 DOI: 10.21608/adjalexu.2024.245158.1428

TARGETING WNT PATHWAY THROUGH miR-142-3P AS A POTENTIAL THERAPEUTIC APPROACH IN ORAL SQUAMOUS CELL CARCINOMA (IN VITRO STUDY)

Yara M. Eldahshan[1*] BDS, Sahar E. Riad[2] PhD, Hagar R. Elkafrawy[3] PhD, Enas M. Omar[4] PhD, Mai M. Saleh[4] PhD

ABSTRACT

INTRODUCTION: Oral squamous cell carcinoma (OSCC) accounts for 95% of oral cancers and is associated with a low 5-year survival rate using the conventional treatments methods. Advancements in oncological research require understanding the molecular mechanisms driving OSCC occurrence and progression to devise more effective therapeutic approaches and improving prognostic outcomes. Among these mechanisms, the WNT/β-catenin signaling pathway is recognized for its contribution in promoting tumorigenesis, cancer progression, and metastasis, making it a potential target for unconventional cancer therapies. MicroRNA-142-3p (miR-142-3p), a constituent of the miR-142 family, has demonstrated tumor-suppressive properties in certain malignancies. It was proposed that miR-142-3p positively modulates WNT signaling by directly targeting the adenomatous polyposis coli (APC) gene. This targeting destabilizes β-catenin, resulting in suppression of WNT pathway activity.

OBJECTIVES: In this current investigation, the aim was to explore the capabilities of miR-142-3p in modulating the WNT pathway and evaluating its impact on the proliferation, migration, and apoptosis of OSCC-4 cells.

METHODOLOGY: MiR-142-3p was transfected into OSCC-4 cell line. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was employed to determine cytotoxicity, while the impact on cell migration was evaluated through scratch wound healing test. Apoptotic effects were evaluated using Annexin-V, while cell proliferation was measured through Ki-67 staining, both analyzed by flow cytometry.

RESULTS: The results indicated that miR-142-3p suppressed OSCC-4 proliferation and migration, demonstrating its anti-proliferative potential. It didn't induce early apoptosis however, it enhanced late-stage apoptosis in a dose-dependent manner.

CONCLUSIONS: miR-142-3p exhibits a promise as a potential therapeutic option for OSCCs.

KEYWORDS: Oral squamous cell carcinoma, WNT pathway, Epithelial mesenchymal transition, miR-142-3p.

RUNNING TITLE: WNT Pathway Targeting via miR-142-3p in OSCC

1-BDS 2014, Faculty of Dentistry, Pharos University, Alexandria, Egypt
2-Professor of Oral Pathology, Oral Pathology Department, Faculty of Dentistry, Alexandria University, Alexandria, Egypt
3-Lecturer of Medical Biochemistry, Center of Excellence for Research in Regenerative Medicine and Applications (CERRMA), Faculty of Medicine, Alexandria University, Egypt
4-Lecturer in Oral Pathology, Oral Pathology Department, Faculty of Dentistry, Alexandria University, Alexandria, Egypt

Corresponding author:
yara.eldahshan92@gmail.com

INTRODUCTION

Oral squamous cell carcinoma (OSCC) represents the most prevalent type of oral and oropharyngeal cancers, amounting to about 95% of cases (1). It is ranked as the sixth most prevalent cancer globally, contributing to approximately 300,000 new cases annually (2). Despite advancements in therapeutic modalities, the overall survival rate for OSCC persists to be below 60%, with prognosis significantly influenced by the TNM staging (2).

The canonical WNT significantly impacts the development and progression of several varieties of human malignancies (3). It has been identified to have a pivotal role in triggering the epithelial-mesenchymal transition (EMT) process and the maintenance of Cancer Stem Cells (CSCs) (4-5). As a result, there has been a growing interest in targeting this pathway as a potential avenue for therapeutic intervention for neoplastic conditions characterized by elevated rates of both mortality and morbidity (3).

Epithelial-mesenchymal transition significantly contributes to the local recurrence and lymph node metastasis of OSCC, correlating with a lower patient survival outcome (6). Investigations have revealed the participation of MicroRNAs (miRNAs) in orchestrating EMT regulation at the post-transcriptional level (7). They play a critical role in EMT regulation, not solely through influencing key transcription factors, but also by interacting with the pathways that govern differentiation and transformation (8).

