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MICRORNA-145 INHIBITOR INDUCED PROLIFERATION OF HUMAN ORAL SQUAMOUS CELL CARCINOMA BY UPREGULATING C-MYC AND DOWNREGULATING CASPASE-3 GENES: AN IN-VITRO STUDY

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ABSTRACT

Background: Oral cancer has contributed to a tremendous death rate and the total survival rate is expected to be about 50% after 5 years. MicroRNAs are associated with oral carcinogenesis due to their ability to regulate gene expression. To limit tumor growth, miR-145 influences signaling pathways by targeting tumor-specific genes.

Objective: This research was conducted to investigate the potential impact of miR-145 on the human oral squamous cell carcinoma cell line (OECM-1), and Human Oral Fibroblast cell line (HOrF) as negative control cells. Also, to investigate its impact on the expression of C-Myc and Caspase-3 genes.

Material and Methods: In this study, there were four groups. **Group I:** untreated OECM-1, **Group II:** treated OECM-1, **Group III:** Untreated HOrF, and **Group IV:** treated HOrF. MiR-145 inhibitor was transfected into cell lines. Methyl Thiazole Tetrazolium assay was used to analyze the vitality and the cell proliferation rate of the cell lines. Furthermore, the expression of C-Myc, and Caspase-3 was assessed in OECM-1 and HOrF cell lines using SYBR Green-based qPCR.

Results: The findings demonstrated a significant increase in the cell viability and the proliferation rate after transfection of OECM-1 cells with miR-145 inhibitor. In addition, miR-145 inhibitor transfected OECM-1 cells enhanced C-Myc gene expression and decreased Caspase-3 gene expression.

Conclusion: Suppression of miR-145 caused a higher proliferation rate in Human Oral Squamous Cell Carcinoma (OECM-1) by increasing C-Myc gene expression and downregulating the level of Caspase-3 gene.

KEYWORDS: Oral squamous cell carcinoma, MiR-145, C-Myc, Caspase-3.

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INTRODUCTION

Oral squamous cell carcinoma (OSCC) is a cancerous condition that originates in the oral mucosa's stratified squamous epithelium and may progress to any part of the mouth, including the lips and even the oropharynx [1].

OSCC is responsible for more than 90% of all oral cancers. It ranks sixth globally in terms of incidence and eighth in terms of mortality [2].

The excessive consumption of tobacco, alcohol, and betel nuts seems to affect the evolution of OSCC [3].

According to the most recent worldwide statistics, there were 354,864 cases and 177, 384 deaths of oral cancer in 2023 [4].

The accumulation of genetic and epigenetic changes is the main driving factor of oral carcinogenesis which is an intricate and multi-step process. The process of tumorigenesis and tumor growth is aided by genetic abnormalities, such as point mutations, deletions, amplifications, and chromosomal rearrangements, cumulatively occurring [5].

Alongside genetic and epigenetic pathways, microRNAs (miRNAs or miRs) have been demonstrated to affect a variety of physiological processes and to be significant modulators of carcinogenesis in a variety of malignancies [6].

MiRNAs are noncoding, single-stranded RNAs that range in length from about 19 to 24 nucleotides. Many biological processes rely on them. The approximately 8,000 miRNAs that make up the human miRNA base are thought to be the main regulators of nearly 30% of all genes [7].

MiRNAs negatively influence messenger RNAs (mRNAs) either by actively encouraging the target mRNA's degradation or by hindering translation [8].

Since miRNAs and their targets are not precisely matched, they have the ability to regulate a broad spectrum of genes [9].

In this regard, miRNAs are showing great potential as promising biomarkers for diagnostic and monitoring purposes in OSCC [10].

Because they specifically target tumor suppressor genes and oncogenes, miRNAs are categorized as either tumor suppressors (oncosuppressors) or oncogenic (oncomiRs), which trigger tumor progression [11].

MiRNAs have been found to be associated with oral carcinomas due to their role in disruption of the cell cycle, cell proliferation, tumor progression, metastasis, cell cycle arrest, and chemoresistance [7].

The miR-145 gene has a 4.08 kb length and resides in chromosome 5 at position 5q32-33. This location is often deleted through carcinogenesis. Proximity to a crucial tumor-vulnerable area suggests a possible link between the miR-145 gene location and the initiation of malignancies [12].

MiR-145 consequently influences related signaling pathways hence suppressing tumor development, spread, and invasion. Additionally, it can enhance sensitivity to chemotherapeutic drugs and induce apoptosis [11].

