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Hsa-mir-556-5p: a prognostic factor in oral squamous cell carcinoma

Behnaz Raei[1], Yousef Seyedena[1], Mojgan Alaeddini[2], Nazanin Hosseinkhan[3] and Mehrdad Hashemi[1,4,*]

1 Department of Genetic, Faculty of Biological Sciences, North Tehran Branch, Islamic Azad University, Tehran, Iran.
2 Dental Research Center, Dentistry Research Institute, Tehran University of Medical Sciences, Tehran, Iran.
3 Endocrine Research Center, The Institute of Endocrinology and Metabolism, Iran University of Medical Sciences, Tehran, Iran.
4 Farhikhtegan Medical Convergence Sciences Research Center, Farhikhtegan Hospital, Tehran, Iran.

*Corresponding author: Farhikhtegan Medical Convergence Sciences Research Center, Farhikhtegan Hospital Tehran Medical Sciences, Islamic Azad University, Tehran, Iran. E-mail: drmehashemi@gmail.com

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Abstract

Background: Around 5% of tumors develop in the head and neck area, with nearly 50% of those appearing in the oral cavity. The sixth highest number of cancer-related deaths is attributed to oral cancer. These figures illustrate the severity of the disease and emphasize the importance of raising awareness and providing early screening to detect and manage it. The treatment and forecast for individuals with oral squamous cell carcinoma (OSCC) remain a significant challenge. This highlights the need for more effective treatments and strategies to improve the prognosis for individuals diagnosed with OSCC. Over the past few years, there has been notable progress in using microRNAs (miRNAs) as diagnostic and prognostic markers for cancer, making them a highly useful and valuable tool.

Methods: For this research, we cultured and maintained cell lines for hypopharyngeal cancer (FaDu), oral cancer (Cal 27), and PDL cells. Next, we employed quantitative real-time polymerase chain reaction (qRT-PCR) to verify the levels of expression of the possible biomarker, has-miR-556-5p.

Results: During the discovery phase, we identified hsa-miR-556-5p as being differentially expressed, with a statistically significant increase in its expression level. More recently, we used real-time PCR to confirm that hsa-miR-556-5p is markedly up-regulated in oral squamous cell carcinoma (OSCC). Our study suggests that hsa-miR-556-5p has the potential to be a new and innovative biomarker for OSCC.

Conclusions: The candidate miRNA can be chosen as a promising biomarker for predicting patient outcomes and can even aid in detecting the disease at an early stage in medical settings.

Keywords: Oral squamous cell carcinoma, Hsa-miR-556-5p, real-time PCR

1. Introduction

The prevalence of conventional oral squamous cell carcinoma (OSCC), which is a prevalent cancer type in the head and neck region, has been observed to rise in several countries, particularly in younger demographics [1-3]. Approximately 350,000 new cases and 170,000 fatalities in 2018 were attributed to arising from oral squamous cell carcinoma (OSCC) in various parts of the oral cavity, including the alveolar ridge, buccal mucosa, the floor of the mouth, palate, tongue, and similar regions. The majority of the global instances of OSCC are detected in Asian populations [4, 5]. Traditionally, Lifestyle-related habits, like smoking and consuming too much alcohol, have been linked to the formation of oral squamous cell carcinomas (OSCCs) [6]. The habit of chewing betel quid is often associated with the disease in regions where oral squamous cell carcinoma (OSCC) is prevalent, especially in South and Southeast Asia. This habit is associated with approximately 50% of OSCC cases in these areas [7]. The primary ingredient of the betel quid is areca nut, and this is a known carcinogen. In some communities, smokeless tobacco is also included in the quid. Human papillomavirus (HPV) has also been identified as a major risk factor for head and neck squamous cell carcinoma (HNSCC) [8]. While HPV infection is responsible for approximately 60% of oropharyngeal carcinoma cases, its involvement in OSCC is not as certain, and only up to 25% of OSCC cases are linked to HPV infections [9] [4]. Various populations also exhibit differences in anatomical locations where cancers originate. For instance, OSCC occurring in the tongue and floor of the mouth are widespread among Caucasian populations, whereas those in the tongue and buccal mucosa are more common among the Asian populace. These

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disparities could be attributed to differences in behavioral patterns. For instance, the practice of chewing betel quid, which involves placing it in the buccal mucosa for an extended duration, exposes that particular area to the carcinogens in the quid [1, 2].

