Available online at www.medicinescience.org

ORIGINAL ARTICLE

Medicine Science International Medical Journal

Medicine Science 2020;9(1):160-3

Identification of chemoresistance-associated miRNAs in hypopharyngeal squamous cell carcinoma

Elif Pala[1], Tuba Denkceken[2]

[1]*SANKO University Faculty of Medicine, Department of Medical Biology, Gaziantep, Turkey*
[2]*SANKO University Faculty of Medicine, Department of Biophysics, Gaziantep, Turkey*

Received 23 September 2019; Accepted 14 January 2020
Available online 28.02.2020 with doi: 10.5455/medscience.2019.08.9158

Abstract

This study aimed to determine candidate miRNAs that could help to evaluate whether patients with hypopharyngeal squamous cell carcinoma (HSCC) would benefit from docetaxel, cisplatin, and 5-fluorouracil (TPF) induction chemotherapy. We downloaded the expression profiles of HSCC patients from the Gene Expression Omnibus database. miRNA profiles were analyzed via Principal Components Analysis (PCA), followed by Linear Discriminant Analysis (LDA). The Database Annotation for Visualization and Integrated Discovery tool was utilized for enrichment analysis; STRING and Cytoscape were used for network construction. We detected that hsa-miR-15b-5p, hsa-miR-93-5p, and hsa-miR-130a-3p might act as crucial regulators in chemoresistance of HSCC, and they may play as prognosticators and therapeutic targets in the future.

Keywords: HSCC, chemoresistance, component analysis, functional enrichment analysis, protein-protein interaction, miRNA-mRNA regulatory network

Introduction

Hypopharyngeal carcinoma (HPC) arises from a subsite of the upper aerodigestive tract, which accounts for 0.8-1.5% of head and neck tumors with poor prognosis [1]. Hypopharyngeal squamous cell carcinoma (HSCC) is a histologically dominant tumor subtype, often diagnosed in the advanced stage, and the 5-year overall survival for all stages is approximately 30%. Induction chemotherapy with docetaxel, cisplatin, and 5-fluorouracil (TPF) has been developed and is considered an option to total laryngectomy, and this treatment has significantly enhanced the outcomes in HSCC to sustain the normal physiological function of the larynx [2-4]. Understanding the genetic differences among patients sensitive and resistant to induction chemotherapy will contribute to the knowledge of the molecular basis of HSCC. Identification of these highly relevant gene biomarkers can help to prevent unnecessary medication and may allow higher expectations of the desired individualized treatment.

***Coresponding Author:** Elif Pala, SANKO University, Faculty of Medicine, Department of Medical Biology, Gaziantep, Turkey,
E-mail: epala@sanko.edu.tr

Materials and Methods

Collection and inclusion criteria of studies

We searched the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm. nih.gov/geo/) by using the following keywords: "hypopharyngeal squamous cell carcinoma" (study keyword), "Homo sapiens" (organism), "Expression profiling by array" (study type). The inclusion criteria were: (1) expression levels of tissues from the experimental group compared to the control group, (2) adequate information to perform the analysis. This study is the descriptive cross-sectional study and the GSE85608-GPL21572_series_matrix.txt.gz microRNA expression profile was downloaded from the GEO database which consists of 12 docetaxel, cisplatin, and 5-fluorouracil (TPF)-sensitive patients (aged 56.3±9.68 years) and nine resistant controls (aged 59.2±12.42 years). Receiver Operating Characteristic (ROC) analysis with a discriminant function score produced an areas under the ROC curve (AUC) of 0.926 for separating chemosensitive from resistant samples, as seen in fig. 1. Minimal required sample size is calculated as 4 per group by using AUC value (0.926), Type I error (Alpha, Significance) value 0.05 (5%), Type II error (Beta,1-Power) value 0.20 (power is 80%) and null hypothesis value 0.5 [5]. This calculation shows that the number of the minimum sample size used in our study is sufficient.

