Exosomes in cancer theranostic: Diamonds in the rough

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\textbf{ABSTRACT}

During the last 10 years, exosomes, which are small vesicles of 50–200 nm diameter of endosomal origin, have aroused a great interest in the scientific and clinical community for their roles in intercellular communication in almost all physiological and pathological processes. Most cells can potentially release these nanovesicles that share with the parent cell a similar lipid bilayer with transmembrane proteins and a panel of enclosed soluble proteins such as heat shock proteins and genetic material, thus acting as potential nanoshuttles of biomarkers. Exosomes surface proteins allow their targeting and capture by recipient cells, while the exosomes’ content can modify the physiological state of recipient cells. Tumor derived exosomes by interacting with other cells of the tumor microenvironment modulate tumor progression, angiogenic switch, metastasis, and immune escape. Targeting tumor-derived exosomes might be an interesting approach in cancer therapy. Furthermore, because a key issue to improve cancer patients’ outcome relies on earlier cancer diagnosis (metastases, as opposed to the primary tumor, are responsible for most cancer deaths) exosomes have been put forward as promising biomarker candidates for cancer diagnosis and prognosis. This review summarizes the roles of exosomes in cancer and clinical interest, focusing on the importance of exosomal heat shock proteins (HSP). The challenges of clinical translation of HSP-exosomes as therapeutic targets and biomarkers for early cancer detection are also discussed.

\textbf{KEYWORDS}
cancer diagnosis; cancer therapy; exosomes; heat shock proteins

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\textbf{ARTICLE HISTORY}
Received 15 August 2016
Revised 13 October 2016
Accepted 17 October 2016

\textbf{Introduction}

For many years, researchers thought that intercellular communications were ensured only by hormones, cytokines or neurotransmitters. However, it is now well established that cells can communicate by means of extracellular vesicles (EVs). EVs are a generic name for all vesicles that are small spherical structures surrounded by a lipid bilayer (of similar structure to that of cell membranes) and that contain hydrophilic soluble components. Through the extracellular vesicles, cells can transfer information from the plasma membrane or internal compartments.\textsuperscript{1} There are different EVs: (i) directly formed and released from the cells’ plasma membrane e.g microparticles,\textsuperscript{2} microvesicles,\textsuperscript{3} or exosomes,\textsuperscript{4} (ii) with an endocytic origin and release in the extracellular media by exoyctosis called exosomes,\textsuperscript{5} (iii) that present several characteristics of exosomes but differ by certain biophysical properties, i.e. exosomes-like vesicles,\textsuperscript{6,7} (iv) release by cells in apoptosis and called apoptotic vesicles.\textsuperscript{8} Recently, a new type of EVs have been described in gastrointestinal stromal tumors called spheresomes.\textsuperscript{9} All these vesicles types differ in their subcellular origin, their biophysical and/or biochemical properties, their receptors composition, and their content in soluble proteins and genetic material. As they contain some nanoliters of cytosol and expose at the outer space the same proteins than the parental cell, they are also considered as nanosized cells with a functional role in many biological processes. Among the different EVs, exosomes have been particularly studied since they have been shown to play a role in many physiological and pathological processes.\textsuperscript{10,12} Exosomes are cup-shaped nanovesicles that represent a distinct class of membrane vesicles, with a density of 1.13–1.19 g/ml and a diameter of 50–200 nm. These vesicles form a bioactive cargo since they carry genetic material including DNA, mRNA and miRNA, and numerous proteins, notably heat shock proteins, known to play important roles in immunity and cancer.\textsuperscript{10,13,14} The exosome, thanks to its a lipid bilayer, act like a nanoshuttle protecting these
molecules from their degradation in the extracellular medium. In this review we provide a comprehensive overview of the interest of heat shock proteins contained in exosomes in cancer diagnosis and therapy.

