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The biology and function of extracellular vesicles in nasopharyngeal carcinoma (Review)

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Abstract. Extracellular vesicles are a heterogeneous group of membrane-enclosed vesicles, which play an important role in intercellular communication. Increasing number of studies have shown that tumor-derived extracellular vesicles might be involved in the transfer of oncogenic cargo (proteins, lipids, messenger RNA, microRNA, non-coding RNAs and DNA) through which cancer cells could shape the tumor microenvironment and influence tumor progression. Nasopharyngeal carcinoma-derived extracellular vesicles have also reported to facilitate tumor proliferation, metastasis and immune escape. Moreover, nasopharyngeal carcinoma-derived extracellular vesicles might serve as biomarkers for early diagnosis and therapeutic targets. The present review provides information on the biological and clinical significance of extracellular vesicles in tumors, especially in nasopharyngeal carcinoma.

Contents

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1. Introduction

Intercellular communication is an essential hallmark of multicellular organisms. In recent years, the intercellular transfer of extracellular vesicles (EVs) have been discovered as a remarkable new system for cell-to-cell communication (1,2). EVs are a heterogeneous group of membrane-enclosed vesicles ranging from 30 to 1,000 nm in size released by a variety of cells including cancer cells into the extracellular milieu (3). Then, they can diffuse to neighboring cells or be carried to distant locations where they may induce signal transduction or mediate the horizontal transfer of molecular information in recipient cells (4). Subsequent studies have shown that EVs reflect the biological function of parental cells by containing a variety of cargos such as proteins (including transmembrane and enclosing cytosolic proteins), lipids, messenger RNA (mRNA), microRNA (miRNA), non-coding RNAs and DNA (5). They can be detected in many human body fluids, including plasma (6), cerebrospinal fluid (7), urine (8), bronchoalveolar lavage (9), malignant ascites (10), saliva (11), semen (12), nasal lavage fluid and ascites (13).

The composition and function of EVs depend on their originating cells. Tumor-derived EVs have been recently discovered to be involved in the transfer of oncogenic cargo through which cancer cells can shape the tumor microenvironment and influence tumor progression and metastasis (14-17). Moreover, EVs derived from stromal cells in the tumor microenvironment may contribute to tumor progression through the transmission of their cargo to tumor cells (18,19). This functional role of EVs in cancer development as well as their ability to be easily isolated from body fluids such as serum makes them an attractive candidate for biomarker development.

The present review will provide an overview of EVs in nasopharyngeal carcinoma (NPC), with a focus on their role in reprogramming tumor microenvironment and influencing tumor progression. The potential role of the molecules within EVs in NPC diagnosis and therapeutic targets will also be addressed.

2. Exosomes and microvesicles

Depending on the mode of release and the size, EVs are currently classified into two general types: exosomes and microvesicles

(MVs) (20). They are distinct in biogenesis, morphology, molecular composition, size and buoyant density (20,21). In the present review, we provide a brief description of exosomes and MVs prior to a discussion on their roles in NPC.

A population of EVs formed from the inward budding of intercellular endosomes result in multivesicular bodies (MVBs), which are known as exosomes. After inward budding, the MVBs fuse with the plasma membrane, releasing the exosomes into the extracellular space (22). Another release process involves the direct shedding by budding from the plasma membrane and this process forms MVs (23). In addition to the differences in the mode of release, the size of the vesicles is also used for characterization. Although different scales are used, MVs are from 100 to 1,000 nm and exosomes are smaller with a diameter of 30 to 100 nm (24). EVs are mostly isolated from body fluids and the supernatants of cultured cells by performing differential ultracentrifugation (24). Then, varied sized classes of EVs can be efficiently separated using their different floatation velocity (25). Currently, with the interest in their potential use in therapy or as biomarkers for disease, commercially available kits are being developed and marketed. Further characterization of isolated EVs requires immunoblotting, mass spectrometry and imaging techniques. In general, the specific EV marker proteins are typically used to show the purity and enrichment, besides, mass spectrometry approach is used to profile EV protein compositions. The morphology and size can be determined by electronic microscopy. The number and size distribution of EVs can be measured by nanoparticle tracking analysis, flow cytometry and tunable resistive pulse sensing (26). However, the distinctive features, properties and functional roles of each subtype are still under investigation.