Data processing and Statistical analysis

miRNA tissue expression profiles of 12 TPF-sensitive patients and nine resistant controls were downloaded (GSE85608) from GEO database. miRNA profiles were analyzed via Principal Components Analysis (PCA), followed by Linear Discriminant Analysis (LDA) [6, 7]. PCA was performed to reduce the number of predictor variables for the differentiation of chemosensitive/ resistant samples. Then LDA was used for separation while maximizing the variation between the groups and minimizing separation within each group. The miRNA tissue expression profiles were analyzed for the statistical analysis. In our study the entire data matrix of GSE85608 microRNA expression profile was [21x4603]. Firstly, we performed a PCA to reduce the dimension of observed variables into a relatively smaller number of components while maintaining as much information or variance. The PCA was applied to 4603 microRNA expression profiles of 21 centered and scaled (zero mean value and a standard deviation of one) data set. Whole data matrix "X" (P×M) contains the number of patients (P=21) and the number of microRNA expression profiles (M=4603). This matrix is separated into several principal components (PCs). The result of PCA is a product of PC scores "S" and PC loadings "L" matrices plus the residue "R": S=X. L→X=S.L'+R=s1l'1+s2l'2+s3l'3+……snl'n+R where X: (P x M) initial data matrix (21x4603); S: Weight matrix, eigenvalue, score [s1, s2, s3…, sn]; L': Eigenvector matrix, principle component factor (loading), variance; [l'1, l'2, l'3…., l'n]; Residual matrix (containing noise) and n is the number of computed PCs. Secondly, the most significant ($p<0.05$) components are determined for the differentiation of samples. Then these significant PCs are used as the input variables of LDA. To prevent overfitting, the cross-validation was performed by using the "leave-one-out" technique. The AUC as well as the sensitivities and specificities for the optimal cut-points are calculated using the discriminant function scores which is obtained by LDA. All statistical analyses were done by using open source R Studio software program with the version of '1.1.456' [8].

Target Prediction

All miRNA names were standardized according to miRBase v.22 via miRNAmeConverter available in Bioconductor R-package [9]. Then, MultiMiR package was utilized to predict targets of miRNAs that include 14 databases. This package was used to predict targets of miRNAs by DIANA-microT, ElMMo, MicroCosm, miRanda, miRDB, PicTar, PITA and TargetScan databases with the criterion of primary score listed in top 35 [10]. Targets of each miRNA were obtained by minimum one validated and three predicted algorithms, and these genes were chosen for the following analysis.

Functional Enrichment Analysis

The Database Annotation for Visualization and Integrated Discovery (DAVID) was utilized for enrichment analysis [11]. We used the DAVID database to implement Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis on significant miRNA targets. The species was limited to "Homo sapiens" and the $p < 0.05$ cut-off was considered as significant.

Protein-protein Interaction (PPI) Network Construction

The targets were mapped by STRING with a confidence score>0.7 as a cut-off criterion to estimate the PPI information [12], and then interactions were viewed with Cytoscape [13]. The genes with a node degree>35 were considered as hub genes.

miRNA-Hub Genes Network Construction

miRNA-hub genes regulatory network was constructed. The miRNAs with a degree>5 were considered to play an essential role in discriminating chemosensitive/resistance samples.

Results

Identification of Differentially Expressed miRNAs (DEMs)

PCA was performed by 1st finding the direction having the most significant variance (PC1: 21%), and after that finding following directions (PC2:8%, PC3:6%, PC4:6%, PC5-21 collectively provides 5% or less of variance however yet contributed significantly). The paired two-sample t-test on all component scores showed that there were two (PC6 and PC13) most significant ($p<0.05$) components for discriminating samples. The p-values of these components were calculated as 0.007476 for PC6 and 0.03598 for PC13. These two vital components were applied as the input variable of LDA. The cross-validation was conducted by using the leave-one-out technique to prevent over-fitting [14]. The Areas Under the ROC Curve (AUC) was computed, and the specificities and sensitivities for the optimal cut-points were determined with the discriminant function scores, which was acquired by LDA [15]. The classification based on the discriminant score gave a sensitivity of 100% and specificity of 91.7% in discriminating chemosensitive/resistant samples with an accuracy of 95.2% and AUC of 92.6% (Figure 1). Top 100 DEMs were obtained from PC6 and PC13 loading scores.

Target Prediction

MultimiR acquired 1108 target genes according to our criterion for further analysis.

Functional Enrichment Analysis

For 1108 targets, we listed the top 10 KEGG pathways, which revealed that targets were mostly enriched in cancer (Table 1).