Diminished levels of miR-145 expression have been associated with many cancers, including gastric, ovarian, breast, pancreatic, and lung cancers. According to reports, miR-145 prevents the progression of tumors in certain cancers, including gliomas, gallbladder cancer, and OSCC [13].

The C-Myc gene is a critical member of the Myc family of oncogenes. It is located on chromosome 8q24. C-Myc is linked to numerous malignancies' occurrence, growth, medication resistance, invasion, and metastasis [14].

Downregulation of miRNAs with tumor suppressor properties, miR-145, caused overexpression of proto-oncogenes like C-Myc [15].

Apoptosis, cell cycle progression, and tumor cell proliferation are all impacted by amplification or enhanced expression of the C-Myc gene [16].

Overexpression of miR-145 decreased Bcl-2 and C-Myc, which stop cancer cells from replicating by triggering apoptosis and impeding the cell cycle at the G1/S checkpoint [17].

Post transcriptionally, miR-145 hinders tumor development, angiogenesis, and invasion by targeting and controlling vascular endothelial growth factor-A (VEGF-A) and N-RAS [18].

The Caspase-3 gene is a critical modulator of apoptosis that is triggered in apoptotic cells by both intrinsic (mitochondrial) and extrinsic (death ligand) routes [19].

Caspase-3 is encoded by the human CASP3 gene. It belongs to the family of cysteine-aspartic acid proteases. The human Caspase-3 gene, which has seven exons totaling 2,635 base pairs, maps to chromosome 4 at q33–q35 [20].

They are normally represented as inactive zymogens, but upon stimulation, autolytic cleavage occurs, and they become completely active. When miR-145 is upregulated, it triggers the Caspase-3 cascade and causes apoptosis [21].

Furthermore, miR-145 can efficiently down-regulate genes linked to metastasis and stemness, such as CD44, SOX-2, and Matrix metalloproteinases (MMP-9, MMP-2), and up-regulate genes involved in apoptosis, such as Bax, Caspase-3, and Caspase-9 [22].

MATERIAL AND METHODS

Ethical Approval

The research protocol with protocol number (4-586/2022) was approved by the Research Ethics Committee of the Faculty of Dentistry, Minia University at meeting number (87).

I. Material

Cell lines:

Human Oral Squamous Cell Carcinoma cell line (OECM-1) and Human Oral Fibroblast (HOrF) were obtained from the Veterinary Serum and Vaccine Research Institute (VACSERA) in Cairo, Egypt.

Reagent:

MiR-145 inhibitor was obtained from Qiagen, Hilden, Germany.

At Global Research Labs, situated in Medical Centre 2, Nasr City, Cairo, Egypt, this investigation was conducted.

Study Design

In this study, we had four groups

Group I: Untreated OECM-1.

Group II: Treated OECM-1.

Group III: Untreated HOrF.

Group IV: Treated HOrF.

II. Methods

1. Transduction of OECM-1 cells with MiR-145 Inhibitor

Prior to experimenting, OECM-1 cells were placed in a 96-well culture plate and allowed to incubate for one day. Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Thermoscientific, Germany) was used to seed approximately 1 x 10^4 OECM-1 cells in 200 μL.

The medium was supplemented with some antibiotics like streptomycin (10 mg), 1% penicillin G sodium (10.000 UI), amphotericin B (25 µg) (PSA) along with 10% fetal bovine serum. All of these came from Gibco, Thermoscientific, Germany.

Achieving 70% confluence required one day of incubation at 37 °C in a 5% CO_2 environment on culture plates. A complex was created the next day by dispensing 0.5 μL of miR-145 inhibitor into 3 μL of RNase-free water. Once introduced to the cells, this resulted in a final miRNA inhibitor concentration of 50 nM.

The transfection complexes were prepared by mixing 24.25 μL of Roswell Park Memorial Institute culture media (RPMI) without serum with 0.75 μL of HI Perfect Transfection Reagent (cat no: 301704).

The complex was then incubated at 15-20°C for 10 minutes. After 25 μL of the complex has been introduced, 175 μL of DMEM medium is subsequently inserted into each well. The MTT assay was used to verify the viability of the transfected OECM-1 cells, which were maintained at 37 °C in 5% CO_2 for 48 hours subsequent to transfection.

