Up-regulation of miR-21 in Oral Squamous Cell Carcinoma

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Introduction: Oral squamous cell carcinoma (OSCC) is the most abundant dysplasia in the oral cavity that aberrant expression of microRNA, which plays an important role in cancer progression. The microRNA-21 expression is known as oncogenes or tumor suppressor factor in malignancy formation of several cancers. In this study, OSCC tissues was used to investigate the expression of mir-21 in malignancy causes. **Materials and Methods**: Normal samples and OSCC tissues were collected from Amir Alam and Taleghani Hospitals (Tehran, Iran) as fresh and frozen samples. The Expression level of miR-21 in OSCC and normal tissues were determined by qRT-PCR. To find the targets that related to mir-21 was used databases, including TargetScan, GenTrail2, GO and STITCH online websites. **Results**: Up-regulation of MiR-21 was found significantly in OSCC tissues compared to normal tissues (Fold-change=5.54). Targets of miR-21 derived from the TargetScan and GeneTrail2 analysis determined the most significant biological processes associated with the epithelialization, differentiation and morphogenesis. Overexpression of miR-21 could reverse this process and promote the cells to stemness and metastatic state. Importance of miR-21two targets (epidermal growth factor receptor and programmed cell death 4) previously demonstrated in OSCC invasion, metastasis and differentiation. **Conclusion**: Our findings indicated that miR-21 act as an oncomiR in OSCC and may be considered as a biomarker for the development of OSCC treatment.

Keywords: Oral squamous cell carcinoma (OSCC); miR-21; Bioinformatics

Introduction

One of the most common cancers in the oral cavity is squamous cell carcinoma (SCC). Oral squamous cell carcinoma (OSCC) is recognized as an aggressive properties and metastasis of lymphatic tissues. The OSCC is sixth cancer with the prevalence of 5 to 17 percent among advanced cancers and 5 years survival rate of the cancer is less than 70%. The prevalence of OSCC in developed countries is included 3% of all cancers. Prevalence of the cancer in Afghanistan, Pakistan and India reported more than 50% of all the human cancers but there are no specific reports of its prevalence in Iran. The common risk factors in oral cancer, includes sex, age, smoking and alcohol consumption (1).

The fundamental characteristics of cancer are deregulations of gene expression. Misregulation of microRNA has led to progression of OSCC. Micro-RNAs have recently used as biomarkers in the early detection of cancers. The length of micro-RNAs is about 22 nucleotides that are considered as non-coding genes (2). Micro-RNAs are considered as oncogenes or tumor suppressor by altering cancer-associated genes expression. Therefore, micro-RNAs are used as biological markers for diagnosis, prediction and treatment of cancers (3).

In the present study, we focused on the expression of MiR-21 in OSCC tissue samples to determine its role as an oncogene or tumor suppressor gene. MicroRNA-21 was the first introduced micro-RNA and was detected as an oncogene in breast, prostate, gastric and lung cancers (4). Up-regulation of miRNA-21 in the breast has been shown to associate with apoptosis and cell growth (5). However, expression of miR-21 in OSCC among the Iranian population has not been studied. This study investigated the expression level of miR-21 in oral cancer tissues in comparison with normal samples.

Materials and Methods

Sampling:

Eighteen cancerous tissues, i.e., OSCC grade I and II and normal tissue samples, were taken from tumors bank of pathology Department of Amir Alam Hospital, Tehran, Iran. Fresh tissues collected from Department of oral and maxillofacial surgery of

Table 1. Nine enriched prominent among 1887 pathways in the GO-biological process database

GO NO.	Name	Number of hits	q-value
GO:0060562	epithelial tube morphogenesis	86	2.10e-22
GO:0021537	telencephalon development	71	1.89e-21
GO:0048863	stem cell differentiation	75	2.29e-20
GO:0001701	in utero embryonic development	85	8.63e-20
GO:0060070	canonical Wnt signaling pathway	73	5.03e-19
GO:0003390	dendrite development	56	2.23e-17
GO:0060560	developmental growth involved in morphogenesis	58	6.67e-16
GO:0021953	central nervous system neuron differentiation	52	7.58e-16
GO:0048562	embryonic organ morphogenesis	68	9.62e-16

