The anticancer effect of EGFR-targeting artificial microRNA controlled by SLPI promoter in nasopharyngeal carcinoma

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Abstract

Background: This study intends to use artificial microRNA (recombinant adenovirus vector) targeting epidermal growth factor receptor (EGFR) to inhibit the overexpressed EGFR in nasopharyngeal carcinoma, thereby inhibiting the proliferation and metastasis of nasopharyngeal carcinoma.

Method: The research group verified the expression of EGFR in nasopharyngeal carcinoma through databases, clinical tissues, and cellular pathways. The team first tested the transfection of the recombinant adenovirus by fluorescence microscopy. After adenovirus treatment with different multiplicity of infection (MOI), EGFR level and cell viability in cells were examined by Western blot and MTT assay. Next, the effects of adenovirus (Ad)-SLPI-EGFR on cell proliferation, migration, apoptosis, and related proteins were sequentially examined by EdU, scratch, Transwell, and Western blot. In vivo experiments were performed to evaluate the biological function of EGFR in nasopharyngeal carcinoma.

Result: All three validation pathways showed the increase in EGFR expression in nasopharyngeal carcinoma. Transfection tests showed that the SLPI promoter was specific in CNE2 cells. With the increase in MOI, the inhibition of EGFR expression and cancer cell viability by Ad-SLPI-EGFR was enhanced. Meanwhile, Ad-SLPI-EGFR effectively reduced the proliferation and metastasis of CNE2 cells and affected the expression of related proteins. Furthermore, Ad-SLPI-EGFR inhibited the invasion and metastasis of nasopharyngeal carcinoma in vivo.

Conclusion: Ad-SLPI-EGFR inhibits the expression of EGFR in nasopharyngeal carcinoma cells, and finally achieves the purpose of inhibiting the proliferation and metastasis of cancer cells, which may provide novel targeted intervention for the treatment of nasopharyngeal carcinoma.

KEYWORDS  
epidermal growth factor receptor, nasopharyngeal carcinoma, recombinant adenovirus, secretory leukocyte peptidase inhibitor
1 | INTRODUCTION

Nasopharyngeal carcinoma is a special head and neck malignant tumor originating from the nasopharyngeal epithelium, with very special biological behavior (including regional, ethnic, and familial aggregation). For example, nasopharyngeal carcinoma is rare in the global tumor incidence, accounting for only 0.7% of all tumors in 2020. But in Southeast Asia, especially China, nasopharyngeal carcinoma is a relatively common head and neck tumor. Among the new cases in 2020, more than 70% of the patients are from the southeastern coast of China, Southeast Asia, and the Mediterranean region.

At present, the treatment of nasopharyngeal carcinoma is a comprehensive treatment based on radiotherapy. Although early nasopharyngeal carcinoma is very sensitive to radiotherapy, due to its special biological behavior, 20% of patients are still in locally advanced stage or have distant metastasis at the time of treatment. Unfortunately, even with standard treatment, this subset of patients has a poor prognosis. After comparing a large number of clinical treatment results, it is found that similar treatment for patients with the same pathological stage will also have completely different consequences. Widespread heterogeneity drives us to molecularly type patients and treat them symptomatically.

At present, with the rapid rise of molecular targeted drugs, mining disease-specific molecular targets has played a key role in improving the survival of tumor patients and reducing toxic and side effects. But in nasopharyngeal carcinoma, such targeted drugs are limited. Epidermal growth factor receptor (EGFR) is one of the few targeted factors. EGFR has been shown to be abnormally expressed in various head and neck squamous cell carcinoma (HNSC) tumors, including nasopharyngeal carcinoma, and is a potential biomarker for diagnosis and prognosis. EGFR-targeted drugs have also been shown to be effective in different types of malignant tumors. However, with the deepening of clinical research, scientists have found that EGFR-targeted drugs have little effect on HNSC such as nasopharyngeal carcinoma. Limited efficacy, drug resistance, and toxic and side effects have all become limitations of clinical treatment. Therefore, it is particularly important to develop a targeted intervention with better targeting and durable efficacy.