To confirm the validity of the transfection experiment, the AllStars siRNA negative control, cat no.1027280, was employed as a negative control for the miRNA inhibitor. Additionally, the has-miR-1 mimic (catalog number MSY0000416) was implemented as a positive control. Both were from Qiagen, Hilden, Germany. The un-transfected cells were utilized for normalization.

To make sure that inhibiting miR-145 wouldn't have an impact on the viability of healthy cells, the same procedure was carried out on normal human oral fibroblast cells.

2. Methyl Thiazole Tetrazolium Assay for Vitality and Proliferation Assessment

Methyl Thiazole Tetrazolium (MTT) was done using The Vybrant® MTT Cell Proliferation Assay Kit obtained from Thermo Fisher, Germany with cat no M6494, was used to perform the MTT assay. The viability of the miR-145 inhibitor transfected OECM-1 and HOrF cells was evaluated.

After the incubation period, 100μL of the media was taken out and replaced with fresh media. Each well received 20 μL of 4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide solution (1 mg/mL) (Invitrogen, ThermoScientific, Germany). This was followed by a four-hour incubation period at 37 °C in a 5% CO_2 environment.

Lastly, 100 µL of SDS-HCL (sodium dodecyl sulphate with hydrochloric acid) was added to each well after the MTT solution was discarded. Cell viability was evaluated by measuring the optical density at 570 nm with a spectrophotometer (ELx 800; Bio-Tek Instruments Inc., Winooski, VT, USA).

3. Real-time Polymerase Chain Reaction for Gene Expression Analysis

a. Cell Harvesting from Culture Media

Once the cells had grown in a monolayer 6 cell-culture well, they were trypsin-lysed and collected as a pellet of cells; this was followed by washing twice in phosphate buffer saline (pH 7.4).

A rotor-stator homogenizer termed Tissue Ruptor II, manufactured by Qiagen in Hilden, Germany, was used to break up and simultaneously homogenize approximately $1x10^6$ cells in 700 µL of Qiazol lysis. Depending on the size and toughness of the sample, this homogenizer can complete these steps in 15–90 seconds.

b. Total RNA and MiRNAs Extraction and Purification

The resulting mixture was loaded onto an RNeasy Mini spin column after the tissue homogenate had been treated with ethanol. Following complete RNA binding to the RNeasy silica membrane and successful impurity removal, high-quality RNA was extracted in RNase-free water.

RNA was extracted and purified using the RNeasy Mini kit (cat. no. 74104) from Qiagen in Hilden, Germany. This process adhered to the guidelines provided by the manufacturer (23).

c. Reverse Transcription to Synthesize cDNA

The reverse transcription process was used to produce CDNA using the miScript RT Kit from Qiagen in Hilden, Germany.

To begin the first strand cDNA synthesis, a mixture of 4 μl of 5x miScript HiFlex buffer,2 μl

of miScript reverse transcriptase mixture, 2 μl of 10x miScript dNTP mixture, and 7 μl of RNase-free water was mixed on ice to create the reverse transcription master mix.

With a gentle up-and-down pipetting motion, 5 μl of RNA sample was added to each tube. The tubes were then loaded into the Thermal Cycler, sourced from Biometra in Germany, after a short centrifugation to remove any air bubbles and settle the contents.

It took one cycle of 37 ºC for 60 minutes and 95 ºC for 5 minutes to reverse transcribe and synthesize the first strand of cDNA. Cold storage of the cDNA product of the reverse transcription at -20ºC was performed prior to amplification.

d. MiR-145 Expression Analysis

The estimation of miR-145 expression levels was achieved by amplifying the miRNA extract using a miScript primer assay [Hs_miR-145, cat no: 218300, assay ID: MS00003528].

Amplification of the miRNAs was accomplished using the miScript SYBR Green Master Mix (Qiagen, Hilden, Germany). The RUN6 Primer Assay (cat no. 218300; assay ID: MS00033740) was implemented.

The following ingredients were added to prepare the PCR reaction mix: 10x miScript Universal (1μL), 5μL of 2x miScript SYBR Green Master mix, template cDNA(1μL), 10x miScript Hs_miR-145 Primer Assay(1μL), template cDNA(1μL), and RNase-free water. These components were defrosted at room temperature (15-25ºC).

