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ORIGINAL ARTICLE

Evaluation of hsa-mir-675-5p expression and its diagnostic and prognostic relevance in oral cancer

Goran Stojković[1,2], Marija Savić Veselinović[3], Nikola Todorović[1], Bojana Bukurov[1,2], Miljan Folić[1,2], Tjaša Ivošević[1,4], Katarina Zeljić[3]

[1] University of Belgrade, Faculty of Medicine, Belgrade, Serbia

[2] Hospital for Otorhinolaryngology and Maxillofacial Surgery, University Clinical Center Serbia, Belgrade, Serbia

[3]University of Belgrade, Faculty of Biology, Belgrade, Serbia

[4]Center for Anaesthesiology and Resuscitation, University Clinical Center of Serbia, Belgrade, Serbia

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Correspondence to:
Katarina Zeljić
University of Belgrade, Faculty of Biology,
3 Studentski trg, 11000 Belgrade, Serbia
E-mail: katarina.zeljic@bio.bg.ac.rs

Summary

Introduction: Oral cancer is the most common subtype of cancer in the head and neck region, with an increasing incidence worldwide. Unfortunately, no specific biomarkers are used in everyday clinical practise. Small non-coding RNA molecules, microRNA (miRNA), are considered sensitive biomarkers for early diagnosis as well as prognosis in patients with oral cancer. Especially, microRNA derived from the H19 locus are poorly investigated for their potential role in oral cancer.

Aim: The aim of this study was to evaluate expression of hsa-miR-675-5p in tumor and non-tumor tissues of oral cancer patients and to associate it with pathohistological features.

Material and Methods: The study group consisted of 35 patients with oral cancer. Tumor and surrounding non-tumor tissues were taken from each patient**.** Relative expression was measured using the quantitative reverse transcription - real time PCR method**.**

Results: The relative expression of hsa-miR-675-5p was lower in oral cancer tumor than in non-tumor tissue suggesting its tumor suppressive role. hsa-miR-675-5p has diagnostic potential for sensitive distinction of tumor and non-tumor tissues in oral cancer patients. There was no difference in overall survival rates between patients with low and high hsa-miR-675-5p expression, confirming that hsa-miR-675-5p cannot be used as a prognostic biomarker in patients with oral cancer.

Conclusion: hsa-miR-675-5p can be considered as a potential diagnostic but not a prognostic molecular biomarker in oral cancer.

Keywords: oral cancer, hsa-miR-675-5p, biomarker

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INTRODUCTION

Epidemiological data worldwide indicate a troubling increase in the incidence of oral cancer (1). While surgical removal of tumor remains a mainstay treatment, recurrence rates among the patients with negative resection margins can be as high as one-third (2). To reduce the risk of recurrence post-surgery, current histopathological procedures should be improved by adding a molecular approach to analyze tissue changes.

MicroRNAs (miRNAs) represent a class of small, non-coding RNA molecules, typically 18-24 nucleotides in length, involved in posttranscriptional regulation by either mRNA degradation or repression of translation of target gene (3, 4). With over 2 000 miRNAs identified in humans to date, their regulatory role extends over vital biological processes such as: cell proliferation, differentiation, development, apoptosis and immune response. The focus of research into miRNAs in the biomedical field is based on the fact that miRNAs have promising biomarker potential in various pathologies, including cancer (5, 6). Notably, their stability in tissue and liquid biopsies uncerscores their utility as molecular biomarkers (6). In our previous study, we delineated three-miRNAs signature (miR-31-3p, miR-139-5p and miR-30a-5p) for diagnostic use in oral cancer and also identified miRNAs (miR-135b-5p, miR-18a-5p and miR-30a-5p) indicative of poor survival (7). It is important to continue the search for molecular biomarkers to improve future diagnostic, prognostic and therapeutic practice in oral cancer patients.

The IGF2-H19 locus (11p15.5), paternally imprinted and harboring both coding and noncoding genes, is frequently deregulated in cancer (8), yet its involvement in oral cancer remains elusive. Of interest is hsa-miR-675, expressed from the *H19* gene within the IGF2-H19 locus, with documented increased expression of hsa-miR-675-3p in esophageal cancer (9, 10) and underexpression of hsa-miR-675-5p in adrenocortical carcinoma (11). Given its dysregulation across cancer types, its potential implication in oral cancer can be assumed also. To our knowledge, status of hsa-miR-675 in oral cancer remains unexplored, leaving its significance unresolved. Furthermore, the biomarker potential of hsa-miR-675 remains ambiguous.

Our objective was to characterise H19 derived hsa-miR-675 through a bioinformatic approach and assess relative levels of hsa-miR-675 in publicly available databases and clinical samples of oral tumor and surrounding non-tumor tissues. We aim to associate these findings with pathohistological features and survival outcomes in oral cancer patients.

MATERIAL AND METHODS

Exploration of hsa-miR-675 using a bioinformatic approach

hsa-miR-675 was functionally analysed by DIANA-miRPath v4.0 web-based software (http://www.microrna.gr/miRPathv4) (12). Both forms of hsa-miR-675, -5p and -3p, were searched. TargetScan v8.0 was used for gene target selection. To determine significantly enriched molecular pathways with target genes predicted to be regulated by the miRNA of our interest, the Kyoto Encyclopedia of Genes and Genomes (KEGG) was used under the criteria of pathway union, p value threshold of 0.05, and false discovery rate (FDR) correction. If p value was less than 0.05, pathway was considered significantly enriched. Heatmap of significantly enriched pathways was constructed by DIANA-miRPath v4.0.

