical roles are unrelated to protein quality control and are played in a variety of locations (shown with a yellow star). The chaperonin (small dark-pink cylinder) is typically in the mitochondrion matrix (1) in which it assists in the folding of intra-mitochondrial proteins, for instance those of the electron transport system, and therefore it is a vital molecule for maintaining cell viability and organismal physiology under normal conditions and in the face of stress. Consequently, Hsp60 chaperonopathies are usually serious conditions. The non-canonical functions of Hsp60 displayed in a variety of locations beyond the mitochondria, for example in the cytosol (2), are also vital. One example is regulation of apoptosis, which can be in either direction pro- or anti-apoptotic with implications for carcinogenesis. The anti-apoptotic effect of Hsp60 helps cancer cells to become immortal. In the plasma-cell membrane (3), Hsp60 can be recognized by immune cells and antibodies and generate immune reactions that damage the cell, which is a welcome event if the target cell is a cancerous one, but it is a pathogenic reaction when the cell is a normal one, a vascular epithelial cell, for instance, generating autoimmune conditions. Hsp60 exits cells (4) via different mechanisms and can, thus, reach molecules in the extracellular space and other cells nearby and far away, the latter via blood free or on particles, such as platelets, red cells, and microvesicles, like exosomes. In this manner, Hsp60 plays a role in intercellular communication, as illustrated by the microglia activation that occurs in some neurodegenerative diseases via the Hsp60-TLR-4-NF-κB signaling pathway.

2. Hsp60 Chaperonopathies

Hsp60 is multifaceted and plays diverse physiological roles but when abnormal in structure and/or function it can become pathogenic and cause diseases, the Hsp60 chaperonopathies [63,64].

In genetic chaperonopathies, there are pathogenic variants, e.g., missense mutations, in the Hsp60 gene, whereas in the acquired chaperonopathies the gene is normal but the Hsp60 protein is altered structurally and/or functionally. Genetic chaperonopathies are infrequent and typically have an early clinical onset, while the acquired ones are more common, mostly occur in adults, and are often associated with other pathological conditions, especially age-related diseases [65,66]. Another classification of practical value sorts the chaperonopathies according to quantitative parameters pertaining to concentration and functionality into by defect, by excess, and by mistake or collaborationism (Table 1) [65,66].

Table 1. Classification of chaperonopathies according to pathogenic mechanism.

Chaperonopathies	Mechanism	Example	References
Excess	Quantitative variation, in which a gene is dysregulated or overexpressed. Qualitative variation, with a gain of function.	e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease.	[65]
Defect	Quantitative variation with gene downregulation. Qualitative variation, due to structural defect (genetic or acquired)	e.g., Charcot–Marie–Tooth disease, Spastic paraplegia, Hypo-myelinating leukodystrophy	[65]
Mistake or collaborationism	The chaperone is normal but the pathway in which it is involved may promote cell pathology.	e.g., certain tumor types, autoimmune conditions, prion disease	[65]

Acquired chaperonopathies can be caused by aberrant post-translational modifications (PTMs) that have an impact on the structure/function of the chaperone molecule. The Hsp60 amino acid sequence contains various critical sites that can be affected by PTMs [67]. Possible PTMs are phosphorylation, O-GlcNAcylation, nitration, acetylation, S-nitrosylation, citrullination, methylation, oxidation, biotinylation, and ubiquitination [51]. Hsp60 PTMs can have beneficial or deleterious effects. For instance, Hsp60 hyperacetylation in the course of anti-osteosarcoma treatment lead to death of the malignant cells [68]. Hyperacetylation disrupted the Hsp60/p53 complex, restored replicative senescence, and diminished or stopped tumor growth [69]. Phosphorylated Hsp60 on the surface of breast cancer cells induced α3β1 integrin activation, resulting in enhanced motility and adhesion of these cells [70]. Tyrosine phosphorylation of Hsp60 helps malignant cells to escape immune surveillance by NK and CD8 T cells [71].

Chaperonopathies by mistake include all those pathological conditions in which a chaperone is normal as far it can be determined by current methodologies but contributes to the initiation and/or progression of disease, as it has been observed in various types of cancer, and autoimmune, inflammatory, and neurologic disorders [66].

