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Exosome-derived microRNAs in oral squamous cell carcinomas impact disease prognosis

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ABSTRACT

Objectives: microRNA (miRNA) expression patterns have provided new insight as biomarkers of prognosis as well as novel therapeutic targets for several neoplasms. However, the role of exosomal miRNA in the prognosis of oral squamous cell carcinoma (OSCC) has not yet been completely clarified. Paired primary tumor and normal oral epithelial cells from OSCC patients were obtained, and the exosomal miRNA profiles between them were compared by miRNA microarray analysis. The miRNA levels in the serum exosomes of OSCC patients were verified by real-time quantitative reverse transcription PCR (qRT-PCR) analysis. Finally, the biological functions and the potential as a prognostic marker of the selected miRNA candidates were analyzed in the OSCC cells and patients, respectively.

Results: Exosomal miR-155 and miR-21 were significantly upregulated, and exosomal miR-126 was dramatically downregulated in the primary OSCC cells and the serum of OSCC patients. In the analysis of oncogenic behaviors, coculture with either miR-155-rich or miR-21-rich exosomes could promote cell proliferation and invasion accompanied with downregulation of PTEN and Bcl-6 tumor suppressors. Moreover, treatment with miR-126-rich exosomes inhibited oncogenic behaviors and oncogene EGFL7 expression in OSCC cells. Finally, exosomal miR-126 was reduced in the serum of the late-staged OSCC patients, and downregulation of blood exosomal miR-126 was associated with poor survival in OSCC patients.

Conclusion: Exosomal miR-155 and miR-21 are oncogenic miRNAs which suppress PTEN and Bcl-6 expression, and exosomal miR-126 acts as a tumor suppressor which downregulates EGFL7 in OSCC. Furthermore, blood exosomal miRNAs may serve as biomarkers for the diagnosis and prognosis of OSCC.

Introduction

Oral squamous cell carcinoma (OSCC) is the sixth most prevalent malignancy in the world [1], occurring at the lips, tongue, buccal mucosa, mouth floor, gingiva, hard palate, and retromolar trigone [2]. The prognosis of OSCC is usually poor with a high recurrent rate and frequent metastases [3]. Although great progress has been made for the therapeutic strategy during the past decades, the prognosis of OSCC has no significant improvement [4]. Rapid tumor growth and recurrence remain the big challenges for OSCC. Thus, exploration of biomarkers for tumor progression would be helpful to understand tumor development and to find novel therapeutic targets for OSCC.

Exosomes are small (30–100 nm) vesicles that have been recognized as important mediators of intercellular communication [5]. Many cell types can produce exosomes, including dendritic cells, B cells, T cells, mast cells, epithelial cells and tumor cells [6]. Exosomes of tumors may drive the formation of pre-metastatic niche and determine organotropic metastasis through integrins [7]. Exosomes play such biological and pathological roles in intercellular communication through their cargo molecules, which include protein and genetic materials, such as

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microRNA (miRNA) [8,9]. miRNAs are small non-coding RNAs that mediate destabilization and/or translational repression of target messenger RNA (mRNA) molecules and thus reduce the final protein output. Increasing evidences have linked miRNAs to cancer development and progression. miRNAs upregulated in some cancers to promote oncogenesis by targeting tumor suppressor genes are known as "oncogenic miRNAs (oncomiRs)", whereas miRNAs downregulated in cancers are thought to be "tumor suppressor miRNAs (TS-miRNAs)" [10].

In this study, the expression profile of exosomal miRNAs was comprehensively investigated using miRNA microarray analysis in human primary OSCC cells and non-neoplastic squamous cells isolated from paired keratinized gingival tissue. According to the microarray data, the potential candidates for further analyses were preliminarily selected. Moreover, we analyzed serum specimens from a cohort of patients with OSCC and conducted an investigation to systematically identify serum exosomal miRNAs which could potentially serve for prognostic prediction of OSCC.

Materials and methods

Patients and sample collection

Paired tissue samples were obtained from primary tumor and gingival tissues in 3 OSCC patients. In addition, serum samples (n = 35) from OSCC patients were collected. All serum and tissue samples were obtained from patients who underwent primary tumor resection. The serum samples were derived from the residual blood for the purpose of clinical examination during the preoperative and follow-up phases. All patients were diagnosed with OSCC and closely followed up every 1 month for the first year, every 2 months for the second year, every 3 months for the third year, and every 6 months for the fourth year and later postoperatively. The primary culture cells from paired tissue samples were used for microarray analysis and the serum samples were used for further validation by real-time quantitative reverse transcription PCR (qRT-PCR). Additional serum samples were also obtained from healthy individuals (n = 11) as control for qRT–PCR. All tissue and blood samples were collected from Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan. Hematoxylin-eosin-stained sections of each were reviewed by pathologists. Written informed consent was obtained from all patients and healthy individuals. The study was approved by the Chang Gung Medical Foundation Institutional Review Board (No. 103-3528B).