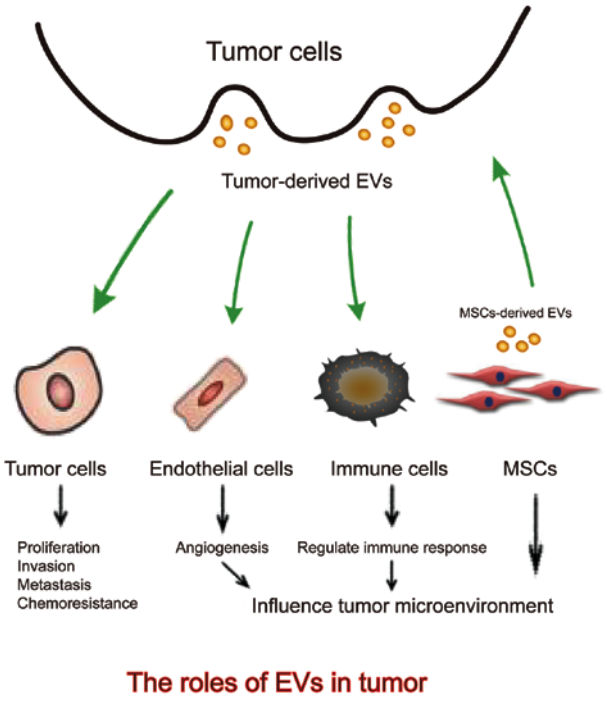

Figure 1. The roles of EVs in tumor. Tumor cell-derived EVs contain specific biologically active components and could transfer them to the recipient cell. They could be transferred to tumor cells, immune cells and endothelial cells to induce a variety of tumor malignant biological properties including tumor proliferation, invasion, metastasis, angiogenesis and regulate immune response and confer chemoresistance. MSCs in the tumor microenvironment could also secret EVs to influence tumor progression.

3. The roles of EVs in tumors

EVs contain specific biologically active components that could be transferred from the original cell to the recipient cell to trigger downstream signaling events. They could directly influence the recipient cells via cell surface interactions or by manipulating the local and distant biological environment (27,28). The above shows that EVs are implicated in a variety of tumor malignant biological properties including modulating tumor proliferation, invasion and metastasis, influencing angiogenesis, regulating immune response and conferring chemoresistance (29-32) (Fig. 1). Better understanding the duality roles of EVs in intercellular communication will help to gain further knowledge on the carcinogenic process.

EVs facilitate tumor metastasis. Metastases refer to the concept that primary tumors infiltrate through the basement membrane and disseminate to the distant organs (33). Studies have reported that the ability for a tumor cell moving to metastatic colonization is not random (34). Tumor cells travelling through the microcirculation is multistage and complex, and more and more scientists have found that tumor microenvironment plays an essential role in this progression. Metastasis occurs when the tumor microenvironment is well suited and tumor cells degrade connective tissue, extracellular matrix and basement membrane components (35,36). As this progression is via circulation, EVs also perform important roles in nearly all the steps. EVs might transfer the signals to other tumor cells and make EMT occur, which might help them to invade the distant tissues easily. Then, EVs might be uptaken by other cells to make the microenvironment more suitable for metastatic cells arrest. Besides, EVs might also regulate inflammation response pathways to help cell metastasis (15-19).