Other examples of chaperonopathies by mistake are those autoimmune conditions in which human Hsp60 acts as auto-antigen. This situation has been described for various autoimmune conditions such as Behçet's disease [72,73], diabetes mellitus [74], systemic lupus erythematosus and vasculitis-associated systemic autoimmune disorders [75–78], atherosclerosis [79–82], and rheumatoid arthritis [83]. The pathogenic autoimmune mechanism for some of these disorders is triggered by the presence of Hsp60 on the plasma-cell membrane where it becomes a target accessible to autoantibodies and, thereby, leads to apoptosis [75,76]. However, in other situations Hsp60 has shown cytoprotective activ-

ity, which reiterates the concept that this chaperonin can play apparently opposing roles (Table 2).

In view of the above considerations, it becomes evident that (1) the kind of activity the Hsp60 chaperonin undertakes depends on its context, i.e., it is determined and/or modulated by the composition of its surroundings, namely, by the receptors and interactors within its reach; and (2) Hsp60 has not only canonical functions, which pertain to protein quality control, but also displays other functions unrelated to protein homeostasis that are also important in health and disease. Therefore, advances in the treatment of Hsp60 chaperonopathies ought to include the development of modulators of the chaperonin in situ. Two types of chaperonin inhibitors are currently being investigated: type I that block the binding and hydrolysis of ATP, and type II that bind cysteine covalently [84]. Natural and synthetic compounds potentially useful have already been identified with various degrees of anti-cancer, anti-inflammatory, or anti-autoimmune potency [63,84].

3. Hsp60 in Carcinogenesis

An indication of Hsp60 involvement in carcinogenesis is its altered expression and localization observed in certain human cancers [47,49,85]. However, the exact role of Hsp60 in cancer remains undefined and seems to change depending on the molecular and cytological context (Table 2). Hsp60 was found increased in various malignancies: cervical and ovarian [86–90], breast [91,92], colorectal [93], lung [94,95], prostate [96], gastric [97,98], and thyroid cancers [99,100]; and in leukemias [101], and glioblastoma multiforme [102–104]. The data in general suggest that Hsp60 is actively involved in carcinogenesis as a pro-tumorigenic factor, because its increase and location changes positively correlated with tumor development and malignancy. Moreover, in some cancers, the Hsp60 increase was also associated with a heightened resistance to anti-cancer drugs and other treatments [89,105], and with metastasization and angiogenesis [106,107]. It has to be emphasized that the quantitative variations of Hsp60 in cancer cells, particularly its increase, may reflect the heightened need of the chaperone system by the malignant cells with their rapid and intensive metabolism and proliferation. Thus, the Hsp60 quantitative patterns observed would be the consequence of the disease but not a distinct etiological factor. We argue that even so, Hsp60 aids the tumor in what we call a chaperonopathy by mistake or collaborationism because the tumor depends on its help. What is the value of this concept? It puts Hsp60 in the stage's center and presents it as a target for developing treatment strategies aimed at inhibiting-blocking this collaborator with the enemy from the inside. For example, Hsp60 downregulation by chemical compounds suppressed cancer-cell proliferation and tumor progression and enhanced the beneficial effects of anti-cancer treatments [62,69,90,108–112]. It was observed that Hsp60 knockdown inhibited tumor progression by altering mitochondrial homeostasis and inactivating the mTOR pathway, in ovarian cancer and glioblastoma [90,109]. In colorectal cancer, Hsp60 inhibition promoted the tumor-suppressive activity of insulin-like growth factor binding protein 7 (IGFBP7) [108]. Treatment of a neuroblastoma cell line with curcumin caused cell death by diminishing the cellular level of Hsp60 [111]. It was suggested that this cytotoxic effect was induced through the downregulation of survivin, whose expression was shown to be positively correlated with the expression of CCAR2 and Hsp60 in neuroblastoma tissues and cell lines [113]. Thus, it is becoming clear that the positive correlation between Hsp60 overexpression and increase in cancer cell proliferation and survival depends on the interaction of the chaperonin with proteins involved in cell cycle and apoptosis. In cancer cells lines, Hsp60 played a cytoprotective and pro-survival role by stabilizing the mitochondrial level of survivin and blocking p53-mediated apoptosis [62,113], or by inhibiting the intracellular isoform of clusterin [114]. In other cases, the pro-tumorigenic role of Hsp60 involved blocking the caspase-dependent apoptosis through the negative regulation of mitochondrial permeability transition [102], or the inhibition of pro-caspase 3 proteolytic activation [115,116].