Network of hsa-mir-675 (-5p and -3p forms) - gene interactions were constructed and visualised by miRNet v2.0 software (https://www.mirnet.ca) (13). miRTarBase v8.0 and TarBase v8.0 were used as a resource of validated interactions between hsa-miR-675 and target genes. On all target genes was performed enrichment analysis using hypergeometric test and KEGG database. If adjusted p value was less than 0.05, pathway was considered significantly enriched.

The University of California Santa Cruz (UCSC) Xena platform (http://xena.ucsc.edu/) was used for visual analysis of hsa-miR-675 expression in head and neck squamous cancer (HNSC) compared to normal tissue using the Genomic Data Commons - The Cancer Genome Atlas (GDC TCGA) database (14). The GDC TCGA HNSC database contains phenotype data for 612 samples. After filtering out samples and restricting the search to oral cancer cases of white ethnicity with data on hsa-miR-675 expression, 254 samples remained **(Figure 1A)**. Of these, 235 were primary tumors and 19 were solid normal tissue. There were 18 matched samples of primary tumors and normal tissue. The hsa-miR-675 stem loop expression data and relevant demographic (gender, age, alcohol consumption, smoking history) and clinical data (histological grade, TNM status, clinical stage, overall survival) were downloaded from the UCSC Xena platform for further statistical analysis. Kaplan-Meier curve of overall survival were generated in UCSC Xena and compared by log-rank test. For survival analysis hsa-miR-675 expression was classified into low and high based on median value.

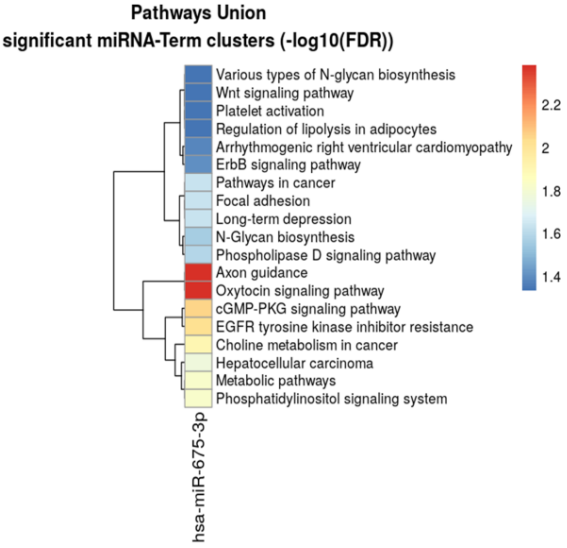
Patients and biological samples

Tissue samples from oral cancer patients were collected in period between 2018 and 2020. This same series of patients (N=35) was used for analysis in our previously published study, with demographic and pathohistological

(A)

KEGG pathway	Target genes (N)	p-value
Axon guidance	61	**1.64578E-05**
Oxytocin signaling pathway	54	**2.45035E-05**
cGMP-PKG signaling pathway	56	**7.7691E-05**
EGFR tyrosine kinase inhibitor resistance	31	**0.000113062**
Choline metabolism in cancer	37	**0.000180689**
Metabolic pathways	375	**0.000276854**
Phosphatidylinositol signaling system	35	**0.00031311**
Hepatocellular carcinoma	54	**0.000401065**
Focal adhesion	62	**0.000628199**
Long-term depression	24	**0.000744748**
Pathways in cancer	140	**0.000741836**
Phospholipase D signaling pathway	48	**0.000909317**
N-Glycan biosynthesis	20	**0.001053068**
ErbB signaling pathway	29	**0.001611169**
Arrhythmogenic right ventricular cardiomyopathy	27	**0.001840491**
Wnt signaling pathway	50	**0.002372702**
Platelet activation	41	**0.002431426**
Regulation of lipolysis in adipocytes	22	**0.002188501**
Various types of N-glycan biosynthesis	16	**0.002567327**

(B)



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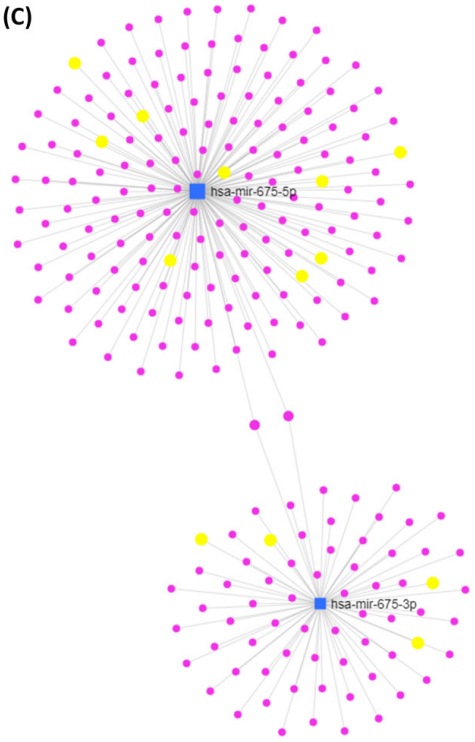