MicroRNAs are a subset of small, RNA molecules composed of a single strand that are noncoding in nature, with a length ranging approximately from 21 to 23 nucleotides (9). They show their influence over a range of signaling pathways including WNT, Notch, TGFb, and Hedgehog (7). miRNAs show two-sided behavior, functioning in the capacity of tumor suppressors or oncogenes during EMT and metastasis processes (7). Studies have indicated that miRNAs have the capacity to regulate the expression of over 30% of critical genes that play pivotal role in vital physiological processes like cell differentiation, proliferation, programmed cell death, invasion, metabolic equilibrium, and survival (10-13). Circulating miRNAs present a promising potential as biomolecular indicators for both cancer diagnosis and prognosis, regardless of tumor stage or the presence of genetic mutations (14). These miRNAs, which maintain their structural integrity and can be detected in various bodily fluids, present a valuable opportunity for detecting and predicting various diseases and conditions (15).

Among the miRNAs utilized as biomarkers, miR-142-3p stands out for its distinct ability to perform both oncogenic and tumor suppressor roles in different human cancers (16-17). For instance, in hepatocellular carcinoma cell lines, it exhibits the capability to suppress the migration and invasion through the regulation the RAC1 gene (18). Meanwhile, cervical cancer cells experience inhibition of proliferation and invasion via miR-142-3p's interaction with FZD7 (19). However, its function shifts to that of an oncogene in non-small-cell lung carcinoma cells, where it suppresses TGFβ-induced growth inhibition via modulation of TGFβR1 (20). Some studies have indicated a connection between miR-142-3p and cellular activities such as migration, proliferation, and apoptotic responses in cases of renal cell carcinoma (21) and esophageal squamous cell carcinoma (22).

Isobe et al. revealed that miR-142 and miR-142-3p were involved in the initiation of the canonical WNT pathway in breast cancer stem cells (23). A study conducted by Hu et al, revealed that miR-142-3p has a direct inhibitory impact on β-catenin by targeting its protein translation. This interaction on a molecular level result in the suppression of cellular proliferation (24).

Although several in vivo and in vitro investigations have explored the influence of miR-142-3p in various cancer types, including its impact via the WNT pathway and other signaling routes, it's worth mentioning that none of these investigations have explored the therapeutic potential of this miRNA in OSCC. Thus, this current study aims to investigate the influence of miR-142-3p on OSCC cell proliferation, migration, and apoptosis, with a focus on its interactions with the WNT pathway.

MATERIAL AND METHODS

Cell lines and treatment

The human OSCC-4 cell line was purchased from ATCC (American Type Culture Collection). The study was conducted at the Center of Excellence for Research in Regenerative Medicine and Applications (CERRMA) at Alexandria Faculty of Medicine. Experiments were conducted in accordance with the guidelines approved by the Research Ethics Committee and the Medical Ethics Committee (IRB NO: 00010556-IORG 0008839), Faculty of Dentistry, Alexandria University, Egypt. The cells were cultivated in DMEM/high-glucose (Dulbecco's modified Eagle medium; Lonza; containing 0.2 mmol/ml L-glutamine) supplemented with 100 IU/ml penicillin, 100 µg/ml streptomycin Lonza and 10% fetal bovine serum (FBS; Lonza). Cells were maintained in a humidified 37 °C, 5% CO2 incubator. Cells were monitored daily for their growth and morphology using the phase-contrast inverted microscope (CKX41SF; Olympus). Media was changed every 2 days, and cells were passaged on reaching 80%–90% confluence by suspension with 0.25% (w/v) trypsin-EDTA (Lonza), then plated in T75-cm2 flasks for maintenance or in 6- or 96-well plates according to the experiment conducted.

Cell Transfection

The OSCC-4 cells were subjected to transfection with the hsa-miR-142-3p mimic (Catalog# 4427975, Thermofisher Scientific, USA) using HiPerFect Transfection Reagent 0.5 ml (Catalog# 301704, QIAGEN, Germany) following the manufacturer's guidelines. Briefly, HiPerFect Transfection Reagent (1µM) was added to miRNA mimic (20 and 40 nM) diluted with serum-free medium (10% of total final volume). This complex was incubated for 15 minutes before being added to the cells. At 24 h or 48 h post-transfection, downstream experiments were performed.