Taleghani Hospital, Tehran, Iran. The samples include 60% females and 40% males in the range of 30-60 years old. This study was approved by research ethics committee of Shahid Beheshti University of Medical Sciences (No. IR.SBMU.RETECH.REC.1396.126)

Real-Time PCR:

The tissues were used for RNA extraction using TRIzol reagent followed by PureLink RNA Mini Kit according to the manufacture protocol. The optical density of extracted RNAs (at 260/280 nm) was evaluated by BioPhotometer (Eppendorf AG, 22331 Hamburg) for quality control. Samples also were randomly selected and ran on the agarose gel in order to observe the 18s and 28s bands.Processes of cDNA reverse transcription were carried out using the thermal cycler, an Agilent kit protocol and the U6-F (AAATTGGAACGATACAGAGAAG) was used as the control. The miR-21 forward primer was as follows GTAGCTTATCAGACTGATGTTGA. Real-Time PCR performed using SYBR Green and Eva Green. In present study, Eva Green fluorescent color used to determine the quality and quantity of the qRT-PCR products. Real-Time-PCR approach was made by ABI applied Biosystems and StepOne software (v2.1) to determine the quantity of expression. The quality of micro-RNA expression was investigated using melt-curves in order to study dimers. This analysis can be significantly amplified gene expression, product concentration and purity of the product achieved in each sample. The best products did not contain any dimers and in the following step read cycle threshold (CT). In order to the comparison of MiR-21 expression in cancer tissues and control, we selected ΔΔCT (CT cancer – CT normal) as an accurate method and presented relative fold change in gene expression level as $2^{\Delta\Delta CT}$.

Statistical and Bioinformatics Analysis:

For statistical analysis, Prism and SPSS software were used. Comparison between two groups performed by applying t-test in which the statistical significance rate was assumed $p<0.05$. Software used in this study included the Gene Runner and Oligo design to design primers.

Bioinformatics analysis studied ran as the following steps: MiR-21-related targets were extracted from TargetScan (http://www.TargetScan .org), to find gene ontology (GO) used GeneTrail2 (https://genetrail2.bioinf.uni-sb.de) (6). GO of biological process categories related to targets of miR-21 in GeneTrail2 web server was selected with the lowest q value, To identify the drug-protein interaction used STITCH database (http://stitch.embl.de), Also used drug bank (http://www.drugbank.ca) to analyze the drugs related targets.

Results

OSCC and surrounding normal tissues were collected and extracted RNAs to determine the expression of miR-21 in cancer tissue and compare to normal one. In Figure 1A, represented the bar charts based on the amount drawn ΔCT of cancer and normal samples that were a significant difference between the two groups. In figure 1B, miR-21 in OSCC tissues was up-regulated with $P<0.01$ and fold change was 5.54.

TargetScan web server was used for bioinformatics analysis to find the miR-21 target genes. 3667 targets of miR-21 were extracted from TargetScan. The molecular mechanism was studied using Genetrail2, which represented 1887 significant records. The result of the miR-21 target in GO analysis determined the specified biological process remarkable in Table 1. STITCH analysis of target genes of miR-21detremied CI-1033 as a drug with inhibitor function that is significantly associated with target genes. Furthermore, analysis of CI-1033 in the drug bank online database revealed its administration in esophageal squamous cell cancer.

