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Matrix metalloproteinase 13-containing exosomes promote nasopharyngeal carcinoma metastasis

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Key words

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Nasopharyngeal cancer (NPC) is an endemic type of head and neck cancer with a high rate of cervical lymph node metastasis. Metastasis is the major cause of death in NPC patients. Increasing evidence indicates that exosomes play a pivotal role in promoting cancer metastasis by enhancing angiogenesis and ECM degradation. Matrix metalloproteinase 13 is an important kind of matrix proteinase that is often overexpressed in various tumors and increases the risk of metastasis. However, little is known about the potential role of MMP13-containing exosomes in NPC. In this study, we found that MMP13 was overexpressed in NPC cells and exosomes purified from conditioned medium (CM) as well as NPC patients' plasma. Transwell analysis revealed that MMP13-containing exosomes facilitated the metastasis of NPC cells. Furthermore, siRNA inhibited the effect of MMP13-containing exosomes on tumor cells metastasis as well as angiogenesis. The current findings provided novel insight into the vital role of MMP13-containing exosomes in NPC progression which might offer unique insights for potential therapeutic strategies for NPC progressions.

Nasopharyngeal cancer (NPC) is an endemic type of head and neck cancer. Most patients are diagnosed at an advanced stage and 70–90% of patients present with cervical lymph node metastasis.[1] Nasopharyngeal cancer patients with metastasis have a higher rate of treatment failure and mortality.[2] Therefore, it is crucial to explore the mechanisms of tumor metastasis and discover valuable factors for early diagnosis and novel therapeutic strategies for metastasis.

As noted, exosomes play an important role in promoting progression of NPC by increasing its invasive potential.[1] Exosomes are nano-sized (50–100 nm in diameter) membrane-bound vesicles released by a variety of cell types,[2] especially abundantly by tumor cells.[3] Exosomes can also be found in human body fluids including blood, urine, and breast milk.[4–6] Exosomes can remove proteins from one cell to another and act as potential carriers of protumorigenic factors in intercellular communication.[7,8]

Tumor exosomes originating from mammary, lung, colon, prostate, and ovarian cancers have been proved to promote the establishment and metastatic spread of the primary tumor.[9–13] Exosomes appear to be derived to form the premetastatic niche, and facilitate the growth and invasiveness of tumor cells.[14]

It has been documented that tumor exosome-containing protein, mRNA, and miRNA may participate in tumor progression.[1] A growing number of MMPs have been detected in exosomes, which can modulate the tumor microenvironment by degradation of the ECM and basal membranes as an essential process for tumor metastasis.[3]

Matrix metalloproteinases are a group of zinc-dependent proteolytic enzymes. They stimulate tumor angiogenesis and establish metastatic foci at secondary sites.[15,16] Numerous reports have documented the overexpression of MMPs in several kinds of advancing tumors that are associated with poor prognosis.[17,18] Matrix metalloproteinase 13 is an important kind of MMP that is often overexpressed in various tumors[19,20] and has been documented to increase the risk of metastasis of breast carcinomas, squamous cell carcinomas of the head and neck, and melanomas.[18,20,21] Matrix metalloproteinase 13 has the capacity to degrade several ECM components,[22] promote angiogenesis in the chorioallantoic membrane,[18] as well as act as a stromal mediator in the maintenance of the angiogenic response of developing skin carcinomas.[18]

However, the expression of MMP13 in NPC and tumor-derived exosomes, as well as the importance of their interactions

with the stromal microenvironment during metastasis, was not clear. Our study aimed to investigate the effect of MMP13-containing exosomes on NPC progression. We found MMP13 overexpressed in tumor-derived exosomes. Moreover, in exosomal cell–cell transmission of MMP13, exosomes can also facilitate cross-talk between tumor cells and stromal cells, which support the metastatic cascade and promote angiogenesis.

Materials and Methods

Cell lines and clinical specimens. Nasopharyngeal cancer cell lines CNE1, CNE2, 5-8F, 6-10B and immortalized normal nasopharynx epithelial NP69 cells were received as a kind gift from the Sun Yat-Sen University (Guangzhou, China) and the Xiang-Ya School of Medicine, Central South University (Changsha, China). The NPC cell lines were cultured in RPMI-1640 medium supplemented with 10% FBS (Gibco BRL, Grand Island, NY, USA) that had been ultrafiltered through a membrane of 0.22 μm pore size (Millipore, Billerica, Massachusetts) to exclude bovine exosomes. NP69 cells were cultured in defined keratinocyte serum-free medium (Invitrogen, Carlsbad, CA, USA). Human skin fibroblast (HSF) cells were cultured in DMEM (Invitrogen); HUVECs were cultured in DMEM low glucose (HyClone, Logan, UT, USA). All cell lines were incubated at 37°C containing 5% CO_2.

Blood samples were obtained from newly diagnosed NPC patients and healthy donor at the Affiliated Hospital of Nantong University (Nantong, China). Samples were taken in the morning when patients were in the fasting state.

This study was approved by the Institute Research Ethics Committee of Nantong University. The experiments were undertaken with the understanding and written consent of each patient.

Exosome isolation and purification. Exosomes were isolated from CM and plasma as previously described.[3,23] The isolation method included a penultimate centrifugation step to remove small cell debris and then ultracentrifugation at 100 000 *g* for 1 h to generate an exosome pellet (Type 90 Ti rotor; Beckman Coulter, Fullerton, CA). The pellets were then washed once with PBS.

