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CSN6 promotes malignant progression of oral squamous cell carcinoma by down-regulating TIMP-2

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Abstract. – OBJECTIVE: This study was designed to investigate the expression characteristics of CSN6 in oral squamous cell carcinoma (OSCC), and to further explore the mechanism of how it promotes the malignant progression of this cancer.

PATIENTS AND METHODS: The expressions of CSN6 and TIMP-2 in tumor tissue samples and adjacent normal ones collected from 36 OSCC patients were detected via quantitative Real Time-Polymerase Chain Reaction (qRT-PCR), and the interplay between their expression levels and the clinical indicators or prognosis of OSCC patients was analyzed as well. Meanwhile, the expressions of CSN6 and TIMP-2 in OSCC cell lines were further verified via qRT-PCR. In addition, CSN6 overexpression and knockdown models were constructed using lentivirus in OSCC cell lines, CAL-27, and Tca8113. At the same time, transwell and cell wound healing assays were conducted to uncover the impact of CSN6 on the function of OSCC cells. Finally, the potential mechanism was explored using Luciferase reporter gene and recovery experiments.

RESULTS: In this work, qRT-PCR results revealed that the level of CSN6 in tumor tissues of OSCC patients was remarkably higher than that in adjacent normal ones. Compared with patients with low expression of CSN6, those with high expression CSN6 had a higher incidence of lymph node or distant metastasis and a lower overall survival rate. *In vitro* experiments revealed that silencing CSN6 remarkably attenuated the invasive, as well as migration capacities of OSCC cells while overexpression of CSN6 conversely enhanced those. Subsequently, in OSCC cell lines and tissues, TIMP-2 expression was remarkably reduced, which was negatively correlated with CSN6 level. Bioinformatics and Luciferase reporter genes demonstrated that CSN6 can target the corresponding sites of TIMP-2 promoter. In addition, cell recovery experiments suggested the existence of a mutual regulation between CSN6 and TIMP-2, which may synergistically modulate the malignant progression of OSCC.

CONCLUSIONS: The above results indicated that CSN6 was remarkably upregulated both in OSCC tissues and cell lines, which is remarkably relevant to the incidence of lymph node or distant metastasis and poor prognosis of OSCC patients. Additionally, we verified that CSN6 may promote OSCC malignant progression by regulating TIMP-2.

Key Words:
CSN6, TIMP-2, Oral squamous cell carcinoma, Malignant progression.

Introduction

Oral cancer is a malignant tumor occurring in the oral cavity. The epithelial derived tumor cells are the most common, especially squamous epithelial cells, accounting for 80% of oral and maxillofacial malignancies, ranking 7th in malignant tumors[1,2]. In China, oral squamous cell carcinoma (OSCC) is mostly found in areas including the tongue, buccal mucosa, gums, and palate[3,4]. Among the traditional treatment methods, surgery remains the first choice for the treatment of OSCC[5,6]. Although satisfactory progress has been made in surgical and intraoperative simultaneous repair methods, the results are still not satisfactory due to the particularity of the oral cavity[6,7]. Therefore, how to prevent and treat oral cancer is one of the hotspots of modern medical research[7]. With the development of gene therapy and the application of modern medicine such as cell and molecular biology, increasing specific tumor markers have been found in the occurrence and progression of tumors. Therefore, these specific tumor markers are conducive to promoting early diagnosis and treatment of cancer and optimizing the quality of life of cancer patients[8,9].

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COP9 signaling complex (CSN), first discovered in Arabidopsis mutants, is a highly conserved multi-subunit protein complex related to the regulation of plant phytogenesis[10,11]. In mammals, CSN consists of eight subunits, named CSN1-CSN8[12,13]. As an important subunit of CSN, CSN6 is involved in a variety of biological functions such as embryonic development, cell cycle regulation, and signal transduction[14,15]. In recent years, with the deepening of the research on CSN6, its correlation with tumor has become a research hotspot[16,17]. CSN6 is mainly involved in the occurrence and development of tumors *via* regulating ubiquitin-mediated protein degradation, affecting protein stability[17,18]. According to literature reports[16-18], CSN6 is highly expressed in a variety of malignant tumors (such as breast cancer, ovarian cancer, and colon cancer), so it is expected to be a potential prognostic marker of OSCC.

Currently, metalloproteinase tissue inhibitor 2(TIMP-2), matrix metalloproteinase 9(MMP9), and MMP2 pathways have been found to play a crucial role in progression of malignant tumors[19,20]. The expression of TIMP-2, as a natural inhibitor of MMP2 and MMP9, was found to be reduced in many tumors, including OSCC, but the reason still remains unclear[21,22]. It was found through literature reports and bioinformatics analysis that TIMP-2 may serve as one of the potential target genes of CSN6. Since CSN6 and TIMP-2 have not been reported in OSCC, we aimed to investigate the expression of CSN6 and TIMP-2 in OSCC cell lines and tissues and analyze the interplay between CSN6 and TIMP-2. Meanwhile, their impacts on the prognosis of OSCC patients were also explored to further provide a reliable basis for clinical auxiliary diagnosis and targeted therapy of OSCC.

Patients and Methods

Patients and OSCC samples

Tumor tissue samples and adjacent ones of 36 patients with OSCC were collected. All specimens were obtained from tissue samples from oncology, stomatology, and needle biopsy. In addition, all patients did not receive anti-tumor treatments. The study was approved by the Ethics Committee of the hospital and all patients had signed informed consent. All patients were followed up, for collection of general conditions, clinical symptoms, and imaging examination. This research was conducted in accordance with the Declaration of Helsinki.

Cell Lines and Reagents

Four human OSCC cells (Fadu, SCC-25, CAL-27, Tca8113) and a normal human oral cell line (Hs 680.Tg) were purchased from ATCC (American Type Culture Collection; Manassas, VA, USA), while Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were purchased from Life Technologies (Gaithersburg, MD, USA). Cells were cultured in DMEM medium containing 10% FBS in a 37°C, 5% CO_2 incubator.

