Extracellular Vesicles for Non-Melanoma Skin Cancer

Subjects: Dermatology

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Standard non-melanoma skin cancer (NMSC) treatment involves surgery, recently combined with chemotherapy or immunotherapy in cases of advanced tumors. EVs, including exosomes, are integral to carcinogenesis, and are found in NMSC releasing mediators impacting tumor progression. Nevertheless, the precise intercellular signaling role of NMSC-derived EVs remains unclear.

Keywords: non-melanoma skin cancer ; basal cell carcinoma ; cutaneous squamous cell carcinoma ; exosomes ; extracellular vesicles ; biomarker ; treatment ; diagnosis ; prognosis

1. Introduction

Non-melanoma skin cancers (NMSCs) represent approximately 30% of human cancers [1]. Numerous population-based studies have demonstrated that the incidence rates of the two main NMSC types, namely basal cell carcinoma (BCC) and cutaneous squamous cell carcinoma (cSCC), are rising [2]. The underlying pathogenesis of NMSC has not yet been fully elucidated, but a variety of factors, in addition to environmental exposure and UV radiation, are associated with an increased risk of developing NMSC [2]. Although the current available treatment options, including surgery and radiotherapy, are proven to be effective for the majority of NMSC cases, those at advanced stages and presenting metastatic tumors require systemic therapy, such as immunotherapy, epidermal growth factor receptor (EGFR) inhibitors, and platinum-based chemotherapy [1]. cSCC has a stronger malignant tendency to develop metastases or local recurrence [2], while BCC follows a less aggressive clinical progression; however, left untreated, both are related to significant morbidity [3]. Immunotherapy is currently considered the most effective option for unresectable NMSC [1]. The anti-PD1 inhibitors pembrolizumab and cemiplimab have been approved as the optimal treatment options for locally advanced and metastatic cSCC [1][4]. Cemiplimab has recently received FDA approval for advanced BCC, which was formerly treated with Sonic-Hedgehog inhibitors [1]. Even though immunotherapy appears promising, its potentially fatal adverse effects require careful selection of eligible patients [1]. In this context, other treatment options are researched.

Extracellular vesicles (EVs), such as exosomes, apoptotic bodies, microvesicles and oncosomes, have been reported as main determinants of the pathogenesis and progression of melanoma, and thus are currently explored as diagnostic and prognostic biomarkers [5]. Exosomes are small membranous vesicles (sEVs) with the ability to transfer their cargo among cells [6] and have a critical role in both physiological and pathological processes, such as carcinogenesis [3][7]. Exosomes are released by all cell types, including tumor cells, and widely exist in all body fluids such as blood, urine, saliva, cerebrospinal fluid, and amniotic fluid [6][8][9]. NMSC cell-derived exosomes generate and release mediators that modulate tumor growth and potential metastases [5]. However, the intercellular signaling role of EVs in NMSC is largely unknown [7].

2. EV Structure and Function

Exosomes are nano-sized EVs, which differ in morphology, biological properties, biogenesis, and functional roles from other larger types of EVs [4][6][8][10]. Both exosomes and other EVs carry and deliver information through biologically active molecules and mediators and regulate skin homeostasis or disease pathogenesis [8][11]. A phospholipid bilayer with protein markers on the surface, dependent on each cell function, forms their membrane [8][10]. These protein markers can be used to differentiate tumor-derived exosomes [6].

Depending on their intracellular origin, EVs can be categorized as exosomes or microvesicles [11]. Exosome biogenesis follows certain stages. After the infolding of the cytoplasmic membrane generates early endosomes, they integrate molecules, such as DNA, RNA, proteins, and lipids, into multi-vesicular bodies (MVBs). The fusion of MVBs with the cytoplasmic membrane leads to the release of exosomes into the extracellular space [10]. On the other hand, microvesicles are formed through direct budding from the plasma membrane [11]. The size of exosomes typically ranges between 40 and 120 nm, while microvesicles vary more in size, from 50 to 1000 nm [12]. Due to the size overlap and the lack of specific markers, distinguishing between the two types is difficult without observing the formation process.

However, determining the biogenesis pathway of EVs remains challenging, except when live imaging techniques are employed [13].