Electron microscopy. Purified exosome pellets were fixed with 2.5% glutaraldehyde and then centrifuged at 100 000 *g* to remove the glutaraldehyde. The pellets were then negatively stained by 3% aqueous phosphotungstic acid and fixed on copper mesh Formvar grids. Samples were observed using the JEOL Transmission Electron Microscope (JEM-1230; JEOL, Tokyo, Japan).

Western blot assay. Equal amounts of proteins were separated on SDS-PAGE gel and transferred to PVDF membranes and blocked with 5% non-fat milk. The membranes were incubated with primary antibody against MMP13, β-actin, CD63 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), CD9 (Abcam, MA, USA), Vimentin, E-cadherin, N-cadherin (Cell Signaling Technology, Danvers, MA, USA). The membranes were then incubated with secondary antibody (Santa Cruz Biotechnology). Protein bands were visualized using ECL reagents.

***In vitro* metastasis assays.** For the invasion assay, the Transwell system (24 wells, 8-μm pore size; Millipore) and Matrigel were used according to the manufacturer's protocols. Aliquots of 3×10^5 cells were seeded into the upper chambers precoated with Matrigel. After incubation for the indicated times, the cells at the bottom of the insert were fixed, stained, and counted in five random 100× fields per well under a microscope (Olympus, Tokyo, Japan).

For the migration assay, 5×10^4 cells were seeded into the upper chambers without a Matrigel coating. Other processes were followed as for invasion assays.

Quantitative real-time PCR. Total RNA was extracted using TRIzol reagent (Invitrogen) from NPC cells and NP69 cells. The quantitative PCR was carried out according to the instructions for Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, USA). The amplification program included an initial denaturation step at 95°C for 10 min followed by 40 cycles at 95°C for 15 s, one cycle at 60°C for 10 s, and elongation at 72°C for 10 s. The sequences of the MMP13 primers were: forward, GCAGTCTTTCTTCGGCTTAG; and reverse, AGGGTCCTTGGAGTGGTC; GAPDH served as control. The experiment was carried out in triplicate. Relative quantification of mRNA levels was carried out by the comparative *Ct* method using *GAPDH* as the reference gene and the formula $2^{-\Delta\Delta Ct}$.

Immunocytochemical analysis of MMP13. Cells cultured on glass coverslips were fixed with 4% paraformaldehyde. Cells were incubated with antibody overnight, washed with PBS, and incubated with FITC-labeled secondary antibodies (EarthOx, USA, 1:1000) and the nuclei (blue) were labeled with Hoechst (Invitrogen Life Technologies;1:1000). The cover slips were then observed under an Olympus camera.

***In vitro* tube formation assay.** The HUVECs were seeded at 1.5×10^4 cells/well of 96-well plates precoated with Matrigel and cocultured with exosomes for indicated times. Images of the capillary-like tube networks were obtained using an inverted microscope.

siRNA transfection. The sequence of the most effective MMP13-targeted siRNA (sense, 5′-GGAGAUAUGAUGAUACUAAdTdT-3′; antisense, 5′-UUAGUAUCAUCAUAUCUCCdTdT-3′) was chosen from four individual siRNAs. We used a scrambled-sequence siRNA duplex as a negative siRNA control. All siRNAs were designed and synthesized by Biomics Biotechnologies (Nantong, Jiangsu Province, China).

Transmission electron microscopy. According to the manufacturer's instructions, PKH67 (Sigma-Aldrich Co., St. Louis, MO, USA)-labeled exosomes were added to HUVECs and CNE2 cells. The cells were fixed and then processed as introduced immunocytochemical analysis. Images were collected with a TCS SP-5 confocal microscope (Leica Microsystems, Wetzlar, Germany), captured under 400 Hz with an image resolution of 512 × 512 pixels, and then analyzed by Leica Application Suite 2.02.

Cell viability assay. Cells were seeded into 96-well plates and assessed by CCK8 assay (Beyotime Institute of Biotechnology, Haimen, People's Republic of China). The absorbance of each well was read on a microplate reader (F-2500 Fluorescence Spectrophotometer; Hitachi) at 450 nm.

Statistical analysis. Each experiment was carried out at least three times; data were presented as mean ± SEM, where differences were evaluated using Student's *t*-test. A value of $P < 0.05$ was considered statistically significant.

Results

High level of MMP13 in exosomes enhanced metastasis. Based on several reports indicating that exosomes were present in different biological fluids from both tumor patients and healthy subjects,[24] we purified exosomes from the plasma of NPC patients (NPC exo) and healthy donors (normal exo). Electron microscopy confirmed the presence of exosomes in size