Subsequently, 2 μL of RNase-free water was added until the total volume reached 10 µl for each well's reaction.

e. C-Myc and Caspase-3 Genes Expression Analysis

The QuantiTect SYBR Green PCR Kit (cat no. 204141), QuantiTect primer assay [Hs_C-Myc] (with cat no. 249900 and ID: QT00035406), QuantiTect Primer Assay [Hs_CASP3] (ID: Q00186333] and the Hs_ACTB_1_SG QuantiTect Primer Assay (β-actin) cat no. 249900, assay ID: QT00095431 were used to measure the expression levels of the C-Myc and Caspase-3 genes after miR-145 amplification.

The PCR reaction mix was prepared using the following steps to achieve an entire volume of 18 µl per well: 10 µl of 2x QuantiTect SYBR Green PCR Master Mix, 2 µl of 10x QuantiTect Primer Assay, 2 µl of 10x Universal Primer, and 4 µl of RNase-free water. 2 µl of template cDNA was added to yield an overall volume of 20 µl.

After careful mixing, the reaction mix was placed into rotor-disc wells in the right proportions and sealed with heat-sealing film. All samples were investigated using the 5-plex Rotor-Gene PCR Analyzer manufactured by Qiagen in Germany.

The RT-PCR software was utilized to perform the activation step of the Hot Star TaqDNA Polymerase at 95ºC for 15 minutes. Subsequently, there were a total of 40 repetitions for each of the three procedures: denaturation at 94°C for 15 seconds, annealing at 55 °C for 30 seconds, and extension 70°C for duration of 30 seconds.

For miR-145, the levels of RUN6 were utilized as a reference gene to standardize the expression levels. However, for C-Myc and Caspase-3, β-actin was employed as a housekeeping gene.

The comparative expression level (fold change) relative to the calibrator (negative control sample) was determined after normalization to the internal control using equation 2-ΔΔCt test control.

4. Statistical Analysis

A one-way analysis of variance (ANOVA), and Tukey's multiple comparison were utilized to find the groups with significantly different means. The ***P***-value ≤0.05 was considered statistically significant.

RESULTS

1- Methyl Thiazole Tetrazolium Assay for Vitality and Proliferation Assessment

The percentage of proliferation index was calculated based on the proliferation of OECM-1 cells transfected with miR-145 (treated cells), and OECM-1 (untreated cells). The findings revealed that, OECM-1 treated cells with miR-145 inhibitor exhibited a considerable increase in cell proliferation (141.8%) when compared to the untreated cells (99.9%), as shown in table 1 and figure 1.

However, to determine if suppressing miR-145 would be detrimental to normal cells, we transduced the HOrF cells with the anti-miR-145. The acquired data presented that after miR-145 was suppressed, no significant change occurred in normal cells (vitality percentages were 95.8% and 100.4% respectively), as presented in table 1 and figure 1.

2- Real-time Polymerase Chain Reaction for Gene Expression Analysis

- MiR-145 Expression Analysis

The expression of miR-145 gene in OECM-1 and HOrF cells was evaluated using SYBR Green qPCR. It was also measured in OECM-1 after transduction with a miR-145 inhibitor.

When OECM-1 cells were compared to HOrF cells, results revealed a noteworthy decrease in miR-145 expression, the inhibitor caused more downregulation in the level of miR-145 in OECM-1 transfected cells, as illustrated in table 2.

TABLE (1) MTT assay for OECM-1 and HOrF cells transfected with miR-145 inhibitor for 48 hours

Experiment	Transduction with miR-145 inhibitor			
Drug	**MiR-145 inhibitor**		**Incubation: 48 hours**	
Cells	**OECM-1**		**HOrF**	
	OECM-1	**MiR-145 inhibitor**	**HOrF**	**MiR-145 inhibitor**
Average of OD	2.38	3.37	2.57	2.43
Viability (%)	99.9%	141.8%	100.4%	95.8%

OD: optic density at 570nm.