Cell cultures

The tissue specimens were sent to the cell culture laboratory in 10-ml culture media (Dulbecco's Modified Eagle's Medium [DMEM]: Gibco, Bethesda, MD, USA; pH 7.2) supplemented with 10% heat-inactivated fetal calf serum (FCS), 100 U/ml penicillin, 100 μg/ml streptomycin and 0.5% amphotericin B (Gibco) to prevent growth of microorganisms. The tissue specimens were washed and disinfected in a 10% povidone iodine solution for one to two minutes and then washed in culture media. The tissue specimens were cut into pieces, approximately 11 mm in size, and placed in the culture plate (T-25 flask, Corning, New York, USA) using a sterile needle of the dental injector. Tissue pieces were left in the culture plate for 15–20 min and then the culture media were gently dropped on the tissue pieces. After waiting for three to four hours, the culture plate was flooded with 5 ml culture media and then incubated at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. The old culture medium was replaced with a fresh one twice a week. When the keratinocytes, which were squamous in shape, started to multiply around the tissue sample to a diameter of 2–5 mm, the culture medium was changed to EpiLife culture medium supplemented with human keratinocyte growth factors (Cascade Biologics, Portland, OR, USA), 125 μg/ml gentamycin and 1 μg/ml amphotericine B (Sigma-Aldrich, St Louis, MO, USA), with a calcium concentration of 0.06 mM (Sigma-Aldrich). Thus, fibroblast overgrowth was eliminated and prevented. The culture was fed every other day with the EpiLife culture medium. After about 10 days, when the primary cell culture reached a 70–80% confluence, oral mucosa keratinocytes were harvested with a solution of 0.025% trypsin-EDTA (Cascade Biologics) at 37 °C. After four to five minutes, trypsin-EDTA activity was inhibited with an equal volume of 0.0125% trypsin inhibitor. Primary cultured keratinocytes were used from the third through the fifth passages in a T-75 flask. Moreover, OSCC cell lines SCC4 and FaDu were purchased from Taiwan Bioresource Collection and Research Center, Hsinchu, Taiwan and cultured in DMEM or RPMI supplemented with 10% FBS without antibiotics–antimycotics.

Isolation of exosomes

Exosomes were isolated from the serum and the culture medium using a Total Exosome Isolation Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol.

Microarray analysis for miRNAs

Exosomal miRNA expression profiles in the culture medium of primary culture cells derived from the paired oral epithelial tumor tissues (n = 3) and keratinized gingival tissues (n = 3) were examined using a miRNA microarray analysis system (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's protocol.

qRT–PCR analysis for miRNAs

Exosomal miRNA expression was assayed using qRT–PCR in serum sample of OSCC patients. After isolation and quantitation of the exosomal RNA, 5-μg RNA was used for a reverse transcription reaction with the Taqman MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). qRT-PCR was performed using a 7500 Fast Real Time PCR system (Applied Biosystems) with TaqMan® Universal PCR Master Mix, No AmpErase® UNG (Applied Biosystems). miRNAs specific primers (hsa-miR-21, ID: 000397; hsa-miR-205, ID: 000509; hsa-miR-155, ID: 002287; hsa-miR-126, ID: 002228; hsa-miR-20a, ID: 000580; hsa-miR-32, ID: 002109; hsa-miR-362, ID: 001273; hsa-miR-101, ID: 002143; RNU6B as internal control, ID: 001093) (Applied Biosystems) were used in this study.

qRT–PCR analysis for specific genes

Total RNA was isolated from cultured OSCC cells using Quick-RNA MiniPrep Kit (Zymo Research, Orange, CA, USA). Briefly, 2 μg of total RNA was used for the reverse transcription reaction with Superscriptase II (Invitrogen) using oligo-dT and random primers. cDNA was used as template for qRT-PCR analysis. Amplification and detection were performed by TaqMan probes (Applied Biosystems) or SYBRR Green master mixes (Thermo Fisher Scientific, Inc, Waltham, MA, USA) in a 7500 Fast Real-Time PCR System (Applied Biosystems). The probe and primer sequences used in this study were listed in Table 1.

Cell proliferation assay

Cells were supplemented with fresh medium containing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich) and incubated for 2 h at 37 °C. After medium removal, the viable cells were dissolved with 100 ml of DMSO and determined by reading optical densities in a microplate reader (Dynex Technologies, Inc., Chantilly, VA) at an absorption wavelength of 570 nm.

Cell invasion assay

Cells were seeded in triplicate in the upper compartment of the chamber (2.5×10^4 cells in 50 μL per well) and supplemented with

Table 1
The probes and primers for qRT-PCR analysis.