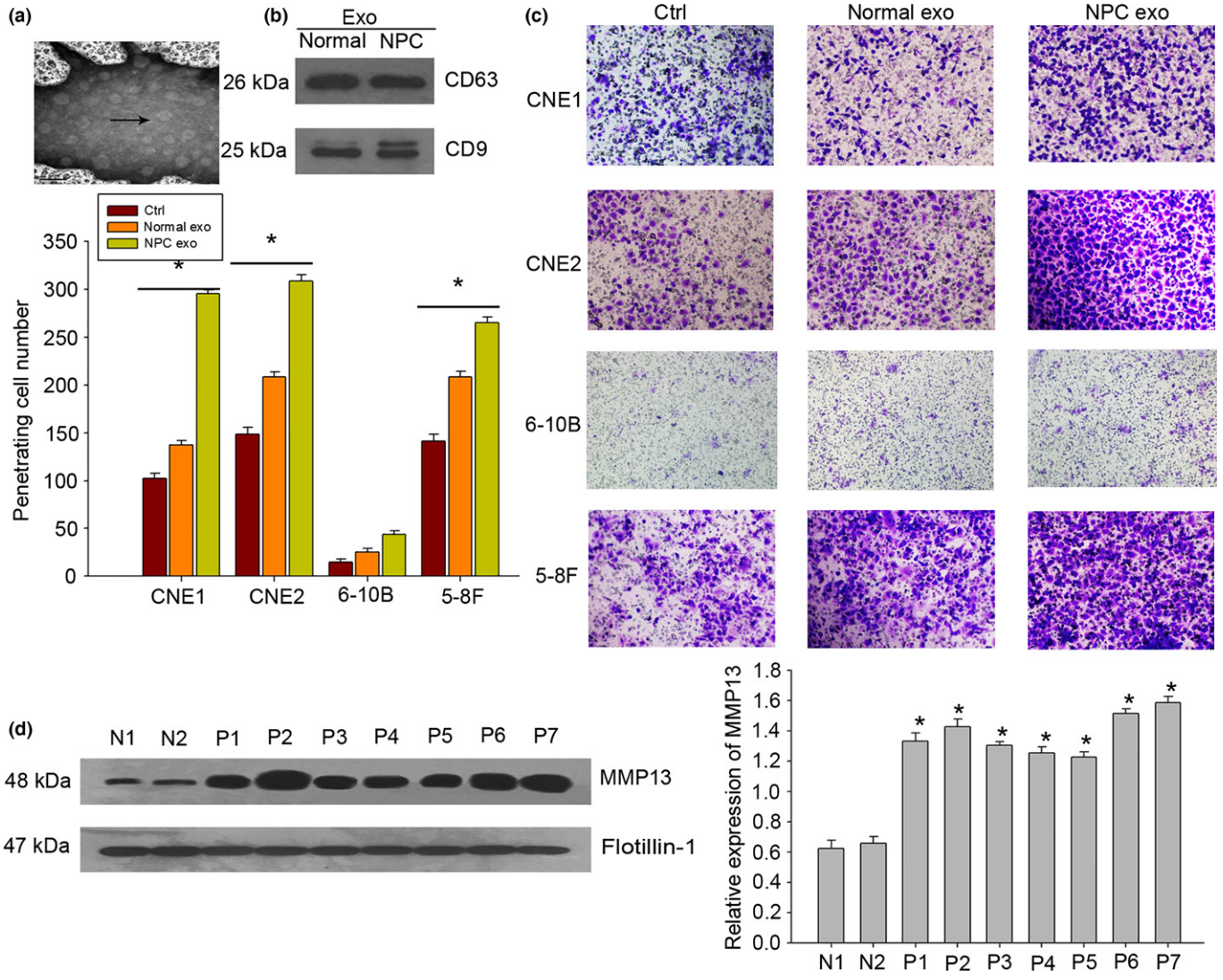

Fig. 1. Matrix metalloproteinase 13 (MMP13) is highly expressed in nasopharyngeal cancer (NPC) patient-derived exosomes and stimulates migration of NPC cells. (a) Representative electron microscopy images of exosomes purified from plasma (scale bar = 200 nm). (b) Western blot analysis of exosomal markers CD63 and CD9. (c) Exosomes from the plasma of NPC patients (NPC exo) facilitated CNE1, CNE2, and 5-8F cell migration. Images of cells adhered to the lower surface of the filter insert from a representative experiment are shown. The numbers of migratory cells were quantified using Image J software. Cells in five randomly selected areas were counted and statistical analyzed. (d) MMP13 protein level in exosomes purified from plasma samples from NPC patients and healthy donors. All experiments were carried out in triplicate and presented as mean ± standard. $*P < 0.05$.

(Fig. 1a) and Western blot analysis showed the known exosomal markers tetraspanins CD63 and CD9 were highly enriched (Fig. 1b). Interestingly, we found NPC exo increased migration of CNE1, CNE2, and 5-8F cells compared to normal exo, but not 6-10B (low tumorigenesis and metastasis) cells (Fig. 1c).

As the production of MMPs has been shown to be critical in the process of tumor metastasis, we then investigated whether these exosomes purified from NPC patients were enriched in MMP13. We found that in all cases, MMP13 was enriched in NPC exo compared to healthy donors (Fig. 1d).

Taken together, these findings suggested that exosomes from NPC patients' blood enhanced NPC metastasis and MMP13 might play an important role in the exosome-mediated transformation.

Circulating exosomes interacted with stromal cells to mediate tumor microenvironment. Emerging evidence suggests that most tumor cells have the ability to produce various proteases involved in the remodeling of the ECM, either by themselves or through tumor–stromal cell interactions. Our findings on the exosome-associated molecules motivated further studies on the functional role of exosomes in cross-talk between malignant cells and cells of the tumor stroma.

After preconditioning with NPC exo but not normal control, we found a time-dependent upregulation of MMP13 in HSF (Fig. 2a). To mimic the tumor microenvironment, we used the CM of HSF cocultured with exosomes to find whether MMP13-bearing exosomes can be potent phenotypic modifiers of their microenvironment. The number of invasive cells in Transwell invasion assays was higher in CM of NPC exo-treated HSF cells than control (Fig. 2b).

The results above suggested exosomes from NPC patients had unique inherent properties that allowed tumor cells to invade through interaction with stromal cells such as HSF cells.

