The Integrated Analysis of Differential Long Non-Coding RNAs Expression Profiles in Extracellular Vesicles Derived From Chemoradioresistant and Parental Nasopharyngeal Carcinoma Cells

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Abstract

The patients with nasopharyngeal carcinoma (NPC) suffer from poor outcomes after chemoradiotherapy. Extracellular vesicles (EVs) play crucial roles in regulating cancer progression and chemoradioresistance. To investigate the functional role of chemoradioresistant cell-derived EVs for parental cells, and long non-coding RNAs (lncRNAs) expression profiles in EVs secreted by chemoradioresistant cells. The effect of chemoradioresistant NPC cell-derived EVs on migration and invasion abilities of parental cells were detected by Transwell assays. Human cancer lncRNA PCR array was performed on EVs released from chemoradioresistant NPC cells (CNE1R and CNE2R) and parental cells (CNE1 and CNE2). The concurrent chemoradioresistance of CNE1R and CNE2R was significant higher than that of CNE1 and CNE2. Transmission Electron Microscopy (TEM), Nanoparticle Tracking Analysis (NTA), and western blotting results showed the CNE1, CNE2, CNE1R and CNE2R -derived EVs were successfully obtained. The chemoradioresistant cell-derived EVs remarkably promoted migration and invasion ability of parental cells with different differentiation. Notably, a total of 91 differentially expressed (DE) lncRNAs were regulated in EVs by chemoradiotherapy. Among them, the 4 upregulated (MALAT1, FAM212B-AS1, LINC-PINT and H19) and 7 down-regulated (SNHG16, CDKN2B-AS1, ZFAS1, CCAT1, SNHG6, GAPLINC and TUG1) DE lncRNAs associated with chemoradioresistance, were identified in EVs derived from chemoradioresistant cells with different differentiation. We found the EVs derived from chemoradioresistant NPC cells regulated an aggressive phenotype of NPC cells, and identified an altered lncRNAs expression pattern in chemoradioresistant NPC cell- derived EVs.

Introduction

Nasopharyngeal carcinoma (NPC) belongs to a squamous-cell carcinoma that located in the nasopharyngeal epithelium [1]. This carcinoma has a distinctive ethnic and geographic distribution, which has higher occurrence in southern China than in the rest of the world [2]. EBV infection and lymphoepithelial-like histologic are notable characteristics of NPC [2]. Non-keratinizing, undifferentiated NPC (WHO type III) is the most common subtype, and accounts for 63–95% of all NPC cases worldwide [3]. Prognosis is poor once the NPC cells migrate even if this carcinoma obtained advanced therapies [4]. The metastatic NPC is mainly caused by many factors such as Epstein-Barr virus infection, heredity and environmental factors [5, 6]. These factors can result in disruption of cell junctions, epithelial-mesenchymal transition (EMT), invasion, and clonogenicity [7]. Although the application of radiotherapy or chemotherapy techniques have contributed to the improvement in the local control of NPC, chemoradioresistance is a major impediment to achieve longterm survival [8]. A majority of the NPC patients receiving radiotherapy treatment confront with local recurrence and distant metastasis within 1.5 years for radioresistance [9].

Long non-coding RNAs (lncRNAs) are a class of non-protein-coding transcripts above 200 nucleotides in length [10]. lncRNAs can function as competing endogenous RNAs and antisense RNAs, and are proved to act as either promoters or suppressors in various carcinomas. The lncRNAs mainly act in epigenetic level, transcriptional level and posttranscriptional level, among other processes [11]. The recent studies

have showed the lncRNAs are abnormal various types of cancers treated with chemoradiotherapy. For instance, lncRNAs with aberrant expression values between the two groups of LARC patients have been identified in local advanced rectal cancer (LARC) with different neoadjuvant chemoradiotherapy downstaging depth score [12]. Wei Xiong et al has identified 2,662 differentially expressed lncRNAs in 5-FU-based colorectal cancer HCT116 cells relative to those in parental HCT116 [13]. However, the abnormal lncRNA expression profile in NPC receiving chemoradiotherapy, and in released EVs of that is still known few.