Gene	Probe	Forward primer	Reverse primer
PTEN	CCAGTGCTAAAATTCA	CCTTCTCCATCTCCTGTGTAATCAA	GTTGACTGATGTAGGTACTAACAGCAT
Bcl-6	TGGCCTCGCCGGCTGACAG	AGCAAGGCATTGGTGAAGACA	ATGGCGGGTGAACTGGATAC
EGFL7		GGGATGGCAGGGAGATACTTG	CTGGCGTGGGACTTGGTG
β-actin		CCCATCTATGAGGGTTACGC	TTTAATGTCACGCACGATTTC

DMEM serum-free media. The lower compartment was filled with 30 μL of DMEM media containing 10% CS serum media. A polycarbonate filter (8-μm pore size Nucleopore; Costar, Cambridge, MA) was coated with 0.1% gelatin to allow cell adhesion, and the upper and lower compartments were separated by the coated filter. After incubation for 24 h in a humidified 5% CO_2 atmosphere chamber at 37 °C, cells on the upper side of the filter were removed. Migrated cells on the lower side were fixed in absolute methanol and stained with 10% Giemsa solution (Sigma). Finally, the fixed cells were photographed by microscope with digital images system (Olympus, Tokyo, Japan) and counted as mean ± SD per high-power field by the average of five different high-power fields.

Statistical analysis

Differences between the groups were statistically evaluated using the unpaired Student's *t* test. The results are presented as mean ± SD. Survival curves were plotted according to the Kaplan–Meier method and the generalized log-rank test was applied to compare the survival curves. $P < 0.05$ was considered statistically significant. GraphPad Prism 7.0 (GraphPad Software, San Diego, CA) was used for statistical analysis.

Results

Differential expression of miRNA in the secreted exosomes between human primary OSCC cells and non-neoplastic squamous cells

miRNA expression profile was analyzed by miRNA array in the secreted exosomes of human primary OSCC cells and squamous cells isolated from paired keratinized gingival tissue (Table 2). Thirty-one miRNAs were downregulated with at-least 2-fold change in the exosomes of OSCC cells. Twenty-six of them were downregulated in 2 to 5 folds, and five miRNAs were downregulated in 5 to 10 folds. Moreover, twenty-one miRNAs were upregulated (2 to 5 folds) in the exosomes of OSCC cells. Based on the above results, the potential of exosomal miRNAs in the serum from OSCC patients to act as a biomarker was further validated in this study. It has been reported that miR-155 [11], miR-21 [12], miR-205 [13], miR-126 [14], miR-20a [15], miR-32 [16], miR-362 [17] and miR-101 [18] showed clinical significance in OSCC. By qRT-PCR analysis, exosomal miR-155, miR-21 and miR-205 were significantly upregulated in the serum of OSCC patients (Fig. 1). Furthermore, exosomal miR-126, miR-20a, miR-32, miR-362 and miR-101 were significantly downregulated in the patient serum with OSCC (Fig. 1). Taken together, the expression profile of selected miRNAs was consistent in the microarray and qRT-PCR analyses, and exosomal miRNA in the serum could be potentially used as a non-invasive tumor marker of OSCC.

Table 2
Comparison of fold change (tumor/normal) in exosomal miRNA levels between paired primary tumor and normal cells from OSCC patients.

Fold change	Number	miRNA
−5 ~ −10	5	hsa-miR-126c, hsa-miR-190, hsa-miR-20a, hsa-miR-32, hsa-miR-362-3p
−2 ~ −5	26	hsa-let-7a, hsa-miR-101, hsa-miR-1257, hsa-miR-1259, hsa-miR-1266, hsa-miR-1297, hsa-miR-130a, hsa-miR-203, hsa-miR-30, hsa-miR-379, hsa-miR-411, hsa-miR-424, hsa-miR-582-5p, hsa-miR-587, hsa-miR-590-5p, hsa-miR-592, hsa-miR-593, hsa-miR-609, hsa-miR-644, hsa-miR-648, hsa-miR-769-3p, hsa-miR-886-5p, hsa-miR-889, hsa-miR-9, hsa-miR-922, hsa-miR-95
2 ~ 5	21	hsa-miR-155, hsa-miR-1205, hsa-miR-1274b, hsa-miR-1275, hsa-miR-1277, hsa-miR-138–2, hsa-miR-182, hsa-miR-187, hsa-miR-188-3p, hsa-miR-200a, hsa-miR-204, hsa-miR-208b, hsa-miR-543, hsa-miR-548e, hsa-miR-571, hsa-miR-589, hsa-miR-597, hsa-miR-605, hsa-miR-92a-2, hsa-miR-21, hsa-miR-205

Exosomal miR-155 and miR-21 as oncomiRs in OSCC

It has been reported that miR-155 [19] and miR-21 [20] promoted oncogenic behaviors in OSCC cells. Thus, we selected these two miRNAs (miR-155 and miR-21) upregulated in the exosomes of the patient serum with OSCC to perform further functional analysis. In this study, SCC4 cells acted as packaging cells for production of miR-rich exosomes, and FaDu OSCC cells severed as target cells for validation of oncogenic function (Fig. 2A). miR-rich exosomes were collected in the culture medium of SCC4 cells after miR mimics transfection for 48 h. Treatment with miR-155- or miR-21-rich exosomes promoted miR-155 or miR-21 upregulation in FaDu cells, respectively (Supplementary Fig. 1), and exosomal miR-155 or miR-21 significantly promoted cell proliferation of FaDu cells (Fig. 2B). Moreover, cell invasiveness was promoted in the target cells after treatment with miR-155- or miR-21-rich exosomes (Fig. 2C). Previous studies indicated that miR-21 and miR-155 overexpression could downregulate PTEN and Bcl-6 tumor suppressors, respectively, to promote the oncogenic behaviors of OSCC cells [21,22]. By qRT-PCR analysis, miR-21- or miR-155-rich exosomes from SCC-4 cells significantly downregulated both PTEN and Bcl-6 mRNA in FaDu cells (Fig. 2D). Taken together, miR-21- or miR-155-rich exosomes may promote oncogenesis by downregulating PTEN and Bcl-6 in OSCC cells.