We obtained similar results in HUVECs, that MMP13-bearing exosomes increased MMP13 levels in HUVECs (Fig. 2c) and promoted their proliferation (Fig. 2d). As shown in

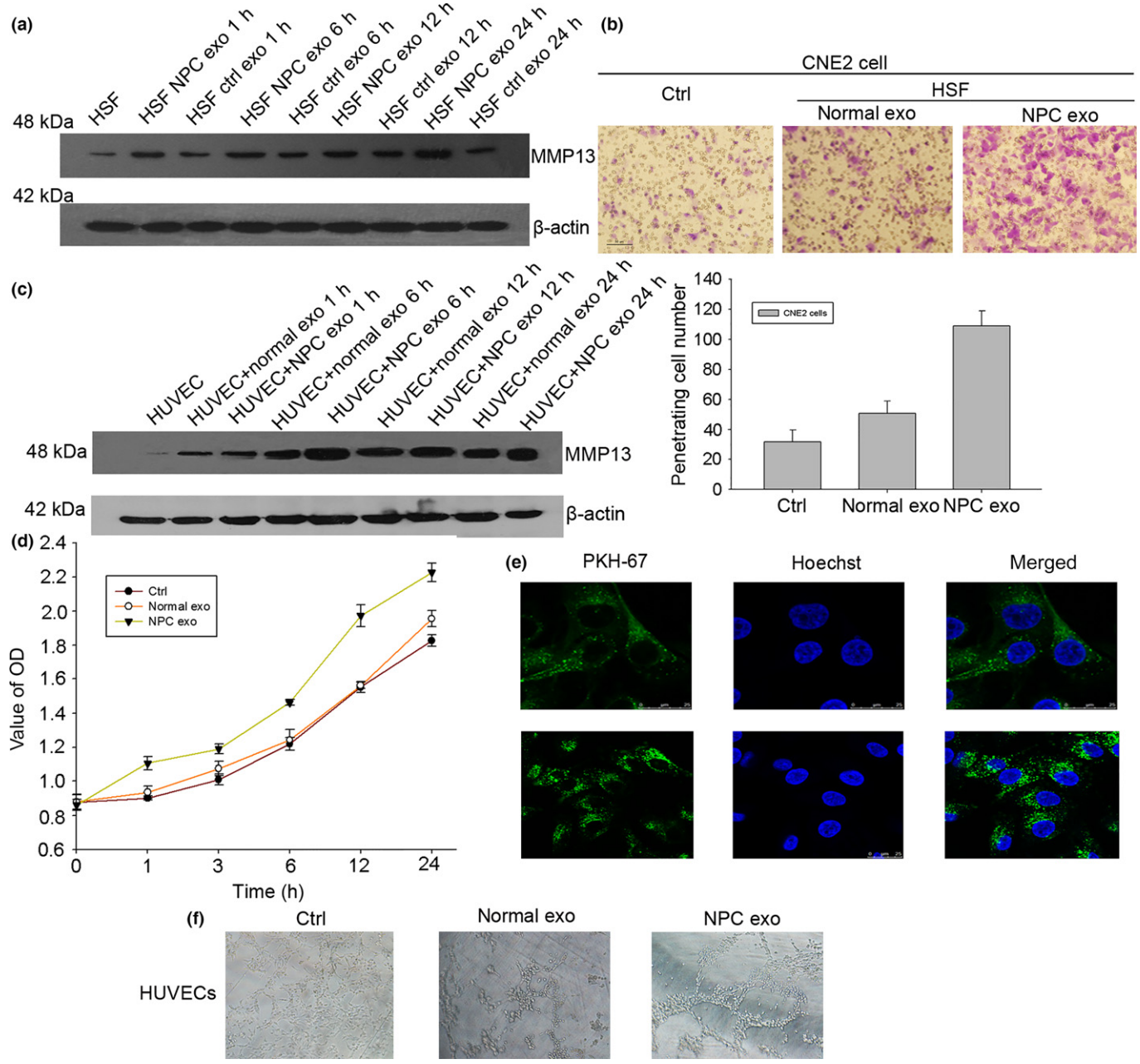

Fig. 2. Circulating exosomes from nasopharyngeal cancer (NPC) patients interact with stromal cells to mediate tumor microenvironment. (a,c) Human skin fibroblast (HSF) cells (a) and HUVECs (c) cocultured with exosomes from the plasma of NPC patients (NPC exo) induce MMP13 protein upregulated with time-dependency. (b) Conditioned medium from HSF cells cultured with NPC exo enhances invasion of CNE2 cells. Data represented as mean number of invasive cells per HPF. Compared with the parental control. (d) Effects of NPC exo on HUVEC proliferation. (e) Confocal microscopy analysis of PKH67-labeled (green) NPC exo uptake by HUVECs after coculture for 3 h. Top images show high magnification; bottom images show low magnification. (f) HUVECs cultured for 24 h in the absence (Ctrl) or presence of normal and NPC exo and then grown on Matrigel. Shown are representative photomicrographs of tubes from the different treatment groups. All experiments were carried out in triplicate and presented as mean ± standard. $*P < 0.05$.

Figure 2(e), incubation of fluorescent NPC exo (green) with HUVECs resulted in a time-dependent transfer of fluorescence to HUVECs. Exosomes from the plasma of NPC patients were significantly more potent in stimulating tube-forming capacity of HUVECs (Fig. 2f).

Taken together, we found MMP13-containing exosomes facilitate NPC progression and the cross-talk between tumor and stromal cell in the microenvironment.

Nasopharyngeal cancer cells released exosomes to increase tumor cell metastatic properties that contained high levels of MMP13. To determine the role of MMP13 in cancer cells and to confirm the effect of MMP13 in NPC progression, we investigated the MMP13 level in NPC cells. As shown in Figure 3(a), NPC cells, especially CNE2 cells, contained high levels of MMP13 compared to NP69 cells. Quantitative RT-PCR (Fig. 3b) and immunofluorescence (Fig. 3c) experiments confirmed the results.