Table 1. Enriched KEGG pathways of top 10 differentially expressed genes obtained from DAVID

TERM	COUNT	P-VALUE
Endocytosis	43	$1.7e^{-9}$
Pathways in cancer	56	$1.9e^{-8}$
Pancreatic cancer	18	$4.2e^{-7}$
HTLV-I infection	35	$3.0e^{-5}$
Melanoma	16	$3.4e^{-5}$
PI3K-Akt signaling pathway	43	$3.6e^{-5}$
FoxO signaling pathway	23	$3.8e^{-5}$
Hepatitis B	24	$4.4e^{-5}$
Proteoglycans in cancer	29	$6.9e^{-5}$

Signaling pathways regulating pluripotency of stem cells	23	$7.5e^{-5}$

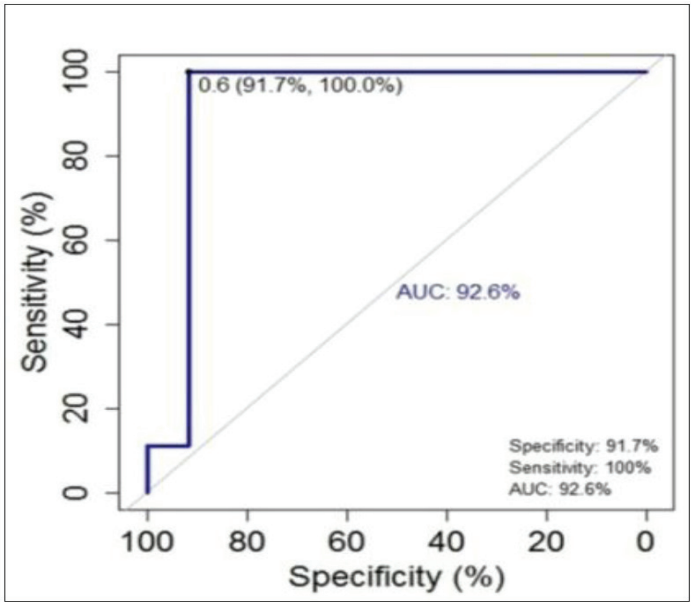

Figure 1. Receiver Operating Characteristic curve, comparing chemosensitive/ resistant samples

PPI network and identification of hub genes

The 1108 targets were utilized to set the PPI network by STRING. Consequently, we examined the STRING results by using Cytoscape, and 28 genes in the PPI network were recognized as hub genes (Figure 2). These hub genes included UBC, EGFR, MAPK1, RAC1, PTEN, HSPA8, BTRC, PPP2CA, SMURF1, RBBP7, SMAD3, STAT3, ITCH, CDC27, TGOLN2, CCND1, UBE2D2, ACTR2, PIK3R1, RAB5A, CUL3, VEGFA, CUL2, UBE2B, CLTA, UBE2V1, ESR1, and CUL5.

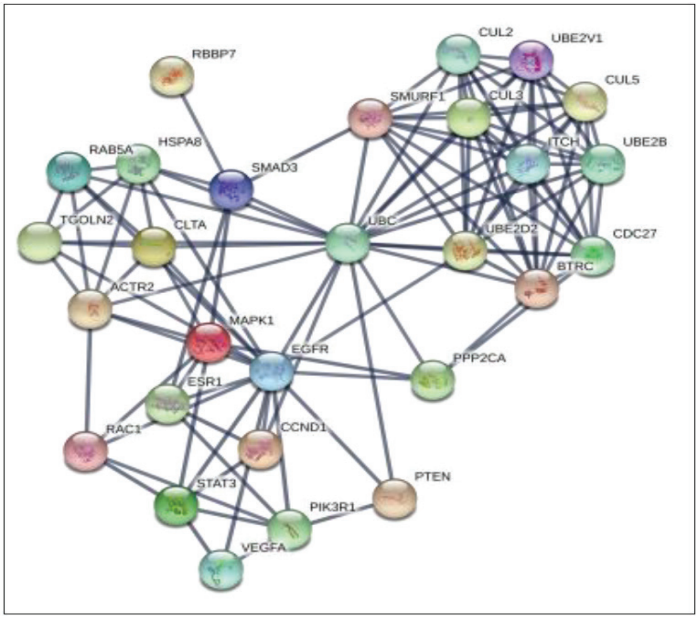

Figure 2. PPI network of 28 hub genes

Construction of miRNA-hub genes regulatory network

Twenty-eight hub genes and their corresponding ten miRNAs made 41 miRNA-hub gene pairs in the network. The relationship between miRNAs and hub genes are shown in Figure 3. miRNAs; hsa-miR-15b-5p, hsa-miR-93-5p, and hsa-miR-130a-3p are considered to be potential key miRNAs.