EVs, including exosomes, are characterized by carrying a load of biologically active molecules and mediators. These include lipids, proteins, amino acids, metabolites, and nucleic acids, notably microRNA (miRNA), non-coding RNAs (ncRNAs), long non-coding RNAs (lncRNA), and circular RNAs (circRNA) [8][11]. Through the release of these components, EVs enable intercellular communication between both cancer and normal cells, via direct contact or receptors [5][10]. As a result, specific genetic information is transferred from cancer to benign cells, contributing to cancer progression and metastasis [8].

3. EVs Role in Tumorigenesis

Tumor cells release a large quantity of EVs that are critically involved in cancer pathogenesis, progression, and metastasis [5][8][11]. Tumor-derived exosomes (TEXs or TDEs) mediate between tumor cells and the tumor microenvironment (TME) [14] and can induce tumor progression and even metastasis by supporting tumor initiation, angiogenesis, epithelial-to-mesenchymal transition (EMT), matrix remodeling, and immune modulation [9]. These processes are modulated largely by the cargo of miRNAs transported via exosomes. Specifically, the overexpression of miRNAs induces cancer development, whilst miRNA underexpression is related to an inability to suppress the expansionary tendency of tumors [11][15].

Overmiller et al. also demonstrated that SCC-derived EVs can alter the TME by inducing the proliferation of local fibroblasts [7]. TDEs are also implicated in both pro- and anti-tumor immune responses. Immune escape is induced by modulating the expression of IL-6, leading to deregulation of dendritic cell maturation. Dendritic cells are an essential immune system cell population. The anti-tumor defense mediated by natural killer cells (NK) is also activated by IL-6 expression modulation. NK cells, stimulated by TDEs and the pro-inflammatory cytokines that are secreted, induce tumor cell apoptosis [5]. On the other hand, TDEs can also decrease NK cells within the TME and thus induce immune suppression [5].

4. EVs as Therapeutic Targets in cSCC

In view of the role of EVs in tumorigenesis and metastasis, EVs and their cargo could be potential therapeutic targets. In fact, in head and neck SCC (HNSCC), Teng et al. hypothesized that targeting exosomes derived from irradiated HNSCC cells would downregulate the AKT pathway, and thus radiosensitivity could be enhanced. They also reported the ability of many exosome inhibitors such as GW4869 to disrupt the lipid composition, indomethacin, or Ras inhibitors [6].

4.1. Circ-CYP24A1

A potential target that has recently gained attention due to its specificity and stability both as a diagnostic biomarker and therapeutic target is circRNA, a non-coding RNA whose expression is enriched and stable in exosomes. It is postulated that there is an association between circRNAs and the development of multiple human tumors, such as gastric cancer. CircRNAs can induce the progression and peritoneal metastasis of gastric cancer by translocation to the target cells via exosome secretion [16]. Zhang et al. deployed RNA sequences to form expression profiles of exosomal circRNAs in cSCC. They compared the plasma exosomes derived from five cSCC patients with five healthy samples, showing an upregulation of 25 and a downregulation of 76 circRNAs in cSCC, among the 7577 differentially expressed circRNAs [3]. Upregulated circRNAs, such as circ-CYP24A1, were principally involved in the immune response, while downregulated circRNAs were modulators in metabolic pathways in cancer cells and RNA transportation. A correlation between the tumor's clinical characteristics and circ-CYP24A1 was also reported. The inhibition of exosomal circ-CYP24A1 was shown to possibly also affect SCC progression by suppressing the tumor's locally invasive and metastatic dynamic [3]. These data indicated that the exosomal circ-CYP24A1, among other exosomal circRNAs, might be considered a potential therapeutic target to restrain the development, migration, and invasion of cSCC [3].