MTT Cytotoxicity Assay

The MTT assay was carried out to investigate the possible cytotoxic effects of the miR- 142-3p in a dose-dependent manner within a concentration range of 0 to 40 nM (25).

The OSCC-4 cells were seeded in a 96-well plate at a cell density of 7000 cells per well until they reached confluence at 24 hours. Subsequently, the cells were transfected with different dose combinations of miR-142-3p (0, 5, 20 and 40 nM) and transfection reagent (0, 0.7 and 1 µM) diluted with serum-free medium (10% of total final volume). After an incubation period of 24 hours, each well received 100 µL of MTT solution (0.5 mg/mL in DMEM) and was

subsequently subjected to an additional incubation period at 37 °C for 3.5 hours. After discarding the MTT solution, 100 µl of dimethyl sulfoxide (DMSO) was added to each well to solubilize the formazan crystals generated by viable cells.

The absorbance levels of treated and untreated control wells were measured using spectrophotometry at 550 nm using an ELISA microplate reader (Infinite F50, TECAN, Switzerland).

Grouping

All the following downstream experiments were performed on the following groups of cells:

Group I: Untreated OSCC-4 cell line served as the negative control with no treatment received.

Group II: OSCC-4 cell transfected with (20 nM) and HiPerFect transfection reagent (1 µM)

Group III: OSCC-4 cell transfected with (40 nM) and HiPerFect transfection reagent (1 µM)

Scratch Wound Healing Test (Migration Assay)

The scratch wound healing test was performed to assess the anti-proliferative potential of different doses of miR-142-3p (20 nM, 40 nM) (26).

The OSCC-4 cells were cultured in 6-well plates at a density of 25×10^4 cells per well in complete media (DMEM/high-glucose, containing 0.2 mmol/ml L-glutamine; Lonza, supplemented with 100 IU/ml penicillin; Lonza, 100 µg/ml streptomycin; Lonza and 10% FBS; Lonza) until they reached 80- 90% confluence after 24 hours. Subsequently, a sterile 200-µL pipette tip was used to create controlled scratches in the cell monolayer. After washing with Phosphate-Buffered Saline (PBS) to eliminate detached cells, the scratched cells were transfected with varying doses of miR-142-3p (20 nM and 40 nM) and HiPerFect transfection reagent (1 µM) diluted with serum-free medium (10% of total final volume), while untreated scratched wells served as controls.

The migration of cells within the scratch zone was monitored and measured at 0, 24, and 48 hours. An inverted microscope was used to capture five images of the wound region in each well at a magnification of x100. To calculate both the cell migration rate and the percentage of wound closure, Image J software (version 1.53C, NIH, USA) was used for the measurement of the width of the wound gap horizontally.

Flow cytometry-based analysis of apoptosis through Annexin-V/propidium iodide assay

Annexin assay was conducted to assess anti-apoptotic capabilities of different concentrations of miR-142-3p on early and late apoptosis. Conjugated protein annexin V-FITC binds to cell surfaces expressing early apoptosis marker phosphatidylserine. Propidium iodide (PI), a non-cell-permeable DNA dye, stains necrotic cells, while PI- and annexin V-FITC-stained cells indicate early necrosis and later stages of apoptosis (27).

The apoptotic influence of miR-142-3p was assessed through Annexin-V assay and flow cytometry using a BD FACS Calibur™ flow cytometer (San Jose, USA). OSCC-4 cells (2×10^5) were cultivated in six-well plates (Corning, NY), and allowed to adhere for duration of 24 hours in a 5% CO2 incubator maintained at 37 °C. Following this, the cells were transfected with miR-142-3p concentrations of 20 nM and 40 nM and HiPerFect transfection reagent (1 µM) diluted with serum-free medium (10% of total final volume) for a period of 48 hours. Both control and treated cells underwent trypsinization, PBS rinsing, and subsequent centrifugation at 2000 rpm for 5 minutes.