Exosomal miR-126 as a TS-miRNA suppressing oncogenesis in OSCC cells

In miRNA microarray analysis, miR-126 was downregulated in OCSS cells, and miR-126 transfection could inhibit oncogenic processes in OSCC cells [14]. To study the role of miR-126-rich exosomes in the oncogenesis of OSCC cells, SCC4 cells served as packaging cells for the production of miR-126-rich exosomes, and FaDu OSCC cells acted as target cells for the evaluation of tumorigenic role (Fig. 3A). miR-126-rich exosomes were collected in the culture medium of SCC4 cells after miR-126 mimics transfection for 48 h, and the treatment with exosomal miR-126 significantly inhibited cell growth accompanied with miR-126 upregulation in FaDu cells (Fig. 3B and Supplementary Fig. 2). Furthermore, the cell invasion of FaDu cells was suppressed by treatment with miR-126-rich exosomes (Fig. 3C). Previous research indicated that miR-126 can suppress oncogenic processes of OSCC cells by downregulating EGFL7 oncoprotein [23]. Thus, whether miR-126-rich exosomes affected EGFL7 expression of OSCC cells was investigated in this study. In qRT-PCR analysis, exogenous miR-126-rich exosomes from SCC4 cells significantly downregulated EGFL7 mRNA level in FaDu cells (Fig. 3D). Taken together, exosomal miR-126 is a TS-miRNA to suppress the oncogenic behaviors and signaling in OSCC cells.