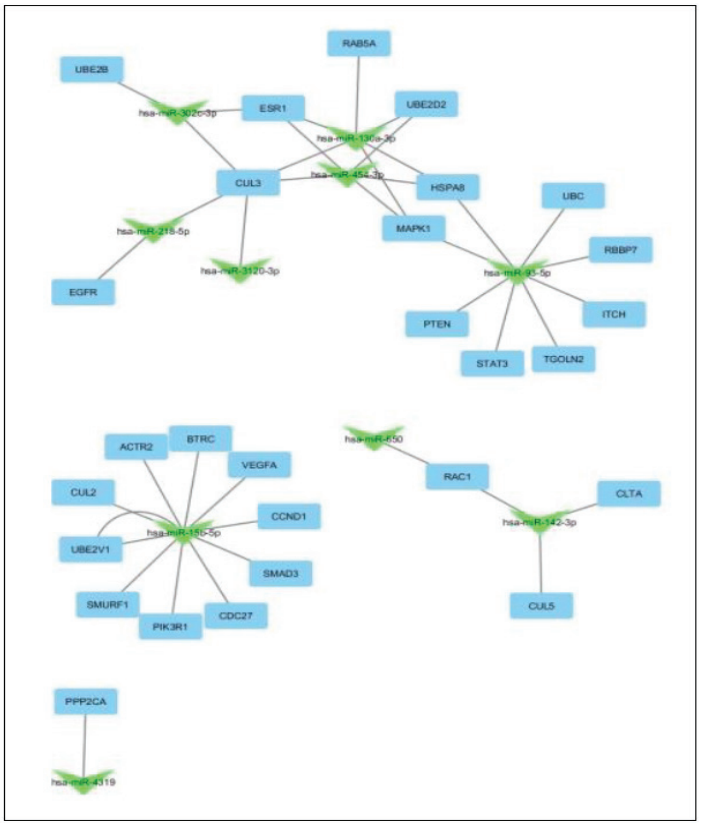

Figure 3. miRNA-hub genes regulatory network. Blue corresponds to the hub genes and green to the miRNAs

Discussion

MicroRNAs (miRNAs) are small non-coding oligonucleotides which are capable of negatively regulating expression of mRNAs by inhibiting protein translation [16]. In recent years, miRNA profiling data sets have increased rapidly with the development of high-throughput techn iques, paving the way for bioinformatics studies. In this study, we detected hsa-miR-15b-5p, hsa-miR-93-5p, and hsa-miR-130a-3p as crucial regulators in chemoresistance of HSCC by using multivariate statistical analysis and bioinformatics approaches.

hsa-miR-15b-5p was shown to be differentially expressed in head and neck squamous cell carcinoma and concluded that this miRNA might be a potential biomarker for individualized treatment in this disease [17,18]. In a study, the different miR expression profiles between laryngeal squamous cell carcinoma and the surrounding normal tissues were compared using miR array. Among differentially expressed miRNAs, miR-93 was upregulated [19]. In a multidrug resistance study, microarray analysis showed that miR-93 was downregulated in multidrug-resistant Hep2/v cells compared with Hep-2 cells [20] and also hsa-miR-130a-3p was shown to be relevant for a response to cisplatin in esophageal squamous cell carcinoma [21]. Moreover, hsa-miR-130a-3p was highlighted in hepatocellular carcinoma drug resistance [22,23], and in-vitro as well as in-vivo studies showed that overexpression

of this miRNA promoted the invasion, migration, and proliferation of nasopharyngeal carcinoma cells [24].

Conclusion

In conclusion, by using bioinformatics approach, we suggested that hsa-miR-15b-5p, hsa-miR-93-5p, and hsa-miR-130a-3p might act as crucial regulators in chemoresistance of HSCC, and they might serve as prognosticators and therapeutic targets in the future.

Competing interests
The authors declare that they have no competing interest.

Financial Disclosure
There are no financial supports

Ethical approval
No ethic approvell is needed to this research.