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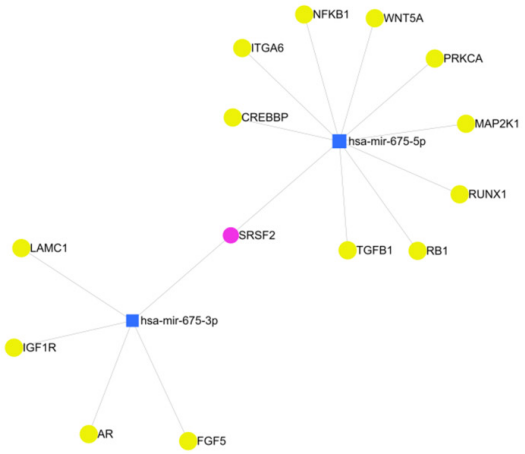

(E)

KEGG	Target genes (N)	p value	Adjusted p
Melanogenesis	9	**0.0000196**	**0.00196**
Prostate cancer	7	**0.000333**	**0.0167**
Pathways in cancer	13	**0.00074**	**0.0247**
TGF-beta signaling pathway	6	**0.00169**	**0.0422**
Prion diseases	3	**0.00381**	**0.0762**
Chronic myeloid leukemia	5	**0.005**	**0.0833**

Figure 1. Significantly enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways for hsa-miR-675-3p presented as list **(A)** and heatmap **(B)**. Intensity of colours represents p value (-log10(FDR)). Target genes (N) – number of genes in the pathway. hsa-miR-675-5p and hsa-miR-675-3p gene interaction networks **(C)**. Yellow circles represent genes involved in significantly enriched pathways in cancer **(D)**.
Blue squares represent miRNA, circles represent genes regulated by miRNA form. Genes names are given along to yellow circles.
List of significant KEGG enriched in genes from gene-miRNA interaction network **(E)**. KEGG with significant p and adjusted p values are presented.
p<0.05 are in bold.

characteristics of the study group detailed therein (7). Ethical approval for the collection and utilization of biological samples for research purposes was granted from the Ethics committee of the Faculty of Medicine, University of Belgrade (approval number: 1550/VII-6).

Patients diagnosed with oral cancer provided informed consent for their patricipation in the study. During surgical tumor excision, specimens from both tumor and surrounding non-tumor tissue (approximatelly 2 cm from macroscopically identified tumor margins) was taken for research, immersed in RNA later solution (Invitrogen, USA) and stored at -80°C. Tissue verification and tumor staging were in accordance with the guidelines outlined in the Union for International Cancer Control Staging Manual, 8th Edition.

Quantification of hsa-miR-675-5p relative expression

The *mir*Vana™ kit (Invitrogen, USA) was used to isolate total RNA from collected biological samples. RNA at a concentration of 20ng/µl was used for cDNA synthesis with the TaqMan™ MicroRNA Reverse Transcription Kit (Invitrogen, USA) according to the manufacturer's recommendations. TaqMan™ MicroRNA Assay was used for hsa-miR-675-5p (ID 002005) quantification by real time PCR. Normalization was done by RNU6B (ID 001093) as an endogenous control. Cycle Treshold (Ct) values were used to calculate the relative expression of the hsa-miR-675-5p target using the comparative ΔCt method (ΔCt=Ct of target – Ct of endogenous control). Ct values were measured in triplicate for each sample, and the average value was used for further analysis. Relative expression, presented as $2^{-\Delta Ct}$, is used for statistical tests.

Statistical analysis

The collected data were analysed and graphically displayed using GraphPad Prism software, version 9. Chi-square ($\chi 2$) test was used for association of categorical variables. The normality of the data was tested using the Shapiro-Wilk test. If the continuous data were normally distributed, a parametric t-test was performed, for non-normally distributed paired data, the Wilcoxon rank test was performed. Receiver Operating Curve (ROC) was used to evaluate diagnostic potential of analysed miRNA. Association of gene expression with patients overall survival was estimated by Kaplan-Meier survival curve and log-rank test. Cox regression analysis was performed to estimate hazard risk depending on hsa-miR-675-5p expression. All p values were two-tailed and if p value was less than 0.05, results were considered significant.

RESULTS

Bioinformatic exploration of hsa-miR-675

Nineteen KEGG pathways showed significant enrichment in genes predicted to be regulated by hsa-miR-675, with metabolic pathways and pathways in cancer being the most enriched **(Figure 1A, 1B)**. Using miRNet v2.0, we constructed a network of interactions between hsa-miR-675-5p and hsa-miR-675-3p, and target genes. Network analysis revealed that hsa-miR-675-5p regulates a larger number of genes (163 genes) compared to hsa-miR-675-3p (70 genes) **(Figure 1C)**. Further analysis using the hypergeometric test and the KEGG database confirmed significant enrichment in cancer with the largerst number of genes affected (genes *RUNX1*, *MAP2K1*, *PRKCA*, *RB1*, *WNT5A*, *CREBPP*, *ITGA6*, *NFKB1*, *TGFB1*) **(Figure 1D, 1E)** affirming the involvment of hsa-miR-675 in carcinogenesis. This justifies the selection of hsa-miR-675 for further analysis in the open transcriptomic database for oral cancer as well as in clinical samples.