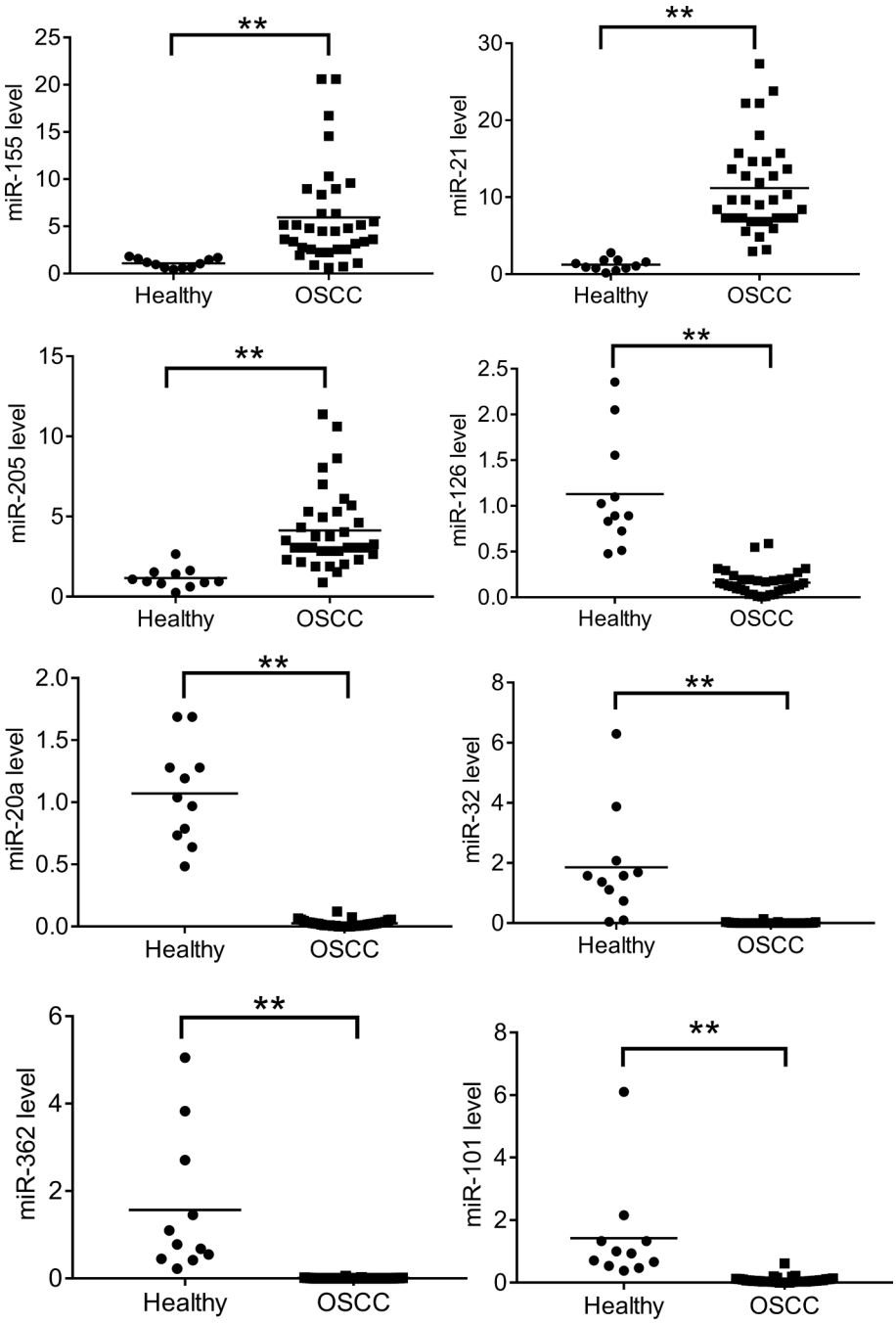

Figure 1. Differential exosomal miRNAs expression in the serum of healthy peoples and OSCC patients. qPCR analysis for exosomal miR-155, miR-21, miR-205, miR-126, miR-20a, miR-32, miR-362, and miR-101 were performed in the serum of healthy persons and OSCC patients. n = 11 in the healthy group; n = 35 in the OSCCs group. All data were mean ± SD (**$p < 0.01$).

The correlation of serum exosomal miRNA with tumor staging and prognosis in OSCC patients

The correlation of serum exosomal miRNA with tumor staging and prognosis of OSCC was investigated in this research. The exosomal miRNAs were extracted from the serum of OSCC patients, and the level of TS-miRNA miR-126 was evaluated. By qRT-PCR analysis, exosomal miR-126 was downregulated in the serum of the OSCC patients, and downregulation of miR-126 was associated with advanced tumor stages (Fig. 4A). To investigate the effect of exosomal miR-126 on the survival of the OSCC patients, we referred the previous study to define miR-126^{High} (miR126 above median) and miR-126^{Low} (miR126 below median) in the serum of the OSCC patients [24]. In Kaplan–Meier analysis, low exosomal miR-126 expression in serum was significantly associated with poor overall survival in OSCC patients (Fig.4B), but exosomal miR-126 expression in serum did not significantly impact the disease-free survival in the OSCC patients (Fig. 4C). Accordingly, we demonstrated for the first time that serum exosomal miRNA may correlate with tumor staging and prognosis of OSCC.

Discussion

Although a few serum miRNAs were proposed as prognostic biomarkers of OSCC, these studies were limited by studying too few miRNAs, using an inadequate inclusion or exclusion criteria, employing a small study population, or having no independent validation [25,26].