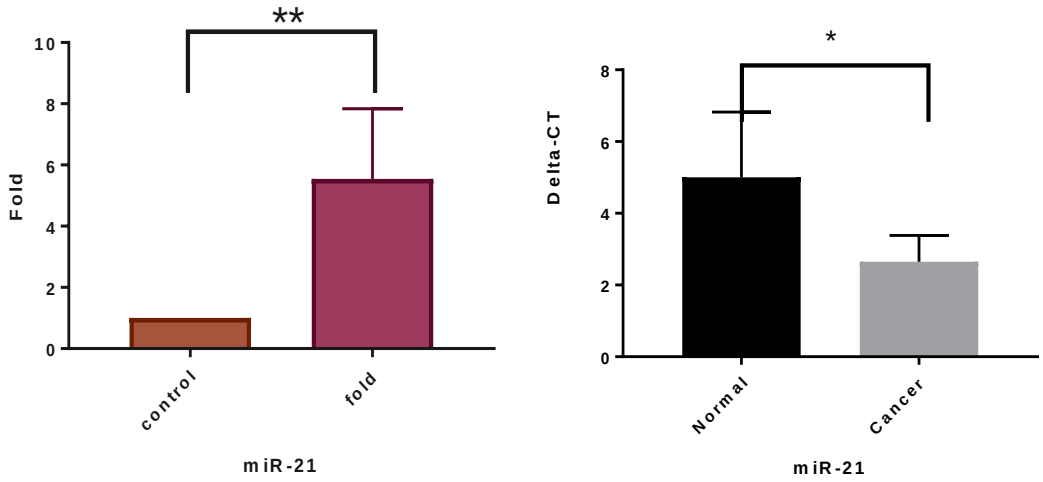

Figure 1. A) The difference in the expression of miR-21 in both normal and cancer tissues. (*P<0.05); B) The expression of miR-21 in tissues OSCC (**P<0.01)

Discussion

Head and neck squamous cell carcinoma (HNSCC) including cancers of the oral cavity, larynx and hypopharynx are considering as the sixth most common cancer worldwide (7). Oral squamous cell carcinoma (OSCC) is the most common type of these cancers and has the highest rate of expansion and nodal metastasis. Despite easy accessibility to OSCC in the mouth, studies have shown that more than 50% of patients' malignancies are diagnosed at an advanced stage, and the lack of early diagnosis and prognostic treatment is intensely perceived in this pathological condition. Progress in understanding the molecular mechanisms controlling oral cancer can improve the diagnosis, treatment and even prevention (8). Micro-RNA-21 a well-studied micro-ribonucleic-acid is involved in the regulation of cell proliferation, apoptosis and epithelial to mesenchyme differentiation and affecting cancer progression (9). Different studies programmed on miRNA in oral cancer. Results determined the up-regulation of miR-21 in OSCC tissue, in comparison with normal tissue (10). The bioinformatics analysis of investigations reviewing the target genes related miR-21 in the GO analysis determined the up-regulating of miR-21 effect on overexpression of transmembrane receptors. Furthermore, it can be involved in transducing signaling pathways lead to tumorigenesis (11).

Detecting metastasis and clinical staging is essential in cancer treatment. For detecting of the cancer, there are techniques including imaging and pathology, but both of them applied when patients are in metastatic stages. Recently, a scientific report tried to administrate molecular signatures such as genotype, protein, mRNA and spatially miRNA profiles to recognize primary and metastasis stages in head and neck cancer (12). In our study, we detected the relation between overexpression of miR-21 and cancer progression. MiR-21 was overexpressed in tumor tissue in compared with normal surrounding tissue. Thus by regulating their target genes disrupted the biological process mentioned in table 1 and led to change the normal process to neoplastic progression.

Programmed cell death 4 (PDCD4) and epithelial growth factor receptor (EGFR) were recognized as crucial genetic targets for miR-21 that their relationship with this microRNA is proved (13, 14). Therefore, choosing a treatment protocol regarding this relationship could guarantee the efficiency of therapy in OSCC. Misregulation of PDCD4 and EGFR are significantly occurred in squamous cell carcinoma. Programmed cell death 4 has been strongly related to the metastasis and progression of malignant oral tissues and regulated by miR-21 (14). The miR-21 could bind to PDCD4 and thus can regulate it. The PDCD4 reducing level was proved this relationship hypothetical between miR-21 and PDCD4. EGFR is a highly expressed receptor involved in the signaling pathways that related to proliferation and survival of most cancer cells, including OSCC (15, 16). The results suggest that CI-1033 as a tyrosine kinase inhibitor related to target genes of miR-21 could be inhibited OSCC as the prescription for a variety of solid tumors including prostate, breast, lung, and brain (17, 18).

Conclusion

It can be concluded that the expression levels of miR-21 in OSCC tissues can be a prognostic factor to better recognize the early pathologic state of OSCC. Necessitate of adding other microRNAs and making a panel of meaningfully changed miRNAs is strongly mentionable, but this study could be a definite step and confirmed that miR-21 could be used as a biomarker for the diagnosis and treatment of oral cancer. Eventually, it is better to perform accurate investigations on big sample size from the oral cavity or blood, tissues and even saliva. Moreover, the Authors suggest more in-vitro investigations on the role of CI-1033 on oral cancer treatment.

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Conflict of Interest: 'None declared'.

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