Exploring hsa-miR-675 in the open GDC TCGA HNSC database via UCSC Xena **(Figure 2A)** revealed no significant difference in expression between primary tumor samples and solid normal tissue among oral cancer patients (p=0.504, t-test Welch's correction), **Figure 2B**. Similary, no significant difference was found in matched tumor and non-tumor tissue from 18 oral cancer patients (p=0.277, t-test Welch's correction), **Figure 2B**.

ROC analysis on whole group including 254 samples, indicated that hsa-miR-675 was not effective as a diagnostic biomarker for distinguishing oral tumor from non-tumor tissue (AUC=0.538, 95% CI=0.421-0.654, p=0.579), **Figure 1C**. The same trend persisted when analyzing a subset of matched samples (AUC=0.620, 95% CI= 0.427-0.813, p=0.217). Furthermore, Kaplan-Meier curve analysis showed no significant difference in overall survival between patients with low and high expression of hsa-miR-675 (p=0.169, log-rank test), **Figure 2D**, indicating that hsa-miR-675 cannot be considered as a prognostic biomarker.

Table 1 presents the association of hsa-miR-675 expression with demographic and pathohistological features, revealing significant associations with the location of primary tumor (p=0.011, $\chi 2$ test), histological grade (p=0.013, $\chi 2$ test), tumor stage (p=0.013, $\chi 2$ test) and tumor size (p=0.019, $\chi 2$ test), categorized into low and high expression groups based on the median expression level.

Relative expression, diagnostic and prognostic potential of hsa-miR-675-5p

In our study group, the relative expression of hsa-miR-675-5p was significantly higher in non-tumor than in tumor samples from oral cancer patients tissue (p=0.006, Wilcoxon signed rank test), **Figure 3**. The expression