**Discovery of exosomes**

The term “exosomes” was first used in 1981 by Trams et al. to appoint small vesicles secreted by several cell types in the extracellular media. In 1983, Johnstone et Pan discovered with the help of electron microscopy that these vesicles derived from multivesicular bodies (MVBs) and have an endocytic origin. At the time, exosomes generated a poor interest since they were considered as a mean to eliminate obsolete proteins. But in 1996, Raposo et al. discovered for the first time that these nanovesicles secreted by antigen-presenting cells (APCs) bore functional peptide–MHC complexes. This article opened a new field in the study of these interesting nanovesicles. Two years later, it was demonstrated the release of exosomes by dendritic cells (DCs) and the ability of tumor peptide-pulsed DC-derived exosomes to suppress growth tumor in vivo. Following pioneer studies showing the potential role of exosomes in the regulation of immune responses, myriad of articles have been published related to the immune function of exosomes and their role in cancer. Furthermore, in addition to immune cells, many other cell types have been described as exosome secretory cells such as epithelial cells, neurons and tumor cells.

Exosomes can be isolated from cell culture supernatants and can be found in numerous body fluids such as blood, urine, saliva, bronchoalveolar fluid, seminal fluid, amniotic fluid, breast milk, tumor effusions and cerebrospinal fluid.

**Biogenesis**

The biogenesis of the exosome starts with the invagination of the plasma membrane leading to the endosome formation. Endosomes can differentiate in multivesicular bodies (MVBs), which are endocytic structures formed by the budding of an endosomal membrane into the lumen of the compartment. This leads to the formation of small vesicles called intraluminal vesicles (ILVs), future exosomes. Then, the fusion of these MVBs with the plasma membrane provokes the release of the ILVs in extracellular space, and become exosomes (Fig. 1). Although the biological function of MVBs was
interpreted for many years to be a late step in the degradation pathway toward lysosomes, we now know that MVBS have an alternative fate participating in the exocytic fusion of their external membrane with the plasma membrane. This phenomenon allows the excretion of exosomes by exocytosis into the extracellular space. The mechanisms underlying the sorting of the intraluminal vesicles are not yet fully understood, but 2 ways of exosome sorting have been proposed, dependent or independent on Endosomal Sorting Complex Required for Transport (ESCRT) signals. This complex consist of 4 soluble protein ESCRT-0, ESCRT-I, ESCRT-II and ESCRT-III. ESCRT is involved in the process of membrane invagination to the formation of ILVs and in the selection of proteins integrating these vesicles. Concerning ESCRT-independent signals that regulate exosomes secretion we can name the ceramide pathway, intracellular Ca$^{2+}$ levels, p53 status, Rab protein family, Syndecan-Syntenin-ALIX proteins, high level of heparanase and pH.

How the exosome penetrates into the recipient cell is still a debated issue. Three mechanisms have been proposed based on indirect evidences and in vitro studies: (i) direct contact between surface membranes of vesicles and cells, (ii) endocytosis of exosomes, and (iii) fusion between the membranes of the cell and the exosome. Once the exosome penetrates into the host cell, its content is released in the plasma membrane or in the cytoplasm (Fig. 1).

**Composition**

The composition of exosomes allows their discrimination from other EVs’ family members. The exosome membrane composition is the same than that of the mother cell but present specific enrichments and they contain proteins, lipid and genetic material (Fig. 1). All exosome components described are listed in Exocarta website. Proteins present in exosomes include adhesion proteins such as tetraspanins (CD9, CD63, CD81) and integrins (LFA-1), immunostimulatory molecules (MHC I/II), cytoskeleton molecules (actin, myosin, tubulin), membrane trafficking proteins (Rab GTPases such as Rab 5 and annexin), proteins involved in MVB formation (ALIX, TSG101), intracellular signaling proteins (Go, 14-3-3, syntenin), lipid raft associated proteins (fotillin-1), enzymes (pyruvate kinase, GAPDH), certain ligands such as FAS-L. Finally, several HSPs have been retrieved in exosomes lumen (HSP27, HSP60, HSP70, HSP90) and in exosome membrane (HSP70, HSP60 and HSP90). Some of these proteins are specifically enriched in exosomes compared to cell lysate and are classically used as exosome markers (CD9, CD63, CD81, ALIX, TSG101). Nevertheless, very recently, Kowal et al. compared the composition of EVs subtypes and revealed that although exosomes are enriched in CD9, CD63 and CD81, only TSG101 allows to distinguish exosomes from other EVs subtypes. Finally, numerous studies have revealed that some proteins within the exosomes are dependent on the cell type secreting them while others are independent from the parental cell. These different types of proteins become incorporated into exosomes during exosome formation and serve as cargo for cell–cell communication. Besides, protein exosomes are enriched in lipids such as saturated phospholipids (i.e., phosphatidyl-ethanolamin, phosphatidyl-serin, phosphatidyl-choline), sphingolipids (e.g. ceramids), and cholesterol. These lipid compositions confer to exosomes an exceptional rigidity compared to a plasma membrane. Additional components are found in exosomes including genetic materials such as mRNA (mRNA), transcripts, microRNA (miRNA), and small non coding RNA (snRNA, tRNA).

