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Konstantinos Seretis * , Elena Boptsi , Anastasia Boptsi

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Review

Exosomes as Novel Diagnostic and Therapeutic Agents for Non-Melanoma Skin Cancer: A Systematic Review

Konstantinos Seretis *,†, Eleni Boptsi † and Anastasia Boptsi

Department of Plastic Surgery, Medical School, University of Ioannina, 45100 Ioannina, Greece
* Correspondence: drseretis@uoi.gr
† K. Seretis and E. Boptsi share first authorship.

Abstract: Standard Non-Melanoma Skin Cancer (NMSC) treatment involves surgery, recently combined with chemotherapy or immunotherapy in case of advanced tumors. Exosomes, integral to carcinogenesis, are found in NMSC, releasing mediators impacting tumor progression. Nevertheless, the precise intercellular signaling role of NMSC-derived exosomes remains unclear. This review aims to elucidate their potential role in NMSC diagnosis and treatment. A systematic review encompassed literature searches in electronic databases from inception to September 2023, based on certain inclusion and exclusion criteria, addressing NMSC-derived exosomes, their molecular cargo and their implications in the diagnosis, prognosis, and treatment of NMSC. Key components were identified. Extracellular vesicle (EV) proteins and RNA emerged as diagnostic biomarkers in EV-based liquid biopsy. Circular RNA CYP24A1, known for its molecular stability, holds promise as a diagnostic biomarker. Long noncoding RNAs (lincRNA-PICSAR) and Desmoglein 2 (DSg2) are linked to drug resistance, serving as prognostic biomarkers. EV mediators are actively investigated for their potential role as drug delivery agents. In conclusion, this systematic review showed that NMSC-derived EVs display promise as therapeutic targets and diagnostic biomarkers. Further research is imperative to fully comprehend exosome mechanisms and explore their potential in cancer diagnosis and treatment.

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

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1. Introduction

Non-Melanoma Skin Cancer (NMSCs) represents approximately 30% of human cancers [1]. Numerous population-based studies demonstrate that the incidence of the two main NMSC types, namely the basal cell carcinoma (BCC) and cutaneous Squamous Cell Carcinoma (cSCC), is rising [2]. The underlying pathogenesis of NMSC has not yet been elucidated, but a variety of factors, in addition to environmental exposure and UV radiations, are associated with an increased risk to develop NMSC [2]. Although the current available treatment options, including surgery and radiotherapy, are proven to be effective for the majority of NMSC, advanced stages and 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 metastasis 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]. Anti-PD1 inhibitors, pembrolizumab and cemiplimab, have been approved as the optimal treatment 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

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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]. The 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 cells-derived exosomes generate and release mediators that modulate tumor-growth, and potential metastasis [5]. However, the intercellular signaling role of EVs in NMSC is largely unknown [7].

Aim of this systematic review is to explore and summarize the current evidence, regarding the possible role of exosomes in NMSC.

2. Results

A PRISMA flowchart of the included studies is presented in Figure 1. Through the applied search strategy 888 articles were retrieved, of which 245 duplicates were removed. The remaining articles were screened based on title and abstract, resulting in 16 articles being sought for retrieval. For one article there was only an abstract available, thus 15 full texts were eventually assessed for eligibility. Based on the inclusion criteria, 8 studies were included in this systematic review.

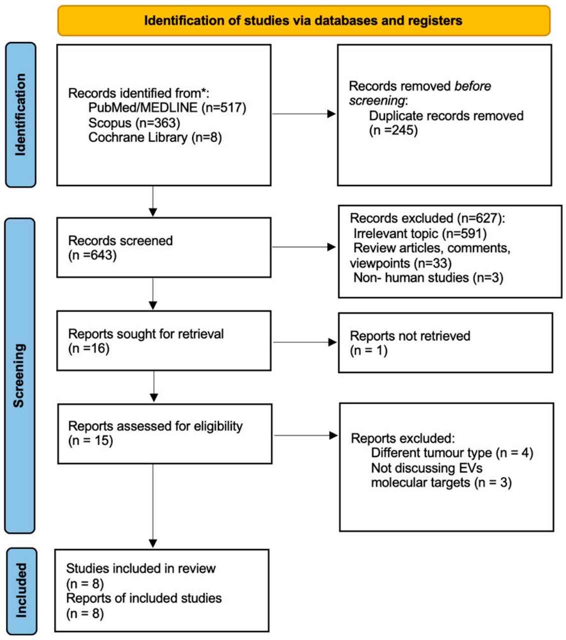

Figure 1. PRISMA flowchart for study selection.