With the development of molecular biotechnology, researchers have discovered that natural microRNAs (miRNAs) can be processed into small RNAs in cells and inhibit gene expression by specifically degrading mRNA and protein translation, which is a hot spot in the current research on gene expression regulation. Therefore, in this study, an adenovirus with higher conductivity and lower pathogenicity was selected as the vector, and the artificial miRNA sequence targeting EGFR was inserted into the adenovirus to synthesize a recombinant adenovirus vector. In this way, we explored the effect of recombinant adenovirus targeting EGFR inhibition on the proliferation and metastasis of nasopharyngeal carcinoma cells.

2 | METHODS

2.1 Collection of clinical samples

The collection of clinical patient samples has been approved by the Experimental Research Ethics Committee of Zhejiang Provincial People’s Hospital. Our team collected a total of 32 groups of nasopharyngeal carcinoma tissues and adjacent tissues. After obtaining patient consent, investigators collected samples after tumor resection and store them in a constant temperature (−80°C) environment.

2.2 Cell culture

The following 4 types of cells were used in the study (purchase routes are included in parentheses): (1) human normal nasopharyngeal immortalized epithelial cell line NP69 (No. CL0592, FENGHUIBIO, China); (2) nasopharyngeal carcinoma cell line 5-8F cells (No. CL0011; FENGHUIBIO), SUNE1 cells (No. 170299-61-3, ShanghaiHonsun Biological Technology Co., Ltd, China), TW01 cells (Shanghai BinSuiBio, China), CNE2 cells (No. BFN60800633, BLUEFBIO, China); (3) human umbilical vein endothelial cells (HUVEC; No. BFN6021653, BLUEFBIO); (4) tool cells HEK-293 (No. CL-0001, Procell, China). The cell culture conditions in this study were set at 37°C with 5% CO2. The growth medium was uniformly prepared by mixing basal medium (89%), fetal bovine serum (10%; 164,210, Procell), and penicillin–streptomycin solution (also known as double antibody; 1%; PB180120, Procell). The basal medium was uniformly purchased from Procell Life Science & Technology Co., Ltd. NP69 and 5-8F cells were used in RPMI-1640 basal medium (PM150110); SUNE1, TW01, CNE2, and HUVEC cells were used in DMEM basal medium (PM150210); HEK-293 cells were used in MEM basal medium (PM150467).

2.3 Adenovirus transfection

According to research needs, we first inserted the SLPI promoter sequence into the pDC312 vector (BioVector NTCC, China) and harvested the pDC312-SLPI plasmid. According to Chen’s method, an artificial miRNA sequence targeting EGFR and a GFP sequence were inserted into the pDC312-SLPI plasmid, respectively. In this study, AdMaxTM Adenovirus Vector Creation Kits (Microbix Biosystems Inc., Canada) were used for viral packaging of the obtained pDC312-SLPI-EGFR and pDC312-SLPI-GFP recombinant adenovirus plasmids. The resulting adenoviruses were named Ad-SLPI-EGFR and Ad-SLPI-GFP (control virus), respectively. Ad-SLPI-EGFR or Ad-SLPI-GFP were transfected into HEK-293 cell lines by transfection reagent (Invitrogen™ Lipofectamine 2000, 11,668,027). The purified virus stock was properly stored and used for subsequent infection of CNE2 and HUVEC cells. According to the experimental purpose, we set different multiplicity of infection (MOI; 50, 100, 200pfu/cell). After 72h of infection, the handlers observed the green fluorescent
signal in the cells using a fluorescence microscope (200x; BX43F, OLYMPUS).