Extracellular vesicles (EVs) released from various types of cells, can be secreted to the extracellular space through fusion with plasma membrane, and contribute to intercellular communication [14]. Classically, EVs can be further classified on the basis of their biogenesis, size and biophysical properties, i.e., exosomes, microvesicles, and apoptotic bodies [15]. EVs carry amounts of bioactive proteins, mRNA, small RNA and lipids, and transfer their contents to recipient cells to involve in tumor cell progress [16], can play crucial role in phenotype alteration of sensitive cancer cells. For instance, NPC exosomes can regulate biological behaviour of NPC cells [17, 18]. MiR-9 exists in human 5-8F cell-derived exosomes [19]. Decrease of miR-9 expression via promoter methylation results in increase of CXCR4 expression, stimulating biological behavior (e.g. proliferation, migration and invasion) of NPC cells via the activation of MAPK pathway [20]. NPC exosomes modulates epithelial-mesenchymal transition of cells via delivering mitotic cytokines (e.g., TGF- β), resulting in resistance to chemotherapy [21]. However, the lncRNA expression profile from NPC-derived EVs is wanting. Whether the chemoradiotherapy-induced NPC EVs regulate aggressive phenotypes of NPC cells is on the way.

In our study, we explored the functional role of chemoradioresistant NPC cells-derived EVs on parental NPC cells with different differentiation, and for the first time identified differentially expressed (DE) lncRNAs related with chemoradioresistance in chemoradioresistant NPC cell-derived EVs via PCR array. The effect of chemoradioresistant NPC cell-derived EVs on migration and invasion abilities of parental cells with different differentiation were evaluated. The shared lncRNAs associated with chemoradioresistance were further identified in EVs derived from chemoradioresistant cells with different differentiation.

Material And Methods

Cell culture

Human NPC cell lines CNE-1 (well differentiation) and CNE-2 (poor differentiation) used in this study were obtained from China Center for Type Culture Collection (Wuhan, China), cultured in RPMI-1640 medium (GIBCO), added with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 mg/ml streptomycin (Invitrogen, Carlsbad, CA, USA) in 5% CO₂ at 37°C.

Establishment of concurrent chemoradioresistant NPC cell lines

When CNE-1 and CNE-2 cells were up to the confluence of 60%, they were cultured in the complete medium containing 0.05ug/ mL cisplatin, and were exposed to 6Gy irradiation using a 6-MV X-ray linear accelerator (ELEKTA, Beijing, China) at 200 cGy/min dose rate. After 24h, the cells were cultured in the fresh complete medium containing 0.05ug/ mL cisplatin (Sigma-Aldrich, Saint Louis, MO, USA). When the cell density reached 80-90%, they were further passaged and cultured. The next chemoradiotherapy was performed when the cell growth was in stable state for a total of 10 times. The CNE-1 and CNE-2 cell lines treated with chemoradiotherapy were named as CNE1R and CNE2R, respectively.

Colony formation assay

Each cell (CNE-1, CNE-2, CNE1R and CNE2R) were respectively inoculated in 6-well plates at a density of 300, 400, 800, 1000, 2000, 4000 cells/well and cultured in 200 μ L cell culture medium for 24h. Subsequently, the cells were removed the original medium and cultured in the fresh complete medium containing 0.05ug/ mL cisplatin. At the same time, these cells were respectively treated with gradient doses of irradiation (0 Gy, 2 Gy, 4 Gy, 6 Gy, 8 Gy and 10 Gy) according to the gradient cell numbers under the above radiation condition. After 14 days, the cells were washed, fixed for 30 min using methanol, and stained with 0.1% crystal violet (Sigma, MO, USA) for 10 min. The number of clones >50 was counted under a microscope (DMIRB, Leica, Germany) to calculate the plating efficiency (PE) (%) that is equal to (number of colonies/inoculated cell number) $\times$ 100%. Surviving fraction (SF) that is equal to number of colonies/ (number of cells seeded $\times$ PE) $\times$ 100%. We plotted the cell survival curve using the multi-target single-hit model: $SF=1-(1-e^{-D/D_0})^N$, and the radiobiological parameters D_0 (the dose that gave an average of one hit per target), D_q (quasi-threshold dose) and SF_2 (the SF following exposure to 2 Gy radiation) were determined using the survival curve. The experiments were repeated in triplicate.