**Clinical interest of exosomes in cancer**

Researches on exosomes have considerably increased over the past decade. Although different areas of research are interested in exosomes, most scientific publications are related to cancer. Exosomes have been reported to be involved in all stages in cancer development: (i) tumorigenic transformation, (ii) tumor growth, (iii) angiogenesis, (iv) modulation of immune responses, and (v) induction of mechanisms to acquire therapy resistance. The impact of exosomes in clinical research is demonstrated by the fact that there are already 19 clinical trials ongoing (web site https://clinicaltrials.gov/). Among them, 13 involve the study of exosomes as cancer diagnosis biomarkers whereas the others use the exosomes for cancer therapy purposes. Thus, exosomes has emerged as potential biomarkers and therapeutic targets in cancer.

**Exosomes as biomarkers**

It is well established that the earlier the cancer is diagnosed, the better the survival rate. Although numerous works have been consecrated to early cancer diagnosis, today there is not yet a reliable detection non-invasive method. The main reasons for this are: first of all, in general there is a poor patients compliance, which make difficult to draw any conclusions from the clinical studies. For example, in France, in 2014, the participation rate for breast cancer screening was only of 52.1%. Secondly, actual detection methods, mainly based on medical imaging, have the limitation of tumor detection at an
early stage. Finally, certain publications have shown that some imaging approaches can have undesirable side effects and favor the appearance of tumors. For instance, mammography has been related to cancer apparition—about 1 to 20 for 100,000 mammograms.55 For all these reasons, it is necessary to develop more performing diagnosis methods. Exosomes appear to be powerful circulating biomarkers.56-58 These vesicles, reported to be stable and biologically active in human blood plasma up to 3 months, can reveal potential diagnostic information through their examination in body fluids, known as liquid biopsies.59 They are great potential tools for providing noninvasive, sensitive and economically justifiable new diagnosis methods in oncology.60 The main advantage of quantifying tumor-derived exosomes compared to circulating tumor cells (CTCs) is that exosomes are found in large amounts compared to CTCs (e.g.: $5.32 \times 10^8$ exosomes per $10^6$ cells in the 24 h period, determined by Nanoparticle Tracking Analysis, NanoSight LM10).61 Furthermore, exosomes can be quantified non-invasively in urines and other human fluids.

