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EXOSOMES, KEY FACTORS IN THE REGULATION AND DEVELOPMENT OF THE TUMOR NICHE

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ABSTRACT

Aim of the study Exosomes are small extracellular vesicles containing genetic material, proteins and lipids. They function as potent signaling molecules between cancer cells and the surrounding ones, contributing to the formation of the tumor microenvironment (TME). Exosomes derived from tumor and stromal cells are involved in all stages of cancer progression and play a crucial role in the development of resistance to treatments. Also, because of their function as mediators in cell-to-cell communication, they constitute an essential part of the therapeutic resistance mechanism associated with TME. In this article, we aimed to analyze the current techniques for the isolation and profiling of exosomes, as well as their contribution to TME interactions and therapeutic resistance. We also discuss the emerging clinical applications of exosomes, considering them as biomarkers, direct therapeutic targets, and artificial nanocarriers. A deep understanding of the TME requires a detailed analysis of exosomes and their contents, which is a promising direction for the development of effective clinical applications.

Key words: Exosomes, tumor microenvironment, therapeutic resistance, biomarkers, cellular communication

INTRODUCTION

The tumor microenvironment is not simply a passive background, but an active participant in the support and development of cancer. Comprised of various cell types in a variety of functional niches, the TME modulates a multitude of cell-cell interactions. These interactions orchestrate reprogramming in cancer-permissive environments and can have significant effects on cancer development, progression, and treatment success[1]. Understanding the tumor microenvironment is essential for the development of innovative and effective anticancer therapies. However, TME has been identified as a major source of treatment resistance, particularly due to its inherent heterogeneity and adaptability [2]. Cells in the TME exchange information through various signaling networks, ranging from juxtacrine interactions such as desmosomes and cell-cell junctions to secreted factors such as cytokines, chemokines and extracellular vesicles such as exosomes [3].

The tumor stroma, the important component of the TME, includes fibroblasts, endothelial cells and pericytes, which form the connective tissue surrounding the tumor. Also, activated fibroblasts, called tumor-associated fibroblasts (CAF - Cancer-Associated Fibroblasts), play a crucial role in remodeling the extracellular matrix and supporting tumor growth, by releasing growth factors, cytokines and proteases that favor the migration and invasion of tumor cells .

The immune system plays an ambivalent role in the tumor microenvironment, having both anti-tumor and pro-tumor effects. Immune cells can be classified into two categories: cells with antitumor activity and cells that promote cancer progression. Antitumor immune cells include cytotoxic T lymphocytes (CTL), natural killer (NK) cells,

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and M1 macrophages, which have the ability to destroy tumor cells and produce cytokines that stimulate the immune response against cancer. Pro-tumor immune cells include regulatory T lymphocytes (Treg), M2 macrophages, and myeloid-derived suppressor cells (MDSCs), which contribute to the suppression of the immune response and promote tumor growth, angiogenesis, and metastasis. (4)

The extracellular matrix (ECM), the third component of the TME, is a complex network of proteins, collagen, elastin, and glycoproteins that form the supporting structure of tumor and stromal cells. Changes in ECM composition and stiffness may facilitate tumor invasion into adjacent tissues and metastasis. In addition, proteases secreted by tumor or stromal cells, such as matrix metalloproteinases (MMPs), degrade the ECM, contributing to its remodeling and tumor invasiveness.

Tumors require a constant supply of oxygen and nutrients to sustain rapid growth. Tumor angiogenesis is the process by which the tumor stimulates the formation of new blood vessels from the existing vasculature using growth factors such as vascular endothelial growth factor (VEGF). However, these vessels are often immature, tortuous, and dysfunctional, leading to hypoxic areas within the tumor, contributing to malignant progression and treatment resistance. [5]

Exosomes and other extracellular vesicles highlight the complexity of intercellular dynamics in the interactions that make up the TME. The aim of this study is to elucidate the biogenesis and function of cancer cell- and TME-derived exosomes and their ability to mediate paracrine signaling and influence tumor progression. The tumor microenvironment promotes growth, progression, invasion and treatment resistance by potentiating important oncogenic pathways in cancer cells. Exosomes are a fascinating component of TME signaling, a current research topic that could lead to exciting clinical therapeutic applications.