However, others have reported that in some types of cancer, Hsp60 was decreased, downregulated not increased or, if increased, caused tumor suppression not enhancement [117,118]. For instance, Hsp60 was decreased in hepatocellular carcinoma (HCC) tissue compared to peritumor tissue, and this pattern positively correlated with high serum AFP (alpha-fetoprotein) level and poor overall survival. Conversely, increased Hsp60 inhibited invasion and migration of HCC cells both in vitro and in vivo, correlating with a better prognosis [118].

All the above observations (Table 2) make clear that Hsp60 has different roles in carcinogenesis that deserve investigation to elucidate the molecular mechanisms involved, particularly those determining whether the chaperonin acts against or for the cancer cell.

Some data in the literature support the assumption that Hsp60 acts as a pro-tumor protein, considering its role in the modulation of anti-apoptotic factors. It is known that during tumorigenesis, cells undergo complex transcriptional events that lead to a dysregulation of numerous factors. In this scenario, transformed cells show the typical phenotype of the proteotoxic stress, in which the consequent Hsp60 overexpression is a key event because of its central role in the regulation of protein homeostasis. Conversely, the observation that, in several types of tumors, such as lung cancer, Hsp60 levels are reduced, further demonstrate that its functioning is not only related to protein folding and the maintenance of protein homeostasis, but is complex and dependent on the cell and tissue type, its molecular interactors and its localization inside or outside the mitochondria and the cell. Thus, the multifaceted and at times contradictory functions of Hsp60 in cancer are still poorly understood and deserve active investigation, considering the importance of the chaperonin for survival of cells, normal or malignant.

Table 2. Hsp60 in human cancers.

Cancer	Hsp60 Level, Location and/or Status	Effect	Reference
Lung carcinoma	Presence of Hsp60 on cancer-cell plasma-cell membrane and on membrane of cancer cell-derived exosomes	Possible involvement in cell-to-cell communication and anti-tumor immune response stimulation	[47,48]
	Decrease of intracellular Hsp60 level and increase of Hsp60 acetylation level after doxorubicin treatment	Hsp60/p53 complex dissociation and restoration of cancer-cells replicative senescence	[69]
	Decrease of intracellular Hsp60 level after CubipyOXA treatment	Dissociation of the Hsp60/pro-Caspase-3 complex and cancer-cell apoptosis	[116]
	Increased Hsp60 level	Positive correlation with cancer progression and poor prognosis	[94,96]
Oral cancer	Presence of Hsp60 on cell surface	Interaction with gamma-delta T cells and transduction of anti-cancer immune response	[52]
Osteosarcoma	Hyperacetylation and loss of mitochondrial Hsp60 after Geldanamycin treatment	Decreased viability and augmented cancer-cell death	[68]
Breast cancer	Increase of phosphorylated surface Hsp60	α3β1 integrin activation and enhancement of cancer cells motility and adhesion	[70]
	Increased cytosolic Hsp60	Enhanced cancer-cell proliferation and reduced apoptosis; positive correlation with worse disease-free survival and poor prognosis	[91,92]
Bronchial carcinoma	Decreased Hsp60 level	Positive correlation with bronchial cancer development and progression	[85,119]
Cervical cancer	Increased Hsp60 level	Positive correlation with cancer progression and malignancy	[86–110]