Transfection

The control group (NC and Anti-NC) and the CSN6 overexpression and knockdown groups (CSN6 and Anti-CSN6) containing the CSN6 lentiviral sequence were purchased from Shanghai Jima Company, Shanghai, China. Cells were grown to a cell density of 30-40%, and transfection was performed. After 48 h, cells were harvested for quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) analysis and cell functional assays.

Transwell Assay

After transfection for 48 h, cells were digested, centrifuged, and resuspended in medium without FBS, and then the density was adjusted to 5 x 10^5 cells/mL. 200 μL of cell suspension (1 x 105 cells) was added to the upper chamber, while 700 μg of a medium containing 20% FBS was added to the lower chamber. According to the different migration abilities of each cell line, cells were put back into the incubator and continued to culture for a specific time. The transwell chamber was clipped, washed 3 times with 1 x phosphate-buffered saline (PBS), and placed in methanol for 15 min cell fixation. After the chamber was stained in 0.2% crystal violet for 20 min, the cells on the upper surface of the chamber were carefully wiped off with a cotton swab. The perforated cells stained in the outer layer of the basement membrane of the chamber were observed under the microscope, and 5 fields of view were randomly selected.

Cell Wound Healing Assay

After the proportion of cells in the bottom of the chamber >90%, a horizontal line is scratched using a tip of 100 μg pipettor, with the back line of the plate as the reference line, perpendicular to

the ground. After the scratches were completed, the prepared PBS solution was used for washing, the removed and necrotic cells were taken out, the medium containing 10% serum was added again, and the cells were again placed in the original cell culture incubator for culture. At 6 h and 24 h after the test, each group of cell plates was observed and photographed under a microscope (Olympus, Tokyo, Japan).

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted from tissue samples using the TRIzol (Invitrogen, Carlsbad, CA, USA) method. 2 μg of total RNA was added to the 20 μg system for cDNA synthesis with reference to the AMV reverse transcription kit. Real Time-PCR was performed using 2xSYBR Green PCR Master Mix (TaKaRa, Otsu, Shiga, Japan), with cDNA as a template, using 0.4 mol/L primer for amplification. Each sample to be tested set up three parallel samples. The primers were subjected to PCR amplification using β-actin as an internal reference. Data analysis was performed using ABI Step One software (Applied Biosystems, Foster City, CA, USA). The following primers were used for qRT-PCR reaction: CSN6: forward: 5'-ACAGTACAAGCAGGTGTTCAGT-3', reverse: 5'-TGGCGGCTATCTGTCTTTGG-3'; TIMP-2: forward: 5'-GGAAGTGGACTCTGGAAACGA-3', reverse: 5'-CTCGGCCTTTCCTGCAATGA-3'; β-actin: forward: 5'-CCTGGCACCCAGCACAAT-3', reverse: 5'-TGCCGTAGGTGTCCCTTTG-3'.

Western Blot

The transfected cells were lysed using cell lysis buffer, shaken on ice for 30 min, and centrifuged at 14,000 x g for 15 min at 4°C. Total protein concentration was calculated by bicinchoninic acid (BCA) Protein Assay Kit (Pierce, Rockford, IL, USA). Western blot analysis was performed according to standard procedures as follows: the extracted proteins were separated using a 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and subsequently transferred to a polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA), followed by incubation with primary antibodies against CSN6, TIMP-2 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and the secondary antibodies anti-mouse and anti-rabbit purchased from Cell Signaling Technology (Danvers, MA, USA).

Dual-Luciferase Reporter Assay

The wild type and mutant binding sites were synthesized and subcloned into the CSN6 basic plasmid vector. 293T cells were seeded on a 24-well cell culture plate for subsequent experiment. This study used a Dual-Luciferase reporter system (Promega, Madison, WI, USA) to assess Luciferase activity.

Statistical Analysis

Data analysis was performed using Statistical Product and Service Solutions (SPSS) 19.0 statistical software (SPSS IBM, Armonk, NY USA). The difference in expression between CSN6 and TIMP-2 in tumor and adjacent tissues of OSCC patients was analyzed by analysis of variance followed by Post-Hoc Test (Least Significant Difference). The relationship between the expression of CSN6 and TIMP-2 and its clinical parameters in OSCC patients was analyzed by Chi-Square test. The relationship between CSN6 and TIMP-2 expression and prognosis of OSCC patients was analyzed by Kaplan-Meier method. Data were statistically significant with mean ± standard deviation and $p< 0.05$.

Results

CSN6 was Upregulated in Human OSCC Tissues and Cell Lines

We detected by qRT-PCR that the expression level of CSN6 in tumor tissues of OSCC patients was remarkably higher than that in adjacent tissues, and the difference was statistically significant (Figure 1A and 1B). Consistently, CSN6 was also found significantly higher in the OSCC cell line than that in Hs 680.Tg, the normal human oral cell line (Figure 1C).

CSN6 Expression was Correlated with Metastasis and Overall Survival Rate of OSCC Patients

Chi-square test was used to analyze the interplay between CSN6 expression and some clinical indicators, such as age, gender, pathological stage, and distant metastasis of OSCC patients. As shown in Table I, CSN6 expression was markedly associated with the incidence of lymph node or distant metastasis; however, no significant correlation was found between CSN6 expression and age, gender, and pathological stage of OSCC patients. In addition, to explore the interplay between CSN6 expression and the prognosis of

Table I. Association of CSN6 and TIMP-2 expression with clinicopathologic characteristics of oral squamous cell carcinoma.

