Extracellular Vesicles in Malignant Melanoma

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Different types of cells, such as endothelial cells, tumor-associated fibroblasts, pericytes, and immune cells, release extracellular vesicles (EVs) in the tumor microenvironment. The components of EVs include proteins, DNA, RNA, and microRNA. One of the most important functions of EVs is the transfer of aforementioned bioactive molecules, which in cancer cells may affect tumor growth, progression, angiogenesis, and metastatic spread. Furthermore, EVs affect the presentation of antigens to immune cells via the transfer of nucleic acids, peptides, and proteins to recipient cells.

Keywords: extracellular vesicles (EVs) ; melanoma ; angiogenesis ; metastasis ; invasion ; drug resistance ; immune system ; therapeutic application

1. Melanoma

Human skin is the first layer of defense, protecting us from external factors and providing control of body temperature and storage of moisture and fat. Skin cancer is a common malignancy, with three major types (basal cell carcinoma, squamous cell carcinoma, and melanoma), which have different precursor cells. Basal cell carcinoma and squamous cell carcinoma are classified as non-melanoma skin cancers [1]. Malignant melanoma which is derived from melanocytes is the most aggressive, invasive, and life-threatening skin cancer [2]. Risk factors for melanoma development include fair skin and exposure to sunlight while ultraviolet light exposure is one of the main causes of the development of melanoma [3]. According to the World Health Organization, approximately 132,000 new cases of melanoma are diagnosed each year worldwide. Especially in white (Caucasian) people, melanoma is becoming more common, mostly due to their less pigmented skin, which renders this population more susceptible to ultraviolet light exposure [4].

Metastasis is a multistep process. The first step involves tumor cells invading the basal membrane and entering the blood vessels. Then, these tumor cells circulate in the blood stream until their attachment at the site of metastasis and initiate subsequent extravasation. Finally, they colonize and grow in distal host organs. In melanoma, vascular invasion occurs predominantly via lymphatic vessels. Vascular invasion is associated with factors indicative of a poor prognosis, including stage, increased Breslow thickness, and ulceration [5]. Cells can communicate by different types of EVs, which include exosomes, apoptotic bodies, and microvesicles (MVs) [6]. EVs are important mediators of intercellular communication between cells and distal organs and are crucial to cancer progression [7].

2. EVs Derived from Melanoma and Their Role in Cancer Progression

Biological information between adjacent tumor cells can be transmitted through tumor-derived EVs in a paracrine manner. This signal transduction between malignant cells not only promotes cancer growth and metastasis but also can interfere with normal signaling pathways [8]. Tumor cells may metastasize to distant organs in the body and regulate the tumor microenvironment to form pre-metastatic niches; in these cases, tumor-derived EVs may be potential biomarkers for tumor progression and invasion [9]. In addition, tumor-derived EVs are expected to be used as carriers for cell-free vaccines and for the delivery of specific tumor therapeutic molecules. In this section, we focus on the role of tumor-derived EVs in melanoma development and metastasis and their potential applications in advancing the diagnosis and treatment of melanoma and personalized medicine.

2.1. Growth and Angiogenesis

The literature indicates that the addition of EVs to a human cell culture enhances EV production and supports cell proliferation [10]. The biodistribution of cancer-derived EVs in tumor tissues is an important factor in determining the role of EVs in tumor proliferation [11]. In vivo experiments have shown that B16BL6 melanoma cells secrete and absorb B16BL6 cell-secreted EVs to induce their own proliferation and inhibit their own apoptosis, promoting tumor progression [12]. EV uptake by target cells relies on the integrity of plasma membrane microdomains, namely lipid rafts, which are known to be enriched with cholesterol. Scavenger receptor type-B1 (SR-B1) is a high-affinity receptor for mature high-density

lipoproteins (HDLs), and SR-B1 maintains cholesterol equilibrium, uptakes extracellular material, and promotes cell signaling [13][14]. The expression of SR-B1 in melanoma enhances EV formation and cellular uptake, promoting a metastatic phenotype. SR-B1 is associated with the expression of microphthalmia-associated transcription factor (MITF) and the regulation of proto-oncogene mesenchymal-to-epithelial transition (MET) factor. SR-B1 is a key molecule for regulating EV uptake and cancer growth [15]. Wnt Family Member 5A (WNT5A) regulates the release of EVs containing the immunomodulatory cytokine IL-6 and proangiogenic factors IL-8, VEGF, and MMP2 from melanoma cells (MeWo, SKmel28, A2058, A375, and HTB63). This effect increases angiogenic processes and facilitates metastatic spread [16]. Hood et al. indicated that melanoma EVs can boost endothelial angiogenic responses to create a premetastatic niche [17]. A previous report indicated that miR-155 in melanoma-derived EVs can induce reprogramming of fibroblasts into CAFs (cancer-associate fibroblast) and trigger the proangiogenic switch of these CAFs [18].