Table 2. *Cont.*

Cancer	Hsp60 Level, Location and/or Status	Effect	Reference
Ovarian cancer	Increased Hsp60 level	Positive correlation with cancer progression and severity (poor prognosis and resistance to anti-cancer treatment)	[89,90,105]
Colorectal cancer	Increased Hsp60 level	Positive correlation with cancer progression and malignancy	[93,120]
	IGFBP7-dependent down-regulation of intracellular and extracellular Hsp60 level	Involvement in tumor suppressive activity of IGFBP7	[108]
Prostate cancer	Increased Hsp60 level	Positive correlation with tumor progression and hormone resistance	[96]
Gastric cancers	Increased Hsp60 level	Positive correlation with cancer progression, invasiveness, and poor overall survival.	[97,98]
Leukemia	Presence of Hsp60 on the cell surface	Activation/maturation of dendritic cells and generation of potent anti-tumor T-cell response	[53]
	Increased Hsp60 level	Positive correlation with lower complete remission rate and shorter survival	[101]
Glioblastoma multiforme	Increased Hsp60 level	Cytoprotective and pro-survival role	[102]
	Decreased Hsp60 level	Reduced cancer cell proliferation and tumor growth	[109,112]
Hepatocellular carcinoma	Decreased Hsp60 level	Positive correlation with cancer progression and poor prognosis	[118]
	Increased exosomal release of Hsp60 after anti-cancer treatment	Activation of anti-tumor immune response	[121]
Pancreatic cancer	Increased Hsp60 level	Positive correlation with cancer-cell proliferation and tumor growth and progression	[111]
Bladder carcinoma	Increased Hsp60 level	Positive correlation with resistance to anti-cancer treatment	[105]
	Decreased Hsp60 level	Positive correlation with higher tumor stage and cancer recurrence	[122]
Renal cell carcinoma	Decreased Hsp60	Disruption of mitochondria homeostasis and positive correlation with cancer progression	[117]
Large bowel cancer	Increased intracellular and exosomal Hsp60 level	Positive correlation with tumor development and progression	[123]
Thyroid cancers	Increased intracellular and exosomal Hsp60 level	Positive correlation with tumor progression	[99,100]

3.1. Hsp60 in Extracellular Vesicles in Carcinogenesis

Extracellular vesicles (EVs) are membranous particles with a diameter of 30–150 nm, which are found in blood, urine, cerebrospinal fluid, breast milk, and saliva, and are released by normal and tumor cells. The International Society for Extracellular Vesicles (ISEV) classify EVs considering their biogenesis pathways and specific markers received from the cells in which they originate, into exosomes, microvesicles, oncosomes, and apoptotic bodies; and considering size into "small EVs" (sEVs) and "medium/large EVs" (m/lEVs) [124]. However, considerable morphological and biochemical heterogeneity exists among the EVs and, to complicate matters even more, many publications do not provide detailed descriptions and/or use non-standardized terminology. Because of this, we will use here EVs and exosomes as synonyms. EVs are involved in physiological and pathological processes as mediator of cell-to-cell communication by carrying proteins,

lipids, and nucleic acids (DNA, mRNA, and miRNAs), that affect recipient cells and modify their functions [125–131]. Their content may vary depending on the cell type that produces them, but in general EVs carry a range of diverse proteins, such as tumor susceptibility gene 101 (TSG101); integrins; and tetraspanins such as CD9, CD53, CD63, CD81, and CD82 [132,133]. EVs are thought to play a role in the remodelling of the pericellular microenvironment that is crucial in maintaining tumor growth and recurrence [123,134]. MicroRNAs transported by EVs are being considered the main players in the modulation of target cell functions [135,136]. Therefore, tumor-derived EVs are attracting attention from scientists interested in molecules that might be used as biomarkers for diagnosis and patient follow up. In addition, EVs are candidates for delivering anti-cancer compounds to specific target tissues because their content can be modified; they show some tissue-specificity; and their immunogenicity is low when they are isolated from the same patient, thus presenting slow risk of generating anti-EV immunity [137–139].

Our research group provided the first evidence that tumor cells actively release Hsp60 via exosomes, through a secretion mechanism that requires the translocation of the chaperonin molecule to the plasma-cell membrane and its association with lipid rafts (Figure 2) [47,48]. The exosomal release of Hsp60 by human tumor cells can be enhanced by anti-cancer treatment. For instance, histone deacetylase inhibitors SAHA causes cell cycle arrest and death in a human lung-derived carcinoma cell line; this cytotoxic effect was associated with generation of oxidative stress, mitochondrial damage, and diminution of the intracellular level of Hsp60, which became nitrated and was released via exosomes [110]. It has been suggested that the released Hsp60 interacts with the immune system, generating an anti-tumor response that potentiates the effect of SAHA. In this manner, exosomal Hsp60 could modulate the tumor microenvironment and, from the practical standpoint it could be consider for use as a diagnostic and prognostic biomarker [123]. Indeed, it was found that the plasma levels of exosomes with Hsp60 diminished after anti-cancer treatment that caused tumor mass reduction in bowel cancer and thyroid papillary carcinoma [100,123]. Additionally, treating hepatocellular carcinoma with anti-cancer drugs caused the release of a larger amount of exosomes containing Hsps, including Hsp60, which was followed by an enhanced anti-tumor immune response mediated by natural killer cells [121].