Parameters	No. of cases	CSN6 expression		*p*-value	TIMP-2 expression		*p*-value
		Low (%)	High (%)		High (%)	Low (%)	
Age (years)				0.298			0.616
<60	13	8	5		4	9	
≥60	23	10	13		9	14	
Gender				0.505			0.729
Male	18	10	8		6	12	
Female	18	8	10		7	11	
T stage				0.298			0.825
T1-T2	23	13	10		8	15	
T3-T4	13	5	8		5	8	
Lymph node metastasis				**0.034**			0.064
No	24	15	9		11	13	
Yes	12	3	9		2	10	
Distance metastasis				**0.015**			0.052
No	23	15	8		11	12	
Yes	13	3	10		2	11	

OSCC patients, we collected relevant follow-up data and plotted Kaplan-Meier survival curves, and it was revealed that high expression of CSN6 was remarkably relevant to the poor prognosis of OSCC ($p<0.05$; Figure 1D).

Effects of CSN6 on Cell Invasiveness and Migration

To figure out the influence of CSN6 on OSCC cell migration and invasion capacity, we constructed CSN6 overexpression model in CAL-27

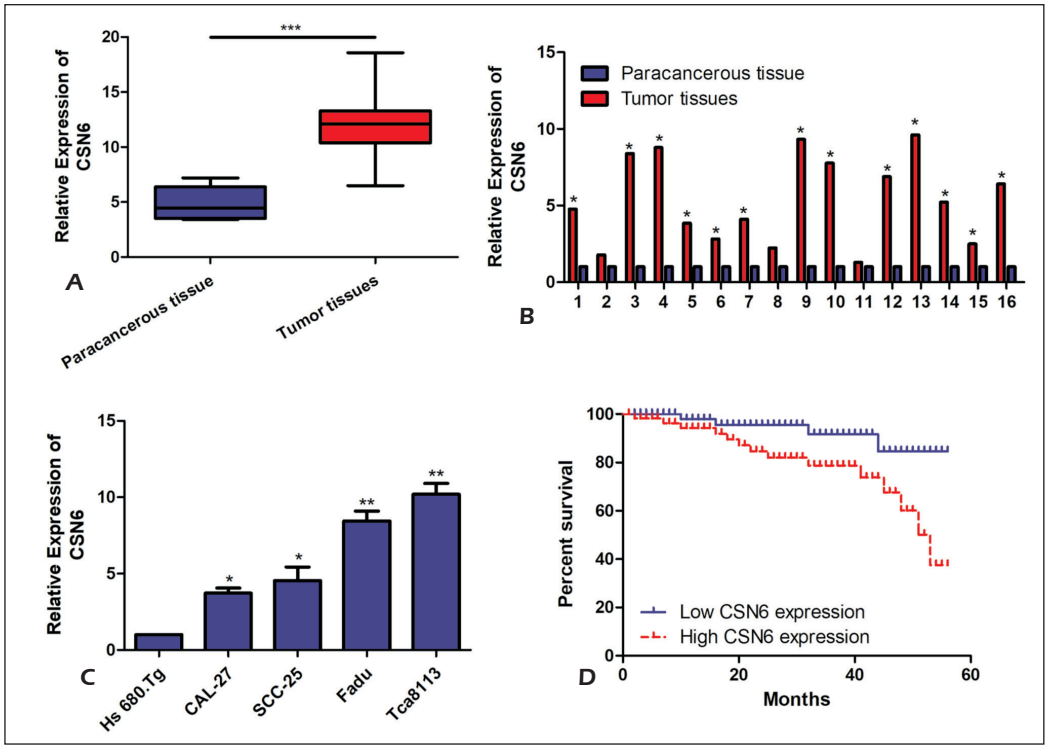

Figure 1. CSN6 is highly expressed in OSCC tissues and cell lines. **A-B**, QRT-PCR was used to detect the difference of CSN6 expression in tumor tissues and adjacent tissues of patients with OSCC. **C**, QRT-PCR was used to detect the expression level of CSN6 in OSCC cell lines. **D**, Kaplan Meier survival curve of patients with OSCC based on CSN6 expression is shown; the higher the expression of CSN6, the worse the prognosis of OSCC patients. Data are shown as mean ± SD, *$p<0.05$, **$p<0.01$, ***$p<0.001$.

cell line and knockdown model in Tca8113 cell line, and then verified the transfection efficiency by Western Blot and qRT-PCR experiments (Figure 2A). Subsequently, transwell invasion and cell wound healing assays revealed that compared with control group, overexpression of CSN6 markedly enhanced cell ability to metastasize, which was inhibited by CSN6 silencing (Figure 2B and 2C).

CSN6 Can Bind to TIMP-2

Western Blot and qRT-PCR experiments revealed that TIMP-2 expression increased in CSN6-silencing group, while the opposite result was observed in CSN6 over-expressing group (Figure 3A and 3B). To further validate the effect of CSN6 on TIMP-2, CSN6 and TIMP-2 were co-transfected into CAL-27 and Tca8113 cell lines for Luciferase reporter