Previous studies showed the molecular composition of exosomes mirrors the specialized functions of the original parental cells, therefore, we next examined whether exosomes

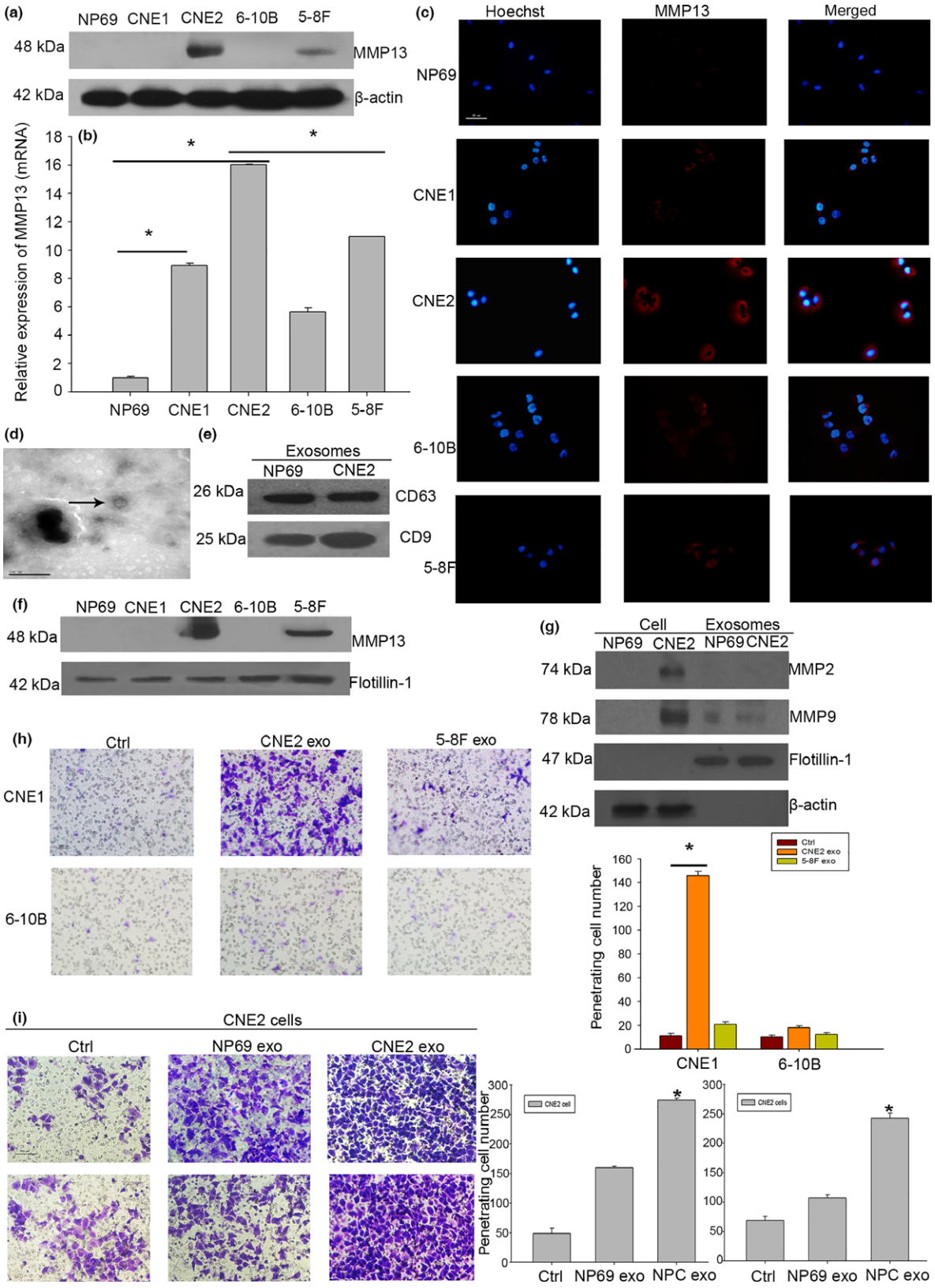

Fig. 3. Exosomes purified from CNE2 (CNE2 exo) increase tumor cell metastatic properties that contained high levels of MMP13. (a) Western blot, (b) quantitative RT-PCR, and (c) immunostaining analysis of MMP13 levels in NP69 cells and nasopharyngeal cancer cells. (d) Electron microscopic and (e) Western blot analysis confirmed the presence of exosomes purified from conditioned medium (CM) of cells. (f) MMP13 levels in exosomes purified from CM. (g) Transwell analyses indicated that CNE2 exo facilitated higher CNE1 cell migration compared to 5-8F exosomes. (h) CNE2 exo significantly facilitated CNE2 cell migration and invasion. (i) Analysis of the expression of MMP2 and MMP9 in CNE2 cells and exosomes by Western blot. All experiments were carried out in triplicate and shown as mean ± standard. $*P < 0.05$.