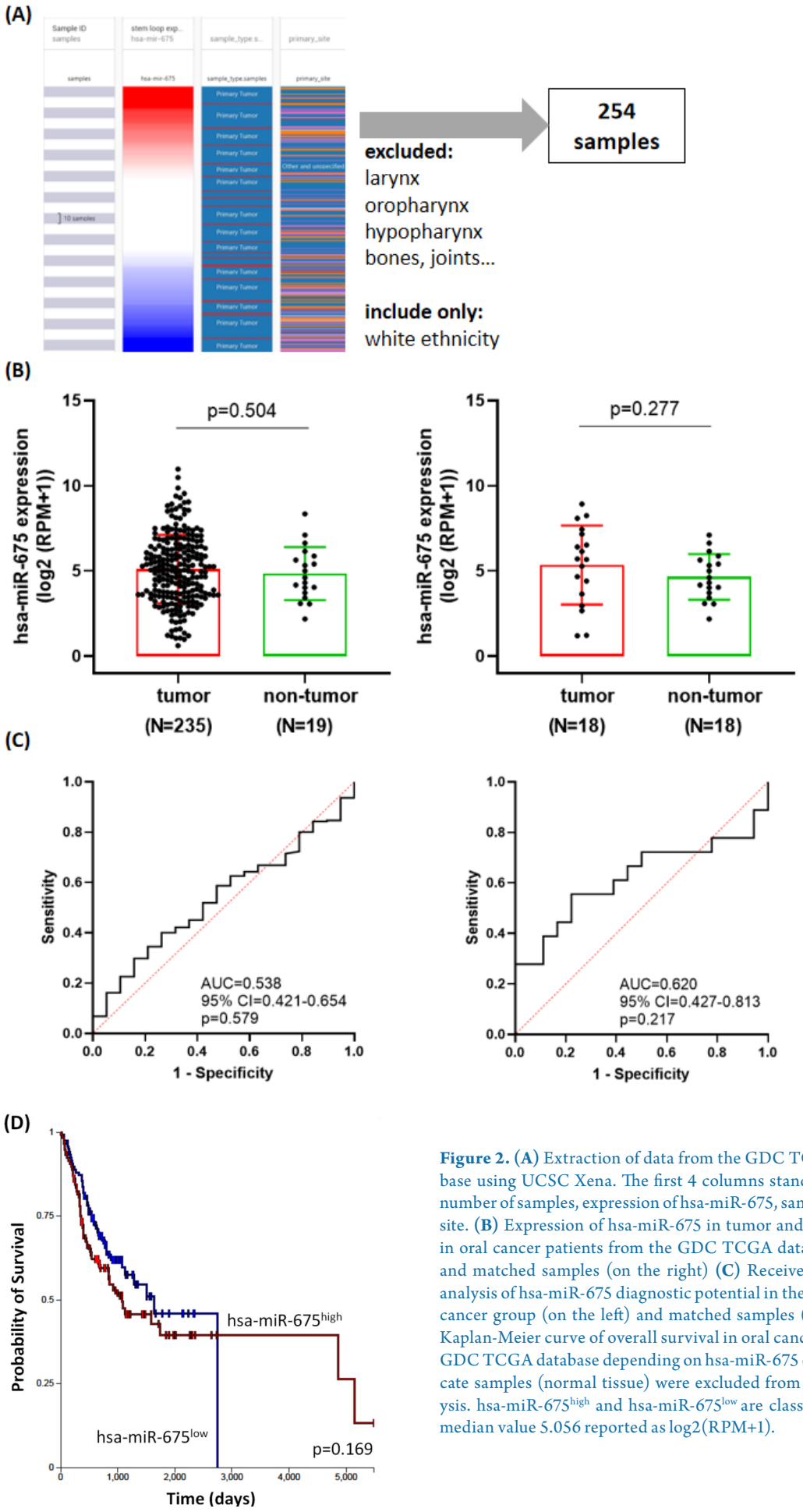

Figure 2. **(A)** Extraction of data from the GDC TCGA HNSC database using UCSC Xena. The first 4 columns stand for, respectively, number of samples, expression of hsa-miR-675, sample type, primary site. **(B)** Expression of hsa-miR-675 in tumor and non-tumor tissue in oral cancer patients from the GDC TCGA database (on the left) and matched samples (on the right) **(C)** Receiver operating curve analysis of hsa-miR-675 diagnostic potential in the GDC TCGA oral cancer group (on the left) and matched samples (on the right) **(D)** Kaplan-Meier curve of overall survival in oral cancer group from the GDC TCGA database depending on hsa-miR-675 expression. Duplicate samples (normal tissue) were excluded from the survival analysis. hsa-miR-675^{high} and hsa-miR-675^{low} are classified according to median value 5.056 reported as log2(RPM+1).

Table 1. Association of hsa-miR-675 expression in oral cancer with demographic and pathohistological features of oral cancer patients from GDC TCGA database downloaded from the UCSC Xena

GDC TCGA demographic and pathohistological features		Relative expression of hsa-miR-675 in tumor tissue				
		hsa-miR-675^{low} N=118		hsa-miR-675^{high} N=117		
		N	%	N	%	p
Sex	male	76	64.4	79	67.5	0.614
	female	42	35.6	38	32.5	
Age (years, median)	≤61	63	53.4	65	55.6	0.739
	>61	55	46.6	52	44.4	
Smoking habits	nonsmoker	64	54.2	59	50.4	0.559
	smoker + ex smoker	54	45.8	58	49.6	
Alcohol consumption	no intake	37	31.4	38	32.5	0.854
	moderate + high	81	68.6	79	67.5	
Location of primary tumor	base of tongue	15	12.7	9	7.7	**0.011**
	floor of the mouth	22	18.6	19	16.2	
	gum	8	6.8	1	0.9	
	lip	2	1.7	1	0.9	
	palate	3	2.5	2	1.7	
	other and unspecified parts of tongue	44	37.3	71	60.7	
	other and unspecified part of mouth	24	20.3	14	12	
Histological gradus	well differentiated	25	21.9	12	10.4	**0.013**
	moderately differentiated	59	51.8	76	66.1	
	poorly differentiated	26	22.8	27	23.5	
	anaplastic	4	3.5	0	0	
	grade can not be evaluated / NA	4	-	2	-	
T staging	T1, T2	24	20.3	41	35	**0.013**
	T3, T4	90	76.3	73	62.4	
	NA	4	3.4	3	2.6	
Tumor size	≤2cm	16	14.5	22	19.1	**0.019**
	2-4cm	24	21.8	41	35.7	
	>4cm	70	63.6	52	45.2	
N staging	N0	56	50.9	57	49.5	0.842
	N1	54	49.1	58	50.5	
	NA	8	-	2	-	

hsa-miR-675^{high} and hsa-miR-675^{low} are classified according to median value 0.056 reported as log2(RPM+1);
NA – not available; GDC TCGA – Genomic Data Commons The Cancer Genome Atlas; N – number of cases; N0 – without regional lymph node involvement; N1- regional lymph node involvement; p<0.05 is presented in bold