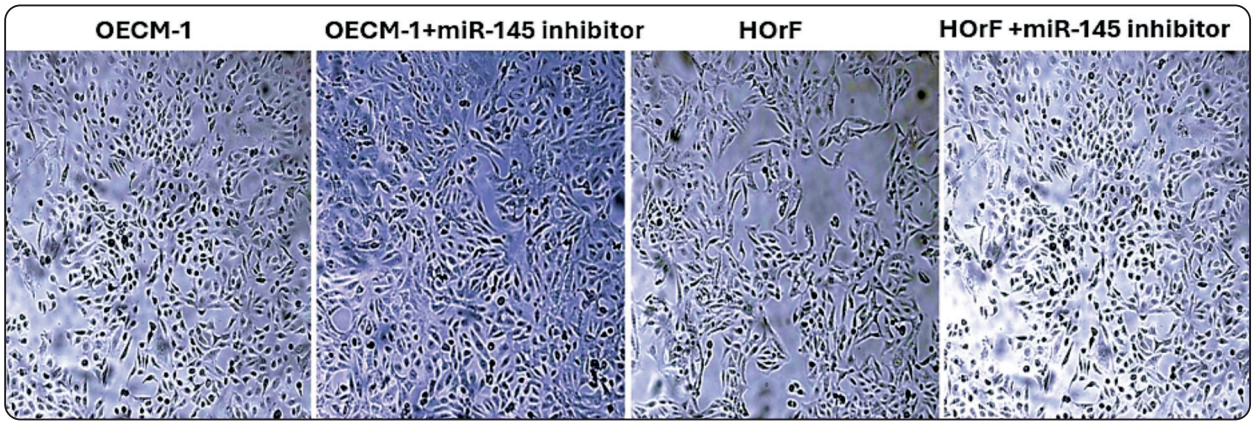

Fig. (1). Photomicrographs of OECM-1 and HOrF cells provided with anti-miR-145, taken with a digital camera from Labomed, USA, called the Vega (LC-6 USB 3.0 COLORFUL CMOS DIGITAL CAMERAS, 5MP).

TABLE (2) MiR-145 gene expression

Serial	Sample code	Fold change [MiR-145]
1	HOrF	1.003
2	OECM-1	0.312
3	MiR-145 inhibitor	0.141

- C-Myc and Caspase-3 Genes Expression Analysis

The target genes of miR-145 "C-Myc and Caspase-3" were evaluated in HOrF cells, and OECM-1 cells following transduction with miR-145 inhibitor.

The collected data showed a significant increase in C-myc expression in OECM-1 transfected with miR-145 inhibitor when compared to OECM-1 untransfected cells.

Furthermore, a greater expression level of C-Myc gene was strongly linked to OECM-1 compared to HOrF Cells, as shown in table 3.

Regarding Caspase-3 "Tumor suppressor gene", its expression level was assessed in OECM-1 cells after transduction with miR-145 inhibitor. MiR-145 inhibition caused a decrease in Caspase-3 gene expression, compared to untreated cells, as presented in table 4.

TABLE (3) C-Myc gene expression

Serial	Sample code	Fold change [C-Myc]
1	HOrF	1.011
2	OECM-1	3.689
3	MiR-145 inhibitor	6.233

TABLE (4) Caspase-3 gene expression

Serial	Sample code	Fold change [CASP3]
1	HOrF	1.01
2	OECM-1	0.529
3	MiR-145 inhibitor	0.157

Statistical Analysis

As illustrated in tables (5 and 6) and figures (2 and 3), the descriptive statistics of ANOVA test and Tukey multiple comparison test showed comparative analysis of C-Myc and Caspase-3 genes.

TABLE (5) Comparative analysis for C-Myc expression in the studied groups

Group	Mean±SD	Range	Statistics
HOrF	1.01±0.199	0.80 – 1.20	
Untreated OECM-1	3.69±0.05	3.63 – 3.73	***P***=0.0002 **
Treated OECM-1	6.23±1.05	5.39 – 7.41	

Table (6): Comparative analysis for Caspase-3 expression in the studied groups

Group	Mean±SD	Range	Statistics
HOrF	1.01±0.162	0.908 – 1.20	
Untreated OECM-1	0.52±0.06	0.467 – 0.574	***P***=0.0001 **
Treated OECM-1	0.157±0.03	0.122 – 0.180	

By comparing the means of different groups using Tukey's multiple comparison test, we were able to track the experiment-wise error rate. *P*-value <0.05 was considered statistically significant, as shown in table 7 and 8.

TABLE (7) Post-Hoc multiple comparison analysis for the C-Myc expression in the studied groups

Tukey's multiple comparison test	Mean Diff.	95.00% CI of diff.	*P*-value
OECM-1 vs. miR-145 inhibitor	-2.544	-4.096 to -0.9917	0.006*
OECM-1 vs. HOrF	2.678	1.126 to 4.230	0.004*
MiR-145 inhibitor vs. HOrF	5.222	3.670 to 6.774	0.0001**

CI: Confidence Interval.