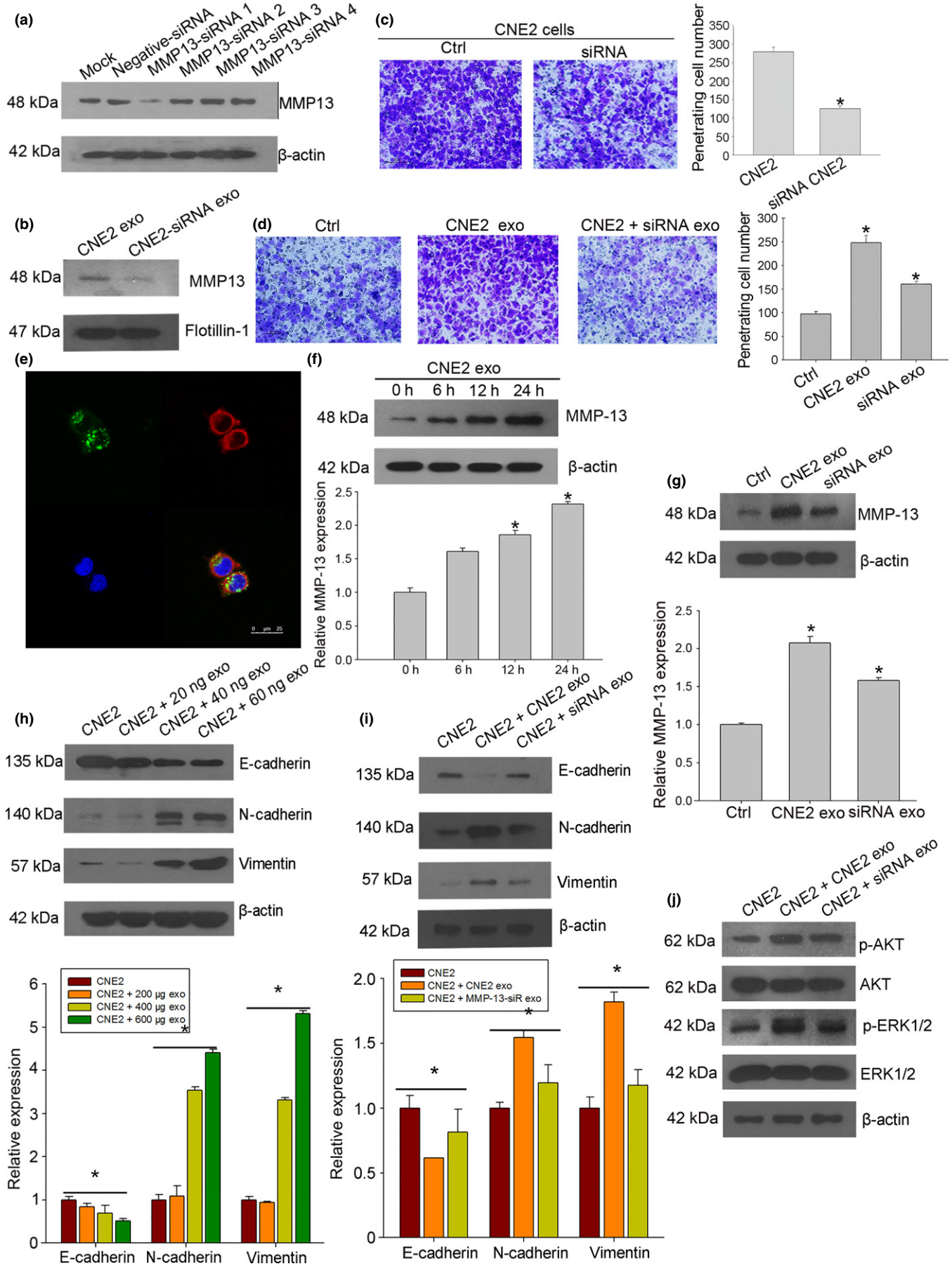

Fig. 4. Matrix metalloproteinase 13 (MMP13) mediates CNE2 cell metastatic properties through epithelial–mesenchymal transition (EMT). Cells transfected with MMP13 siRNA and knockdown efficiency detected by Western blot. Following treatment with MMP13 siRNA1, the level of MMP13 in both CNE2 (a) and exosomes purified from CNE2 (CNE2 exo) (b) were downregulated. (c) MMP13 knockdown inhibited the migration of CNE2 cells. (d) Exosomes from MMP13 siRNA-treated CNE2 cells suppressed the invasiveness of CNE2 cells. (e) Confocal microscopy analysis of PKH67-labeled (green) CNE2 exo uptake by E-cadherin-labeled (red) CNE2 cells after coculture for 3 h. (f) CNE2 cells cocultured with CNE2 exo for 6–24 h induced MMP13 protein upregulated with time-dependency. (g) Western blot analysis showed MMP13 siRNA exo downregulated the MMP13 level in cocultured CNE2 cells. (H,I) Expression of EMT markers was determined by Western blot in CNE2 cells cocultured with CNE2 exo and MMP13 siRNA exo. (j) Western blot analysis of pAKT and pERK1/2 levels in CNE2 cells cocultured with exosomes. All experiments were carried out in triplicate and presented as mean ± standard. $*P < 0.05$.

purified from CM contain MMP13. Electron microscopy and immunoblot analysis confirmed the presence of exosomes purified from both cell lines (Fig. 3d,e). Significant increase in MMP13 was detected in CNE2 and 5-8F exosomes (Fig. 3f). We further investigated whether MMP13 overexpressing exosomes could motivate further metastatic potentiality of recipient cells. We found exosomes purified from CNE2 (CNE2 exo) facilitated more CNE1 cells (well-differentiated NPC cells) migrated compared to 5-8F cells. However, neither CNE2 exo nor 5-8F exosomes had an effect on 6-10B cells (Fig. 3g). The data above suggested exosomes purified from the well-characterized CNE2 cells might participate in NPC progression. As shown in Figure 3(h), CNE2 exo could also significantly facilitate CNE2 cell migration and invasion. We further explored whether the effects of CNE2 exo were mediated by MMP13 or other MMPs. As showed in Figure 3(i), MMP2 and MMP9 were overexpressed in CNE2 cells compared to normal NP69 cells. However, there was no meaningful evidence in exosomes. The results might indicate that exosomes carried selected proteins such as MMP13.