Biogenesis and characterization of exosomes

Exosomes are small extracellular vesicles (<150 nm) that are formed by a dynamic endocytic process [6]. In the process of endosomal maturation, intraluminal vesicles are formed by ESCRT-dependent and -independent processes [7]. The double invagination at the level of the plasma membrane forms endosomes and intraluminal vesicles, subsequently resulting in a lipid bilayer membrane having the same orientation as the plasma membrane of the original cell. This orientation and structure is an essential element for the ability of exosomes to mediate intercellular interactions. Exosomes are then able to be taken up by other cells, where they can unload their contents and influence various biological processes such as cell migration, inflammation and immune response.

Exosomes isolation techniques

Isolation of exosomes from biological fluids (such as blood, urine, amniotic fluid, etc.) or cell culture media is essential for their research and clinical use. There are several techniques for isolating exosomes, each with advantages and limitations depending on the purity and quantity of exosomes desired. In tissue culture models, exosomes are traditionally isolated from the original medium using high-speed differential ultracentrifugation, which includes steps to purify cells, cellular debris, and larger microvesicles [8].

This technique is the most common, but its yield is unstable, purity can be compromised, and ultracentrifugation can destroy exosomes. Another separation method is low-speed centrifugation using polyethylene glycol, but it

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is unclear whether this method affects the functionality of the purified exosomes. Several commercial exosome isolation kits are widely used, but their ability to produce pure and functional exosomes is still poor. In addition, antibody and filter-based enrichment methods can produce pure populations of exosomes without the need for aggressive centrifugation.

In recent years, methods combining acoustics and microfluidics have been developed. These methods include non-contact separation of exosomes from cell culture media and biological fluids [9]. Acoustic fluidics methods use acoustic waves in microfluidic devices to perform size separation from whole blood [9]; while fluidic technologies such as ExoTIC (Exosome Total Isolation Chip) use nanoporous membranes for further enrichment and purification of extracellular vesicles having a size of 30-200 nm. If widely available, acoustic or fluidic methods may be the most accurate approach for isolating functional and intact exosomes in reproducible quantities.

Exosomes can be isolated from conditioned medium of ex vivo cultured tissues or directly from tissues. When isolating directly from tissue, it is essential to use gentle tissue separation to minimize damage to cellular integrity, which can lead to contamination of cellular vesicles[10]. Identifying exosomes is not an easy step because these particles are very small. Although it is possible to roughly estimate their number by protein quantification, direct counting is best done by nanoparticle tracking analysis, using techniques such as NanoSight.

These techniques use emitted light and Brownian motion to accurately determine the size and amount of exosomes in suspension. Flow cytometry can also measure exosomes indirectly by binding them into larger structures. The purity and quality of exosomes is best assessed by electron microscopy, where the classic "cup"-shaped structure and lipid bilayer should be observed[8]. Electron microscopy can detect signs of destruction of exosomes and other macromolecular structures due to the high speed of ultracentrifugation. Purity of exosomes can also be assessed by the presence or absence of protein markers. Exosomes can contain snapshots of the cell of origin, meaning that many proteins present in the cell are, to some extent, also present in exosomes. In general, the absence of cellular contamination can be confirmed by examining the presence of exosomal structural molecules such as the tetraspanins TSG101, CD81 and CD9 and the absence of histoneproteins[10].

Each exosome isolation technique has advantages and disadvantages in terms of purity, yield, and time required. Choosing the appropriate method depends on the type of sample, the purpose of the experiment, and the available resources. For certain clinical applications, such as exosome-based diagnostics, fast and easy-to-use methods such as polymer precipitation or microfluidics are often preferred.

The composition of exosomes

As we already mentioned, exosomes are small extracellular vesicles released by almost all types of cells, measuring 30-150 nm. They facilitate intercellular communication by transporting biological molecules such as proteins, lipids and nucleic acids. Major components include membrane proteins, adhesion proteins, transport and fusion proteins, and cytosolic proteins. The lipid membrane is rich in sphingolipids, ceramides, cholesterol and phospholipids (11).