2.1. General Study Characteristics

The 8 studies included were conducted in the USA (3), China (3), Austria (1). Additionally, a multicenter study involved centers in Austria, Japan, UK, Chile, USA. All studies were published after 2017. Study characteristics are summarized in Table 1. All studies were prospective either clinical (5) or experimental (3) studies. Among the eight studies, five analyzed patient samples, while three analyzed cell lines. Patient samples were sourced either from serum or tissues, including skin from the healthy controls. The role of exosomes as prognostic biomarkers, diagnostic biomarkers or therapeutic agents was examined in two, three, and six studies, respectively (Table 1).

Table 1. Study characteristics.

Author, Year	Target Molecule	Source	Study group	Control group	Incubation	Exp. method	Analysis method	Outcome	Function
Overmiller A. et al. (2017)	Dsg-2-CTF	Cell lines	-	-	A431/GFP, A431-Dsg2/GFP, HaCaT/GFP, HaCaT-Dsg2/GFP, primary NHK	In vitro	Western blot	Overexpression in SCC-derived EVs resulted in increased EVs secretion; inhibition of proteolysis of Dsg2 resulted in reduced EVs secretion	Therapeutic target
Chang et al. (2017)	miR197	Serum	9 MBCC patients	9 non MBCC patients	NHK, human skin fibroblasts	In vitro	PCR	SS upregulation in MBCC patients; no impact on proliferation noted in fibroblasts and keratinocytes	Therapeutic target, prognostic biomarker
Sun et al. (2017)	Ct-SLCO1B3	Tissue	RDEB-SCC patients	-	RDEB, RDEB-SCC, NHK	In vitro and in vivo	PCR	Expression of Ct-SLCO1B3 only in RDEB-SCC derived EVs	Diagnostic biomarker
Zhao Z. et al. (2020)	ALA-PDT exosomes	Cell lines	-	-	SCCs (human A431, mouse PECA, primary mice SCCs), fibroblasts 3T3, DCs	In vitro	Western blot	Stimulation of DCs maturation and fibroblasts' TGF-β1 secretion, leading to an anti-tumor immune response	Treatment
Wang et al. (2020)	lnc-PICSAR	Serum	30 cSCC patients	30 healthy patients	NHEK, A431, HSC-5 cells	In vitro and in vivo	PCR	Elevated in cSCC cells and DDP-resistant cSCC cells	Prognostic biomarker, therapeutic target
Flemming J. et al. (2020)	Dsg-2	Cell lines	-	-	A431/GFP, A431-Dsg2/GFP, A432-Dsg2cacs/GFP	In vitro and in vivo	Western blot	Inhibited palmitoylation of Dsg-2 corellates with reduced sEVs secretion and attenuated tumor development	Therapeutic target

Zhang Z. et al. (2021)	circ-CYP24A1	Serum	5 cSCC patients	5 healty patients	A431, SCL-1 cells	In vitro	PCR	Upregulated in cSCC EVs; inhibition leads to attenuation of the tumor's metastatic dynamic	Therapeutic target, diagnostic biomarker
Zauner R et al. (2023)	miRNA (expr. profile)	Tissue	6 RDEB-cSCC	4 healthy patients, 5 RDEB	-	In vitro	PCR	51 miRNAS found significantly up-regulated and 74 down-regulated in RDEB-cSCC compared to RDEB	Diagnostic biomarker

Expr.: Expression, Exp: Experimental, SS: Statistical significant, Dsg2: desmoglein 2, CTF: C terminal fragment, A431: epidermoid carcinoma cell, GFP: green fluorescence protein, HaCaT: normal keratinocytes, NHK: normal human keratinocytes, cSCC: cutaneous squamous cell carcinoma, EVs: extracellular vesicles, MBCC: metastatic basal cell carcinoma, RDEB: recessive dystrophic epidermolysis bullosa, ALA-PDT: 5-aminolevulinic acid photodynamic therapy, DCs: dendritic cells, NHEKs: Normal human epidermal keratinocytes, DDP: cisplatin, HSC-5: human skin squamous cell carcinoma, lnc-PICSAR: long noncoding RNA p38 inhibited cutaneous squamous cell carcinoma-associated lincRNA, Dsg2cacs: unpalmitoylated Dsg2, sEVs: small extracellular vesicles.