These results suggested an inherent difference in the basal levels of exosome-associated MMP13, which might play a vital role in increasing NPC metastatic properties.

Matrix metalloproteinase 13 mediated metastatic properties of CNE2 cells through epithelial–mesenchymal transition. Our findings that MMP13 is sorted into exosomes motivated further evaluation of the functional impact of CNE2 exo on progression of NPC and to independently verify whether it's effect on the metastatic properties of CNE2 cells could be attributed to the high level of MMP13. Compared to three other kinds of siRNA, MMP13 siRNA1 resulted in a significant reduction of MMP13 protein expression in CNE2 cells (Fig. 4a), as well as in exosomes purified from CM of MMP13 siRNA1-transfected CNE2 cells (Fig. 4b).

As shown in Figure 4(c), the depletion of MMP13 slightly reduced the migration of CNE2 cells. Similarly, exosomes from MMP13 siRNA-treated CNE2 cells (siRNA exo) suppressed the invasiveness of CNE2 cells (Fig. 4d), suggesting that MMP13 in exosomes was required for metastatic properties.

To further explore the effect of MMP13-containing exosomes, we cocultured CNE2 exo with CNE2 cells. As showed in Figure 4(e), after coculture, large numbers of PKH-67-labeled exosomes could be found in CNE2 cells, and the MMP13 levels were upregulated gradually after 6 h with a time tendency (Fig. 4f). However, the MMP13-targeted siRNA revised the effect above (Fig. 4g).

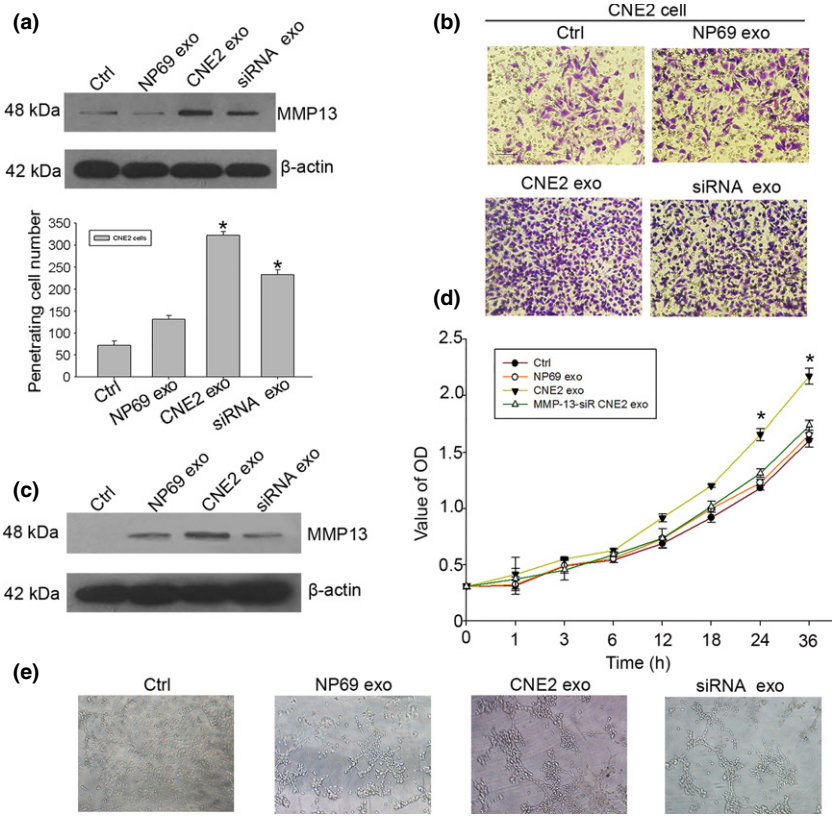

Fig. 5. Exosomes purified from CNE2 (CNE2 exo) containing MMP13 play an important role in interaction with stromal cells. Expression of MMP13 in human skin fibroblasts (HSF) (a) and HUVECs (c) was detected using Western blot after coculture with indicated exosomes. (b) Conditioned medium from HSF cultured with CNE2 exo enhanced invasion of CNE2 cells. (d) CNE2 exo promoted proliferation of HUVECs analyzed by CCK8 assay. (e) HUVECs cocultured with exosomes were seeded into Matrigel and images were taken. All experiments were carried out in triplicate and presented as mean ± standard. $*P < 0.05$.

Emerging evidence suggests cancer cells induced to express mesenchymal phenotype[9] by releasing exosome-like extracellular vesicles carrying tissue factor to facilitate tumor progression. As shown in Figure 4(h), the level of E-cadherin decreased, whereas the expression of N-cadherin and vimentin increased markedly. The results suggested incubating CNE2 cells with CNE2 exo could induce EMT with a dose-dependent tendency. The MMP13 siRNA-suppressed CNE2 cells released exosomes with lower levels of MMP13, which reversed the effect of CNE2 exo-induced EMT (Fig. 4i). Hence, molecular changes confirmed MMP13 contributed to promote the metastatic properties of CNE2 cells by inducing EMT.

We then examined the activation status of AKT and ERK1/2, which are known as downstream mediators of MMP13. As shown in Figure 4(j), treatment with CNE2 exo led to the upregulation of the phosphorylation levels of AKT and ERK1/2, however, if the same cells were challenged with exosomes purified from MMP13 siRNA-suppressed CNE2, the activation status was almost completely abrogated. Therefore, our data suggested that intracellular downstream pathways of MMP13 could be activated by exosomes released by NPC cells.