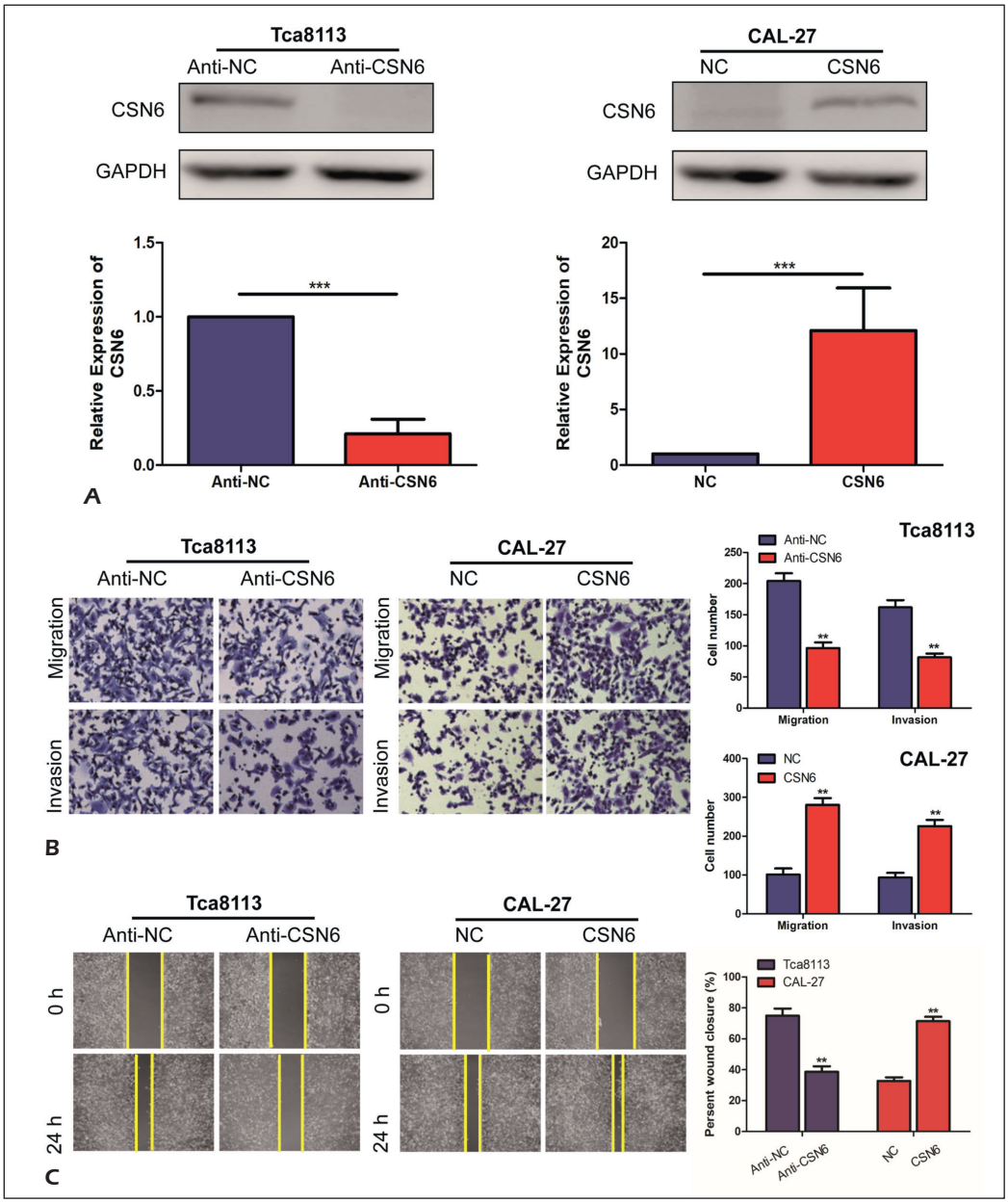

Figure 2. Effects of CSN6 on the ability of OSCC to metastasize. **A**, Transfection efficiency of CSN6 after transfection of CSN6 overexpression or knockout vector in CAL-27 and Tca8113 cell lines was detected by Western Blot and qRT-PCR. **B**, Transwell invasion and migration assays were performed to show the invasion and migration of oral squamous carcinoma cells after transfecting CSN6 vectors in CAL-27 and Tca8113 cell lines (magnification: 40×). **C**, Cell wound healing assay detected the invasive ability of OSCC cells in the CAL-27 and Tca8113 cell lines (Magnification: 40x). Data are shown as mean ± SD, $^{**}p<0.01$, $^{***}p<0.001$.

gene experiments. The results indicated that TIMP-2 could be bound to CSN6 through the binding site (Figure 3C). In addition, qRT-PCR detected that TIMP-2 mRNA expression level was remarkably reduced in tumor tissues of OSCC patients compared with adjacent ones (Figure 3D); therefore, a negative correlation between CSN6 and TIMP-2 was discovered (Figure 3E).