During the last years, improvement in some techniques like mass spectrometry has allowed to better study exosome protein content. First, several studies indicate that tumor derived exosomes carry more proteins than healthy donors-derived exosomes, particularly when compared to patients with an advanced stage disease.62,63 In 2012, Peinado’s team defined a melanoma-specific exosome signature that included tyrosinase-related protein-2 (TYRP2), very late antigen 4 (VLA-4), heat-shock protein-70 (HSP70), an HSP90 isoform and the MET oncoprotein.62 Furthermore, TrkB (Tropomyosin receptor kinase B) expression was detected in exosomes isolated from plasma of glioblastoma patients, suggesting that this receptor may be considered also as a new biomarker for glioblastoma diagnosis.54 It was later on reported that certain proteins were differentially expressed dependently on the melanoma cells from which the exosomes were analyzed, revealing a specific signature for metastatic cell lines.65 In this way, in exosomes from patients with metastatic melanoma, MIA (Melanoma Inhibitory Activity) and S100B can be detected, therefore their quantification presents diagnostic and prognostic utility.66 In 2015, Hoshino et al. revealed that specific integrin expression in exosomes could be used to predict organ-specific metastasis.67 In Non Small Cell Lung Cancer (NSCLC), leucine-rich α-2-glycoprotein (LRG1) was found to be expressed at higher levels in urinary exosomes of NSCLC patients suggesting that LRG1 may be a candidate biomarker for non-invasive diagnosis of NSCLC in urine. (Li et al.,58) More recently, it has been determined a combination of several exosomal proteins (CD151, CD171 and tetraspanin 8) that could be used as a promising diagnostic tool of lung cancer independently of its stage and histology.68 In acute myeloid leukemia, TGFβ1 expression seems to be useful to predict response to immunotherapy.70 Finally, in urological malignancies, exosomes in the urine have been described as robust biomarkers and particularly those expressing survivin for early detection of prostate cancer.71 Other proteins have been described as specific of cancer-derived exosomes compared to healthy donors and seem also candidates as cancer diagnosis tools. This is the case for Claudin, which is present only in exosomes derived from the plasma of women with ovarian cancer,72 for Glypican-1 that appears to allow to distinguish an ovarian cancer with high specificity and sensitivity or for CD9-CD147 that is embedded in colorectal cancer-derived exosomes.73 It has also been reported that 80 percent of the exosomes isolated from NSCLC samples was positive for surface EGFR (epithelium growth factor receptor) by immune staining compared to only 2% of the exosomes in chronic inflammatory lung tissue.74 Finally, as a general marker of cancer-derived exosomes, our team has recently proposed membrane HSP70 that is present in exosomes released by large panel of cancer cells but not by their normal counterparts; see below.

New researches focus on miRNA potential because exosomes offer a miRNA protection from RNases contrary to free circulating miRNA. In 2007, Valadi et al. showed for the first time the transfer of functional miRNAs between 2 cells by means of exosomes.75 miRNAs are a class of 21–25 small non coding but functional RNA that negatively regulates mRNA expression. These small non-coding RNAs plays important roles in cancer,76 explaining why this discovery suggested a new regulatory role for exosomes in cancer. Today, numerous studies have identified different functional exosomal miRNAs and their role in cancer;77,78 and proposed their use as diagnosis biomarkers.79,80,81 For example, in lung cancer 2 miRNAs, miR-21 and miR-155 have been found to be significantly upregulated in recurrent tumors compared to primary tumors.

**Exosomes as immunotherapy agents**

Despite improvements in treatment and longer survival, cancer stays a principal cause of death in the world. Since the discovery of functional MHC-peptides complexes in DCs-derived exosomes,18 many immune functions for exosomes have been described.19 Researchers tend also to find a new way to modulate immune responses against cancer with the help of exosomes: it is called cell-free vaccines. A classical approach consists in loading exosomes derived from DCs with a tumor specific antigen to restore antitumor immunity. For example, André et al.
isolated exosomes from DCs following tumor peptide pulse and their administration in murine tumor models resulted in rejection of established tumors, an action mediated by T-cell activity. It was later shown that vaccination with exosomes containing modified IL-2 could induce a significant regression of a pre-established tumor by targeting the antigen-specific Th1-polarized immune response and cytotoxic T lymphocytes (CTL). More recently, it has been described an alternative approach to prepare exosomes GPI-IL-12 from fusion gene-modified renal cancer cells and to use them for immunization. This modified exosomes-based vaccine can induce an antigen-specific immune response and CTL more efficiently, resulting in more significant cytotoxic effects in vitro.

Chaput et al. demonstrated that isolated DCs-derived exosomes pulsed with Mart1 (Melanoma antigen recognized by T-cells) peptides in vitro were able to activate CTL and in combination with appropriate adjuvants, to induce an antitumor response. In a sarcoma mice model, it was found that OVA (chicken egg ovalbumin) packaged-exosomes allowed a more efficient induction of antitumor immune responses than the native soluble OVA secreted form. Another way to modulate antitumor immune responses is to combine vaccination by exosomes with other molecules. It was found that ascite-derived exosomes combined to GM-CSF in the immunotherapy of colorectal cancer could induce an antitumor cytotoxic T lymphocyte response whereas combined vaccination with tumor antigen loaded DC-derived exosomes with metronic cyclophosphamide, which inhibit Treg function and restore T and NK cell effector functions, could boost NK cell mediated antitumor immunity in lung cancer patients. Finally, recent several studies have shown that HSP-exosomes can also modulate the immune system. This part is discussed in more detail below.