CNE2 exo containing MMP13 play an important role in interaction with stromal cells. Given the observations that exosomes from NPC patients' plasma could mediate the tumor microenvironment by interaction with stromal cells, it was of interest to ascertain the role of MMP13 secreted in exosomes in mediating the tumor microenvironment. In accordance with the findings of NPC exo, CNE2 exo could upregulate MMP13 in HSF cells (Fig. 5a) as well as HUVECs (Fig. 5c). As previously mentioned, NPC exo increased NPC cell invasion, migration, and angiogenesis capacity, and we obtained similar results with CNE2 exo (Fig. 5c–f). Furthermore, to investigate whether MMP13 played a vital role in the cross-talk between cancer and stromal cells, we used MMP13 siRNA CNE2 exo cocultured with HSF cells and HUVECs. As shown in Figure 5(c–f), we found significant degradation compared with the invasive, migration, and angiogenesis ability observed in CNE2 exo. Together, these results strongly indicated that MMP13 secreted in exosomes facilitate the progression of NPC.

On the basis of all of these findings, a model is proposed for the function of the exosome−MMP13 axis in NPC progression. We present evidence for a previously unknown tumor−-stroma communication involving exosome-mediated signaling, increased stromal MMP13 secretion, and enhanced tumor cell metastasis and angiogenesis: the tumor-derived exosome–MMP13 axis.

Discussion

Metastasis is the main factor associated with cancer-promoted death.[10] Research has documented that both the regulation of ECM degradation and the interaction of tumor cells with structural and cellular components of surrounding tissues are essential processes during tumor progression.[11] As the result of a combination of environmental, viral, and genetic factors, NPC is one of the leading causes of cancer-related death and metastasis may be the leading cause of death in NPC patients.[2] Therefore, the identification of a useful biomarker of NPC may be promising for improved clinical management.

Numerous reports had documented the expression of MMPs in several kinds of advancing tumors that were associated with poor prognosis.[12] Matrix metalloproteinase 13 is induced during metastasis of various cancers including squamous cell carcinomas of the head and neck[20,21,29] and is associated with a poor clinical outcome. Thus, MMP13 may serve as a prognostic marker for tumor invasiveness.[30]

This article focuses on the role of exosomes in cell–cell and cell–stroma interactions in facilitating the metastasis of NPC cells. As the molecular composition of exosomes closely reflects the specialized functions of the parental cells of origin,[13] we found MMP13 overexpressed in NPC exo (Fig. 1d) as well as in NPC cells and exosomes purified from CM (Fig. 3a,d). Furthermore, NPC exo increased tumor cell metastatic properties (Fig. 2d).

Stromal cells within tumor play a vital role in malignant transformation progression of solid tumors[14] which respond to tumor cells by the induction of MMPs.[30] Furthermore, exosomes can also interact with stromal cells in the cancer microenvironment, thus impact on tumor cell proliferation, metastasis, and angiogenesis.[1,10,31–34]

Our data further suggested that MMP13-carrying exosomes promote tumor progression through regulating stromal cells. HSF cells that can persistently release MMPs to the microenvironment of tumor were chosen to evaluate the interaction between NPC exo and stromal cells. After preconditioning with NPC exo or CNE2 exo, a time-dependent upregulation of MMP13 in HSF cells was found (Figs 2a and 5a), which might suggest that HSF release more MMPs, including MMP13, to the microenvironment to promote CNE2 cell invasion.

As MMPs also appeared to be essential in angiogenesis,[15] we tested the role of NPC exo in facilitating angiogenesis.

As in HSF cells, the level of MMP13 in HUVECs was gradually upregulated after coculture with NPC and CNE2 exo (Figs 2c and 5c). Cancer exosomes stimulated proliferation (Figs 2d, 5d) and tube-forming capacity of HUVECs (Figs 2f, 5e). As HUVECs did not express MMP13 initially, we hypothesized that exosomes might be internalized by HUVECs and act as cargos for MMP13 transportation from cancer cells to recipient cells. We found that, 3 h after coculture, the PKH67 labeled NPC exo were efficiently internalized by HUVECs (Fig. 2e) and CNE2 cells (Fig. 4e). Uptake of NPC exo by HUVECs and CNE2 cells may play a significant part in tumor progression.

To further prove that MMP13 regulated the metastatic and angiogenesis abilities of CNE2 cells, we treated CNE2 cells with MMP13 siRNA with the reduction of MMP13 protein expression in CNE2 cells and exosomes (Fig. 4a,b), which reversed the effect of CNE2 exo-induced (Fig. 5). Furthermore, CNE2 exo could be taken up by CNE2 cells, which also upregulated the level of MMP13 in it with a time tendency (Fig. 4e–g).

The role of exosomes in EMT promotion has been described recently.[17] In our study, the MMP13 siRNA-suppressed CNE2 cells released exosomes with lower levels of MMP13 which reversed the effect of CNE2 exo-induced EMT (Fig. 4h–i) as well as the upregulation of the phosphorylation levels of AKT and ERK1/2 (Fig. 4j). It indicated that MMP13 might be a new target for NPC therapy.

In summary, this work proved that MMP13-containing exosomes from NPC could mediate the tumor microenvironment, such as facilitating tumor cell migration and invasion by interaction with stromal HSF cells and HUVECs. Our findings provide important and unique insights into the pathogenesis of NPC and underscore the need to explore alternative therapeutic approaches to impair MMP-driven mechanisms of tumor invasion and metastasis.

Acknowledgments

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Disclosure Statement

The authors have no conflict of interest.

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