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2.2. Exosomes 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]. They carry and deliver information through biologically active molecules and mediators and regulate skin homeostasis or disease pathogenesis [8]. Furthermore, a 40-160 nm phospholipid bilayer with protein markers on the surface, dependent on each cell function, forms their membrane [8,10]. Those protein markers can be used to differentiate tumor-derived exosomes [6].

Exosomes biogenesis follows certain stages. After the infolding of the cytoplasmic membrane generates the 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 in the extracellular space [10]. Thus, exosomes are characterized by a load of biologically active molecules and mediators, including 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]. Through the release of those components, exosomes 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].

2.3. Exosome Role in Tumorigenesis

Tumor cells release a large quantity of exosomes, that are critically involved in cancer pathogenesis, progression, and metastasis [5,8]. The tumor-derived exosomes (TEXs or TDEs) mediate between the tumor cells and the tumor microenvironment (TME) [11], and can induce its progression and even metastasis by supporting tumor initiation, angiogenesis, epithelial-to-mesenchymal transition (EMT), matrix remodeling and immune modulation [9]. Those processes are modulated largely by the cargo of miRNAs transported via exosomes. Specifically, the overexpression of miRNAs induces cancer's development whilst its underexpression is related to an inability to suppress the tumor's expansionary tendency [12,13].

Overmiller et al. also demonstrated that SCC-derived EVs can alter the TME by inducing the proliferation of the local fibroblasts [7]. Also, TDEs are implicated with 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, an essential immune system's cell population, while anti-tumor defense mediated by natural killer cells (NK) is also activated. NK cells, stimulated by TDEs and the pro-inflammatory cytokines that are secreted, induce tumor cells apoptosis (5). On the other hand, TDEs can also decrease NK cells within the TME and thus induce immune suppression [5].

2.4. Exosomes as Therapeutic Targets in cSCC

In view of the exosome's role in tumorigenesis and metastasis, exosomes 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. Also, they reported many exosomes inhibitors such as GW4869, that disrupt the lipid composition, indomethacin, or Ras inhibitors [6].

2.4.1. 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 an association of circRNAs with 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 [14]. Zhang et al. deployed RNA-sequence to form expression profiles of exosomal circRNAs in cSCC. They compared the plasma exosomes, derived from five cSCC patients, with five healthy samples, showing among the 7,577 differentially expressed circRNAs, an upregulation of 25 and a downregulation of 76 circRNAs in cSCC [3]. Upregulated circRNAs, 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 could 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].

2.4.2. Desmoglein 2 (Dsg2)

Another key target component seems to be desmoglein 2 (Dsg2), a desmosomal cadherin, that is often overexpressed in cancers, including NMSC, and is associated with poor prognosis in melanoma by promoting tumor angiogenesis [13]. 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 impoverished in miR-146a [7,13]. The active role of Dsg2 in the EVs biogenesis was confirmed by Overmiller et al. when the overexpression of Dsg2 both in non-cancerous and SCC cells was compounded with enhanced EVs secretion. In A431 SCC cells the EVs secretion was even 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 mechanism, the release of cytokines, known for their role in tumor growth, from the exosomes in response to the 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 IL-8 gene was the outcome of the unsuccessful inhibition of NFkB signaling pathway by miR-146a. As a result, the lower levels of miR-146a led to the increased expression of IL-8 [13]. 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 higher in patients with significantly lower expression of IL-8 [13]. Even though more research is required to elucidate the implementation of those ascertainments also in the cSCC, targeting Dsg2 or IL-8 could enhance the susceptibility of SCC cells to immune agents and ameliorate the 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 EVs secretion in SCC cells. In view of this fact, post-translational Dsg2 alteration seems promising as a research field for new treatment strategies [7,15]. Flemming et al. also suggested that besides Dsg2 proteolysis, the palmitoylation of Dsg2 is essential for the release of EVs, which rendered palmitoylacyltransferases (PATs) as possible therapeutic targets [13].