EVs facilitate tumor angiogenesis. Angiogenesis is the new blood vessel formation from pre-existing vasculature. It is a complex and multistep process consisting of proliferation, migration, invasion, adhesion and differentiation of endothelial cells (37). Angiogenesis occurs under various physiological and pathological conditions (38) and pathological angiogenesis could enhance tumor growth and metastasis by providing oxygen and nutrients (39). Intriguingly, previous studies have shown that genetic information can be transferred to human umbilical vein endothelial cells (HUVECs) to induce pathological angiogenesis (40). As EVs contain rich genetic information and can mediate exchange of molecules (41), studies have been performed to know the relationship between EVs and pathological angiogenesis.

EVs might be present in the fluids of the organism and several solid tissues (42). In recent years, there are many reports discussing the relationship between tumor-derived EVs with angiogenesis. According to the study of Kosaka, the EVs from tumor cells contain various pro-angiogenic molecules, such as bFGF, VEGF and TGF-β (43). Futhermore, Kosaka *et al* (44) reported that EVs derived from metastatic tumor cells promoted angiogenesis through enhancing the tube formation and migration of endothelial cells *in vitro*. In addition, our previous study also detected that EVs from NPC cells

are enriched of HAX-1, and could accelerate the proliferation and migration of HUVECs (45), collectively suggesting that EVs might be involved in the communication between tumor cells and HUVECs and play an important role in modulating angiogenesis.

EVs regulate tumor immunology. Immunoescape has been considered as one of the most common occurrences during tumor progression. Tumor cells might employ different methods to evade immune surveillance, accelerating their proliferation, migration and other biological malignant behavior. Increasing evidence shows that the dysregulation of immune system plays an essential role in tumor progression. As EVs can mediate cell-to-cell communication, they are also known to influence the immune system. EVs might contain receptors, proteins, RNA and DNA, which can be uptaken by immune cells and impact the immune microenvironment. This process could evoke immune responses, either make cells escape the immune response or activate immune suppression. Thus, investigating how tumor-derived EVs influence immune system might help us find the mechanism of tumorigenesis and develop new strategies for tumor therapy. Clayton *et al* (46) reported that EVs secreted by tumor cells might contribute to the production of extracellular adenosine and modulate T cells in the tumor environment. Wieckowsk *et al* (47) reported that tumor-derived EVs might be able to induce immune suppression by promoting T regulatory cell expansion and induce the apoptosis of CD8(+) T cells via activating the Fas/Fas ligand pathway. Tumor-derived EVs might also influence macrophages. EVs secreted by prostate cancer contained high level of MFG-E8 (milk fat globule-EGF factor 8 protein), when the macrophages were cocultured with the tumor cells, MFG-E8 expression was elevated and possibly polarized into M2 type tumor-associated macrophages. This type of cells could accelerate tumor progression (48). Besides, EVs might have impact on the complement system. Whitehead *et al* (49) showed that malignant cell-derived EVs could increase complement activation via calcium-sensitive pathways. Taken together, tumor derived EVs have important roles in the immune system.

EVs regulate drug sensitivity. By playing a role in facilitating cell to cell communication, EVs may mediate resistance to cytotoxic insults. In lung cancer, Xiao *et al* (50) found that EVs could regulate the sensitivity of tumor cells to cisplatin (DDP). They treated lung cancer cells A549 with DDP, and more EVs secreted in the supernatant were observed. Besides, in these EVs, the expression levels of some miRNAs and mRNAs associated with DDP sensitivity were dysregulated, transferring DDP resistance to those untreated cells. In breast cancer, drug-resistant breast cancer cells also released EVs to confer adriamycin resistance to sensitive ones by delivering specific miRNAs (51-53). In prostate cancer, Corcoran *et al* (54) treated the cells with EVs derived from sera of patients undergoing docetaxel treatment, and after the treatment, these cells also showed response to docetaxel. These results showed that EVs play an important role in drug resistance and might act as new therapeutic targets.