3.2. Hsp60 and miRNAs Correlations and Implications for Carcinogenesis

MicroRNAs (miRNAs) are a class of small non-coding RNAs that regulate gene expression post-transcriptionally by translational inhibition and/or mRNAs destabilization [140]. MiRNAs regulate most protein coding genes, and thus they virtually control all biological processes [141,142]. MiRNAs dysregulation following amplification or deletion of miRNA genes, abnormal transcriptional control of miRNAs, dysregulated epigenetic changes, or defects in the miRNA biogenesis process, are associated with pathological conditions, including cancer [143,144]. Dysregulated, abnormal miRNAs can affect all hallmarks of cancer (e.g., continued proliferative signalling, evasion of growth suppressors, cell death resistance, invasiveness and metastasization, and angiogenesis) by acting either as onco-miRs or tumor suppressors, with inhibition of tumor suppressive mRNAs or oncogenic mRNAs, respectively [145,146]. For this reason, miRNAs are considered for use not only as diagnostic and prognostic biomarkers, but also as potential targets or agents in anti-cancer treatment [147].

MiR-9 and miR-221 have been classified as onco-miRs because an increase in their levels paralleled increased risk for tumorigenesis and resistance to chemotherapeutics in primary cancers [148–151]. In breast cancer, miR-9 and miR-221 increased levels correlated with poor outcome and promoted tumor progression and aggressiveness by favoring epithelial-mesenchymal transition (EMT) and breast cancer stem cell phenotypes [152]. Therefore, they have been suggested as potential biomarkers for breast cancer progression and targets for treatment.