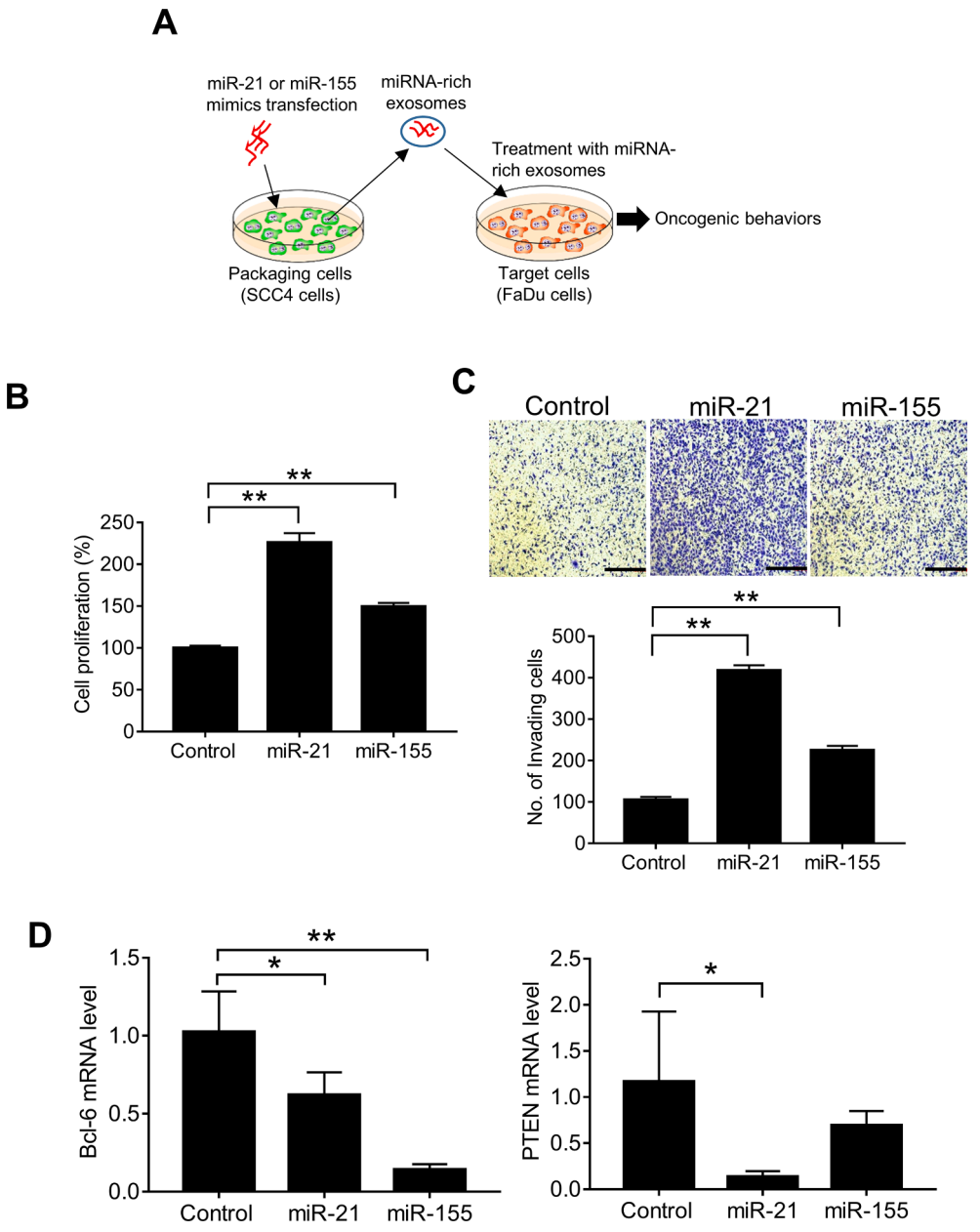

Figure 2. Exosomal miR-21 and miR-155 acting as oncomiRs in OSCC. (A) SCC4 cells were used as packaging cells for the production of miR-rich exosomes, and FaDu OSCC cells were severed as target cells for the validation of oncogenic function. **(B)** MTT assay for cell proliferation in FaDu cells after treatment with miR-21-rich or miR-155-rich exosomes for 72 h. **(C)** Cell invasion assay in FaDu cells after treatment with miR-21-rich or miR-155-rich exosomes for 24 h. Scale bar = 200 μm. **(D)** qRT-PCR analysis for PTEN and BCL-6 in FaDu cells after treatment with miR-21-rich or miR-155-rich exosomes for 48 h. All data were mean ± SD (*$p < 0.05$; **$p < 0.01$).

The aim of this study was to comprehensively select potential exosomal miRNA candidates in the paired normal and cancer cells from OSCC patients using microarray analysis, and further identify specific serum exosomal miRNAs as biomarkers reflecting the progression of OSCC. In this study, we hypothesized that OSCC cells secrete exosomes containing miRNAs in order to transport signals to recipient cells. By miRNA microarray analysis, thirty-one miRNAs were downregulated with at-least 2-fold change in the exosomes of human primary OSCC cells, and twenty-one miRNAs were upregulated (2 to 5 folds) in the exosomes of OSCC cells. Among these miRNAs, miR-155 [11], miR-21 [12], miR-205 [13], miR-126 [14], miR-20a [15], miR-32 [16], miR-362 [17] and miR-101 [18] were associated with the outcome in the OSCC patients. Thus, the expression levels of these eight miRNAs were further validated in the serum of OSCC patients using qRT-PCR analysis, and the results were consistent with the microarray data. miR-155, miR-21 and miR-205 were upregulated in the serum of OSCC patients, and miR-126, miR-20a, miR-32, miR-362 and miR-101 levels were reduced in the serum of OSCC patients. By miRNA-rich exosomes and OSCC cells co-culture assay, miR-21-rich and miR-155-rich exosomes promoted oncogenic behaviors including cell proliferation and cell invasion. Thus, we confirmed that both miR-21 and miR-155 were oncomiRs in OSCC cells. Recent studies also indicated that miR-21 can promote cell invasion, impact disease free survival and act as a biomarker in OSCC [27–29]. Moreover, it has been reported that miR-155 is also an oncomiRs which can promote oncogenic behaviors and impact prognosis in OSCC [19,30,31]. In the gene regulation, we found that both exosomal miR-21 and miR-155 could target BCL6, and PTEN mRNA level was repressed by exosomal miR-21 in OSCC cells. This finding is consistent with or similar to the previous reports [21,22,32]. BC6 is a novel tumor suppressor gene in medulloblastoma by repressing Sonic Hedgehog (SHH) signaling [33], and SHH signaling promotes tumor cell growth and bone destruction in OSCC [34]. Thus, BCL6/SHH axis may participate in the exosomal miR-21- and miR-155-promoted oncogenic processes of OSCC cells. Moreover, PTEN is a well-known tumor suppressor in many types of cancer including OSCC [35], and exosomal miR-21/PTEN axis may play a crucial role in cell proliferation and invasion in OSCC cells.