2.4 | Grouping description

The grouping of experiments in this study is described in detail here. CNE2-control group contained normal cultured CNE2 cells; CNE2-Ad-SLPI-EGFR group contained CNE2 cells infected with recombinant adenovirus Ad-SLPI-EGFR; CNE2-Ad-SLPI-GFP group contained CNE2 cells infected with recombinant adenovirus Ad-SLPI-GFP; HUVEC-control group contained normal cultured HUVEC cells; HUVEC-Ad-SLPI-EGFR group contained HUVEC cells infected with recombinant adenovirus Ad-SLPI-EGFR; HUVEC-Ad-SLPI-GFP group contained HUVEC cells infected with recombinant adenovirus Ad-SLPI-GFP.

2.5 | Quantitative real-time polymerase chain reaction (qRT-PCR)

For the comparison of EGFR expression in nasopharyngeal carcinoma (nasopharyngeal carcinoma tissue vs. paracancerous tissue; normal nasopharyngeal epithelial cells vs. nasopharyngeal carcinoma cell lines), we used a conventional qRT-PCR assay. Samples (tissues or cells) were pretreated as homogenates or suspensions, respectively. After treatment with HaiGene TRIzol solution (B0201), total RNA was extracted from the samples and continued to be processed by Golden 1st cDNA Synthesis Kit (D0401; HaiGene). The SYBR fluorescent dye method was used for qRT-PCR detection. Collected cDNA and prepared primers before experiment (EGFR (5'-3'): (Forward sequence) TGGTTGTCATTGCTGTCG, (Reverse sequence) AGGCCCTCTAGCATTCTTAC; GAPDH sequence (5'-3'): (Forward sequence) TGTGGGCATCAATGGATTTGG, (Reverse sequence) ACACCATGTATTCCGGGTCAAT) were mixed with SYBR Green qPCR Master Mix (Cat. No.: HY-K0501A; MedChemExpress) and sent to 7500 Fast Real-Time PCR Systems (Applied Biosystems®) for testing. The mRNA level of EGFR was calculated according to the 2^ΔΔCt formula.

2.6 | Western blot

WB Super RIPA Lysis Buffer (C2501; HaiGene) was used to extract total protein from adenovirus-transfected CNE2 or HUVEC cells. The loading buffer added after quantification and heating with boiling water were both to reduce protein loss. In electrophoresis and electrophoresis steps, SDS-PAGE gel (prepared before experiment) was an important medium for protein transfer. Cellular proteins transferred onto NC membrane (0.1 NC; Amersham Protran) were called “blot.” A nonfat dry milk solution was used to block the hydrophobic binding sites on the NC membrane. The subsequent antibody incubation process was the key to identifying the target protein. The primary antibody (antibody to the protein to be studied; purchased from Abcam) was fully covered on the blot of the NC membrane (processing temperature: 4°C). The next day, the same secondary antibody (containing HRP label; purchased from Abcam) as the primary antibody was continuously incubated with the washed blot (processing temperature: room temperature). After 1.5 h, the NC membrane was placed in a fully automated digital gel imaging analysis system (Tanon 3500). Before detection, Western BLOT Ultra Sensitive HRP Substrate (T7104Q; Takara), which can enhance the signal, was added dropwise to the blot. It should be noted that the antibody information is listed in the table in detail. Among them, GAPDH was the internal reference gene determined in this experiment.

2.7 | MTT experiment

In this experiment, CNE2 and HUVEC cells were evenly plated in a 96-well plate. We transected CNE2 and HUVEC cells with adenovirus at gradient MOI for 72 h. After the treatment, MTT detection solution (M5655-100MG; Sigma-Aldrich) was added to the cells immediately (MTT dose: 10 µl/well). The culture needs to be terminated after 4 h. At the same time, the DMSO solution was mixed with the above cell solution (DMSO dose: 100 µl/well). The cell mixture was placed in a UV-Vis Spectrophotometer Alpha1860S (LASPEC) to measure the absorbance (OD value) at 570nm.