4.2. Desmoglein 2 (Dsg2)

Another key target component seems to be desmoglein 2 (Dsg2), a desmosomal cadherin. Dsg2 is often overexpressed in cancers, including NMSC, and is associated with poor prognosis in melanoma as it promotes tumor angiogenesis [15]. Flemming et al. and Overmiller et al. reported that Dsg2 is implicated in the release of EVs from SCC keratinocytes enriched with cytokines, such as IL-8, and is impoverished in miR-146a [7][15]. The active role of Dsg2 in the biogenesis of EVs was confirmed by Overmiller et al. when the overexpression of Dsg2 both in non-cancerous and SCC cells was compounded with enhanced EV secretion. In A431 SCC cells the secretion of EVs was also decreased when Dsg2 was

targeted with shRNA [7]. Through the release of EVs, Dsg2 also promoted tumor proliferation in both cutaneous and head and neck SCCs. To explore the underlying mechanisms, the release of cytokines, which are known for their role in tumor growth, from the exosomes in response to increased levels of Dsg2 was investigated. It was reported that IL-8, which also induces tumor progression and immune response, was significantly increased. Considering the downregulation of miR-146a in those cells, the enhanced expression of the IL-8 gene was the outcome of the unsuccessful inhibition of the NFkB signaling pathway by miR-146a. As a result, the lower levels of miR-146a led to the increased expression of IL-8 [15]. Moreover, the correlation between immunotherapy response and IL-8 rate was explored. Following the measurement of IL-8 levels in patients with HNSCC under therapy with nivolumab, an anti-PD1 agent, treatment response rates were found to be higher in patients with significantly lower expression of IL-8 [15]. Even though more research is required to elucidate the implementation of those ascertainments in relation to cSCC, targeting Dsg2 or IL-8 could enhance the susceptibility of SCC cells to immune agents and ameliorate provided therapy.

Overmiller et al. demonstrated that SCC-derived EVs are enriched with Dsg2-C-terminal fragment (Dsg2-CTF), which occurs after the modification of the full length Dsg2 by metalloproteinase 17 (ADAM17). Since ADAM17 levels are increased in cSCC, they hypothesized that during malignant transformation, Dsg2 fractures into a ~95 KDa ectodomain and intracellular CTF, which has an essential role in EV secretion in SCC cells. In view of this fact, post-translational Dsg2 alteration seems promising as a research field for new treatment strategies [7][17]. Flemming et al. also suggested that besides Dsg2 proteolysis, the palmitoylation of Dsg2 is essential for the release of sEVs, which rendered palmitoylacyltransferases (PATs) as possible therapeutic targets [15].

4.3. p38 Inhibited Cutaneous Squamous Cell Carcinoma-Associated lincRNA (PICSAR)

Following the current guidelines for SCC patients to minimize the risk of metastasis or recurrence, surgery is accompanied by radiotherapy, and less often chemotherapy. However, the acquired drug resistance of tumor cells represents a significant impediment to appropriate therapy provision. Extracellular vesicles, secreted from tumor cells, facilitate cancer cell adaptation to microenvironmental conditions and chemoresistance through the transfer of ncRNAs [5][18]. Long noncoding RNAs (lncRNAs), namely noncoding RNAs with >200 nucleotides, participate in the regulation of multiple human cancers, while their deregulation is associated with chemoresistance [18]. Wang et al. investigated the role of PICSAR in the resistance of cSCC cells to cisplatin (DDP), a chemotherapeutic drug commonly used in cSCC treatment. Lnc-PICSAR was elevated in the exosomes derived from SCC patients' serum and SCC cells compared to non-cancerous cells. In addition, lnc-PICSAR levels were higher in DDP-resistant SCC cells than in DDP-sensitive cells [18]. The correlation between lnc-PICSAR and miR-485-5p and REV3L was also studied. Lnc-PICSAR is involved in the regulation of SCC chemoresistance by inhibiting miR-485-5p, which subsequently promotes the expression of REV3L. Based on these data, exosome-mediated lnc-PICSAR could present a potential prognostic biomarker to evaluate the treatment response, as well as a therapeutic target [18].

4.4. miRNA

Recent studies have investigated the role of miRNA in the metastatic potential of BCC, melanoma, breast, prostate, and lung cancer [19][20][21][22]. Chang et al. isolated exosomal miRNA from patients with metastatic BCC (MBCC) and non-metastatic BCC (non-MBCC) and reported that exosomes in patients with MBCC increased the proliferation and invasion ability of fibroblasts [23]. Among the isolated miRNAs, nine were significantly overexpressed in MBCC in comparison to non-MBCC. The role of mir-197 was further investigated, considering its role in non-BCC tumors. Even though mir-197 was found to be present at enhanced levels in patients with MBCC, its inhibition was not correlated to decreased fibroblast and keratinocyte proliferation [23].