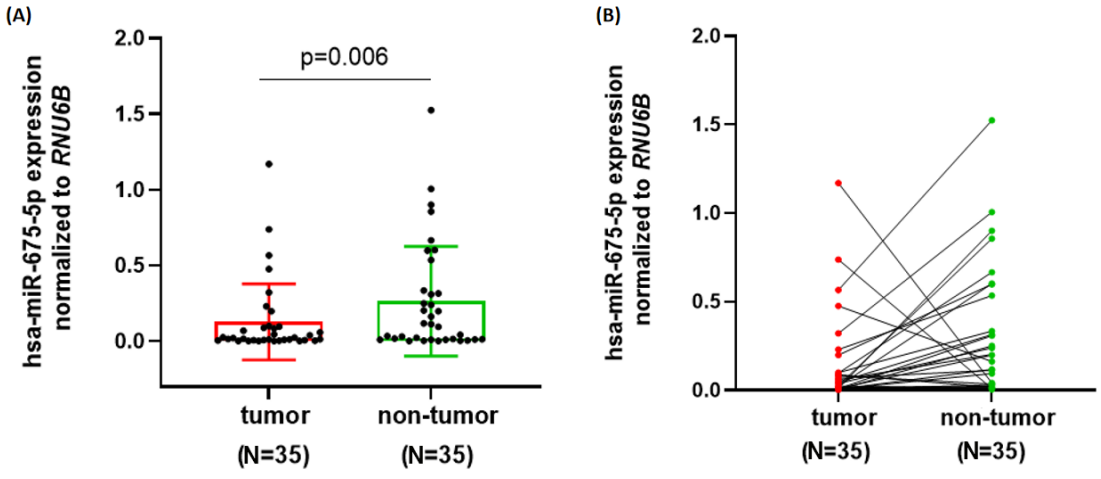

Figure 3. hsa-miR-675-5p relative expression (presented as mean ± SD of $2^{-\Delta Ct}$ value) of oral cancer patients in **(A)** tumor and non-tumor tissue **(B)** paired tumor and non-tumor tissue.
SD – standard deviation

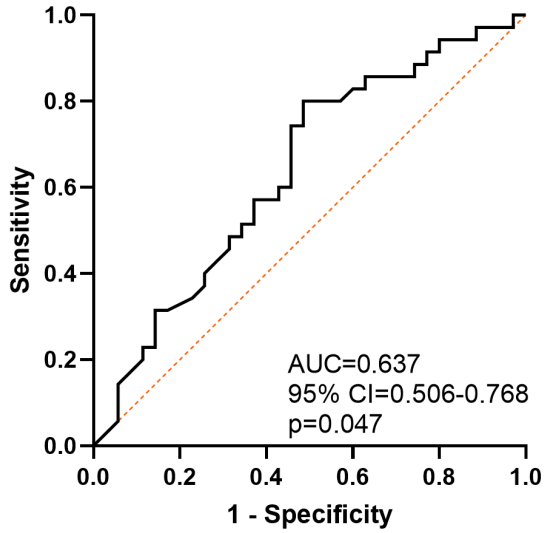

Figure 4. Receiver operating curve analysis of hsa-miR-675-5p diagnostic potential in oral cancer.
AUC – Area under curve
95% CI – 95% Confidence interval

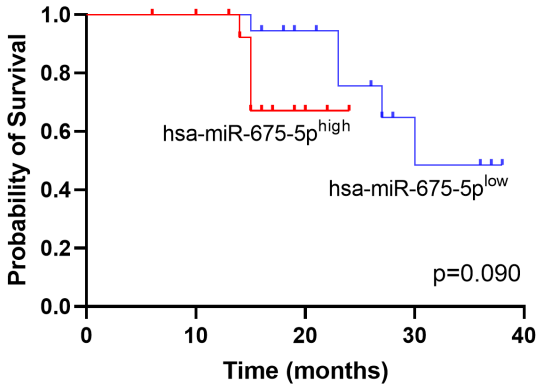

Figure 5. Kaplan-Meier curve of overall survival of oral cancer patients depending on hsa-miR-675-5p expression.
hsa-miR-675-5p^{high} and hsa-miR-675-5p^{low} are classified according to median value 0.021 reported as $2^{-\Delta Ct}$.

level in non-tumor samples was 2-fold higher than in tumor samples. In both groups, relative expression was variable with a mean $2^{-\Delta Ct}$ value of 0.127 ± 0.250 in tumor and 0.264 ± 0.362 in non-tumor tissue.

ROC curve analysis indicated acceptable discriminatory power of hsa-miR-675-5p for distinguishing oral tumor from non-tumor (AUC=0.637, 95% CI=0.506-0.768, p=0.047), **Figure 4**, with a proposed cut-off value of 0.106, estimated using the maximum Youden index (sensitivity + specificity -1) with a specificity and sensitivity of 80% and 51.4%, respectively.

The median value of hsa-miR-675-5p expression in tumor tissue ($2^{-\Delta Ct}$ value of 0.021) was used to classify it into low (hsa-miR-675-5p^{low}) or high (hsa-miR-675-5p^{high}) expression groups. There was no difference in survival between oral cancer patients with hsa-miR-675-5p^{low} and hsa-miR-675-5p^{high} expression (p=0.090, log-rank test), **Figure 5**. Survival rates were not significantly different when patients were stratified by tumor stage and size.

Relative expression of hsa-miR-675-5p and demographic and pathohistological features

Table 2 shows that no significant associations were found between the expression of either low or high hsa-miR-675-5p in tumor tissue and the demographic and pathohistological features of oral cancer patients. Cox regression analysis further indicated that hsa-miR-675-5p was not associated with oral cancer outcome (hazard risk=3.596, 95% CI=0.706-18.315, p=0.123).

DISCUSSION

The search for suitable molecular biomarkers in oral cancer research continues to offer vast potential. With the plethora of genes, non-coding transcripts and their interactions, the design of comprehensive gene panel for testing apperas promising. Nonetheless, focusing on the analysis of a single gene candidate is a valuable starting point for developing a gene panel for diagnostic and prognostic assesments of oral cancer patients. In this study, we investigated hsa-miR-675 as it arises from IGF2/H19 locus, often deregulated in different cancers, but unstudied in oral cancer.