2.2. Migration and Invasion

Studies on melanoma cell migration and invasion and on the underlying molecular mechanisms are essential for improving melanoma diagnosis, prognosis, and therapy. EVs play an important role in this regard and regulate the migratory and invasive capacity of melanoma cells. Several studies have demonstrated that EVs can increase migratory and invasive capacities [19]. EVs derived from melanoma cells have also been shown to increase type I interferon receptor (IFNAR1) and cholesterol 25-hydroxylase (CH25H) in normal cells, thus facilitating EV uptake and pre-metastatic niche development [20]. Matrix metalloproteinases (ADAM) and ADAM with thrombospondin motifs (ADAMTS) are enriched in melanoma-derived EVs. These proteins are critical for degrading the extracellular matrix of cancer cells and increasing metastatic spread [21]. Insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1) is a multifunctional RNA-binding protein that has been linked to the development of a variety of malignancies. According to previous research, EVs derived from IGF2BP1-overexpressing melanoma cells exacerbate EV-induced metastasis [22]. Xiao et al. showed an increase in invasiveness when normal melanocytes were treated with melanoma EVs [23]. Melanoma usually metastasizes to the lungs, bones, liver, and brain and rarely to other organs. The current mechanism of this pattern needs further understanding, but it is likely that EVs play an important role. For example, melanoma cells are exposed to bone-derived soluble factors, which are related to the molecular activation pathway of stromal-cell-derived factor 1 (SDF-1)/CXC chemokine receptor type 4 (CXCR4)/type 7 CXC chemokine receptor (CXCR7). To this end, EVs reprogram the innate osteotropism of melanoma cells by upregulating their CXCR7 expression [24]. These results suggest that melanoma-cell-derived EVs contribute to melanoma metastasis. In addition, adipocytes secrete EVs, which are oxidized by fatty acids and are absorbed by tumor cells, resulting in increased metastasis and invasion of melanoma [25]. EVs from melanoma cells with poor metastatic potential potently inhibit metastasis to the lung and trigger immune surveillance, resulting in the elicitation of a broad range of monocyte (PMO)-reliant innate immune responses. Furthermore, Plebanek et al. suggested that cancerous cells are cleared at the pre-metastatic niche [26].

2.3. Tumor Microenvironment

The interactions of cancer cells with their environment determines whether the primary tumor is contained, metastasizes, or establishes dormant micrometastases. EVs play essential roles in the interstitial transport and intercellular communication within the tumor microenvironment (TME). Metastatic tumor cells show increased ability to sort EV cargo (i.e., proteins and microRNAs) and to release EVs. EV cargo is then transferred to stromal cells, including those that are present in premetastatic niches. Furthermore, EVs promote tumorigenesis and invasion through a variety of mechanisms, resulting in premetastatic niche formation. The following table describes the roles of EVs in the TME [27] (**Table 1**).

Table 1. The mechanisms of tumor microenvironment regulations in cancers.

Method	Mechanism	Reference
pH	**Extracellular acidity may increase the ability of cancer cells to release EVs. The pH of the environment can be used to regulate the release of EVs, affecting the development of the tumor or the control of drug resistance.**	**[28]**
EMT pathway	**During EV-mediated epithelial–mesenchymal transition (EMT)-like processes, the mitogen-activated protein kinase (MAPK) signaling pathway is activated and promotes metastasis. It was demonstrated that melanoma-cell-derived EVs promote the EMT in the tumor microenvironment.**	**[29]**
Inflammatory	**EVs secreted by metastatic melanoma cells spontaneously metastasize to the lungs and brain and activate proinflammatory signals that induce cell inflammation to promote tumor metastasis.**	**[30]**
Metabolism	**miRNA inhibitors of melanoma-derived EVs regulate stromal cell metabolism, inhibit the activity of miR-155 and miR-210, and may contribute to the promotion of metastasis.**	**[31]**

Method	Mechanism	Reference
Immune system	The lipid, protein, DNA, mRNA, and miRNA components in EVs are transferred to recipient tumor cells, affecting many immune-related pathways, leading to the activation, differentiation, and expression of the immune cells and the regulation of the tumor microenvironment, thus affecting tumor development, metastasis, and drug resistance. EVs are regulated and released by the TME and regulate the cell biology of myeloid-derived suppressor cells (MDSCs), including promoting their activation and amplification and enhancing their immunosuppressive functions.	[32][33]