5. EVs as Diagnostic Biomarkers

The stability and the easy collection of EVs from the circulation and body fluids through non- or minimally invasive methods are attractive features of exosomes, demonstrating their potential role as biomarkers of different diseases [6][8]. Although research on the therapeutic possibilities of EVs is at an early stage, their diagnostic role has been already explored by many recent studies that focus on the value and utility of EV-based liquid biopsy. It was reported that an EV-protein and RNAs are effective biomarkers for stage I and II pancreatic cancer screening, achieving excellent rates of both specificity and sensitivity when combined [24]. Similar research has also demonstrated the utility of an EV-RNA for the diagnosis of non-small lung cancer [17][25]. In HNSCC, liquid biopsy recognizes exosomal miRNAs and exosome-derived proteins [6]. In view of the growing demand for early screening and diagnosis, research on cSCC has also turned towards the identification of biomarkers.

Sun et al. studied the expression of Ct-SLCO1B3, an EV tumor marker gene, in patients with recessive dystrophic epidermolysis bullosa (RDEB). It was demonstrated that the gene was expressed only in RDEB-SCC-derived EVs, and thus could be considered a potential diagnostic biomarker, with the perspective that more studies will be conducted to ascertain whether those results apply to the general population [26]. Moreover, Zauner et al. demonstrated that there is a specific miRNA panel that can distinguish RDEB-cSCC from RDEB lesions and healthy skin samples, and thus can be used as a diagnostic biomarker. Based on those findings, they proposed a tumor detection model. However, due to the small available sample, they used miRNA-seq panels of HN-SCC as supplementary data, since the miRNA profiles of the two cancers displayed significant similarities. As a result, three tumor detection models were created which included 33, 10, and three miRNAs that were significantly deregulated in HN-SCC and RDEB-cSCC exosomes. Each model was tested on the HN-SCC training set and then its predictive ability was evaluated both on the HN-SCC set and RDEB-exosome data. They demonstrated that the less complex model that was based on three unique miRNAs could accurately predict tumors. However, clinical research is required to assess the applicability of this model [27].

As mentioned above, besides their role as a therapeutic target, circ-CYP24A1 and linc-PICSAR can serve as diagnostic biomarkers as well. Notably, circ-CYP24A1 is considered an excellent diagnostic biomarker, mostly because of its resistance to the catalytic effect of RNAse [3].

6. EVs' Role in Prognosis

The possibility of biomarkers used to strategically evaluate cancer treatment effects was also explored [8]. In HNSCC, Theodoraki et al. studied the role of circulating exosomes as biomarkers of completely cured or relapsed disease by isolating exosomes from different stages of the treatment timespan: before, during, and after therapy [28]. Regarding cSCC, linc-PICSAR was linked to enhanced chemoresistance, and Dsg2 was hypothesized to participate in causing a decreased immunotherapy response [15][18].

7. EVs as Drug Delivery Systems

EVs, especially exosomes, are also being researched as potential drug delivery agents [8]. Up to this time, drug delivery systems have included peptides, polymers, nanoparticles, liposomes, and vector viruses. However, several issues have emerged regarding the imminent immune reaction to foreign molecules and the questionable success of perfusion into the target cell population. On the other hand, EVs have certain characteristics that render them ideal candidates for drug delivery. In order to fulfill their role as cell-to-cell mediators, the phospholipid bilayer of EVs offers resistance to external degrading forces of the circulatory system, as to protect their molecular cargo, leading to longer circulating half-life [11][29]. Moreover, EVs can infiltrate the blood-brain barrier, thus expanding their target group. Last but not least, since EVs are autologous mediators, the immune response is not induced [11][12]. EVs derived from cancer cells can be used as an excellent drug delivery system not only for chemotherapeutic drugs but for miRNA-based gene therapy, since EVs naturally carry miRNA. Due to increasing interest, the usage of EVs as natural drug carriers has been investigated in many cancer types, among which are HNSCC, breast, colon, gastric, and brain cancers [11][30][31][32]. However, there is still little to no research on whether this therapy can be applied for cSCC patients.

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