EV isolation, quantitation and characterization

EVs isolation and identification referred to the MISEV2018 guidelines [22]. NPC cells were cultured until 80%-90% confluence was reached, and then changed with serum starvation. After incubation for 48 h, the supernatants were centrifuged at 300 g for 10 min to remove dead cells and cell debris. Subsequently, the supernatants were filtered using a 0.22 μ m membrane and concentrated to 1 ml using Amicon Ultra 15 Centrifugal Filter Unit (Millipore, USA). The lipids left was transferred to a fresh microcentrifuge tube. EVs were isolated using the ExoQuick TC kit (System Biosciences, USA) referring to manufacturer's protocol. The isolated EVs were resuspended in PBS. The EVs were fixed using 4% paraformaldehyde and loaded onto a TEM copper grid (Agar Scientific Ltd., Stansted, UK), and then washed with 1% glutaraldehyde, PBS, and distilled water., Next, EVs were incubated with 4% uranyl acetate and observed under TEM (Hitachi H7500 TEM, Tokyo, Japan).

Nanoparticle tracking analysis (NTA) was performed by Nanosight (ZetaView PMX 110, Particle Metrix, Meerbusch, Germany, ZetaView 8.04.02 SP2) to characterize the size distribution and concentration of EVs derived from CNE1, CNE2, CNE1R and CNE2R.

Western Blotting

EV protein quantification was obtained using Pierce BCA Protein Assay Kit (Life Technologies, USA) following the manufacturer's instructions. The proteins were separated by 10% SDS-PAGE and transferred to nitrocellulose membranes. Membranes were incubated with primary antibodies for mouse anti-CD63 (Cat# ab108950, Abcam, 1:1000) and GAPDH (Proteintech, Chicago, UK; 1:1000) at 4°C overnight, and then washed and incubated with secondary antibody (Santa Cruz, Dallas, USA) for 2 hours. Protein bands were visualized using enhanced chemiluminescence reagent (ECL Advance™; GE Healthcare) and images were obtained via a ChemiDoc MP system (Bio-Rad, Hercules, CA, USA).

EV Labelling and Internalization

EVs were isolated from chemoradioresistant NPC cells (1.5×10^6 cells), and suspended in 100ul 1×PBS, and then were mixed with 1ul Dil lipophilic dye (Invitrogen, Carlsbad, CA, USA) for 4 min at 25°C. After adding 1ml FBS to terminate the reaction, the 200ul ExoQuick-TC was added into mixture on ice for incubating 30 min. The labelled EVs were reisolated by ultracentrifugation at 14000 rpm at 4°C for 3 min. Recipient CNE1 and CNE2 cells were incubated with 300ul Dil-labelled EVs for 3 h at 37°C in 5% CO₂, fixed using 4% paraformaldehyde at 25°C for 10 min, washed, and incubated with DAPI (Invitrogen, Carlsbad, CA, USA) for 10 minutes at 25°C, and subjected to fluorescence microscope (Nikon, TE300) with 40× magnification (n = 3).

Migration and invasion assays

For the migration assays, 1×10^5 cells were seeded onto the upper chamber (Cat#353097, FALCON,) added with 500 µl of serum-free DMEM and incubated with or without EVs. Subsequently, the 700 µl DMEM with 10% FBS was supplemented into the lower chambers. After 24 h of incubation, the cells underneath the membrane were fixed using 4% paraformaldehyde and stained with 800µl crystal violet (Cat# C0121; Beyotime, Shanghai, China) for 30 min at room temperature. The number of migrated cells was counted under a microscope. For the invasion assays, the basic steps were the same as above, except that chambers precoated with matrigel (Cat #354480, BioCoat,).

The RNA extraction, cDNA Synthesis Using an RT² First Strand Kit and human cancer lncRNA PCR array

Total RNA derived from NPC cells was obtained using Trizol reagent (Invitrogen, Carlsbad, CA, USA), and its concentration was measured by Nanodrop 2000 (Tiangen, Beijing, China). RNA were performed with agarose electrophoresis for RNA quality control. The cDNA was synthesized using RT² first strand kit by reference to the manufacturer's manual (Cat:330401, Qiagen, Hilden, Germany). The 10 µl reverse transcription mixture was prepared and thoroughly mixed with the genomic elimination mixture (10µl) and performed an incubated at 42°C for 15 min. After the reaction termination at 95°C for 3 min, a 91 µl of RNase-free water was added into each reaction mixture and mixed for following assays.