Exosomes carry various types of RNA, including mRNA, miRNA, lncRNA, and dDNA. They also contain bioactive factors such as cytokines and signaling molecules. The composition of exosomes allows them to modulate immune responses, influence

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metastasis and facilitate tissue regeneration. Their complex composition reflects the cell of origin and plays a central role in the transfer of molecular information between cells, influencing biological processes such as immunity, inflammation, cancer and cell regeneration.

To characterize the protein profile of exosomes, there are several experimental techniques used to identify and analyze the proteins contained in exosomes. These techniques vary according to the specific purpose (detection, quantification, identification) and the complexity of the exosomal proteome, in this sense mass spectrometry is the reference method, while techniques such as Western blot, ELISA and flow cytometry are ideal for validation and quantification of specific proteins.

Exosomal RNA plays a crucial role in intercellular communication and is a key element of exosomes function. Exosomes contain a variety of RNA molecules, including mRNA, miRNA, lncRNA, circRNA, and other types of RNA, each with a role in gene regulation and intercellular communication. Exosomal RNA is extremely stable, due to the protection conferred by the lipid membrane of exosomes. This stability allows exosomes to carry RNA over long distances, protecting it from degradation in the extracellular environment, including the action of ribonucleases. Their high stability makes exosomal RNA a valuable biomarker for disease diagnosis, as it can persist in biological fluids (blood, urine, saliva) for a long time. (12)

The role of exosomes in tumor niche communication and development

In terms of cancer, exosomes play an important role as mediators of intercellular signaling leading to tumor progression [13]. In addition, exosomes may serve as biomarkers for the detection and progression of cancer. Cancer cells secrete exosomes that have been shown to influence cancer progression through the formation of tumor-supporting stroma and the differentiation of fibroblasts into tumor-supporting stromal myofibroblasts. This differentiation depends on the expression of α -smooth muscle actinin and the induction of TGF-β signaling [14]. Cancer-associated fibroblasts (CAF), which play an important role in cancer aggressiveness, also secrete exosomes involved in cancer cell invasion. Thus, exosomes secreted by cancer cells and CAF directly affect both the surrounding stroma and other cells, altering their function and thus promoting tumor progression.

Exosomes derived from cancer cells also exhibit immunomodulatory functions leading to immunosuppression and evasion of immune responses. Clayton et al. showed that proliferation of peripheral blood lymphocytes in response to IL-2 is inhibited by exosomes derived from cancer cells, and NK cell activity is reduced[15].

Yu et al. [15] revealed that TS/A murine mammary tumor cells secrete exosomes that inhibit the differentiation of tumor myeloid progenitor cells into dendritic cells due to IL-6 induction. Taylor et al. demonstrated that exosomes from human ovarian cancer samples suppress the expression of signaling components of T cell activation, such as JAK3 and CD3, inducing T cell apoptosis. In vitro studies have suggested that exosomes derived from cancer cells affect T cell gene expression and induce downregulation of suppressor genes in CD4+ T cells, resulting in loss of CD69 and reduced function. Colorectal cancer cells have been shown to secrete exosomes containing miR-145, which are subsequently taken up by macrophages and cause a shift to the M2 phenotype.

Exosomes secreted by cancer cells may play an important role in angiogenesis, a process essential for cancer progression.

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Melanoma cells have been shown to secrete exosomes containing angiogenesis-regulating factors such as vascular endothelial growth factor (VEGF), IL-6, and matrix metalloproteinase 2 (MMP2) through WNT5A signaling. In addition, depletion of WNT5A resulted in reduced branching of endothelial cells [16]. Umezu et al. [17] found that exosomes derived from leukemic cells (K562) contain miR-17-92 clusters, which are transported to endothelial cells (HUVECs-human umbilical vein-derived cells) and reduce α5 integrin expression. Thus, exosomes may play a role in the communication between leukemia and endothelial cells via miRNA content. Similarly, colon cancer cells promote endothelial cell proliferation via exosomes. They promote endothelial cell proliferation through exosomes and their mRNA content [18]. Metastatic cancer cells secrete exosomes containing miR-105. MiR-105 promotes endothelial cell proliferation by targeting tight junction protein ZO-1.