TABLE (8) Post-Hoc multiple comparison analysis for the Caspase-3 expression in the studied groups

Tukey's multiple comparison test	Mean Diff.	95.00% CI of diff.	*P*-value
OECM-1 vs. miR-145 inhibitor	0.3720	0.120 to 0.623	0.009*
OECM-1 vs. HOrF	-0.4813	-0.73 to -0.230	0.003*
MiR-145 inhibitor vs. HOrF	-0.8533	-1.16 to -0.602	0.0001**

CI: Confidence Interval.

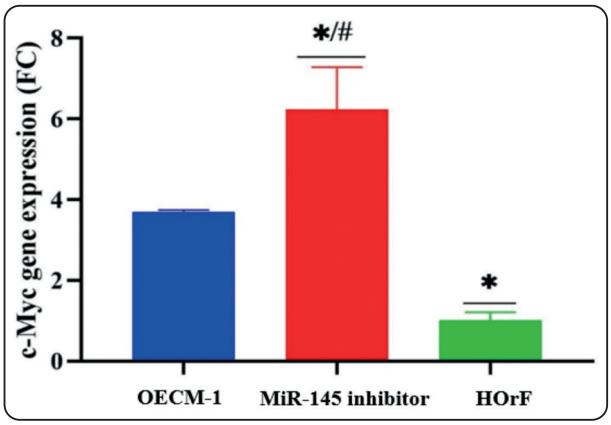

Fig. (2) A significant increase in C-Myc expression (FC) following miR-145 suppression.

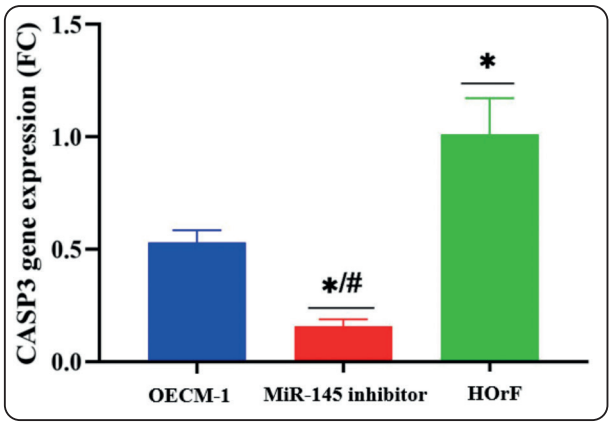

Fig. (3) A significant reduction in Caspase-3 expression (FC) after miR-145 inhibition.

DISCUSSION

Oral squamous cell carcinoma (OSCC) is an extremely aggressive tumor. It represents 90% of all oral cancers. It develops by transforming the oral mucosal epithelium in the lips, tongue, and gums into cancerous cells [2].

The process of oral carcinogenesis is a series of events that start when cumulative genetic and epigenetic modifications impact the surface squamous epithelium. These modifications alter the cellular dynamics from mildly aberrant growth to highly invasive and metastatic malignancy [7].

An upsurge in the field of miRNAs has occurred since multiple studies have shown their critical role in cancer [24].

MiRNAs are known to be significant modulators of gene expression that can change the expression of genes in ways that are either oncogenic or tumor-suppressive [25].

MiRNAs can control numerous genes, possibly hundreds, because of imperfect complementarity when binding to mRNA [26].

MiR-145 is considered noteworthy among the thousands of miRNAs associated with cancer that have been discovered, because of various

investigations examining its biological function and therapeutic significance [27].

Recent data indicates that miR-145 has the potential to be an appropriate therapy and predictive response marker that could increase chemotherapy's effectiveness [28].

It was found that miR-145-5p silences an enormous number of genes that govern all aspects of cancerous activity, including angiogenesis, metastasis, proliferation, differentiation, and resistance to treatment [29].

The identification of miRNA target genes offers information for the creation of novel treatments [13].

By targeting C-Myc, miR-145 inhibits tumor invasion and proliferation while simultaneously inducing cell death [30].

Nuclear protein C-Myc acts as a transcription-related factor and controls numerous essential processes, including metabolism, division, differentiation, adhesion, motility of cells, and angiogenesis [7].

The Caspase family is extremely essential for regulating programmed cell death. Caspase-3 is the most prevalent executioner caspase in apoptosis [12].