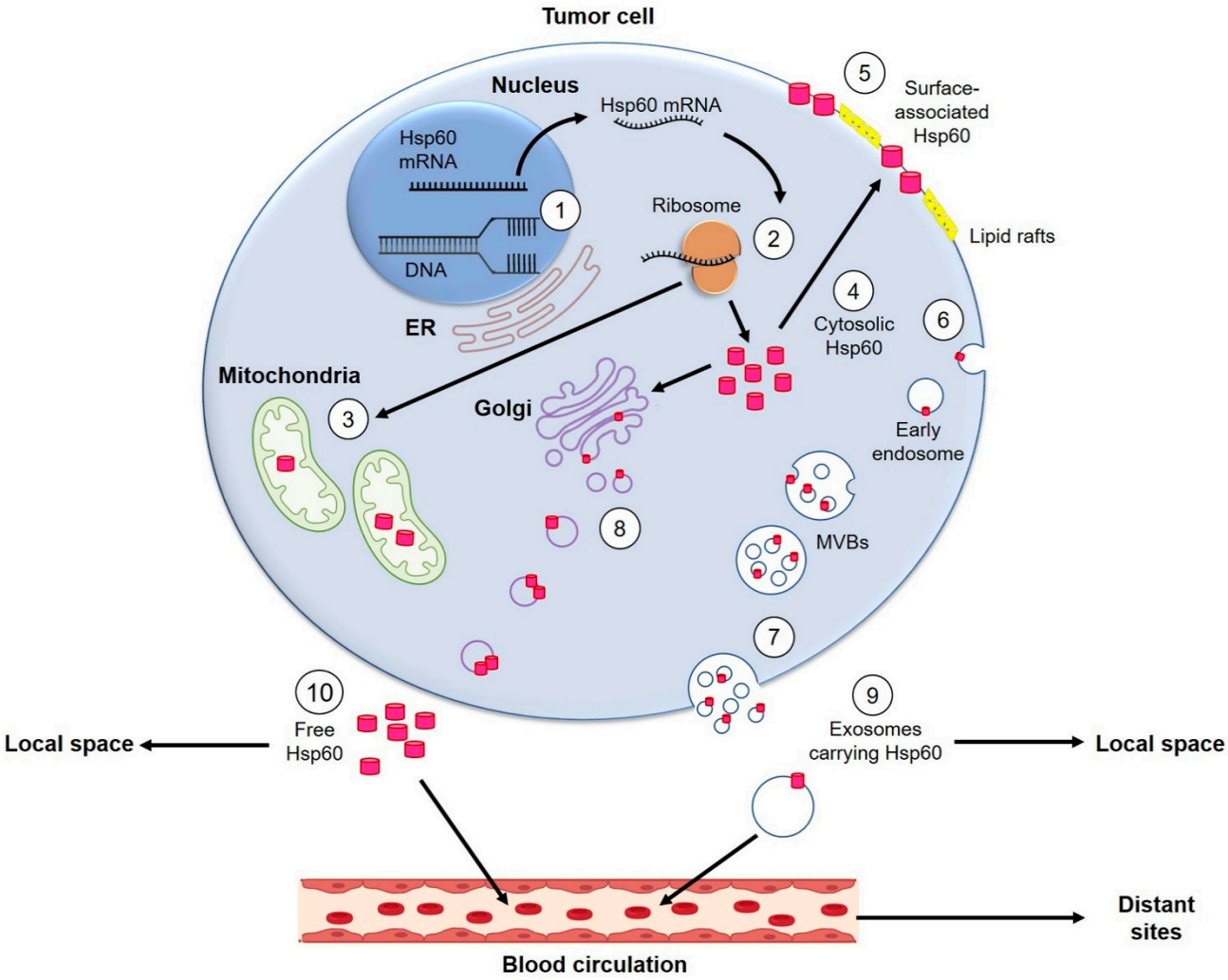

Figure 2. Diagram representing the Hsp60 secreting mechanism. Hsp60 (pink cylinder) is encoded in a single nuclear gene (1). After translation (2), Hsp60 is translocated to the mitochondrion matrix, in which it performs its canonical chaperoning function (3). In tumor cells, Hsp60 accumulates in the cytosol (4), and/or reaches the plasma-cell membrane, near lipid rafts (yellow) (5). The membrane-associated Hsp60 is internalized through a mechanism of endocytosis into early endosomes (6), and then secreted via exosomes after the fusion of multivesicular bodies (MVBs) with the plasma-cell membrane (7). Hsp60 is also exported extracellularly via the classic Golgi-mediated secretion pathway (8). Exosomal Hsp60 (9) and free Hsp60 (10) can reach other cells, e.g., tumor cells and immune cells, nearby or in distant sites via circulation.

miR-30 was found decreased in prostate cancer and acted as tumor suppressor by targeting the EMT-associated gene ERG (Ets-related gene) [153]. Conversely, when increased, miR-30 suppressed EMT and inhibited cell migration and invasion, suggesting it could be used as a therapeutic agent (Table 3) [153].

Hsps, including Hsp60, are also targets for regulation by miRNAs and this has been investigated in various cancers [154–157]. miR-1 and miR-206 increase in rat cardiomyocytes contributed to glucose-mediated apoptosis by diminishing Hsp60 and IGF-1 expression and inhibiting the IGF-1/IGF-1R/PI3 K/Akt pathway [158]. Conversely, treatment of cardiomyocytes with carvedilol, a non-selective β-adrenergic receptor antagonist, inhibited miR-1, which resulted in increased levels of Hsp60 and apoptosis prevention (Table 3) [159].

MiR-29a was found augmented in the serum of breast cancer patients [155]. Downregulation of miR-29a in a breast cancer cell line promoted apoptosis by causing an increase in the level of Hsp60 and a decrease of Hsp27, Hsp40, Hsp70, and Hsp90, suggesting that downregulation of this miRNA is a promising strategy to sensitize cancer cells to chemotherapy [155]. It is likely that the anti-cancer effects observed with the increase

of Hsp60 were related to the known pro-apoptotic role of the chaperonin [56,57], or to its ability to stimulate an anti-tumor immune response when exposed on cell surface or released extracellularly [53,54,121]. The level of miR-644a l was found low in hepatocellular carcinoma tissues and cell lines, and negatively correlated with tumor diameter and TNM (Tumor-Node-Metastases) stages [156]. In vitro, an increase in miR-644a promoted cancer cell apoptosis by inhibiting HSF1, Hsp90, Hsp60, Bcl-2, and Bcl-xL proteins while increasing the levels of BID, BAD, BIM, SMAC, Apaf-1, and cleaved caspases-3 and -9, which are all mediators of cell apoptosis [156]. In view of all these observations, miR-644a was suggested as potential prognostic biomarker and therapeutic target in HCC (Table 3) [156]. It is likely that Hsp60 typically displays a pro-tumorigenic activity that can be suppressed by its miRNA-mediated inhibition [157–159]. A further confirmation of the role of miRNAs in the regulation of Hsp60 level in cancer emerged from a study of the pro-tumorigenic activity of miR-17a in gastric lymphoma [160]. The expression of miR-17 was found significantly higher in gastric lymphoma than in normal tissues, which promoted tumor development, progression, and metastasization by regulating the Hsp60/TNFR2 pathway [160]. Therefore, the latter pathway emerges as a potential target for the diagnosis and treatment of gastric lymphoma (Table 3).