**Exosomes as drug delivery cargos**

From their characteristics and properties, exosomes have been used as natural drug delivery cargos. Indeed, exosomes offers several advantages: (i) from their composition, exosomes are capable to avoid immune response and are less immunogenic than any other drug delivery system; (ii) exosomes can naturally and easily penetrate in a host cell by several means, their nanometric size (50–200 nm) allows them to avoid phagocytosis by the circulating mononuclear phagocytic systems, and the easy extravasation through hyper-permeable blood vessels surrounding tumors, in order to reach tumor tissues and (iv) exosomes’ membrane protects their content from degradation and are very stable. Several means of modifying exosomes composition exists.

The vast majority of exosome-based drug delivery works and reviews describe the therapeutic transfer of interfering RNAs like synthetic siRNAs or miRNAs and therefore will not be discussed here. We will focus on chemical compound, drugs and proteins.

In a zebrafish brain cancer model, exosome-delivered anticancer drugs through the blood brain barrier decreased tumor growth markers and so could be potentially used as a carrier for brain delivery of anticancer drugs. In 2014, Pasucci et al. showed that Mesenchymal Stem Cells (MSC) could incorporate and deliver Paclitaxel to recipient cells through exosomes with increased anti-tumor effects. This study suggests that MSC-derived exosomes could be a new strategy for drug delivery in cancer treatment. Exosomes have also been used as cargos of paclitaxel to increase the effectiveness of the treatment in prostate cancer cells. It has also been shown the effectiveness of targeted exosome-encapsulated doxorubicin for integrin-positive breast cancer cells in inhibition of tumor growth. Recently, Fuhrmann et al. found that exosomes loaded with hydrophilic porphyrins induced a stronger phototoxic effect than the free drug in a cancer cell model (Integrin-positive cancer cells). Zhang’s research group used exosomes derived from different cell types to successfully delivered curcumin to activate myeloid cells, producing anti-inflammatory activity and apoptosis in monocytes. Finally, genetically engineered exosomes expressing high levels of a suicide gene mRNA and protein-cytosine deaminase (CD) fused to uracil phosphoribosyltransferase (UPRT) have been used to treat pre-established nerve sheath tumors (schwannomas) in an orthotopic mouse model and led to tumor regression.

Exosomes seem to be new actors in theranostic oncology. In this context, recent studies have validated a major role for heat shock proteins in exosomes.

**Heat shock proteins and exosomes**

Heat shock proteins are stress proteins subdivided in several families according to their molecular weight: HSP110, HSP90, HSP70, HSP60 and small HSPs. These proteins, very well conserved during evolution, were first discovered in 1962. They represent about 2–3% of cellular proteins. In case of a cellular stress, several of these proteins are overexpressed. A wide variety of stress might induce HSPs expression such as hypoxia, infections, drugs and ischemia. The induction of HSP genes require the activation and translocation to the nucleus of specific transcription factors called “Heat Shock Factors” (HSF). These HSF bind to DNA particular sequences.
named “Heat Shock Elements” (HSE) in the promoter of HSP genes allowing their expression.101

HSPs have been retrieved in all cellular compartments including cytoplasm, nucleus, membrane, mitochondria or endoplasmic reticulum and act as molecular chaperones to maintain cellular homeostasis. HSPs allow the correct refolding of newly-synthesized proteins or incorrectly folded proteins, following physiological conditions or in response to stress.102,103 If they can’t refold correctly the abnormal protein, HSPs can facilitate their proteasomal degradation.104 In case of a cell death stimulus, HSPs are overexpressed and have strong anti-apoptotic properties by associating to different key proteins of the apoptosis transduction signaling pathway.105

HSPs can also be extracellular (membrane-bound or free after secretion).106,107 HSP27,108 HSP70109 and HSP90110 have been found secreted in the extracellular media, and some of them, as already mentioned above, have been shown to be present in extracellular vesicles, notably in exosomes.111-113