EVs as biomarkers in tumor diagnostics. An invasive biopsy of tumor is the golden standard for most tumors, which might be difficult for the early detection in some patients. As reported above, the physiological state of EVs depends on their originating cells and EVs could be detected in human body fluids, such as plasma, cerebrospinal fluid, saliva and urine. This makes them easily isolated and act as new disease biomarkers (24,55,56). Tumor derived EVs contain specific proteins, mRNAs, miRNAs, which could reflect the states of tumor cells, more and more scientists are devoted to utilizing EVs for early diagnosis and assessment of therapeutic responses or prognosis of tumors (57). Studies show that specific proteins in EVs could be used for the early detection of tumors. Guan *et al* (58) reported that the expression level of MDA-9 and GRP78 were higher in EVs derived from metastatic melanoma patients than those without metastases. Thus, MDA-9 and GRP78 in EVs might be useful biomarkers for assessing the prognosis of melanoma. In plasma samples from ovarian cancer patients, EVs exhibited high expression of claudin-4, which might be used as biomarker for ovarian cancer detection (59). Besides proteins, miRNAs in EVs have also described as promising candidates as tumor biomarkers (60). Cazzoli *et al* (61) reported that miR-151a-5p, miR-30a-3p, miR-200b-5p, miR-629, miR-100 and miR-154-3p in EVs might be used to discriminate lung adenocarcinoma and granuloma. Madhavan *et al* (62) showed that miR-1246, miR-4644, miR-3976 and miR-4306 were upregulated in 83% of pancreatic cancer derived EVs, but rarely in control groups and might act as highly sensitive biomarkers. Similar results were also found in prostate cancer, glioblastoma, and colon cancer (63-65). Not only EVs in serum could predict the diagnosis of tumor, EVs in other body fluid might also act as tumor biomarkers. Liu and colleagues (66) reported that miR-21 and miR-146a were upregulated in EVs derived from the cervicovaginal lavage specimens of cervical cancer patients. Therefore, EVs derived from body fluids might take messages of the original cells and provide a new biopsy technique for tumor diagnosis.

EVs as therapeutic targets in tumor treatments. Recently, scientists are devoted to use EVs as the therapeutic approach for tumor treatment. Hiltbrunner *et al* (67) reported that peptide-loaded EVs might be cancer treatment vehicles. They obtained ovalbumin-loaded dendritic cell-derived EVs from $MHCI^{-/-}$ mice, these EVs induce antigen-specific T cells response as wild-type EVs, EVs lacking MHC class I could add tumor infiltrating T cells and increase patients' overall survival. These results confirmed the prospective of using impersonalized EVs for tumors 68). More and more evidence focused on the application of EVs in tumor immunotherapy. Zhang *et al* (69) reported that EVs derived from interleukin (IL)-12 expressing renal cancer cells might express renal cell carcinoma-associated antigen G250 and GPI-IL-12, which could promote the proliferation of T cells and increase the immunogenicity and antitumor effects. This research is a novel way of EV-based vaccine for tumor treatments. Besides, EVs could be isolated from the stromal cells culturing media including MSCs, which might exert similar functions to those of MSCs. MSC-derived EVs could provide anticancer therapy via EV-mediated delivery of anticancer drugs (70). Lou *et al* (71) transfected adipose tissue-derived MSCs (AMSC) with miR-122, which made the effectively package of

miR-122 into secreted EVs. After the communication between AMSCs and HCC cells, the proliferation of HCC cells was inhibited and the tumor cells were sensitive to the chemotherapeutic agents. This research represented a promising strategy for HCC chemotherapy. Shimbo *et al* (72) also reported that microRNA-143 containing MSCs could inhibit the migration of osteosarcoma cells.