The availability of public transcriptomic datasets across various cancer types underscores the importance of leveraging these data. However, conducting expression analysis in own clinical samples remains crucial and justified. In our investigation of hsa-miR-675, we started our exploration with bionformatics approach, initially checking KEGG pathways enriched in genes targeted by hsa-miR-675 and validated gene-miRNA interaction network. Notably, the disruption of signalling pathways, particulary metabolic pathwas and pathways in cancer, aligns with the hallmark characteristics of cancer cells (15). Our findings strongly suggest the involvement of hsa-miR-675 in cancerogenesis, thus validating our choice for further analysis.

Contrary to our expectations, expression analysis of hsa-miR-675 in the GDC TCGA, samples failed to reveal significant differences between cancer samples and solid normal tissues, thereby refuting hsa-miR-675 as a diagnostic and prognostic biomarker in oral cancer. This discrepancy maystem from the composition of the TCGA oral cancer cohort, which predominantely comprised cancer samples, potentially diluting any differences with normal tissues. Furthermore, the limited availability of matched samples highlights the need for robust analyses in more homogenous sample sets. Matched samples were only available for 18 oral cancer samples, representing a relatively modest study group. In a group twice as large as that studied by the GDC TCGA, we found significant

Table 2. hsa-miR-675-5p relative expression association with demographic and pathohistological features of oral cancer patients

Demographic and pathohistological features		Relative expression of hsa-miR-675-5p in tumor tissue				
		hsa-miR-675-5p^{low} N=18		hsa-miR-675-5p^{high} N=17		
		N	%	N	%	p value
Sex	male	14	77.8	12	70.6	0.627
	female	4	22.2	5	29.4	
Age (years, median)	<59	11	61.1	7	41.2	0.238
	>59	7	38.9	10	58.8	
Smoking habits	nonsmoker	4	22.2	6	35.3	0.392
	smoker + ex smoker	14	77.8	11	64.7	
Alcohol consumption	no intake	5	27.8	5	29.4	0.915
	moderate + high	13	72.2	12	70.6	
Oral hygiene	good	11	61.1	8	47.1	0.625
	poor	7	38.9	9	52.9	
Location of primary tumor	tongue	12	66.7	13	76.5	0.096
	hard palate	4	22.2	0	0	
	floor of the mouth	2	11.1	4	23.5	
Histological grade	well differentiated	5	27.8	5	29.4	0.944
	moderately differentiated	9	50	9	52.9	
	poorly differentiated	4	22.2	3	17.6	
Tumor size	≤2cm	1	5.6	1	5.9	0.672
	2-4cm	9	50	6	35.3	
	>4cm	8	44.4	10	58.8	
T staging	T1, T2	8	44.4	6	35.3	0.581
	T3, T4	10	55.6	11	64.7	
N staging	N0	9	50	9	52.9	0.862
	N1	9	50	8	47.1	
M staging	M0	18	100	17	100	-
	M1	0	0	0	0	
Recurrences	no	13	72.2	13	76.5	0.774
	yes	5	27.8	4	23.5	

hsa-miR-675-5p^{high} and hsa-miR-67-5p^{low} are classified according to median value 0.021 reported as $2^{-\Delta Ct}$.
N – number of cases; N0 – without regional lymph node involvement; N1- regional lymph node involvement; M0 – without metastases; M1 – present metastases.

hsa-miR-675-5p differences between tumor and non-tumor tissue. Our results of down-regulated hsa-miR-675-5p in oral cancer tissue are in line with findings reported for adrenocortical carcinoma (11), while opposite to findings in esophageal carcinoma (10) and laryngeal carcinoma and cell lines of head and neck cancer (16). In nasopharyngeal carcinoma and tongue cancer cell line, H19 and hsa-miR-675-5p were upregulated (17, 18). Based on these data, it can be assumed that hsa-miR-675 has dual role, both oncogen and tumor suppressor depending on the tumor type. The fact that hsa-miR-675-5p is less expressed in oral cancer tissue suggest that hsa-miR-675-5p could be considered as a potential therapeutic target in suspicious malignant lesions in oral cavity. These assumptions need to be clarified in future studies.

The data presented herein offer potential for early identification of oral cancer patients, even before clinical signs of the disease appear. Validating these findings entails assesing hsa-miR-675-5p levels across various clinical samples, including premalignant oral changes. Additionally, exploring the presence of hsa-miR-675-5p in body fluids, such as serum, plasma, saliva could confirm its candidacy as a non-invasive biomarker. Incorporating hsa-miR-675-5p into existing three-miRNAs signature panel for testing in oral cancer patients warrants further investigation in larger cohorts (7). All recommended investigations should be performed in a larger study group.

The prognostic potential of hsa-miR-675-5p was not confirmed, as there were no differences in overall survival between the groups with low and high hsa-miR-675-5p expression. The median of relative expression was used for classification since it is most commonly used in survival analysis. However, it is justified to set different cut-off values for the classification. In contrast to our results, in laryngeal carcinoma, high expression of hsa-miR-675 was associated with poor prognosis, disease-free survival and recurrence, indicating the predictive potential of hsa-miR-675 (16).