Oral cancer has been associated with numerous risk factors including human papillomavirus infection [1], chronic local trauma [2], exposure to ultraviolet radiation [2], a low-antioxidant diet [3], immunosuppression [4], and Oral Potentially Malignant Lesions (OPMLs) [5]. However, tobacco smoking and alcohol intake are commonly linked causative factors for OSCC. These two factors are the primary causative factors in 74% of OSCC cases in Western countries [6] [7].

Recently, miRNAs, a type of non-coding RNA, have recently emerged as significant players in disease development, including cancer. These single-stranded, endogenous, non-coding RNAs are 18-24 nucleotides in length and control gene expression, thereby regulating cellular processes such as cell cycle, differentiation, and apoptosis. Abnormal expression of miRNAs has been identified as a crucial factor in the development of several cancers, including OSCC [2]. These molecules can function as either oncogenes or tumor suppressor genes in cancer processes [2]. This study aimed to ascertain and confirm miRNA expressions in cell lines as a means of predicting OSCC cancer.

Methods:

Drugs Paclitaxel and Docetaxel were found to share two miRNAs, hsa-miR-187-5p and hsa-miR-106a-3p, which are a sign of the body's reduced response to the drugs. Prostate cancer's sensitivity to Paclitaxel was linked to hsa-miR-556-5p [3]. A possible circulating biomarker for distant metastasis in gastric cancer (GC) was discovered as hsa-miR-556-5p [4]. In lung adenocarcinoma (LA), hsa-miR-556-5p was identified to be elevated as a miRNA after tumor resection [5]. Uhr et al proposed hsa-miR-187-5p and hsa-miR-106a-3p as potential indicators of drug resistance in breast cancer, and they discovered an association between hsa-miR-556-5p and TAX sensitivity [6]. Chen et al. conducted studies that associated the miRNA hsa-miR-556-5p with gastric cancer (GC) and lung adenocarcinoma (LA) (2020) and Han et al. (2020), respectively. However, further research is required to investigate the regulation of hsa-miR-556-5p in OSCC [4] [5].

Cell culture:

The CAL-27 and FaDu cell lines were cultivated in a medium made up of high-glucose Dulbecco's Modified Eagle's medium (DMEM) from Atocel in Austria, together with 10% Fetal Bovine Serum (FBS) from Invitrogen in the USA, and 1% penicillin/streptomycin from Gibco, Invitrogen Corporation in Grand Island, NY. Afterward, the cells were kept in an incubator at a temperature of 37◦C and with 5% CO2 [7].

Analyzing Gene Expression through RNA Isolation and Real-time PCR:

The cells were subjected to RNA extraction using an RNA X-Plus kit, and the RNA yield was measured by employing a NanoDrop spectrophotometer. cDNA was synthesized using a Bio fact kit, and Real-time PCR was performed by utilizing the Cyber Green method and a specific program with 40 cycles of denaturation, annealing, and elongation. The housekeeping gene and positive control used in this study was β-actin. The relative expression levels were analyzed using Rest 2009 software and calculated based on 2-ΔΔct [3].

Results:

In the present investigation, hsa-miR-556-5p expression was examined in the three cell lines, CAL-27, FaDu, and HGF-PI, using the Sybr green quantitative PCR assay. Each reaction was performed in duplicate. GraphPad Prism 9.5.1 software was used to quantify the expression levels, which showed that FaDu and CAL-27 cell lines had different levels of hsa-miR-556-5p compared to HGF-PI. Figure 1, Figure 2, and Figure 3 display the outcomes. In contrast to the HGF-PI cell line, both FaDu and CAL-27 cell lines showed significantly higher expression levels of hsa-mir-556-5p ($p < 0.05$) as indicated in Figures 1,2 and 3, implying an up-regulation in these two cell lines. The PCR product was analyzed by gel electrophoresis, and a singular band was observed, suggesting the selective and distinct amplification of the desired target b.