4. EVs in NPC

NPC has a variety of incidence rates throughout the world, it is a squamous epithelial cancer arising from the nasopharynx with an incidence of 30-80/100,000 each year in China (73). The high mortality rate of this disease arises from the lack of effective early diagnosis, more importantly, most NPCs are poorly differentiated and have high tendency to metastasize and invade adjacent regions, more than one third of the patients will develop distant metastasis within 4 years (74). The prognosis may be very poor as soon as NPC patients have metastatic disease (74,75). Thus, the identification of the mechanisms associated with NPC early diagnosis, metastasis and prognosis is of great significance. Various research has shown that EVs, which play important roles in tumor progression might also be present in NPC patient's serum and be recognized and taken up by other cells in the microenvironment. The intercellular communication between tumor cells and surrounding cells could facilitate tumor proliferation, metastasis and immune escape. Therefore, the functions of EVs in NPC progression and NPC-derived EVs might serve as biomarkers for early diagnosis and therapeutic targets.

EVs and NPC microenvironment. The notion of tumor-associated microenvironment refers to tumor-promoting and tumor-suppressing cells, soluble molecules and extracellular matrix components (76). It is obvious that tumor cells and stromal cells are in mutual dependence and in a sense, tumor microenvironment has become 'the end of the cancer cell' (77). In the tumor microenvironment, tumors release a variety of factors, which not only support tumor proliferation but also facilitate tumor cells to metastasize to distant organs (78). The factors include single cells, EVs and cytokines and they can influence distant tissues to have negative or positive feedback on themselves (79,80). They might impact distant cell signaling and maintain a better environment for tumor progression.

Many studies have tried to understand the cellular interactions within the tumor microenvironment (81). As important components of tumor stromal cells, mesenchymal stem cells (MSCs) have received much attention in recent years (82). Studies have shown that MSCs could home to primary or metastatic tumor sites and contribute to the formation of the tumor microenvironment (83,84). As paracrine effectors of MSCs, EVs have also been reported to play an important role during the interaction between tumor microenvironment and tumor cells, they could carry membrane and cytoplasmic components and mediate interactions with target cells (85). Previous studies have reported that EVs derived from MSCs might promote renal cancer cell growth and EVs from multiple myeloma (MM) patient bone marrow-derived MSCs promoted MM tumor growth (18,86). A recent study reported that EVs also mediate the interaction between MSCs and NPC cells. The data showed that NPC cells could take up MSC-derived EVs and these EVs could promote tumor proliferation, migration and the process of epithelial-mesenchymal transition (EMT). Moreover, the study showed that FGF19 was highly expressed in MSC-EVs. Besides, MSC-EVs could stimulate NPC progression by activating the FGF19-FGFR4-dependent ERK signaling cascade and by modulating the EMT. These data indicated that EVs have an important role in NPC microenvironment and participate in influencing NPC progression (19).

Hypoxia-mediated release of EVs in the NPC environment. Recent clinical and preclinical findings in NPC suggest that tumor hypoxia which occurs in >80% of NPC tumors playing a key role in NPC progression and resistance to therapy (87-89). Hypoxia, or oxygen deprivation, is one of the most common phenomena in human solid tumors. The lack of oxygen in the inner core of solid tumors, primarily due to increasing distance of tumor cells from blood vessels and the formation of aberrant blood vessels resulting in poor blood flow, is believed to contribute to tumor progression, as well as resistance to chemotherapy and radiotherapy (90,91).

Cancer cells can adapt to a hypoxic microenvironment via multiple cellular mechanisms (92). EVs are among the most significant tumor promoting factors stimulated by hypoxia that influence adjacent tumor microenvironments (93). Hypoxia can remarkably stimulate EVs secretion; for instance, nucleic acids and proteins as transmission signals in EVs in the tumor microenvironment are involved in various functions, such as inducing intratumoral heterogeneity, altering immunological responses, producing cancer-associated fibroblasts and promoting angiogenesis and metastasis (92). Park *et al* (94)found that hypoxia (1% O_2) is insufficient to induce apoptosis; nevertheless, hypoxia can stimulate the release of EVs in human lung cancer cell line A549 and aid angiogenesis by chemo-tactically attracting endothelial cells and fibroblasts and by stimulating stromal cells to release angiogenesis-promoting cytokines. As in skin cancers, hypoxic A431 carcinoma cells released EVs enhancing angiogenesis in a chorioallantoic membrane assay and metastasis. Aga *et al* (95) demonstrated that endogenous hypoxia-inducible factor 1 alpha (HIF1-α) is detectable in EVs and that latent membrane protein 1 (LMP1) could increase the level of HIF1-α in EVs. The present study found that in NPC, hypoxia stimulated MMP-13 expression in EVs in a HIF-1α dependent manner. Moreover, MMP-13 in EVs significantly upregulated vimentin expression, while decreasing E-cadherin level in NPC cells, *in vitro* and *in vivo* (96).