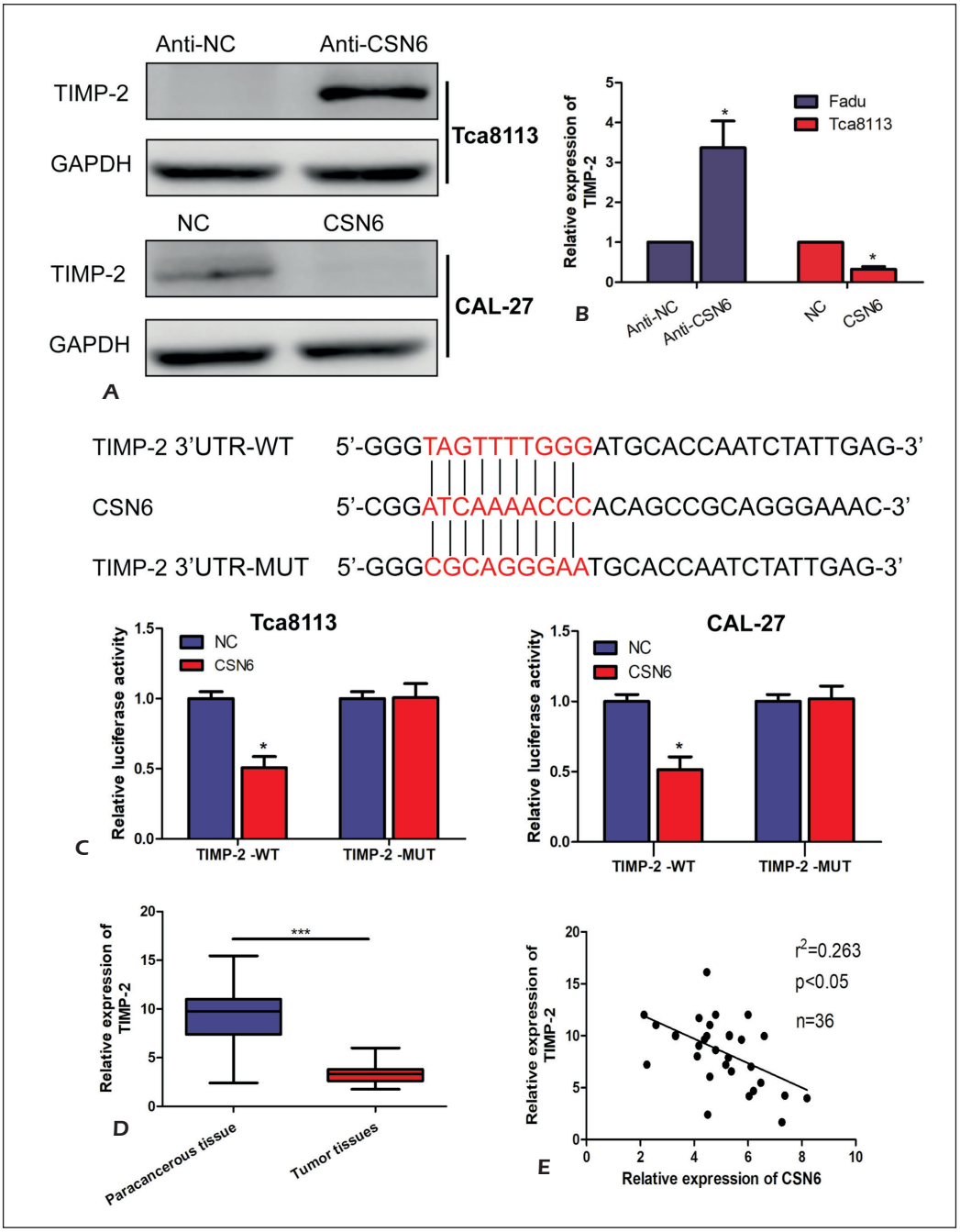

Figure 3. CSN6 can target bind to TIMP-2. **A**, Western Blot detected differential expression of TIMP-2 after overexpression or silencing of CSN6. **B**, Differential expression of TIMP-2 after overexpression or silencing of CSN6 by qRT-PCR. **C**, Dual-Luciferase reporter gene assay verified the direct targeting of CSN6 and TIMP-2. The results of the Dual-Luciferase reporter gene assay in the CAL-27 and Tca8113 cell lines indicated that overexpression of TIMP-2 significantly attenuated the luciferase activity of the wild-type CSN6 vector without attenuating that of the mutant vector ($p>0.05$). **D**, QRT-PCR was used to detect the expression of TIMP-2 in tumor tissues and adjacent tissues of patients with OSCC. **E**, There was a significant negative correlation between the expression levels of CSN6 and TIMP-2 in OSCC. Data are mean ± SD, *$p<0.05$, ***$p<0.001$.

Effects of TIMP-2 on Cell Migration and Invasion Ability

To figure out the influence of TIMP-2 on OSCC cell migration and invasion capacity, we constructed TIMP-2 overexpression model in CAL-27 cell line and knockdown model in Tca8113 cell line, and then verified the transfection efficiency by Western blot and qRT-PCR experiments (Figure 4A). Subsequently, we demonstrated through transwell and cell wound healing assay that compared with NC group, the ability of OSCC cells to metastasize was remarkably reduced after overexpression of TIMP-2, while the opposite ability was observed after knockdown of TIMP-2 (Figure 4B and 4C).

CSN6 Modulated TIMP-2 Expression in Human OSCC Cells

To further explore the relationship between CSN6 and TIMP-2 in OSCC cells, we overexpressed TIMP-2 in OSCC cells with CSN6 knockdown, or downregulated TIMP-2 in OSCC cells with CSN6 overexpression, verified the transfection efficiency by Western blot and qRT-PCR experiments (Figure 5A). Subsequently, the results of transwell invasion and migration experiments suggested that TIMP-2 can counteract the effect of CSN6 on the migration ability of OSCC cells (Figure 5B).

Discussion

Oral squamous cell carcinoma (OSCC) is the most common malignant tumor among oral tumors[1-3]. At present, surgical resection is still the main treatment method for OSCC[4,5]. However, most patients have been diagnosed in advanced stage, and the high incidence of relapse and metastasis results in a poor prognosis. Therefore, effective inhibition of malignant metastasis of tumor cells is the key to the treatment of OSCC[6-8].