Elif Pala ORCID: 0000-0002-1690-3170
Tuba Denkceken ORCID: 0000-0002-4663-5410

References

1. Takes RP, Strojan P, Silver CE, et al. Current trends in initial management of hypopharyngeal cancer: the declining use of open surgery. Head & Neck. 2012;34:270-281.
2. Pointreau Y, Garaud P, Chapet S, et al. Randomized trial of induction chemotherapy with cisplatin and 5-fluorouracil with or without docetaxel for larynx preservation. J NTL Cancer I. 2009;101:498-506.
3. Vermorken JB, Remenar E, Van Herpen C, et al. Cisplatin, fluorouracil, and docetaxel in unresectable head and neck cancer. N Engl J Med. 2007;357:1695-704.
4. Lian M, Shi Q, Fang J, et al. In vivo gene expression profiling for chemosensitivity to docetaxel-cisplatin-5-FU (TPF) triplet regimen in laryngeal squamous cell carcinoma and the effect of TPF treatment on related gene expression in vitro. Acta Otolaryngol. 2017;137:765-72.
5. Goksuluk D, Korkmaz S, Zararsiz G, et al. easyROC: An Interactive Web-tool for ROC Curve Analysis Using R Language Environment. The R Journal. 2016;8:213-230.
6. Abdi H and Williams LJ. Principal component analysis.WIREs Comp Stats. 2010;2:433-59.
7. Lachenbruch PA and Mickey MR. Estimation of error rates in discriminant analysis. Technometrics. 1968;10:1-11.
8. RStudio Team (2016). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA.
9. Haunsberger SJ, Connolly NM, and Prehn JH miRNAmeConverter: an R/bioconductor package for translating mature miRNA names to different miRBase versions. Bioinformatics. 2016;33:592-3.
10. Ru Y, Kechris KJ, Tabakoff B, et al. The multiMiR R package and database: integration of microRNA–target interactions along with their disease and drug associations. Nucleic Acids Res. 2014;42:e133-e133.
11. Huang DW, Sherman BT, and Lempicki RA Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc. 2008;4:44.
12. Szklarczyk D, Gable AL, Lyon D, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res. 2019;47:607-13.
13. Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13:2498-504.
14. Brenning A and Lausen B Estimating error rates in the classification of paired organs. Stat Med. 2008;27:4515-31.
15. Fawcett T An introduction to ROC analysis. Pattern Recogn. Lett. 2006; 27:861-74.
16. Ma L and Weinberg RA MicroRNAs in malignant progression. Cell Cycle. 2008;7:570-2.
17. Ahmad P, Sana J, Slavik M, et al. MicroRNA-15b-5p Predicts Locoregional Relapse in Head and Neck Carcinoma Patients Treated With Intensity-modulated Radiotherapy. Cancer Genom Proteom. 2019;16:139-46.
18. Ramdas L, Giri U, Ashorn C, et al. miRNA expression profiles in head and neck squamous cell carcinoma and adjacent normal tissue. Head Neck. 2009;31:642–54.
19. Cao P, Zhou L, Zhang J, et al. Comprehensive expression profiling of microRNAs in laryngeal squamous cell carcinoma. Head Neck. 2013;35:720-8.
20. Yin W, Wang P, Wang X, et al. Identification of microRNAs and mRNAs associated with multidrug resistance of human laryngeal cancer Hep-2 cells. Braz J Med Biol Res. 2013;46:546-54.
21. Lindner K, Eichelmann AK, Matuszcak C, et al. Complex Epigenetic Regulation of Chemotherapy Resistance and Biology in Esophageal Squamous Cell Carcinoma via MicroRNAs. Int J Mol Sci. 2018;19:499.
22. Xu N, Shen C, Luo Y, et al. Upregulated miR-130a increases drug resistance by regulating RUNX3 and Wnt signaling in cisplatin-treated HCC cell. Biochem. Biophys. Res. Commun. 2012;425:468-72.
23. Li B, Huang P, Qiu J, et al. MicroRNA-130a is down-regulated in hepatocellular carcinoma and associates with poor prognosisMed. Oncol. 2014;31:230.
24. Chen X, Yue B, Zhang C, et al. MiR-130a-3p inhibits the viability, proliferation, invasion, and cell cycle, and promotes apoptosis of nasopharyngeal carcinoma cells by suppressing BACH2 expression. Biosci Rep. 2017;37:BSR20160576.