2.4.3. 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, tumor cells' acquired drug resistance displays a significant impediment in the appropriate therapy provision. Extracellular vesicles, secreted from tumor cells, facilitate cancer cells' adaptation to microenvironments' conditions and chemoresistance through the transfer of ncRNAs [5,16]. 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 [16]. Wang et al. investigated the role of PICSAR in cisplatin (DDP) resistance of cSCC cells, a chemotherapeutic drug commonly used in cSCC treatment. Lnc-PICSAR 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 [16]. 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 the miR-485-5p, which subsequently promotes the expression of REV3L. Based on these data, exosome-mediated lnc-PICSAR could therefore present a potential prognostic biomarker to evaluate the treatment response, as well as a therapeutic target [16].

2.4.4. miRNA

Recent studies investigate the role of miRNA in the metastatic potential in BCC, melanoma, breast, prostate, and lung cancer [17–20]. 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 [21]. Among the isolated miRNAs nine were significantly overexpressed in MBCC in comparison to non-MBCC. The role of mir-197 was further investigated considering their role in non-BCC tumors. Even though mir-197 was found enhanced in patients with MBCC, its inhibition was not correlated to decreased fibroblasts and keratinocyte proliferation [21].

The stability and the easy collection of exosomes 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 the research on the therapeutic possibilities of exosomes is at an early stage, its diagnostic role has been already explored by many recent studies, that focus on the value and utility of the 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 [22]. Similar research has also demonstrated the utility of an EV-RNA for the diagnosis of non-small lung cancer [15,23]. 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 as well has 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 [24]. 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 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 their miRNA profiles displayed significant similarities. As a result, three tumor detection models were created which included 33, 10 and 3 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 3 unique miRNAs could accurately predict tumors. However, clinical research is required to assess the applicability of this model [25].

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].

The possibility of biomarkers used, strategically, to evaluate the cancer treatment effect 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 [26]. Regarding cSCC, linc-PICSAR was linked to enhanced chemoresistance and Dsg2 was hypothesized to participate in decreased immunotherapy response.

Exosomes are being also researched as potential drug delivery agents [8]. Up to this time, drug delivery systems included peptides, polymers, nanoparticles, liposomes or vector viruses. However, several issues emerged regarding the imminent immune reaction to the foreign molecules and the

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questionable successful perfusion in the target cell population. On the other hand, exosomes have certain characteristics that render them as ideal candidates for drug delivery. In order to fulfill their role as cell-to-cell mediators, exosomes' phospholipid bilayer offers resistance to external degrading forces of the circulatory system, as to protect their molecular cargo, leading to longer circulating half-life [12,27]. Moreover, exosomes can infiltrate the blood-brain barrier, thus expanding their target group. Last but not least, since exosomes are autologous mediators, immune response is not induced [12]. Exosomes 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 exosomes naturally carry miRNA. Due to the increasing interest, the usage of exosomes as natural drug carriers has been investigated in many cancer types, among which are HNSCC, breast, colon, gastric, and brain cancer [12,28–30]. However, there is still little to no research on whether this therapy can apply to cSCC patients.

2.5. Prospects for Exosomes in NMSC Anticancer Therapy

NMSC was treated with surgical excision combined with chemotherapy or more recently with immunotherapy. The current progress in the field of molecular biology regarding the role of exosomes and their molecular or protein cargo in cSCC has made the identification and use of exosomes as therapeutic targets appealing, and maybe feasible. Exosomes as a drug delivery system seem very promising. Their ability to escape the immune system's components and their natural targeted property has attracted scientific interest. More isolation methods and novel loading techniques have been developed to meet the increasing demand. Exosomes could be isolated from the patient's own cells and be appropriately modulated with specific markers to target specific cancer cells. In fact, clinical trials with plant- and human tissue-derived exosomes have been carried out in melanoma, lung, and colorectal cancer [6]. Overmiller et al. reported that co-cultivating fibroblasts with A431-Dsg2/GFP cells led to the modification of a higher percentage of fibroblasts into GFP+ ones compared to the cultivation with A431-GFP cells. These results indicate that the invasion of EVs into the adjacent cells is facilitated by Dsg2, and thus EV Dsg2 modification could enhance drug delivery to cSCC cells. Moreover, as mentioned, Dsg2 post-translation modification is a promising therapeutic target. miRNA alteration could be used to target the processed Dsg2, given its importance in gene expression and its reported anti-glioma and anti-hepatocellular carcinoma activity [15].