The results of the RNA extraction from the tissues showed that the RNA concentration was appropriate for all samples, and the A260/A280 absorption ratio indicated a good RNA quality without contamination or degradation. Gene expression differences were calculated using Genex v6.04 software, and statistical analysis was performed using SPSS v21 software. The diagrams were plotted using Graph Pad 7 software. The T-test analysis of the RT-PCR results using specific primers for hsa-miR-556-5p showed that the expression of this miRNA was significantly higher in tumor tissue than in healthy tissue.

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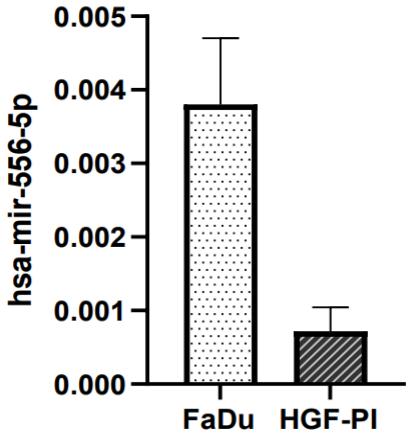


Figure 1. shows the difference between normal cell line (HGF-PI) and FaDu in terms of Hsa-mir-556-5p expression

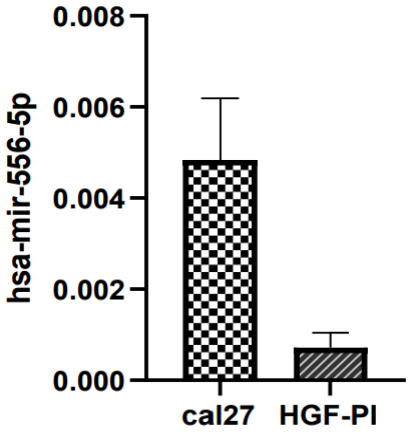


Figure 2. shows the difference between normal cell line (HGF-PI) and CAL-27 in terms of Hsa-mir-556-5p expression.

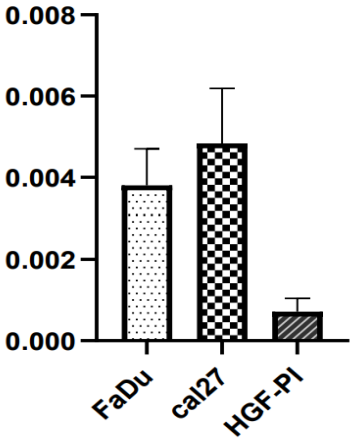


Figure 3. shows the difference between normal cell line (HGF-PI), FaDu, and CAL-27 in terms of Hsa-mir-556-5p expression.

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Discussion:

Oral cancer is a significant health concern worldwide, and early detection is crucial for successful treatment outcomes. The dysregulation of miRNAs has been linked to the development and advancement of various cancers, Such as oral cancer. Over the past few years, the field of research on miRNAs has gained considerable attention as a potential tool for early detection, diagnosis, and prognosis of oral cancer. Our study aimed to evaluate the expression of hsa-miR-556-5p in OSCC patients to assess its potential as a biomarker for early diagnosis.

Our findings showed a significant up-regulation of Hsa-miR-556-5p in OSCC tissues compared to normal tissues. This result is consistent with previous studies that have reported the dysregulation of hsa-miR-556-5p in other cancers, including gastric and lung cancers. The overexpression of hsa-miR-556-5p may contribute to the development and progression of OSCC through various mechanisms, including the regulation of tumor suppressor genes and oncogenes. Therefore, our study supports the potential of hsa-miR-556-5p as a biomarker for the early detection and diagnosis of OSCC.

In conclusion, our study provides evidence of the potential utility of hsa-miR-556-5p as a biomarker for the early diagnosis of OSCC. Further studies are necessary to validate these findings and to explore the mechanisms underlying the dysregulation of hsa-miR-556-5p in oral cancer. Ultimately, the identification of reliable biomarkers for early detection and diagnosis of OSCC is crucial for improving treatment outcomes and reducing mortality rates associated with this malignancy. The study of miRNAs in oral cancer may provide a valuable tool for achieving this goal.

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