2.8 | EdU experiment

Adenovirus (MOI = 50pfu/cell)-transfected CNE2 and HUVEC cells were homogeneously seeded in a 96-well plate. The EdU reagent provided by the kit (C0081S; Beyotime) was pre-diluted to 20µM in cell culture medium (named EdU working solution). Next, CNE2 or HUVEC cells were added with an equal volume of EdU working solution and incubated for 2 h. After removing the medium, EdU-labeled CNE2 or HUVEC cells were fixed and permeabilized. The added Azide 647 Click reaction solution (0.5 ml/well) was fully combined with the cells. After aspirating the reaction solution, the experimenter used Hoechst 33342 (C1025; Beyotime) for CNE2 or HUVEC nuclei staining. Subsequent fluorescence detection was done under a microscope (200x). Proliferating cells represented by red fluorescence and nuclei represented by blue fluorescence were shown by merge plot.

2.9 | Scratch test

Adenovirus (MOI = 50pfu/cell) transfected CNE2 and HUVEC cells were adjusted to suspensions of defined concentrations (2 x 10^5 cells/ml). After tearing off the protective film at the bottom of the ibidi wound healing petri dish (80,209, Germany), the CNE2 or HUVEC cells in suspension were transferred into 2 wells of the insert (81,176, ibidi, Germany) (transfer volume: 70µl). Formal experimental
operation was started after 6 h of adaptive culture. The researcher needs to remove the insert at 0 h and 72 h of the formal experiment and record the distance of the scratch through a microscope (100×). The rest of the time CNE2 and HUVEC cells were routinely cultured.

2.10 Transwell test

Corning® Transwell® Cell Culture Insert (CLS3422) provided an experimental environment for cell invasion, while BD Biosciences Matrigel (356234) simulated the extracellular matrix in vitro. The Matrigel in flow state was spread on the bottom of the Transwell insert (upper chamber). To induce cell invasion, cell growth medium was added to Transwell plates (lower chamber). After Matrigel coagulation, CNE2 or HUVEC cells seeded in serum-free medium were transferred to the upper chamber (cell concentration: 1 × 10⁵ cells/ml; transfer volume: 100 μl). After 72 h, the invasive cells in the lower chamber were collected. The treatment of paraformaldehyde and crystal violet staining solution helped the experimenter to count the cells more accurately under the microscope (×250).

2.11 Inguinal lymph node metastasis model

The inguinal lymph node metastasis model was used to investigate the role of EGFR in lymph node metastasis in nasopharyngeal carcinoma cells as previously described.⁴⁹ Five-week-old BALB/c nude mice (18–20 g) were obtained from Vitalriver Corporation (China). All experimental procedures were approved by the Institutional Animal Care and Use Committee of Zhejiang Provincial People’s Hospital. Mice were randomly divided into three groups (n = 8/group), as following: CNE2-control, CNE2-Ad-SLPI-EGFR and CNE2-Ad-SLPI-GFP groups. On day 0, CNE2 cells suspension (5 × 10⁵ cells) were inoculated into the foot pads of the mice. On day 28, the mice were euthanized, and the primary tumors and inguinal lymph nodes were excised and embedded in paraffin. Sections of the primary tumors were subjected to H&E staining for histological examination. Metastatic tumor cells in the lymph nodes were stained with an anti-pan-cytokeratin antibody (ab7753; Abcam) with immunohistochemical assay.²⁰ Pan-cytokeratin is expressed in all epithelial cells and is a useful marker of epithelial origin cells.

2.12 Statistical analysis

The data analysis method in this study needs to be explained as follows: This study used the Gene Differential Expression module of the Starbase website (http://starbase.sysu.edu.cn/index.php) to analyze the differential expression of EGFR in Head and Neck squamous cell carcinoma (HNSC); in Figure 1B, the expression difference of EGFR in paracancerous tissue (Normal group) and nasopharyngeal carcinoma tissue (tumor group) was compared by paired samples t test; in addition to the results of the above two groups, the rest of the results were uniformly analyzed by one-way analysis of variance. The above analyses were all performed in GraphPad prism software (version 8.0). Regarding statistical significance, this study

![Figure 1](image)