However, there are still unmet challenges. Currently, ultracentrifugation is the gold standard isolation method. However, the low yield and the risk of exosome degradation due to the strong forces applied require further evolution of the existing techniques [27]. By the time purified exosomes can be successfully isolated, the field of miRNA-based therapy can evolve as well. Besides the proper isolation of the exosomes, more hurdles are yet to be exceeded, such as the loading of the modulated miRNA without damaging the EVs membrane and thus leading to the alteration of its desired properties [12]. Moreover, due to their low bioavailability, the prospect of using synthetic mimics is also explored [6].

Zhao et al. reported a novel anti-tumor therapy for premalignant and malignant skin lesions [11]. Photodynamic therapy with 5-aminolevulinic acid (ALA-PDT) consists of a photosensitiser and light sources, which may lead to vascular endothelial cell injury, tumor cell apoptosis, and suppression of tumor metastasis or relapse [11]. The authors reported that exosomes' anti-tumor properties are mediated by the maturation of dendritic cells (DC), and fibroblast secretion of TGF-β1. Dendritic cells are known for their antigen-presenting property that induces immune response. Subsequently, they demonstrated that matured DCs increased the secretion of IL-12 that activates cytotoxic T lymphocytes (CTLs) and resulted in the inhibition of tumor growth. In this study, ALA-PDT exosomes could stimulate the maturation of dendritic cells (DC), which are identified in the TME. Therefore, they demonstrated that ALA-PDT exosome therapy can lead to an anti-tumor immune response [11].

3. Discussion

While non-melanoma skin cancers are often uniformly regarded as non-aggressive tumors, this is true only for small lesions, treated early and adequately. Both BCCs and cSCCs are characterized locally by variable destructive growth and invasion of the surrounding tissues, while regionally less often by lymph node metastasis, and distantly by low rate of metastasis in case of cSCCs [31–33]. Although the mortality rate associated with these tumors is relatively low, they pose a substantial health burden globally, due to their high prevalence. In addition, they are linked to significant morbidity, particularly when affecting cosmetically sensitive areas such as the face, often necessitating challenging reconstructive operations [34–37].

Failure to resect a NMSC completely predisposes to recurrence, stimulates the cancer cells' aggressiveness, and the metastatic potential of SCC [31–33]. Early detection and intervention are thus crucial to minimizing the potential consequences of NMSC, emphasizing the importance of effective prognostic and diagnostic biomarkers in mitigating the impact of NMSC. The identification of tumor-derived exosomes and their validation as biomarkers hold promise in refining risk stratification, guiding guidelines-based treatment decisions, and enhancing overall management strategies for individuals diagnosed with NMSC. Prognostic biomarkers can offer insights into the likelihood of cSCC progression and its potential for metastasis, aiding clinicians in tailoring surgical and oncological interventions to the specific needs of patients. Diagnostic biomarkers, on the other hand, play a crucial role in early detection, allowing for timely and targeted therapeutic management. Research efforts are focused on unraveling the molecular and genetic signatures associated with these tumors, aiming to identify molecular biomarkers that can be reliably assessed through non-invasive methods. Lee et al. reviewed in 2022 the literature on the genomics, transcriptomics, and proteomics of SCC-derived extracellular vesicles, encompassing pre-clinical and clinical studies [15]. Certain molecules showed to be involved in EV-mediated tumor invasion and drug resistance, thus serving as potential prognostic and therapeutic predictors.