These results suggest that exosomes secreted by cancer cells are involved in both angiogenesis and metastasis. Exosomes derived from cancer cells affect the extracellular matrix (ECM) and play an important role in cancer cell migration by promoting the adhesive junction. Fibronectin in these exosomes appears to be particularly important for cell migration. Muetal[19] showed that tumor-derived exosomes can bind to individual components of the ECM, such as hyaluronic acid and laminin.

In addition, these exosomes are rich in proteases that degrade collagen, laminin, and fibronectin to form premetastatic niches. Metastatic potential can be transferred between metastatic and non-metastatic cancer cells via exosomes, as shown by Le et al. [20]. Extracellular vesicles containing miR-200 secreted from metastatic breast cancer cell lines have been shown to alter gene expression and promote mesenchymal epithelial transition (MET) in non-metastatic cells [5]. Another example is pancreatic ductal adenocarcinoma, where exosomes were shown to induce prehepatic metastatic niche formation in naïve mice.

In recipient hepatocytes, secretion of TGF-β and upregulation of fibronectin occurs and a fibrotic microenvironment is formed. Macrophage migration inhibitory factor (MIF) in exosomes antagonizes bone marrow-derived macrophages, leading to metastasis. Exosomal integrins appear to be particularly important in determining organ-specific metastases. Integrins α6β4 and α6β1 are associated with lung metastasis, while integrin αVβ4 correlates with liver metastasis[21].

Exosomes as diagnostic markers and therapeutic targets

In recent years, exosomes have attracted the attention of researchers for their potential in the diagnosis and treatment of some conditions, especially in cancer, neurodegenerative diseases and chronic inflammation. Their use as markers and therapeutic targets has opened new perspectives in precision medicine.

Exosomes reflect the molecular content of the cell of origin, providing information about its physiological and pathological state. They may contain specific proteins, non-coding RNAs (especially microRNAs) and other molecules involved in cell signaling processes. This ability to reflect the state of cells has led to the use of exosomes as biological markers (biomarkers) for various conditions [22].

Towards the use for cancer diagnosis, tumor exosomes carry molecules derived from cancer cells, such as oncoproteins, specific RNAs and tumor DNA. Thus, analysis of exosomal content may provide a non-invasive method for early detection of cancer or monitoring of disease progression. For example, microRNAs in exosomes can be used

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to diagnose different types of cancer, such as breast, prostate, liver or lung. In addition, increased levels of specific exosomes in biological fluids (eg blood, urine) are correlated with the presence and aggressiveness of tumors.

Another direction of use as biomarkers is for the diagnosis of neurodegenerative diseases.

Exosomes originating from the central nervous system (CNS) have been identified in cerebrospinal fluid and even blood, suggesting that they may carry proteins associated with neurodegenerative diseases, such as tau protein and beta-amyloid in Alzheimer's disease. This makes it possible to detect early and monitor the progression of these diseases. Exosomes could also help differentiate between various neurological disorders, which may improve differential diagnosis. [23]

Exosomes are not only passive carriers of molecules, but can actively modulate cellular behavior, either by fusing with other cells or by releasing their intracellular contents. This characteristic has turned them into a promising therapeutic target, especially in the context of chronic and neoplastic diseases.

An important aspect of targeted therapy is inhibiting the release of tumor exosomes or blocking their interaction with healthy cells. Since tumor exosomes are involved in promoting metastasis and treatment resistance, reducing or neutralizing their activity could improve the effectiveness of traditional oncology treatments. For example, inhibitors of exosome release (such as GW4869) have been investigated that block the production of these vesicles by cancer cells, thereby preventing the spread of pro-tumor messages.Exosomes can be genetically engineered to deliver drugs or specific genetic material (such as microRNAs or RNAi) to target cells due to their ability to cross biological barriers such as the blood- brain barrier. This property makes them useful in treating brain diseases or localized cancers that are difficult to access. In addition, exosomes have high biocompatibility and are less immunogenic compared to other drug delivery systems such as synthetic nanoparticles.[24]

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