The immunoregulatory properties of EVs in NPC. Heavy lymphoid infiltration in the primary tumor sites is an important biologic feature of NPC (97). EVs, which act as intercellular vehicles are also reported as important mediators in NPC progression and immune escape. Ye *et al* (98) isolated EVs from the serum of NPC patients and found the concentration of EVs was positively correlated with lymph node stage and poor prognosis of NPC patients. To further confirm the high level of EVs in patients with lymph node metastasis was associated with T-cell immune response, the scientists treated T cells with EVs derived from the supernatant of NPC cells TW03. The results showed that TW03-derived EVs could inhibit the proliferation of T-cell and the differentiation of

Th1 and Th17 and induce regulatory T cells (Treg cells) by altering p-ERK and p-STAT. Besides, NPC derived EVs also have anti-inflammatory effects and increase the expression of proinflammatory cytokines. These findings suggested that EVs might be potential targets for NPC immunotherapy. Some scientists also focused on Treg cells within the tumor site and investigated the mechanisms of Treg recruitment and the interaction between NPC-EVs and Treg cells. The results showed that CCL20 was highly expressed in NPC-EVs, which might play an important role in the recruitment of human Treg into tumor sites. Besides, NPC-EVs could induce the conversion of $CD4^+CD25$-T cells into $CD4^+CD25^+$ Treg, then promote their regulatory phenotype and increase the suppressive function of Treg. The results confirmed that NPC-EVs in the tumor microenvironment could interact with Treg to exert immunoregulatory properties. In a word, NPC-EVs might be a newly defined way to regulate the immune system of NPC (99).

5. Contents of NPC-derived-EVs

EVs are reported to contain a variety of mRNAs, miRNAs and proteins, which play an essential role in tumor malignant behavior. NPC derived EVs also interact with other cells and are involved in NPC proliferation, invasion and metastasis. Thus, the studies highlight the important roles of EVs as they might contribute to NPC progression. Abundant research has been carried out to characterize the content of EVs.

HAX-1 in NPC-derived-EVs. HS1-associated protein X-1 (HAX-1) was identied more than 10 years ago as a novel protein with ubiquitous tissue expression (100). It has been shown that HAX-1 interacts with the 3'-untranslated regions (3'UTR) of a variety of proteins and binds to the 3'-untranslated regions of certain mRNAs involvement in multiple signaling pathways and cellular processes (101-106). HAX-1 is reported to be associated with biological processes such as cell apoptosis, cell motility and endocytosis, so it also plays an important role in regulating tumor cell apoptosis, proliferation and invasion. HAX-1 expression is a predictor of tumorigenesis, growth, progression, invasion, and metastasis of many human malignancies (107,108), and is overexpressed in many tumors (107,109,110) such as esophageal squamous cell carcinoma (111,112), colorectal cancer (113), oral squamous cell carcinoma, lung cancer, lymphoma, melanoma (114), leukemia, myeloma, breast cancer and hepatoma (115). In our previous study, we also found that HAX-1 was highly expressed in NPC tissues compared with normal tissues. Besides, its expression level was correlated with lymph node metastasis and clinical stage of NPC patients, it could also predict poor prognosis (45). As reported, tumor-derived EVs play an important role in tumor progression and metastasis by acting as intercellular communicators (116,117). The roles of NPC-derived EVs in NPC tumor growth, migration and angiogenesis were also confirmed in our previous study (45). Moreover, we found surprisingly that HAX-1 was selectively packaged in NPC-derived EVs. It is highly expressed in EVs derived from NPC patients compared with healthy donors (45). To confirm the role of EVs regulated by HAX-1, we stimulated HUVECs with EVs which contain different levels of HAX-1 protein. As expected, HAX-1-containing EVs could accelerate HUVECs proliferation, migration and angiogenesis. Moreover, the intracellular downstream pathways were also activated during the interaction between recipient HUVECs with HAX-1-containing EVs. So the expression level of HAX-1 in NPC patients derived EVs might be a biomarker for NPC diagnosis and act as a therapy target (45).