On the other hand, a TS-miRNA, miR-126, was identified to be downregulated in the exosomes of OSCC cells. miR-126-rich exosomes

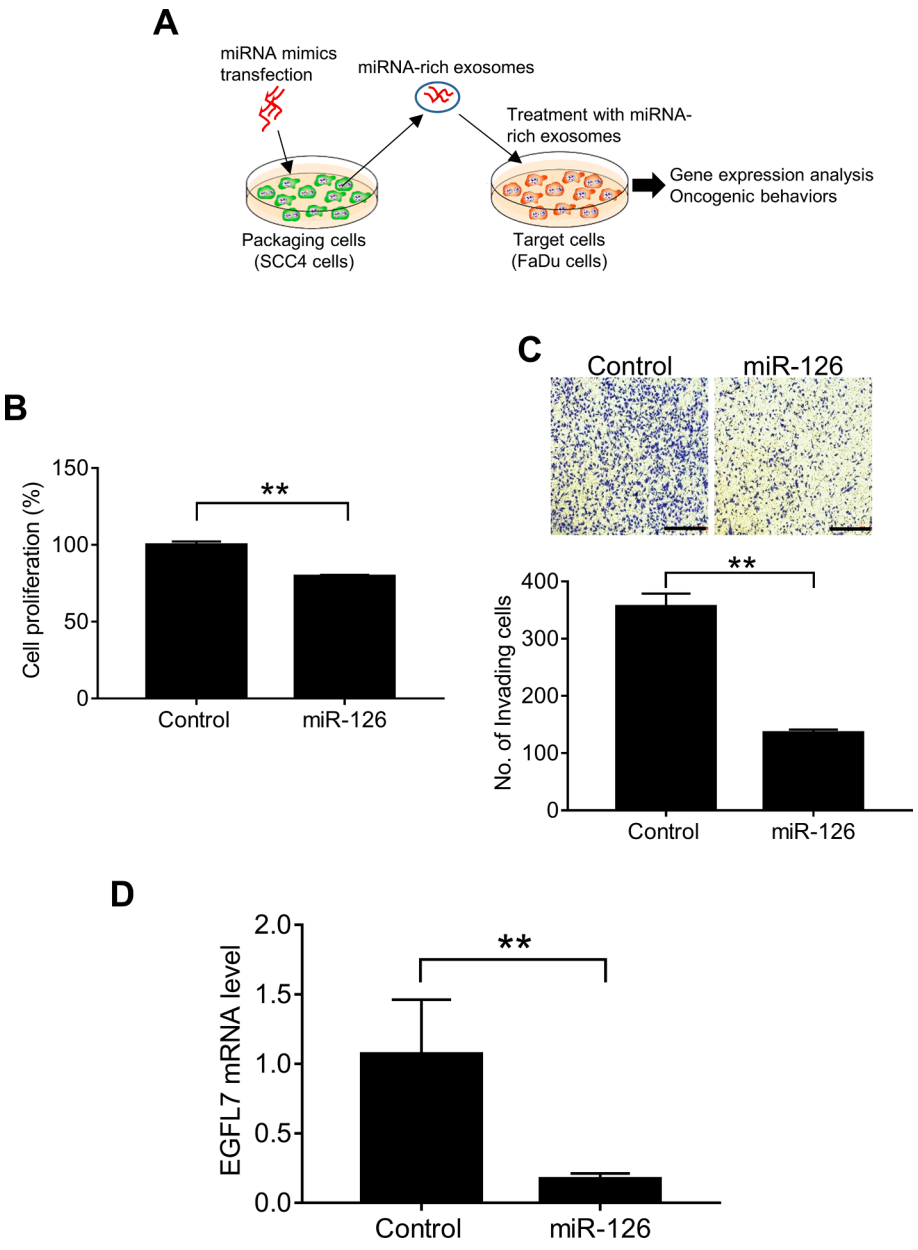

Figure 3. miR-126 acting as a TS-miRNA in OSCC. **(A)** SCC4 cells were used as packaging cells for the production of miR-126 exosomes, and FaDu OSCC cells were severed as target cells for the validation of oncogenic function. **(B)** MTT assay for cell proliferation in FaDu cells after treatment with miR-126-rich exosomes for 72 h. **(C)** Cell invasion assay in FaDu cells after treatment with miR-126-rich exosomes for 24 h. Scale bar = 200 μm. **(D)** qRT-PCR analysis for EGFL7 in FaDu cells after treatment with miR-126-rich exosomes for 48 h. All data were mean ± SD (**$p < 0.01$).

significantly repressed oncogenic processes including cell proliferation and cell invasion in OCSS cells. It has been reported that miR-126 can suppress angiogenesis, lymphangiogenesis and tumorigenesis by targeting EGFL7 in oral cancer [23,36], which is consistent with the result of the present study that miR-126-rich exosomes significantly downregulated EGFL7 mRNA level of OSCC cells. EGFL7 is an oncogene in many types of cancer including OSCC [23,36–40]. In signal transduction, EGFL7 also participates in the activation of VEGF [36], Notch [40] and Wnt [39] signaling. Thus, exosomal miR-126 may affect VEGF, Notch and Wnt pathways mediated by EGFL7 downregulation to inhibit the oncogenic processes of OSCC cells.