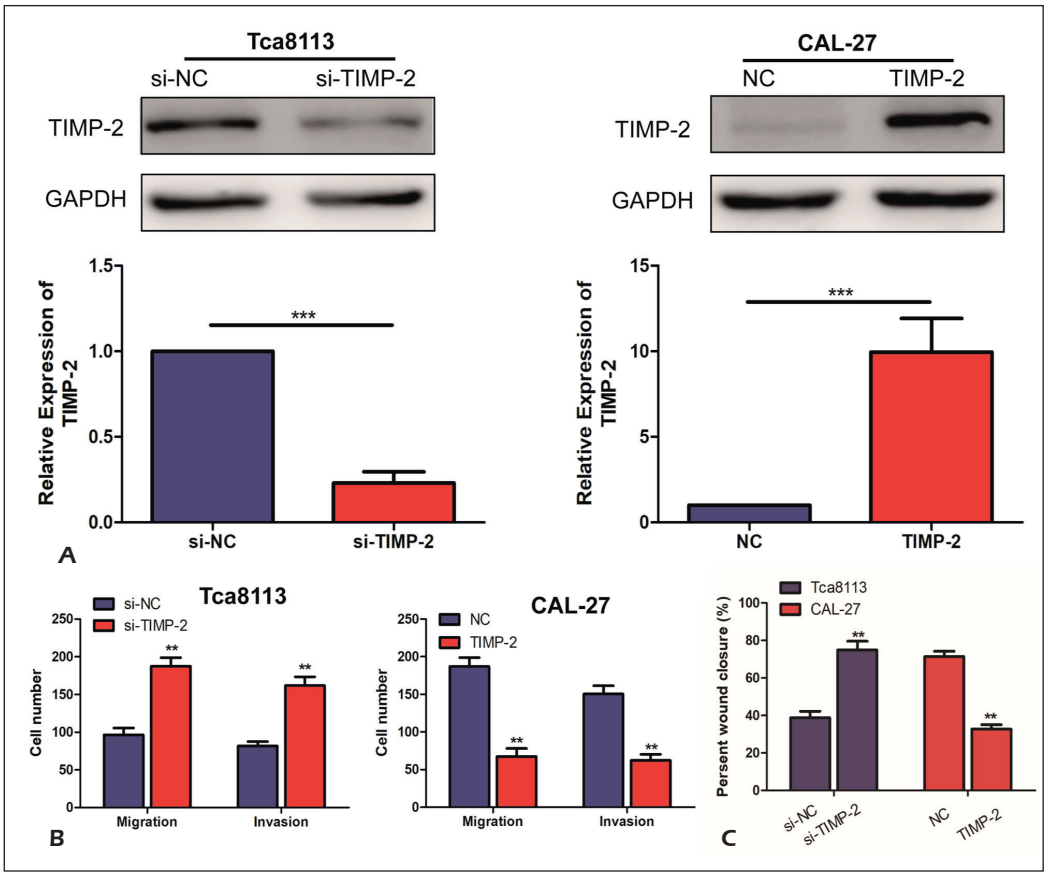

Figure 4. Effects of TIMP-2 on OSCC metastasis. **A**, Western Blot and qRT-PCR validated the transfection efficiency after transfection of TIMP-2 overexpression or silencing vector in CAL-27 and Tca8113 cell lines. **B**, Transwell invasion and migration assays tested the invasion and migration ability of OSCC cells in CAL-27 and Tca8113 cell lines after overexpressing or silencing TIMP-2. **C**, Cell wound healing assay revealed the invasion ability of OSCC cells in CAL-27 and Tca8113 cell lines after overexpressing or silencing TIMP-2. Data are mean ± SD, $^{**}p<0.01$, $^{***}p<0.001$.

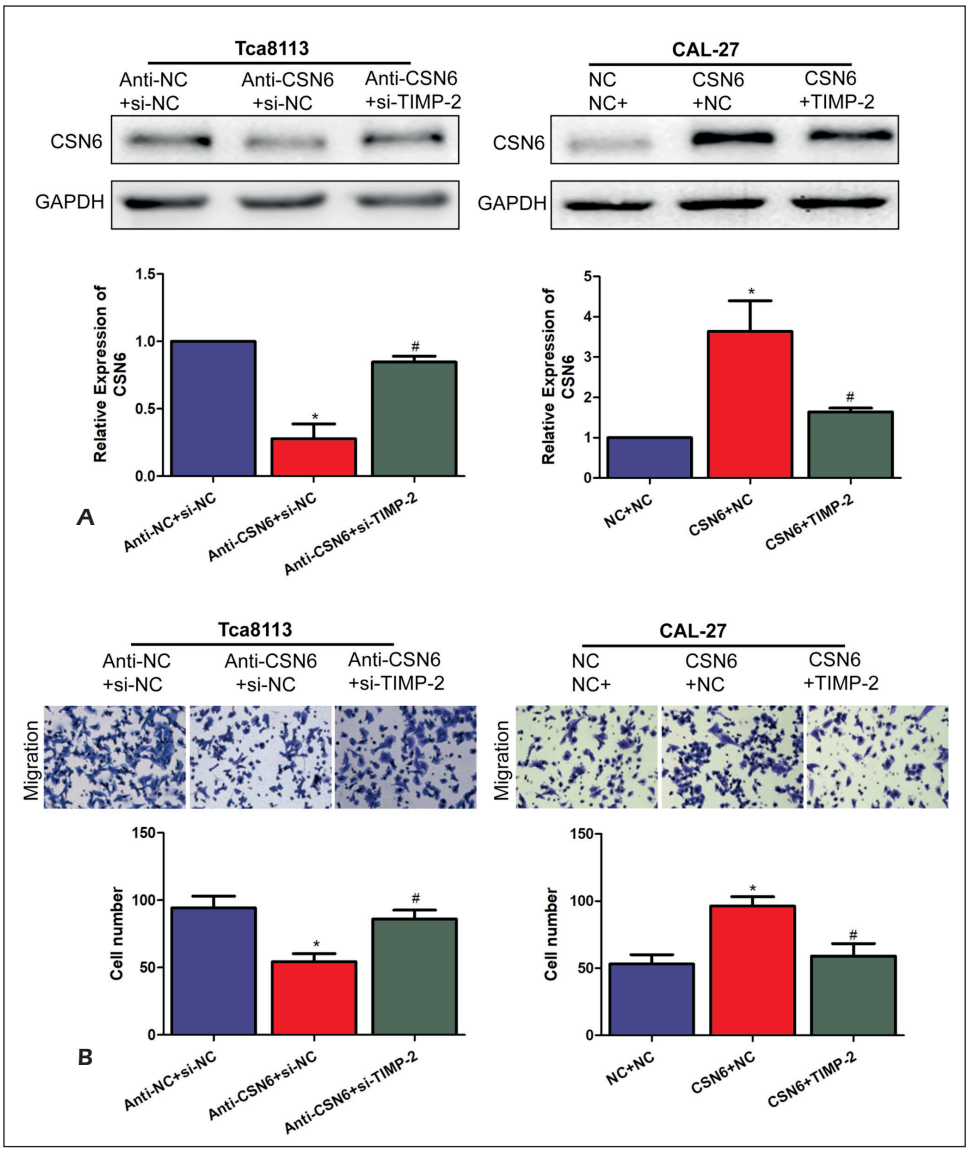

Figure 5. CSN6 regulates the expression of TIMP-2 in OSCC. **A**, CSN6 expression levels were detected by Western Blot and qRT-PCR after co-transfection of CSN6 and TIMP-2 in OSCC cell lines. **B**, Transwell migration assay detected the impact of CSN6 and TIMP-2 on metastasis ability of OSCC cells CAL-27 and Tca8113 cell lines after co-transfection of CSN6 and TIMP-2 (magnification: 40×). Data are shown as mean ± SD, $*^{\#}p<0.05$.