FIGURE 1 Levels of EGFR were confirmed to be elevated in nasopharyngeal carcinoma tissues and cells. (A) The expression difference of EGFR in HNSC was analyzed by Starbase website (http://starbase.sysu.edu.cn/index.php). (B) Differential expression of EGFR in nasopharyngeal carcinoma tissues (Tumor group) and adjacent normal tissues (Normal group) was analyzed by qRT-PCR. **p < 0.001 versus Normal. (C) Differences in EGFR expression in nasopharyngeal carcinoma cell lines and normal cells were analyzed by qRT-PCR. ***p < 0.001 versus NP69. All experiments were repeated three times to average; in qRT-PCR experiments, GAPDH was used as an internal reference gene. EGFR, epidermal growth factor receptor; HNSC, head and neck squamous cell carcinoma; qRT-PCR, quantitative real-time polymerase chain reaction; SLPI, secretory leukocyte peptidase inhibitor.
adopts the internationally recognized $p < 0.05$ as the identification standard.

3 | RESULT

3.1 | The level of EGFR was confirmed to be elevated in nasopharyngeal carcinoma tissues and cells

According to the analysis of the Starbase database, the expression level of EGFR in HNSCs was higher than that in normal controls ($p = 1.1 \times 10^{-5}$, Figure 1A). To validate its results, we compared the levels of EGFR in nasopharyngeal carcinoma tissues and adjacent tissues, as well as in nasopharyngeal carcinoma cell lines and normal cells. The results were obvious, in nasopharyngeal carcinoma tissues and cells, the level of EGFR was higher than that in the control group ($p < 0.001$, Figure 1B,C).

3.2 | Ad-SLPI-EGFR inhibited the expression of EGFR and the viability of nasopharyngeal carcinoma cells in a titer-dependent manner

After successful collection of Ad-SLPI-EGFR and Ad-SLPI-GFP, we compared the transfection of Ad-SLPI-GFP in CNE2 cells (nasopharyngeal carcinoma cells) and HUVEC cells (control cells) by fluorescence microscopy. Obviously, a large amount of green

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**FIGURE 2** Ad-SLPI-EGFR inhibited EGFR expression and nasopharyngeal carcinoma cell viability in a titer-dependent manner. (A) The transfection of recombinant adenovirus was observed by fluorescence microscope (×200). (B) The effect of different titers (MOI = 50, 100, 200 pfu/cell) of recombinant adenovirus Ad-SLPI-EGFR on EGFR expression in CNE2 cells was analyzed by western blot. In this experiment, GAPDH was used as an internal reference gene. ***$p < 0.001$ versus Ad-SLPI-GFP; +++$p < 0.001$ versus Control. (C) The effect of different titers (MOI = 50, 100, 200 pfu/cell) of recombinant adenovirus on the viability of CNE2 and HUVEC cells was analyzed by MTT assay. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus CNE2 control. All experiments were repeated three times to average. EGFR, epidermal growth factor receptor; MOI, multiplicity of infection; SLPI, secretory leukocyte peptidase inhibitor
fluorescence signal of Ad-SLPI-GFP were observed in CNE2 cells (Figure 2A); in HUVEC cells, the green fluorescence signal was very weak (Figure 2A). This result suggests that the SLPI promoter has specificity in nasopharyngeal carcinoma cells.

We next treated CNE2 cells or HUVEC cells with adenovirus at a gradient MOI, respectively. In the Western blot results, Ad-SLPI-EGFR significantly reduced the protein level of EGFR \((p<0.001, \text{Figure 2B})\). In the cell viability assay, Ad-SLPI-EGFR could significantly reduce the viability of GNE2 cells \((p<0.05, \text{Figure 2C})\), but Ad-SLPI-EGFR did not affect the viability of HUVEC cells (Figure 2C). And the results of these two groups of experiments were the same. That is, the higher the titer of adenovirus, the stronger the inhibitory effect of Ad-SLPI-EGFR (Figure 2B,C).