This systematic review focused on the collection, analysis, and synthesis of the available evidence, highlighting the advances in our understanding of the intricate molecular pathways involved in NMSC, and the potential role of tumor-derived exosomes as therapeutic targets, especially in metastatic BCCs and advanced SCCs. Among the strengths of this review, is the rigorous methodology applied, limiting the risk of bias, and therefore enhancing the presented outcomes of interest. In addition, the utilization of a tool to grade the confidence of the reported results further improved the conducted study analysis.

Nevertheless, this review is still subject to limitations. Of note is the relatively small number of included studies, an inherent drawback of a cutting-edge research field. The different molecular pathways involved in BCC and SCC tumorigenesis also increase the heterogeneity, although the outcomes associated with these tumors were presented separately.

Overall, further research is anticipated to shed light in this evolving landscape of NMSC molecular mechanisms, enabling the integration of biomarkers into clinical practice, in order to improve the diagnostic accuracy, the predictive ability of each NMSC clinical course, as well as the availability and efficacy of targeted therapeutic options, even in case of advanced tumors.

4. Materials and Methods

4.1. Study Protocol

A systematic review was conducted using a predetermined protocol established according to the Cochrane Handbook's recommendations [38], registered at PROSPERO database (registration number: CRD42023492207). The review adhered to the updated PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines, presented in the Supplementary Materials (Table S1) [39].

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4.2. Search Strategy

An electronic literature search in MEDLINE (PubMed), Scopus, the Cochrane Library and CENTRAL electronic databases was conducted from inception to September 2023. No time and language restrictions were applied. The completed search strategy is included in the Supplementary Materials (Table S2). This search was supplemented by a review of reference lists of potentially eligible studies.

4.3. Eligibility of Relevant Studies

Studies met the following inclusion criteria: (1) prospective design; (2) evaluation of patients diagnosed with non-melanoma skin cancer or cell lines from such population; (3) reported data on components in extracellular vesicles with diagnostic, prognostic, or therapeutic value; and (4) publication in a peer-reviewed journal. We excluded studies reporting on mucosal SCC, on melanoma or when EVs molecular cargo was not researched. Review articles, duplicate reports, and non-human studies were excluded as well.

4.4. Study Selection

Two reviewers (K.S. and E.B.) independently screened retrieved database files and the full script of potentially eligible studies for relevance. Disagreement was resolved by consensus.

4.5. Data Collection and Risk of Bias Assessment

Data extraction was conducted independently by the 2 reviewers using a standardized form. Discrepancies were resolved by consensus. We extracted data, including the general study characteristics, sample sources, incubation, experimental and analysis methods applied, target molecules studies, and outcomes of interest. Primary outcome was the functional effect of exosomes on the target population of NMSC patients, which will enable their application as prognostic or diagnostic biomarkers or potential therapeutic targets.

The quality of studies was assessed using the SYRCLE's tool specifically designed for in vivo research studies [40] and a customized Cochrane risk of bias tool (Table S3) tailored to address the requirements of in vitro research [41]. The quality assessment of the included studies is available in the Supplementary Materials (Methods, Tables S4 and S5).

4.5. Data Synthesis and Analysis

We provided a narrative summary of the included studies based on the exosomes studied in the literature and the publication date. The outcomes presented were further categorized, according to the functional role of the exosomes, to prognostic, diagnostic or therapeutic targets.

5. Conclusions

The expanding knowledge of the functions and possibilities of exosomes is intriguing. As illustrated in this review, exosomes have an essential role in the pathogenesis and metastasis of cSCC by inducing microenvironmental changes and cellular interaction between cancerous and benign cells. An increasing number of studies focus on exosome-based therapy, reporting potential therapeutic targets including circ-CYP24A1, lnc-PICSAR, translated Dsg2 and miRNAs. However, more research is warranted to elucidate the clinical applicability of those findings.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Table S1. PRISMA 2020 checklist. Table S2. Keywords and MeSH terms used in different databases in the literature search., Table S3. Modified RoB-2 tool., Table S4. Quality assessment of in vivo studies., Table S5. Quality assessment of in vitro studies.

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visualization, K.S.; supervision, K.S. and E.G.L.; project administration, K.S.; funding acquisition, K.S. All authors have read and agreed to the published version of the manuscript.

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