The RT² SYBR Green Master mix was used for human cancer lncRNA PCR array (Yingbiotech, Shanghai, China). Briefly, a real-time PCR mixture was prepared by adding the 650 µl of 2X PCR master mix, 102µl diluted cDNA, and 548µl RNase free water. The membrane on the PCR Array was carefully, and 10 µl of

the PCR mixture was added to each of the wells. Finally, PCR Array was performed in triplicates. GAPDH was used as endogenous control. The $2^{-\Delta\Delta Ct}$ method was applied to calculate the relative lncRNAs expression.

Statistical analysis

All data were expressed as mean $\pm$ SD in three duplicate experiments. GraphPad Prism 8 (GraphPad Software, Inc.) was applied for statistical analysis. Student's T-test was utilized to calculate the differences. The statistically significant differences were considered at $p < 0.05$.

Results

Identification of the chemoradioresistant CNE1R and CNE2R cells

To identify whether CNE1R and CNE2R cells were resistant to concurrent chemoradiotherapy compared to parental cells, we performed colony formation assay. The results showed that the morphology of CNE1R and CNE2R cells presented the cell body elongated, the extended pseudopod and large intercellular space compared with the CNE1 and CNE2 cells (Fig. 1A). With the increase of radiation dose, the number of cell colonies significantly decreased. At the same radiation dose, the number of CNE1 and CNE2 cell colonies were lower than that of CNE1R and CNE2R cell colonies, respectively (Fig. 1B). The SF value of CNE1R and CNE2R was higher than that of CNE1 and CNE2, respectively (Fig. 1C). Additionally, there was an increase in D0, Dq and SF2 in the CNE1R and CNE2R relative to the CNE1 and CNE2, respectively (Table I), indicating an obvious chemoradioresistance were found in CNE1R and CNE2R as compared to CNE1 and CNE2. These results showed that the chemoradioresistant NPC cells with different differentiation were successfully obtained.

Identification of the chemoradioresistant and parental NPC cell -derived EVs

To identify the collected EVs derived from the well and poorly differentiated NPC cells receiving with and without chemoradiotherapy, TEM and NTA were performed. As presented in Fig 2A and 2B, the obtained particles were consistent with EVs in size (100-150 nm) and morphology (round-shaped).

The concentration of CNE1-EVs, CNE1R-EVs, CNE2-EVs and CNE2R-EVs cells were $7.8E+9$ Particles / mL, $1.3E+12$ Particles / mL, $1.2E+12$ Particles / mL, $1.3E+12$ Particles / mL, respectively. There were no remarkable differences between the concentration of released EVs of these cells except for CNE1-EVs. Additionally, chemoradioresistant and parental NPC cell-derived EVs expressed its marker (CD63) protein. The above results revealed we successfully obtained EVs derived from chemoradioresistant NPC cells with different differentiation (Fig. 2C).

The chemoradioresistant NPC cells released- EVs potentiated migration and invasion ability of parental cells

To clarify the functional roles of chemoradioresistant cell-released EVs in parental cells, we first identify whether chemoradioresistant NPC cell -secreted EVs were efficiently taken up by parental cells. As expected, CNE1 and CNE2 taken up DiI-labelled EVs derived from CNE1R and CNE2R (Fig. 3). The further results showed that CNE1R-EVs and CNE2R-EVs significantly promoted the migration and invasion ability of CNE1 and CNE2 cells, respectively (Fig. 4 and Fig. 5), indicating that chemoradioresistant NPC cell-derived EVs facilitated an aggressive phenotype of parental cells with different differentiation, and might involve in progress of NPC through regulating the invasion and metastasis of recipient cells.

The chemoradiotherapy reshaped the lncRNAs profiling of EVs derived from the well and poorly differentiated NPC cells

To further identify the DE lncRNAs regulated by chemoradiotherapy in EVs released by well and poor differentiated NPC cells, we performed the real-time Profiler PCR array. Between CNE1R-EVs vs CNE1-EVs and CNE2R-EVs vs CNE2-EVs, there were 49 and 53 DE lncRNAs, respectively (Supplementary table S1 and S2). Overall, a total of 91 DE lncRNAs were affected by chemoradiotherapy. there were 15 upregulated DE lncRNAs found in the CNE2R-EVs, and 18 upregulated DE lncRNAs identified in the CNE1R-EVs, among which 4 upregulated DE lncRNAs were shared (Fig. 6A). Moreover, a total of 38 downregulated DE lncRNAs were identified in the CNE2R-EVs, and 31 downregulated DE lncRNAs were found in the CNE1R-EVs, among which 7 downregulated DE lncRNAs were shared (Fig. 6B). Hierarchical clustering was utilized to analyze the expressed pattern of the 11 shared lncRNAs (Fig. 6C). Chemoradioresistant NPC cells and parental cells had a high degree of heterogeneity. The similar spectral clustering was observed in NPC cells with or without receiving chemoradiotherapy.