### 3.3 Ad-SLPI-EGFR could significantly inhibit the proliferation, migration, and invasion of GNE2 cells, and affect the level of related proteins

In the cell basic function analysis experiment, adenovirus Ad-SLPI-EGFR significantly reduced the proliferation ability of CNE2 cells \((p<0.001, \text{Figure 3})\), and reduced the migration and invasion of CNE2 cells \((p<0.001, \text{Figure 4A-D})\). Not only that, but Ad-SLPI-EGFR also affected the expression of proliferation and epithelial-mesenchymal transition (EMT)-related proteins at the protein molecular level. Specifically, Ad-SLPI-EGFR prevented the expression of PCNA, N-cadherin, and Vimentin in GNE2 cells and stimulated the up-regulation of E-cadherin \((p<0.01, \text{Figure 5A-E})\). However, in the control cell HUVEC, Ad-SLPI-EGFR did not show the above-mentioned functional inhibition and molecular regulation ability (Figure 3-5). Overall, Ad-SLPI-EGFR could exert a targeted inhibitory effect in nasopharyngeal carcinoma cells.

### 3.4 Ad-SLPI-EGFR inhibited nasopharyngeal carcinoma cell invasion and metastasis in vivo

To confirm the functions of EGFR in nasopharyngeal carcinoma in vivo, a popliteal lymph node metastasis model was constructed by transplanting GNE2 cells stably expressing EGFR into the footpads of nude mice. After 28 days, the primary tumors inguinal lymph nodes were obtained for analysis (Figure 6A). As expected, H&E
staining showed that the primary tumors in the CNE2-Ad-SLPI-EGFR group had a weaker aggressive phenotype with the invasion of tumor cells towards the skin and muscle, when compared to tumors in the CNE2-Ad-SLPI-GFP group (Figure 6B). In addition, the volume of the popliteal lymph nodes \((p<0.01, \text{Figure 6C,D})\) and the pan-cytokeratinative tumor cells (Figure 6E) were greater in the CNE2-Ad-SLPI-GFP group than in the CNE2-Ad-SLPI-EGFR group.
Adenovirus is a non-enveloped DNA virus that is ubiquitous in nature and can infect humans. Adenoviruses consist of a 26–45kb linear double DNA genome with an icosahedral protein capsid. Extensive experimental data show that the use of vectors to introduce genes into cells is more efficient than non-viral transfer methods (such as cationic polymer-DNA complexes). Adenovirus vectors have the advantages of moderate genome size, recombination of depression genes, high MOI for reproduction, wide host range, and high safety. Such vectors can be used not only for vaccine research but also for tumor treatment after being transformed. Therefore, it has broad prospects in the fields of in vivo gene transduction, gene therapy and vaccine development. This is also the reason for choosing adenovirus as the vector in this study.

RNA interference (RNAi) is an important way of post-transcriptional gene silencing in the regulation of gene expression, and it is ubiquitous in a variety of multicellular organisms. The researchers used this feature to synthesize small interfering RNA (siRNA) and short hairpin RNA (shRNA) for gene therapy in vitro. With the vigorous development of molecular technology, artificial miRNA has appeared in the field of researchers as the second-generation shRNA. Artificial miRNA adds the miRNA framework on the basis of retaining the specific sequence structure targeted by shRNA. This can not only target and suppress genes more efficiently, but more importantly, it can be activated by most of the regulators in mammals. With this advantage, we constructed a recombinant adenoviral vector (Ad-SLPI-EGFR) using an artificial miRNA targeting EGFR and the nasopharyngeal carcinoma-specific promoter SLPI. Our experimental results showed that Ad-SLPI-EGFR successfully blocked the protein expression of EGFR. Furthermore, we demonstrated that Ad-SLPI-EGFR has a clear functional inhibition of nasopharyngeal carcinoma cells, but not HUVEC cells. In addition, we performed the work on animal validation of Ad-SLPI-EGFR on metastasis of nasopharyngeal carcinoma in vivo.