2.4. Immune System

The tumor microenvironment controls immune surveillance and anti-tumor immunity [34], mainly through intra- and extracellular signaling. Immunoediting is a complex process that includes intra- and extracellular signals. EVs play an important role in immune escape, both directly and indirectly. The direct modulation of either immune cells or their immature precursors is mostly driven by EV-mediated anti-apoptotic or pro-apoptotic signaling during the melanoma cell migration. The indirect roles of EVs include the expansion and differentiation of negative regulators of the immune system, such as myeloid-derived suppressor cells (MDSCs) and regulatory T lymphocytes (Tregs), thus promoting tumor cell escape from immune surveillance [35][36]. Several effects, i.e., mechanisms link EVs and the immune system (**Table 2**). Studies have shown that EVs secreted by tumor cells protect and maintain the growth of cancer cells, while EVs produced by normal cells, especially stem cells, inhibit tumor growth and suppress cancer progression [37]. Homing of melanoma exosomes to sentinel lymph nodes imposes synchronized molecular signals that effect melanoma cell recruitment, extracellular matrix deposition, and vascular proliferation in the lymph nodes [38]. In addition, tumor-derived EVs were shown to interfere with immunization by inducing loss of antigen expression, suppression of immune effector cells, exchange of nucleic acids, changes in recipient cell transcription, and inhibition of the immune cell response [39]. Other studies point out that tumor cells and tumor-infiltrating immune cells form a highly tolerant microenvironment, increasing tumor growth and allowing metastatic spread. Studies of anti-tumor immunity have explored the host's immune responses and promote the development of new therapies and novel methods for use in future therapeutic methods [40].

Table 2. The effect of tumor-derived EVs in immune systems.

Target	Mechanism	Reference
CD8(+) effector T cells	Melanoma-derived EVs induce immune suppression by promoting T regulatory cell expansion and destroying antitumor CD8(+) effector T cells, thus leading to tumor escape.	[41]
CD4+ T cells	Melanoma-derived EVs may directly activate the mitochondrial apoptotic pathway of CD4+ T cells through the microRNA in the EVs.	[42]
PTEN	Tumor-secreted miR-214 is sufficiently delivered to recipient T cells by EVs specifically targeting mouse peripheral CD4+ T cells. miR-214 downregulates phosphatase and tensin homolog (PTEN) and promotes Treg expansion. Tumor-derived EVs enhance immune suppression and tumor implantation/growth in mice.	[43]
MHC	The major histocompatibility complex (MHC) class I molecules and EVs have an important correlation with the induction of antigen-specific T cell responses and the stable development of tumors.	[44]
PD-L1	Increased tumor surface expression of programmed death-ligand 1 (PD-L1) facilitates tumor cell escape from immune surveillance. PD-L1 interacts with the programmed death-1 (PD-1) receptor on T cells to elicit the immune checkpoint response. Metastatic melanomas release EVs that carry PD-L1 on their surface, which suppresses the function of CD8(+) T cells and facilitates tumor growth.	[45]
PTPN11	Melanoma-derived EVs provide a complex biological load, and the upregulation of tumor tyrosine-protein phosphatase nonreceptor type 11 (PTPN11) expression by B16F0 EVs suppresses T lymphocyte function.	[46]
M1 and M2 macrophages	EVs derived from melanoma in premetastatic lymph nodes trigger angiogenesis in tumors by inducing classically activated (M1) and alternatively activate (M2) macrophage-mediated angiogenesis by inducing endothelial cell proliferation.	[47]
NKG2D	Melanoma-cell-derived EVs downregulate NKG2D expression in natural killer cells to induce immune suppression.	[48]

2.5. Drug Resistance and Clinical Treatment

EVs are involved in the development and regulation of different cancer-related processes. Drug resistance of cancer cells is a huge clinical problem and requires further investigation. Nevertheless, it is known that drug-resistant tumor cells are able to enclose chemotherapeutic agents in EVs and transfer anticancer drugs out of tumor cells. Therefore,

understanding the molecular mechanisms and signaling pathways of EV-mediated drug resistance will help in the design of novel cancer treatments.

A large number of studies indicate that EVs play a crucial role in the development of the drug resistance of cancer cells (**Table 3**). Previous research has indicated that the use of BRAF kinase inhibitors (vemurafenib and dabrafenib) to treat melanoma patients bearing the BRAF-activating mutation V600E results in tumor regression, followed by quick development of drug resistance. Receptor tyrosine kinases (RTKs) are upregulated and activate the PI3K-Akt signaling pathway. EVs from drug-resistant melanoma cells were enriched with the RTK PDGFRβ, and delivering EVs rich in PDGFRβ to metastatic melanoma cells with the BRAF inhibitor-sensitive phenotype activated the PI3K/AKT pathway and resulted in the development of drug resistance [49]. Moreover, a novel truncated form of anaplastic lymphoma kinase (ALK) named ALK^{RES} was found to be secreted in EVs. The transfer of ALK^{RES} to sensitive, ALK-negative melanoma cells caused activation of the MAPK signaling pathway and transferred the characteristics of drug resistance to the recipient cells [50].