Discussion

NPC is considered to be a distinct endemic disease in Southern China [1]. Chemoradiotherapy is a paramount approach as the main treatment strategy for NPC, but the effectiveness of chemoradiotherapy is limited for radiation resistance and drug resistance [23]. Chemotherapy can advance EV secretions in cancer cells, leading to the transfer of chemoresistance-related molecules to the adjacent cancer cells to induce susceptibility to chemotherapy [24, 25]. To elucidate the functional effect of EVs released from chemoradioresistant NPC cells, the chemoradioresistant CNE1R and CNE2R cells were established from their parental cell line. The chemoradioresistant NPC cell-derived EVs were internalized in parental cells, and promoted cell migration and invasion. Subsequently, to delineate the lncRNAs associated with chemoradioresistance, the lncRNA expression profile in EVs derived from chemoradioresistant NPC cells was revealed.

EVs may contribute to chemoradioresistance via transferring genetic information into sensitive cancer cells, and further affect the biological function (e.g. growth, migration, invasion and distant metastasis) of sensitive cancer cells [26, 27]. For instance, recent literature elucidates that exosomal miR-20a-5p derived from radio-resistant NPC cells promoted the adjacent NPC cells radio-resistance through repression of Rab27B by targeting Rab27B 3'-UTR [28]. EBV infection-derived exosomes may activate the

phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathway to promote the stemness and chemoradioresistance of NPC through transferring LMP1 [29, 30]. NPC-exosomes modulates epithelial-to-mesenchymal transition(EMT) of cells through delivering growth factor beta, resulting in resistance to chemotherapy [31, 32]. Here, chemoradioresistant NPC cells-derived EVs facilitated the migration and invasion ability of parental cells, suggesting these EVs promoted an aggressive phenotype of parental NPC cells, and might further affect chemoradioresistance and EMT of parental cells.

Several studies have shown that NPC-derived EVs carry various contents and play crucial roles in progression of NPC [33, 34]. However, the effect of chemoradioresistant NPC-derived EVs on progression of NPC remains unknown via carrying lncRNAs. Linc-ROR facilitates NPC invasion and metastasis via enhancing EMT [35]. H19 has been verified to be involved in NPC metastasis [36]. Knockdown of XIST leads to a remarkable decrease of cell proliferation, a marked increase of radiosensitivity in NPC cells [37]. These findings suggest that chemoradioresistant NPC cells-derived EVs, containing active lncRNAs (i.e. MALAT1, FAM212B-AS1, LINC-PINT and H19), might exert functional role in NPC progression. This will be our future research direction.

Conclusion

In the present study, the chemoradioresistant NPC cells-derived EVs facilitated migration and invasion ability of cells with different differentiation. The 4 upregulated and 7 down-regulated lncRNAs were shared in EVs derived from well-differentiated and poor-differentiated NPC cells receiving chemoradiotherapy. We propose this study provides the clue that secreted EVs of chemoradioresistant NPC cells may carry lncRNAs to affect aggressive phenotype of parental cells.

Abbreviations

Extracellular vesicles: EVs; nasopharyngeal carcinoma: NPC; long non-coding RNAs: lncRNA; Transmission Electron Microscopy:TEM; Nanoparticle Tracking Analysis: NTA; differentially expressed: DE; plating efficiency: PE; Surviving fraction: SF.

Declarations

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Competing interests

The authors declare that they have no competing interests.

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability

Not applicable.

Authors' contributions

Conceptualization and Funding acquisition, C.S. and Y.Y.; Data curation, W.X., R.S., C.L., and C.L.; Formal analysis, J.Q., Q.L., X.W., Q.W., G.X., and R.C.; Investigation, W.X., R.S., C.L., C.L., J.Q., Q.L., X.W., Q.W., G.X., and R.C.; Methodology, W.X., R.S., C.L., and C.L.; Writing – original draft, W.X., R.S., C.L., C.L., J.Q., Q.L., X.W., Q.W., G.X., and R.C.; Writing – review & editing, W.X., R.S., C.L., and C.L.; All authors read and approved the final manuscript.