HSPs have an important function in cancer by acting at different levels. First, they can promote tumor growth by stabilizing oncogenic proteins. For example, HSP90 can stabilize c-Src, STAT3, Raf-1 or HER2/neu.114 Certain HSPs, mainly HSP70 and HSP27, can also increase the resistance to chemotherapy by inhibiting apoptosis.115 Further, some HSPs can promote angiogenesis such as HSP70 and HSP90 that can sequester HIF-a, which is necessary for VEGF production.116 HSP90 is also involved in the VEGF synthesis and may be a potential novel target for anti-angiogenic therapy.117 Moreover, HSPs play a role in metastasis formation; some clinical studies have shown a correlation between the expression of HSP27 and/or HSP70 and the metastatic potential.118-120 Finally, extracellular HSPs can have immunosuppressive functions. Indeed, HSP70 secreted by colorectal cancer cells can activate myeloid-derived suppressor cells and inhibit T cells activation.121

During the last few years there have been a growing interest in extracellular HSPs because of the increasing evidences of their role in the induction of innate immune responses with immunostimulatory or immunosuppressive effects, depending on the nature of the HSP, its localization and cell type.122 Among them, exosomal HSPs seems to modulate the immune response and play anti-tumor functions.13 This is the rational for the use of exosomal HSPs in cancer therapy and diagnosis.

Published data about extracellular HSPs can be confusing as the term “extracellular HSPs” is generally employed for both soluble, membrane-bound and exosomal HSPs. EV-associated HSPs are still quite new in the field of extracellular HSPs and therefore most papers do not unambiguously differentiate between the different forms of extracellular HSPs. Moreover, very often, researchers write about HSPs in “extracellular vesicles” without given any precision about which subtypes of vesicles they are analyzing. To overcome this problem, in this review, we will summarize mainly data about clearly established exosomal HSPs (i.e. studies in which HSPs are determined from previously isolated exosomes -from human body fluids or culture supernatants).

**HSP-exosomes in cancer therapy**

Several studies have shown that certain exosomal HSPs could modulate the immune system (Fig. 2). HSP70-exosomes could stimulate natural killer cells (NK) reactivity.123 When preincubated with HSP70 surface-positive exosomes, NK cells initiated colon tumor cells apoptosis through granulocyte B release.124 It was later on discovered that extracellular HSP70 could also activate macrophages and that this immune modulator effect depended on the ability of HSP70, present on the cell surface, to translocate into the plasma membrane.125 It was suggested that HSP70, release through exosomes derived from stressed cells, constitute a form of intercellular communication in order to inform macrophages and to induce innate immune responses.125 These studies suggested that exosomal HSPs could be used in cancer therapy. In 2006, Chen al. tested the vaccination with exosomes presenting HSP60 and HSP90, derived from lymphoma cells. Researchers found an increase in the anti-tumor immune response involving the induction of IFN production and the activation/maturation of dendritic cells.126 Several years later, myeloma cell derived exosomes were genetically modified to express endogenous P1A tumor antigen and a transgenic form of membrane-bound HSP70. These HSP70-modified exosomes were able to stimulate in vitro DC maturation more efficiently. The researchers used them as a vaccine and found that they stimulate type 1 CD4(+) helper T (Th1) cell responses, P1A-specific CD8(+) CTL responses and antitumor immunity.127 More recently, Li-Hong et al. demonstrated that exosomes derived from resistant anticancer drug-treated Hepatocellular Carcinoma (HCC) cells conferred a higher antitumor response by inducing HSP-specific NK cell responses in vitro and suggested HSP-bearing exosomes could be used as an efficient vaccine for hepatocellular carcinoma immunotherapy.128

In apparent contrast with these results, we discovered an immunosuppressive function of HSP70 at the surface of exosomes. We have shown that all cancer cells analyzed so far have the ability to secrete exosomes with HSP70 in their membrane while normal “non cancerous” cells do not. These tumor-derived exosomes, through membrane-anchored HSP70, can activate Myeloid Derived Suppressor Cells (MDSCs),121,50 which are abundant cells in a cancer context that restrain antitumor immunity and promote
tumor expansion. At the molecular level, the extracellular domain of membrane HSP70 binds to the Toll-Like Receptor 2 at the surface of MDSCs thus activating them. This interaction triggers NF-kB signaling pathway allowing the expression of the inflammatory cytokine IL-6, which binds to its receptor IL-6R in an autocrine manner. This interaction leads to STAT3 phosphorylation via JAK2 pathway, activating survival genes in MDSCs that could exert their immunosuppressive functions.