The occurrence and development of tumors is a complex process in which multiple factors are engaged. Under the combined effects of various pathogenesis (including environmental factors and genetic factors), the mutations of DNA in normal human cells activate proto-oncogenes and impair the function of tumor suppressor genes, resulting in loss of control of normal cell growth and division[23,24]. Although sharing the same genome, malignant cells and normal cells have different gene activity states, resulting in different protein synthesis, which ultimately leads to different cell functions[25,26]. Therefore, detection of tumor markers may provide a more convenient, rapid, accurate, and economical method for early diagnosis and prognosis improvement of tumors[27].

CSN6, as a subunit of COP9, was originally discovered from Arabidopsis mutants, which can simulate the development of seedlings in dark environments due to light[12-15]. Recent attention has been given to the fact that CSN6 as an indicator is often found in esophageal squamous cell carcinoma, breast cancer, pancreatic cancer, and leukemia[16-18]. In this study, different expression levels

of CSN6 and TIMP-2 were observed in tumor and adjacent tissue samples of 36 OSCC patients. qRT-PCR revealed that CSN6 expression was remarkably up-regulated while TIMP-2 was conversely downregulated in OSCC tumor tissues. In addition, CSN6 was observed to be remarkably associated with the incidence of lymph node or distant metastasis and poor prognosis in OSCC patients. Therefore, we concluded that CSN6 acts as a cancer-promoting gene while TIMP-2 plays an anti-cancer role in this cancer. Moreover, the results of transwell invasion, migration, and cell wound healing assays also demonstrated that CSN6 could promote the malignant progression of OSCC and play a pivotal role in OSCC; however, its specific molecular mechanism still remains elusive.

If an oncogene can completely complement its target gene, both mRNA and protein levels will be changed, so as to truly regulate a predicted target gene; if there is no complementary pairing, only the protein level of the target gene will be changed[28,29]. Metalloproteinase-tissue inhibitor 2 (TIMP-2), a natural inhibitor of MMPs, has long been known as one of tumor suppressors[28,29]. Relevant literature reported that the higher the degree of malignancy of the tumor, the lower the protein expression level of TIMP-2, and the stronger the malignant progression of the tumor, indicating that TIMP-2 may play a pivotal role in the metastasis of OSCC cells. We revealed that TIMP-2 was less expressed in tumor tissues of patients with OSCC than that in adjacent tissues, thus inhibiting the invasion and migration of OSCC cells. Bioinformatics method predicted that CSN6 DNA promoter sequence contained a TIMP-2 binding site, and the direct binding between CSN6 and downstream TIMP-2 was verified by Dual-Luciferase reporting assay. The above observations suggested that CSN6 is able to cause a rapid decrease in TIMP-2 expression, thus promoting the malignant progression of OSCC.

Conclusions

In summary, the expression of CSN6 in OSCC tissues and cell lines was remarkably increased, which was significantly correlated with the incidence of lymph node or distant metastasis and poor prognosis of OSCC patients. In addition, this study suggested that CSN6 may promote the malignant progression of OSCC *via* regulating TIMP-2.

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Conflict of Interests

The authors declare that they have no conflict of interest.

References

1) Gigliotti J, Madathil S, Makhoul N. Delays in oral cavity cancer. Int J Oral Maxillofac Surg 2019; 48: 1131-1137.
2) Vatsyayan A, Mandlik D, Patel P, Patel P, Sharma N, Joshipura A, Patel M, Odedra P, Dubbal JC, Shah DS, Kanhere SA, Sanghvi KJ, Patel K. Metastasis of squamous cell carcinoma of the head and neck to the thyroid: a single institution's experience with a review of relevant publications. Br J Oral Maxillofac Surg 2019; 57: 609-615.
3) Jiang S, Dong Y. Human papillomavirus and oral squamous cell carcinoma: a review of HPV-positive oral squamous cell carcinoma and possible strategies for future. Curr Probl Cancer 2017; 41: 323-327.
4) Wang SS, Tang YL, Pang X, Zheng M, Tang YJ, Liang XH. The maintenance of an oral epithelial barrier. Life Sci 2019; 227: 129-136.
5) Olek M, Kasperski J, Skaba D, Wiench R, Cieslar G, Kawczyk-Krupka A. Photodynamic therapy for the treatment of oral squamous carcinoma-Clinical implications resulting from in vitro research. Photodiagnosis Photodyn Ther 2019; 27: 255-267.
6) Chegini S, Mitsimponas K, Shakib K. A review of recent advances in histopathological assessment of head and neck squamous cell carcinoma. J Oral Pathol Med 2019 Aug 6. doi: 10.1111/jop.12943. [Epub ahead of print]
7) Garg M, Tudor-Green B, Bisase B. Current thinking in management of the neck (including contralateral neck) in ipsilateral recurrent or second primary oral squamous cell carcinoma. Br J Oral Maxillofac Surg 2019; 57: 711-715.
8) Lorenzo-Pouso AI, Perez-Sayans M, Rodriguez-Zorrilla S, Chamorro-Petronacci C, Garcia-Garcia A. Dissecting the proton transport pathway in oral squamous cell carcinoma: state of the art and theranostics implications. Int J Mol Sci 2019; 20: 4222.
9) Yap T, Celentano A, Seers C, McCullough MJ, Farah CS. Molecular diagnostics in oral cancer and oral potentially malignant disorders-A clinician's guide. J Oral Pathol Med 2019: (DOI: 10.1111/jop.12920).
10) Milic J, Tian Y, Bernhagen J. Role of the COP9 signalosome (CSN) in cardiovascular diseases. Biomolecules 2019; 9. pii: E217.
11) Li P, Xie L, Gu Y, Li J, Xie J. Roles of multifunctional COP9 signalosome complex in cell fate and implications for drug discovery. J Cell Physiol 2017; 232: 1246-1253.