Ethics approval

Not applicable.

Consent to participate

All the authors listed have approved the manuscript.

Consent for publication

All the authors listed have approved the publication.

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Tables

Table I. The radiobiological parameters of chemoradioresistant NPC cells (CNE1R and CNE2R) and parental cells.

Cells	D0(Gy)	Dq(Gy)	SF2
CNE1	2.0333	1.2629	0.5810
CNE1R	2.3759	1.2926	0.6210
CNE2	1.9539	0.4500	0.4290
CNE2R	2.8114	1.3371	0.6620

Figures

Figure 1

The chemoradioresistant NPC cells showed a higher concurrent chemoradioresistance compared to parental cells. (A) Morphological changes of parental cells (CNE1 and CNE2) and chemoradioresistant NPC cells (CNE1R and CNE2R) (Magnification, 10×). (B) The colony formation assay was used to determine the number of colonies of CNE1, CNE2, CNE1R and CNE2R. These cells were treated with 0.05ug/ mL cisplatin, and with gradient doses of irradiation (0 Gy, 2 Gy, 4 Gy, 6 Gy, 8 Gy and 10 Gy) according to the gradient cell numbers (300, 400, 800, 1000, 2000, 4000 cells/well). (C) Cell survival curve based on concurrent chemoradiotherapy. SF, surviving fraction. *P<0.05, n=3 in each group

Figure 2

Characterization of extracellular vesicles (EVs) derived from well and poor-differentiated NPC cells with or without receiving chemoradiotherapy. (A) Transmission electron microscopy images of EVs derived from well-differentiated (CNE1 and CNE1R) and poor-differentiated NPC cells (CNE2 and CNE2R) with or without chemoradiotherapy, Scale bar, 100 nm. (B) Secreted EVs of chemoradioresistant and parental NPC cells, including well differentiated and poorly differentiated cells, were analyzed for particle size by nanoparticle tracking analysis (NTA). (C) Western blot detected the expression of EV marker (CD63) in the secreted EVs of well-differentiated and poor-differentiated CNE1, CNE2, CNE1R and CNE2R

Figure 3

Internalization of EVs derived from chemoradioresistant NPC cells. Immunofluorescence images of DiI-labelled EVs were taken up by CNE1 and CNE2 cells. The 100ul EVs derived from 5×10⁶ CNE1R and CNE2R cells incubated the CNE1 and CNE2 cells for 24h, respectively. Magnification, ×40

Figure 4

The chemoradioresistant NPC cells - derived EVs promoted migration ability of cells without receiving chemoradiotherapy. (A) Both in well-differentiated and poor-differentiated NPC cells, the chemoradioresistant NPC cells-derived EVs enhanced migration ability of cells without receiving chemoradiotherapy (B) A histogram showing the number of migrated cells The 100ul EVs derived from 5×10⁶ CNE1R and CNE2R cells incubated the CNE1 and CNE2 cells for 24h, respectively. ****P<0.0001. n=3 in each group

Figure 5

The secreted EVs of chemoradioresistant NPC cells facilitated invasive ability of parental cells. (A) Both in well-differentiated and poor-differentiated NPC cells, the secreted EVs of chemoradioresistant NPC cells promoted invasive ability of cells without receiving chemoradiotherapy. The 100ul EVs derived from 5×10⁶ CNE1R and CNE2R cells incubated the CNE1 and CNE2 cells for 24h, respectively. (B) A histogram showing the number of invasive cells ****P<0.0001. n=3

Figure 6

Differentially expressed (DE) long non-coding RNAs (lncRNAs) between EVs derived from parental and chemoradioresistant NPC cells. (A) Venn diagrams illustrating co-expressed up-regulated lncRNAs and co-expressed down-regulated lncRNAs (B) between CNE1 vs CNE2 and CNE1R vs CNE2R. (C) The heat map illustrating 11 DE lncRNAs in CNE1 and CNE2 cells, treated with or without chemoradiotherapy. The red indicates upregulation; the blue shows blue

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TableS1.xlsx](#)
- [TableS2.xlsx](#)