Table 3. The effect of tumor-derived EVs on drug resistance.

Gene ID	Mechanisms	Reference
ALK	**ALK activates the MAPK signaling pathway to target cancer. Combined treatment with the inhibitor of ALK and BRAF can significantly reduce tumor growth and induce apoptosis in melanoma.**	**[50]**
PDGFRβ	**PDGFR*β* is a resistance driver transferred to recipient melanoma cells via EVs, resulting in the activation of phosphoinositide 3-kinases (PI3K)/protein kinase B (PKB) signaling and escape from the MAPK pathway in BRAF-inhibitor-sensitive cells, thus influencing drug sensitivity in the recipient melanoma cells.**	**[49]**

2.6. Small RNA (microRNA)

MicroRNAs (miRNAs) constitute a class of small single-stranded noncoding RNAs (~22 nt in length) that suppress gene expression. miRNAs are transcribed in the nucleus by RNA polymerase II or III. Primary miRNA transcripts (pri-miRNAs) are cleaved through a complex that consists of the endonuclease Drosha and the RNA-binding protein DGCR8. Hairpin pre-miRNAs are exported to the cytoplasm and are cleaved by the endonuclease Dicer to form dsRNA–miRNA duplexes. The complementary strand of the mature miRNA sequence is degraded, facilitating miRNA-induced silencing complex (RISC) formation and targeting the complementary sequences in the 3′ UTR of target mRNAs inhibit translation. miRNAs regulate many physiological and pathophysiological processes, such as growth, differentiation, and cancer progression. miRNAs regulate hundreds of genes; thus, miRNAs can cause complex phenotypic changes [51]. The loss of certain miRNAs facilitates cancer growth, whereas overexpression of other miRNAs promotes cancer progression [52]. miRNAs change the phenotype of melanoma cells and metabolic pathways during melanoma progression. They also affect the extracellular matrix (ECM), which includes fibroblasts, endothelial cells, and immune system cells [53]. miRNAs have different functions in each step of the development of different cancers [54]. Cells have the ability to selectively sort miRNAs into EVs for secretion to nearby or distant targets. Moreover, certain disease states have also identified dysregulated EV-miRNA content, shedding light on the potential role of selective sorting in pathogenesis. The latest findings regarding the roles of EVs-relevant miRNAs in melanoma pathobiology are summarized in **Table 4**.

Table 4. The mechanisms and target locations of microRNA in melanoma.

miRNA ID	EV Origin	Effect	Target Site	Reference
let-7g-5p	**Patient's plasma**	**Increases levels of let-7g-5p in EVs, which is associated with better disease control**	**MAPK**	**[55]**
miR-34a	**Patient's plasma**	**Prevents tumor relapse and blocks tumor cell proliferation**	***β*-catenin**	**[56]**
miR-211	**Melanosome**	**Targets IGF2R and leads to activation of MAPK signaling, which promotes melanoma growth**	**IGF2R**	**[57]**
miR-222	**Melanoma EVs**	**Increases tumor malignancy**	**PI3K/AKT**	**[58]**
miR-155, miR-210	**Melanoma EVs**	**Modulate stromal cell metabolism, which promotes the development of metastasis**	**OXPHOS**	**[31]**
miR-709, miR-2137	**Melanoma EVs**	**Modulate T cell function**	**PD-L1**	**[59]**

miRNA ID	EV Origin	Effect	Target Site	Reference
miR-494	Melanoma EVs	Suppresses tumor growth and metastasis when levels are increased	none	[60]
miR-146a, miR-155, miR-125b, miR-100, miR-125a, miR-146b, miR-99b	Melanoma EVs	Convert myeloid cells into myeloid-derived suppressor cells	CTLA-4, PD-1	[61]
miR-106b-5p	Melanoma EVs	Activates the ERK pathway	EphA4	[62]
miR-205	Melanoma	Regulates E2F-regulated AKT phosphorylation to inhibit the proliferative capacity of melanoma cells	E2F1, E2F5	[63]
miR-182	Melanoma	Suppresses the expression of MITF and FOXO3 and stimulates migration of melanoma cells	MITF and FOXO3	[64]
miR-21	Melanoma	Upon upregulation in melanocytes, increases the proliferation rate and decreases the apoptosis rate	PTEN	[65]
miRNA-342	Melanoma	Targets zinc-finger E-box-binding homeobox 1 (ZEB1) and decreases the proliferation and invasion rates of melanoma cells.	ZEB1	[66]

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