The exact mechanism underlying the involvment of hsa-miR-675-5p in oral cancer development is still un-

known. It has been shown that has-miR-675-5p promotes invasion and metastasis through downregulation of target gene SFN (17). Functional studies, including hsa-miR-675-5p mimic and knock-down, performed firstly in *in vitro* setting, should provide more information on role of this miRNA as well as it's host gene *H19* in cancerogenesis. A previous study reported increase in H19 is associated with an increase in hsa-miR-675 in tongue cancer patients (19). Lower expression of *H19* was found in tumor tissue than in non-tumor tissue of tongue cancer, which is consistent with our results since the majority of patients in our study group had oral cancer localised to the tongue. However, anatomic location of the tumor in oral cavity was not associated with hsa-miR-675-5p expression in our study, which justify considering different anatomic location alltogether in our study.

To fully elucidate the role of hsa-miR-675-5p in oral cancerogenesis, it would be useful to investigate the co-expression between hsa-miR-675-5p and its host *H19* as well as other genes in the IGF2/H19 locus. Since IGF2/H19 is an imprinted locus, it is recommended to determine methylation level in relation to the expression of hsa-miR-675-5p.

Co-expression of this miRNA and their target genes should be analyzed, too. So far, oral cancer literature data indicate that almost all structure genes gained through our bioinformatic approach, involved in significantly enriched pathways in cancer, are up regulated. Higher expression was obtained for mRNA of genes *RUNX1* (20), *MAP2K1* (21), *PRKCA* (22) *ITGA6* (23), *NFKB1* (24), as well as for protein products of genes *PRKCA* (25), *RB1* (26) and *WNT5A* (27). These results are in concordance with our results, if hsa-miR-675-5p is down regulated, it is expected that its target genes are up regulated.

CONCLUSION

The results of our study suggest a potential tumor-suppressive role of hsa-miR-675-5p and a highlight its utillity as a diagnostic biomarker in oral cancer. Further investigations should focus on the measurement of hsa-miR-675-5p in liquid biopsies, including saliva, serum and plasma across a larger cohort of oral cancer patients.

Author contributions:

Investigation: GS, MSV, KZ; Clinical samples collection: GS, NT, BB, MF, TI; Clinical data curation: GS, NT; BB, MF, TI; Research data curation: KZ; Formal analysis: KZ, MSV; Methodology: KZ, MSV; Validation: GS, KZ, MSV; Visualisation: KZ; Funding: GS, KZ; Supervision: KZ; Writing – original draft: KZ; Writing – review and editing: GS, MSV, NT, BB, MF, TI, KZ; Final approval of the article: GS, MSV, NT, BB, MF, TI, KZ.

Ethical approval:

Ethical approval for the collection and use of biological samples for research purposes was obtained from the Ethics committee of the Faculty of Medicine, University of Belgrade (approval number: 1550/VII-6).

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EVALUACIJA EKSPRESIJE, DIJAGNOSTIČKOG I PROGNOSTIČKOG ZNAČAJA HSA-MIR-675-5P KOD ORALNOG KARCINOMA

Goran Stojković[1,2], Marija Savić Veselinović[3], Nikola Todorović[1], Bojana Bukurov[1,2], Miljan Folić[1,2], Tjaša Ivoševič[1,4], Katarina Zeljić[3]

Sažetak

Uvod: Oralni karcinom je najčešći podtip karcinoma u predelu glave i vrata, sa rastućom incidencom širom sveta. U svakodnevnoj kliničkoj praksi se ne koriste specifični biomarkeri za dijagnozu i prognozu ovog entiteta. Mali nekodirajući RNK molekuli, mikroRNK (miRNK), smatraju se dobrim kandidatima za biomarkere za uspostavljanje rane i senzitivne dijagnoze, kao i prognozu kod pacijenata sa oralnim karcinomom. mikroRNK koja se transkribuje sa H19 lokusa do sada nije ispitivana kod oralnog karcinoma.

Cilj: Cilj rada je bilo merenje relativne ekspresije hsa-miR-675-5p u tumorskom i netumorskom tkivu pacijenata sa oralnim karcinomom, kao i ispitivanje asocijacije ove miRNK sa patohistološkim karakteristikama pacijenata.

Materijal i metode: Studijsku grupu činilo je 35 pacijenata sa oralnim karcinomom. Od pacijenta uključenih u studiju prilikom ekscizije tumora uzet je uzorak tkiva tumora i okolnog netumorskog tkiva. Relativna ekspresija je merena kvantitativnom reverznom transkripcijom - PCR metodom u realnom vremenu.

Rezultati: Relativna ekspresija hsa-miR-675-5p bila je značajno niža u tumorskom nego u netumorskom tkivu, što ukazuje na njegovu supresivnu ulogu u tumoru. hsa-miR-675-5p ima dijagnostički potencijal za razlikovanje tumorskog od netumorskog tkiva kod pacijenata sa oralnim karcinomom. Nije bilo razlike u preživljavanju između pacijenata sa niskim i visokim nivoima ekspresije hsa-miR-675-5p, što ukazuje da se hsa-miR-675-5p ne može koristiti kao prognostički biomarker kod oralnog karcinoma.

Zaključak: hsa-miR-675-5p se može smatrati potencijalnim dijagnostičkim, ali ne i prognostičkim molekularnim biomarkerom u slučaju oralnog karcinoma.

Ključne reči: oralni karcinom, hsa-miR-675-5p, biomarker

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