The role of oncomiRs miR-21 and miR-155 and TS-miRNA miR-126 had been studied in OSCC cells, but the role of exosomal miR-21, miR-155 and miR-126 have not been investigated in miRNA-rich exosomes and OSCC cells co-culture system. In this study, we demonstrated that exosomal miR-21, miR-155 and miR-126 also participates in the oncogenesis and gene regulation of OSCC for the first time. However, the detailed role and function of these exosomal miRNAs in oral carcinogenesis have not been completely cleared. The detailed exosomal miRNA-impacted signal transduction and oncogenic processes in OSCC should be further investigated in the future. Furthermore, the biological function of exosomal miR-21, miR-155 and miR-126 in OSCC cells should be further validated *in vivo*. On the other hand, betel quid chewing and cigarette smoking are major risk factors of OSCC in Taiwan [41]. In the future work, we would study whether these risk factors participate in the processes and secretion of exosomal miR-21, miR-155 and miR-126 in OCSS cells.

miR-126 level in OSCC tissues is a good prognostic factor [36], but whether serum miR-126 level impacts prognosis of OSCC patients was not reported. In this study, we found that serum miR-126 level was a good prognostic factor in OSCC patients for the first time. Thus, the development of non-invasive kits for serum miRNAs detection would be potentially useful for the prediction of OSCC patient outcome. Furthermore, whether exosomal miRNAs can serve as a therapeutic target should be evaluated in the future.

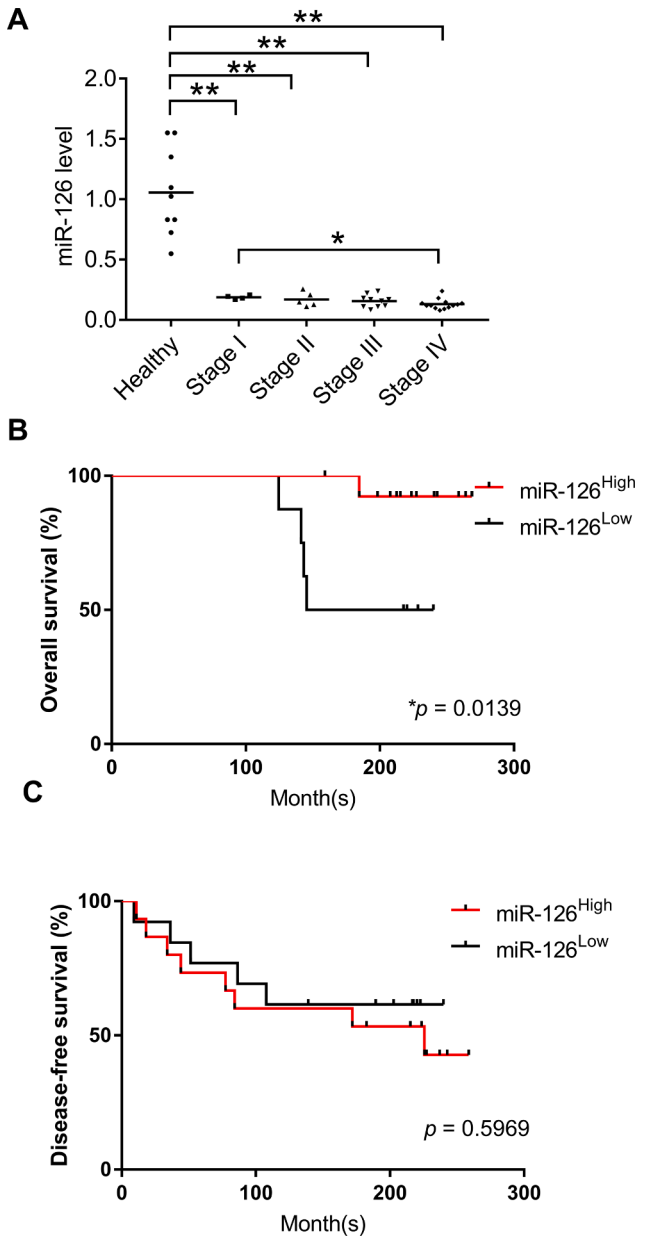

Figure 4. Evaluation of exosomal miR-126 in serum of OSCC patients for tumor staging and prognosis. (A) qRT-PCR analysis for miR-126 in the serum of OSCC patients with different tumor stages. n = 9 in the healthy group; n = 4 in the stage I group; n = 5 in the stage II group; n = 10 in the stage III group; n = 13 in the stage IV group. **(B)** Overall survival analysis in miR-126^{High} and miR-126^{Low} OSCC patients. **(C)** Disease-free survival analysis in miR-126^{High} and miR-126^{Low} OSCC patients. All data were mean ± SD (*p < 0.05, **p < 0.01).

Conclusion

Oncogenic miR-155 and miR-21 from secreted exosome can down-regulate PTEN and Bcl-6 tumor suppressor genes to promote cell proliferation and invasion in OSCC cells, and exosomal miR-126 is a tumor suppressor miRNA which downregulates EGFL7 to repress oncogenic behaviors in OSCC. Furthermore, exosomal miRNAs such as miR-126 in serum may act as non-invasive biomarkers for the diagnosis and prognosis of OSCC.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.oraloncology.2021.105402.

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