Recently, our team has confirmed the release of HSP70-exosomes by cancer cells and their ability to activate MDSC in a small cohort of colon cancer patients. Further, we have developed a peptide aptamer (A8) that binds to the extracellular domain of membrane-bound HSP70, called “TKD.” Membrane HSP70 binds with much higher affinity to A8 than to the TLR2 receptor in the MDSC. As a result, A8 block the capacity of these tumor-derived exosomes to activate MDSC. Thereby, in vivo and in vitro, A8 induce the development of an efficient anti-tumor immune response that was associated to an inhibition of MDSC. In line with our results proposing an HSP70 inhibitor -A8- as an agent that can boost the anti-cancer immune response, HSP90-exosomes have been described as to be involved in the activation of plasmin and cancer cells’ motility in several cancer models. Thus, targeting HSP90 could also represent a way to limit tumor invasion by inhibiting a growing number of proteins that are involved in tumor cell motility.

**HSP-exosomes in cancer diagnosis**

The detection and quantification of exosomal HSPs can provide useful information for establishing new circulating and non-invasive biomarkers (Fig. 2). Our team suggests the use of HSP70-exosomes as a cancer marker because they seem a general feature of cancer cells (but not of “normal” non-cancerous cells) and we have demonstrated that they can be measured in large amounts in biological fluids from cancer patients but not from healthy individuals where they are hardly detected. We have patented an interference biolayer protocol to easily capture HSP70-exosomes isolated from human fluids using as a high affinity ligand our peptide aptamer A8 (WO2015/189395). To move beyond the proof of principle that these tumor-derived exosomes (HSP70-exosomes) can be quantified and might be interesting to follow up cancer patients, we have started a prospective study with the anticancer Center Georges-François Leclerc (CGFL, Dijon, France) in breast, ovarian and
lungs aiming at determining whether the presence of HSP70-exosomes is predictive of the patients’ outcome and whether their detection precedes CTCs and the apparition of metastases.

Finally, HSP60 have also been localized at the membrane of exosomes isolated from blood patients suffering from large bowel cancer, before surgery, but was absent after surgery and in healthy controls. Because of its presence in tumor cells but not in healthy cells, HSP60-exosomes seem to be also interesting biomarkers in cancer, at least for large bowel cancer diagnosis.  

Concluding remarks

In conclusion, there are no doubts that even if there are still many questions remaining to be answered, the relatively young field of exosomes in cancer is gaining greater interest within the scientific and medical communities. There are 2 main limitations in the discussion of the works presented in this review. The first and most important is the lack of standardized protocols for isolation of tumor-derived exosomes; the second is the still partial understanding of the mechanisms involving exosomes functions in cancer. Indeed, there is a Janus faced implication of exosomes in cancer biology explained by the fact that exosomes can transfer both tumor-promoting molecules (e.g.: oncoproteins) and tumor suppressors and can either induce or suppress an immune response. We believed these debated issues could be solved with more precise protocols to isolate cancer exosomes and taking into account the in vivo cancer environmental context.

The available data on exosomes strongly suggest that these diamonds in the rough might represent a revolution in cancer diagnosis and toward a more personalized medicine. Exosomes can be a fingerprint of the parental cell type and of its status. Moreover, they are abundant in body fluids such as blood and urine, therefore representing a precious biomedical tool for non-invasive approaches in cancer diagnosis and cancer patients’ follow up. Furthermore, as nanoshuttles of biomarkers and/or anti-tumor drugs, exosomes open new avenues for the clinical management of cancer.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

This work is supported by the “Investissements d’Avenir” (ANR-11-LABX-0021), the Fondation pour la Recherche Médicale (FRM grant number ECO20160736090 to GC), the Ligue Nationale Contre le Cancer, the Association pour la Recherche sur le Cancer, l’Institut National du Cancer, Centre Georges-François Leclerc, Cancéropole Grand-Est and FEDER.

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