12) Meister C, Gulko MK, Kohler AM, Braus GH. The devil is in the details: comparison between COP9 signalosome (CSN) and the LID of the 26S proteasome. Curr Genet 2016; 62: 129-136.

13) Pick E, Bramasole L. Moonlighting and pleiotropy within two regulators of the degradation machinery: the proteasome lid and the CSN. Biochem Soc Trans 2014; 42: 1786-1791.

14) Xu M, Zhen L, Lin L, Wu K, Wang Y, Cai X. Overexpression of CSN6 promotes the epithelial-mesenchymal transition and predicts poor prognosis in hepatocellular carcinoma. Clin Res Hepatol Gastroenterol 2019 Aug 14. pii: S2210-7401(19)30178-0. doi: 10.1016/j.clinre.2019.07.012. [Epub ahead of print]

15) Jhan JH, Lee YC, Li WM, Chang LL, Hsu WC, Lin HH, Liang PI, Hsu YL, Wu WJ, Lee HY, Li CC, Yeh HC, Ke HL. The prognostic value of CSN6 expression in upper tract urothelial carcinomas. Kaohsiung J Med Sci 2019; 35: 559-565.

16) Choi HH, Lee MH. CSN6-COP1 axis in cancer. Aging (Albany NY) 2015; 7: 461-462.

17) Mao Z, Chen C, Pei DS. The emerging role of CSN6 in biological behavior and cancer progress. Anticancer Agents Med Chem 2019; 19: 1198-1204.

18) Chen J, Shin JH, Zhao R, Phan L, Wang H, Xue Y, Post SM, Ho CH, Chen JS, Wang E, Zhou Z, Tseng C, Gully C, Velazquez-Torres G, Fuentes-Mattei E, Yeung G, Qiao Y, Chou PC, Su CH, Hsieh YC, Hsu SL, Ohshiro K, Shaikenov T, Wang H, Yeung SC, Lee MH. CSN6 drives carcinogenesis by positively regulating Myc stability. Nat Commun 2014; 5: 5384.

19) Peyronnet B, Richard C, Bendavid C, Naudet F, Hascoet J, Brochard C, Senal N, Jezequel M, Alimi Q, Khene ZE, Corlu A, Clement B, Siproudhis L, Bouguen G, Kerdraon J, Manunta A, Game X. Urinary TIMP-2 and MMP-2 are significantly associated with poor bladder compliance in adult patients with spina bifida. Neurourol Urodyn 2019; 38: 2151-2158.

20) Chen MK, Liu YT, Lin JT, Lin CC, Chuang YC, Lo YS, Hsi YT, Hsieh MJ. Pinosylvin reduced migration and invasion of oral cancer carcinoma by regulating matrix metalloproteinase-2 expression and extracellular signal-regulated kinase pathway. Biomed Pharmacother 2019; 117: 109160.

21) Zhang CC, Hoffelt D, Merle U. Urinary cell cycle arrest biomarker [TIMP-2].[IGFBP7] in patients with hepatorenal syndrome. Biomarkers 2019; 24: 692-699.

22) Takahashi Y, Kobayashi T, D'Alessandro-Gabazza CN, Toda M, Fujiwara K, Okano T, Fujimoto H, Asayama K, Takeshita A, Yasuma T, Nishihama K, Inoue R, Qin L, Takei Y, Taguchi O, Gabazza EC. Protective role of matrix metalloproteinase-2 in allergic bronchial asthma. Front Immunol 2019; 10: 1795.

23) Pethe AM, Yadav KS. Polymers, responsiveness and cancer therapy. Artif Cells Nanomed Biotechnol 2019; 47: 395-405.

24) Yousefi B, Mohammadlou M, Abdollahi M, Salek FA, Karbalaei M, Keikha M, Kokhaei P, Valizadeh S, Rezaiemanesh A, Arabkari V, Eslami M. Epigenetic changes in gastric cancer induction by Helicobacter pylori. J Cell Physiol 2019; 234: 21770-21784.

25) Ayala-Cuellar AP, Cho J, Choi KC. Toll-like receptors: a pathway alluding to cancer control. J Cell Physiol 2019; 234: 21707-21715.

26) Jadideslam G, Ansarin K, Sakhinia E, Babaloo Z, Abhari A, Ghahremanzadeh K, Khalili M, Radmehr R, Kabbazi A. Diagnostic biomarker and therapeutic target applications of miR-326 in cancers: a systematic review. J Cell Physiol 2019; 234: 21560-21574.

27) Singh N, Kumar P, Riaz U. Applications of near infrared and surface enhanced Raman scattering techniques in tumor imaging: a short review. Spectrochim Acta A Mol Biomol Spectrosc 2019; 222: 117279.

28) Re A, Joshi T, Kulberkyte E, Morris Q, Workman CT. RNA-protein interactions: an overview. Methods Mol Biol 2014; 1097: 491-521.

29) Higgs PG, Lehman N. The RNA world: molecular cooperation at the origins of life. Nat Rev Genet 2015; 16: 7-17.