SLPI is a serine protease inhibitor (molecular weight 11.7 kDa), which can inhibit the activity of serine proteases such as...
SLPI is mainly secreted by epithelial cells and is an endogenous immune-related protein with broad-spectrum anti-inflammatory effects. The latest research shows that SLPI is both an inflammatory inhibitor and a protease inhibitor and participates in tumorigenesis and metabolism by degrading surrounding tissues. It has been reported that the expression of SLPI is increased in most human tumor cells, such as breast cancer, lung cancer, ovarian cancer, colorectal cancer, and malignant glioma. The differential expression of SLPI has an unnegligible impact on tumor development (e.g., invasion and migration). In this study, the SLPI promoter with nasopharyngeal carcinoma specificity is an important component of the recombinant adenovirus vector. Similar effects have also been reported in squamous cell carcinomas such as laryngeal carcinoma.

**FIGURE 6** Ad-SLPI-EGFR inhibited nasopharyngeal carcinoma cell invasion and metastasis in vivo. (A) A representative image of metastatic popliteal lymph nodes. (B) The H&E staining shows that the primary footpad tumor invaded in the skin and muscle. Scale bar: 100μm. (C) Image of metastatic popliteal lymph nodes. (D) The volume of the popliteal lymph nodes was greater in the CNE2-Ad-SLPI-GFP group than in the CNE2-Ad-SLPI-EGFR. (E) The immunohistochemical staining of pancreatic acinar odes was greater in the skin and mus. Scale bar: 100 or 200μm×100 and ×40 magnification. All data are presented as mean data standard deviation of at least three independent experiments. ***p < 0.001 versus Ad-SLPI-GFP. Ad, adenovirus

EGFR is a glycoprotein receptor on the cell membrane surface, belonging to the ErbB receptor family and it is a receptor tyrosine kinase. EGFR has tyrosine kinase activity. When combined with ligand-EGF, it can phosphorylate tyrosine residues, activate EGFR, and promote the growth and proliferation of tumor cells. A large number of basic research and clinical experiments have confirmed that EGFR-related antibodies and inhibitors can be used for tumor treatment. Therefore, the development of targeted anti-tumor drugs targeting EGFR has been very significant, such as small molecule inhibitors represented by gefitinib, and monoclonal antibodies represented by cetuximab. However, the efficacy of these drugs in nasopharyngeal carcinoma is minimal. High drug resistance and toxic side effects are the main reasons for limiting drug use.
Referring to the latest research technology, this study used pancreatic virus as a vector to insert the nasopharyngeal carcinoma-specific promoter SLPI and the artificial miRNA sequence for EGFR. This makes full use of the tissue affinity and gene transduction efficiency of the vector, while avoiding the restriction of RNAi by the U6 promoter. Finally, we demonstrated that the recombinant adenovirus Ad-SLPI-EGFR effectively down-regulated the synthesis of EGFR protein and further prevented the proliferation and metastasis of nasopharyngeal carcinoma.

5 | CONCLUSION

Ad-SLPI-EGFR inhibits the expression of EGFR in nasopharyngeal carcinoma cells and achieves the purpose of inhibiting the proliferation and metastasis of nasopharyngeal carcinoma. This study provides a new and valuable reference direction for molecular targeted therapy of nasopharyngeal carcinoma.

FUNDING INFORMATION

This work was supported by the Medical Science and Technology Project of Zhejiang Province (No. 202149853, No. 2022KY071) and Zhejiang Traditional Chinese Medicine Scientific Research Fund Project (No. 2021ZA012, No.2022ZA013).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The analyzed datasets generated during the study are available from the corresponding author on reasonable request.

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**SUPPORTING INFORMATION**

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Dong F, Su L, Tan J, Luo H. The anticancer effect of EGFR-targeting artificial microRNA controlled by SLPI promoter in nasopharyngeal carcinoma. *J Clin Lab Anal*. 2022;36:e24729. doi: 10.1002/jcla.24729