To establish a more thorough comprehension of miR-145 possible function in the proliferation rate of OSCC, Methyl Thiazole Tetrazolium assay (MTT assay), Real-time Polymerase Chain Reaction for (C-Myc and Caspase-3) genes Expression analysis, and statistical analysis were implemented in this research.

MTT assay showed that OECM-1 cells transfected with miR-145 inhibitor had a much higher rate of cell proliferation (141.8%) than cells that were not transfected (99.9%).

The observations of the current research concurred with ***Chen et al. 2020*** who found that miR-145 inhibitor-transfected pancreatic cancer (PC) cells proliferated faster than controls in the MTT experiment. This happened as a result of the TGF-ß signaling pathway being triggered by down-regulation of miR-145, which led to improved cell mobility and invasion capabilities [31].

Also, ***Zeinali et al. 2020*** claimed that miR-145 overexpression interfered with cell proliferation, as evidenced by the MTT results. This was accomplished by lowering C-Myc and K-Ras expression in the gastric adenocarcinoma cell line, MKN-45, which had been transfected with miR-145 [32].

Cao et al. 2020 highlighted that miR-145 mimic in cervical carcinoma caused suppression of cellular reproduction and progression of the cell cycle through the downregulation of KLF5, a transcription factor belonging to the Krüppel-like transcription factor (KLF) family, which is involved in cell growth, differentiation, and proliferation [33].

According to the findings of this investigation, it was found that miR-145 showed a tumor-suppressing role, as indicated by its low expression level in OSCC cells compared to normal cells and the much higher rate of proliferation in the miR-145 inhibitor-transfected OSCC cell line. The same findings were revealed ***by Singh et al. 2023*** [34].

It has been established through numerous findings from previous studies that miR-145 is downregulated in a variety of tumors [27].

To clarify the gene regulatory frameworks that miR-145 influences, this work inspected the implication of miR-145 on the expression of C-Myc and Caspase-3 in HOrF cells and OECM-1 cells following miR-145 inhibitor transfection.

The findings revealed that suppression of miR-145 was considerably correlated to increased C-Myc expression levels and reduction in Caspase-3 expression levels in OECM-1 treated compared to untreated groups.

The current study's results matched with previous research by ***Al Rawi et al. 2021*** who demonstrated

that miR-145 was less expressed in oral cancer. It suppressed OSCC invasion by acting on C-Myc and cdk6 [35].

Consistent with the demonstrated findings, the study by ***Zou et al. 2019*** highlighted miR-145's anti-oncogenic function which supported our data. Upregulation of miR-145 by transfecting SGC-7901 cells (gastric cancer cell line) with miR-145 mimic intensified cell death and inhibited cell proliferation by decreasing C-Myc expression [30].

Findings of this research demonstrated a noteworthy decrease in Caspase-3 gene expression in OECM-1cells transfected with an inhibitor of miR-145.

Like these outcomes, prior research stated that miR-145 triggered apoptosis in tongue SCC, as documented by ***Xin et al. 2021***. MiR-145-5p knockdown reduced apoptotic death and decreased Caspase-3 level [36].

Additionally, ***Pan et al. 2018*** established that miR-145 downregulated Bcl-2 and upregulated Caspase-3 and Bax expression, which reduced proliferation and promoted death of NSCLC cells (A549) [21].

The study's observation confirmed ***Gupta et al. 2024*** who verified that miR-145 directly activated BAX/Caspase-3 after DNA damage to induce apoptosis. They confirmed that miR-145 overexpression restricted NSCLC via suppressing C-Myc. It also targeted MALAT1 and BMI1 to eliminate drug resistance [17].

Similarly, ***Du et al. 2017*** reported that miR-145 boosted Caspase-9 and Caspase-3 expression in glioma cells [37].

The findings of the present research were similar to those observed by ***Li et al. 2018***. They reported that via controlling the EGFR downstream signaling pathway, miR-145 suppression by miR-145 inhibitor increased cell proliferation in NSCLC A549 cells and lessened Bax protein, Caspase-3/-9 levels which in turn decreased apoptosis [38].

Finally, statistical analysis verified that there was a statistically significant difference between the groups. The ***P***-value was less than 0.005. The results confirmed the relationship between miR-145 suppression and increased OECM-1 proliferation by increasing C-Myc and decreasing Caspase-3 genes.

CONCLUSIONS

MiR-145 inhibitor produced proliferation of human oral squamous cell carcinoma. MiR-145 inhibitor induced upregulation of C-Myc and downregulation of Caspase-3 genes.

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