Table 3. Examples of miRNAs involved in Hsp60 regulation.

Tissue	miRNAs and miRNAs Status	Effect	Reference
Rat cardiomyocytes	miR-1 and miR-206 high-glucose-dependent up-regulation	Increased cell apoptosis induced by Hsp60 and IGF-1 down-regulation and IGF-1/IGF-1R/PI3 K/Akt pathway inhibition	[158,159]
Breast cancer	miR-29a in vitro down-regulation	Increased cancer cell apoptosis and sensitization to anti-cancer treatment induced by Hsp60 up-regulation	[155]
Gastric Lymphoma	miR-17 higher level	Increased malignancy via regulation of Hsp60/TNFR2 pathway	[160]
Hepatocellular carcinoma	miR-644a in vitro up-regulation	Increased cancer-cell apoptosis induced by Hsp60 inhibition	[156]

4. Concluding Remarks

The involvement of molecular chaperones, including Hsp60, in carcinogenesis has been suggested by various findings [107,161,162]. Molecular chaperones have been found increased in tumor tissues and closely associated with tumor growth and aggressiveness. Along the same lines, decreased levels and expression of chaperones have been found associated with reduced cancer cell proliferation, motility, survival, and metastasization, and with decreased neoangiogenesis and resistance to anti-tumor immune response and treatment [163–167]. The quantitative variations of Hsp60 during carcinogenesis, especially its increase, may be simply the reflection of increased metabolic and proliferative activities of the cancer cell, which would require more chaperonin molecules than a normal cell at its physiological level of metabolism and proliferation. Nevertheless, the role of Hsp60 can still be considered pro-tumoral even if it is not a distinct carcinogenic factor. It would be an example of chaperonopathy by mistake or collaborationism, meaning that the human molecule helps the tumor to grow, proliferate, metastasize, and resist stressors such as anti-cancer drugs. It is crucial to visualize this situation because it then appears very clearly that therapeutic strategies aiming at inhibiting-blocking the chaperonin (negative chaperonotherapy) in tumor cells may offer efficacious ways to defeat cancer. Therefore,

negative chaperonopathy, aiming to inhibit or block pro-tumorigenic molecular chaperones deserves investigation [63,168,169].

The role of Hsp60 in carcinogenesis is not yet fully understood, and its elucidation is complicated because it seems to vary depending on the tumor type, tissue affected, stage, and other unidentified factors. While some reports indicate that Hsp60 displays a protumor role others show the contrary [85,90,98,117–119,122]. While the exact role of Hsp60 in every tumor in which its levels and location changes is being studied, the quantitative variations and tissue distribution of the chaperonin may be used as biomarkers potentially useful for diagnosis, prognostication, and disease monitoring [120,123,170,171]. Additionally, investigations on Hsp60 chaperonotherapy in its various forms should be performed and aiming at standardizing the use the chaperonin as a therapeutic target or agent [63]. Some possibilities are to (i) identify compounds and/or methods that will induce a antitumor immune response by promoting the presence of Hsp60 on the surface of tumor-cells [172]; (ii) develop Hsp60 vaccines exploiting its potential for inducing the production of cytokines by interaction with monocytes and macrophages, and by manipulating its ability to bind and activate TLR-2 in CD4+CD25+ regulatory T cells and TLR-4 in B cells [173]; and (iii) identify compounds able to modulate Hsp60 expression, in order to reduce or enhance its activity in those tumors in which it displays a pro-or anti-tumorigenic role, respectively. Understanding the mode of action of Hsp60 and its regulation by miRNAs in different tissue and tumor types will help in the development of novel therapeutic strategies for cancer therapy [68,112,174]. Moreover, the utilization of advanced drug delivery systems, as exosome-like vesicles promise to be, to deliver molecules, such as miRNAs known to regulate Hsp60, and internalize them in the target cells, should be actively investigated to standardize the particles and their contents as well as their isolation from cancer patients.

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