MMP13 in NPC-derived-EVs. MMPs are members of the metzincin superfamily which comprises zinc- and calcium-dependent enzymes comprising more than 24 subtypes (118). It is widely accepted that MMPs mediate degradation and modify most components of the extracellular matrix (ECM) and the basal membrane (BM), which is critical for cancer invasion and metastasis (119-121). As one of the most important MMP genes, the MMP-13 gene, also known as collagenase-3, is located in chromosome 11q22, spanning approximately 12.5 kb and consists of ten exons and nine introns (122). MMP-13 has the ability to disrupt collagen types I, II, III, IV, VI and X (123). Previous studies have supported that MMP-13 is found to have high expression in various types of tumors, including those from different parts of an individual's body, such as the breast, stomach, head, neck, larynx and colorectum (124). In this sense, upregulation of MMP-13 expression has been associated with increases in invasion and metastasis, and MMP-13 may have a potential influence on risks of cancer development and progression (125,126). Previous evidence showed that MMP-13 acts as a potential intermediate between low expression of microRNA-125b and increasing metastatic potential of non-small cell lung cancer (118). Fan *et al* (127) found that leptin signaling enhances cell invasion and promotes the metastasis of human pancreatic cancer via increasing MMP-13 production. Sedighi *et al* (123) found that MMP-13 level was an accurate diagnostic marker especially to differentiate pre-invasive/invasive lesions from normal controls (sensitivity and specificity: 100%). These findings indicate a potential clinical significance of serum MMP-13 measurement for early detection and prognostic assessment in ESCC patients. In the present study, we first demonstrated that MMP-13 was overexpressed in NPC cells and EVs purified from conditioned medium (CM) as well as NPC patient plasma. Furthermore, MMP-13-containing EVs facilitated the metastasis of NPC cells as well as angiogenesis which provided novel insight into the vital role of MMP-13-containing EVs in NPC progression which might offer unique insights for potential therapeutic strategies for NPC progressions. Then, we further investigated that NPC cells exposed to hypoxia release EVs containing higher level of MMP-13 in HIF-1α dependency that enhances metastases by inducing EMT *in vitro* and *in vivo*. We further found overexpression of HIF-1α and MMP-13 might be involved in the carcinogenesis and development of NPC and they were associated with poor patient prognosis. Thus, MMP-13 overexpression was triggered by hypoxia/HIF-1α as an important mechanism that induced EMT and tumor invasion in NPC (96).

6. Conclusion

Current studies suggest that EVs are important regulators of cell-cell communication. The growing knowledge on their roles in urologic malignancies provides the basis for novel

therapeutic strategies. In addition, nucleic acid and the protein content of EVs hold promise for tumor therapy. For NPC, more and more studies have showed the importance of EVs in tumor proliferation, metastasis, angiogenesis, immune regulation and so on. Besides, EVs could act as potential biomarkers in the early diagnosis of NPC and be used in NPC treatments. However, there are still many questions to be answered including its deeper mechanisms. So further fundamental researches and pre-clinical trials need to be carried out to help better understanding of the role of EVs in NPC. We hope the future studies will give evidence for the large-scale clinical utilization of EVs.

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