Subsequently, cellular samples were collected and subjected to staining using the Annexin V-FITC/propidium iodide procedure, in accordance with the instructions that the manufacturer has provided. The assessment of apoptotic cells was performed by gating 20,000 cells using a flow cytometer. The experimental setup was conducted in triplicate (n = 3), with representative images captured.

Cell proliferation assessment via Ki-67 staining (flow cytometer)

To evaluate miR-142-3p influence on cell proliferation, modifications in Ki-67 transcription of OSCC-4 cell lines were analyzed using flow cytometry (28).

The OSCC-4 cells (2×10^5) were seeded in 6-well plates (Corning, NY), and allowed to adhere for duration of 24 hours in a 5% CO_2 incubator maintained at 37 °C. Cells were then subjected to different miR-142-3p concentrations (20 nM and 40 nM) and HiPerFect transfection reagent (1 µM) diluted with serum-free medium (10% of total final volume) for 48 hours. Post trypsinization and centrifugation, the cell pellet was cautiously treated with 70% cold ethanol (3 mL) dropwise, while simultaneously vortexed for 30 seconds. The cells were then subjected to incubation at -20°C for a 2-hour duration. Subsequent steps included two washes using 4 mL of staining buffer (PBS with 1% FBS, 0.2% Tween 20), followed by a 10 minutes centrifugation at 2000 rpm and supernatant removal (repeated twice). Cells were resuspended in a solution with a density of 1×10^7 cells/mL, afterward 100 µL of this cell suspension, consisting of 1×10^6 cells, was loaded into individual sample tubes.

Cells were stained by Alexa Fluor 488-conjugated Ki-67 antibody (1:50, Cell Signaling Technology, Cat# 11882) and incubated in the dark at room temperature for 30 minutes. After incubation, washing procedure was performed at 2000 rpm for 5 minutes using 2 mL of staining buffer. The final step involved resuspending the samples in 0.5 mL of

staining buffer and were subjected to flow cytometric analysis. Duplicate runs were conducted for each sample, capturing around 10,000 events per tube.

Statistical Analysis

Findings were presented as mean ± standard deviation (SD), obtained from three independent trials, each executed in triplicate. Analysis of the data was carried out with GraphPad Prism version 9.1.0 (GraphPad Software, San Diego, California, USA), statistical significance was defined at a p-value < 0.05. Two-way ANOVA was applied, followed by Tukey's multiple comparisons to analyze and assess statistical significance for flow cytometric, scratch wound healing, and cell viability analyses. The same program was used for illustrations and graphs designing.

RESULTS

Cell viability Assay (MTT Assay)

The data obtained revealed that the observed differences in cell viability when comparing treated and control cells were not statistically significant (Figure 1).

Scratch wound healing assay (Migration Assay)

At the initial time point (0 h), no statistically significant differences were detected in the gap observed within the scratch lines among the groups (p > 0.5), indicating consistency.

After 24 hours, untreated OSCC-4 cells showed 66.03% closure of the cell-free area. Cells treated with 20 nM had a slight reduction in width of the cell-free area at -1.622%, while 40 nM-treated cells displayed more reduction at -6.375%.

After 48 hours of treatment, untreated OSCC-4 cells closed the cell-free zone by 86.67%. Cells treated with 20 nM reduced the width moderately to 33.68%, while 40 nM-treated cells showed a more pronounced reduction to 34.62% (Figure 2).

Flow Cytometry-Based Apoptosis Detection with Annexin-V and Propidium Iodide

The cells transfected with 20 nM of miR-142-3p showed no significant change (p > 0.05) in early apoptosis (8.090 %) compared to untreated OSSC-4 cells (9.015%). Similarly, 40 nM of miR-142-3p didn't significantly impact early apoptosis (8.163%).

Conversely, the annexin assay revealed notable variations in late apoptosis percentages among the examined groups. Untreated OSSC-4 cells showed a late apoptosis rate of (0.300 %). Treating with miR-142-3p at 20 nM significantly raised (p < 0.0001) the late apoptosis rate to (2.455 %), while at 40 nM concentration, late apoptosis further increased to (3.423 %) (Figure 3, 4).

Cell proliferation assessment via Ki-67 staining (flow cytometer)

The results displayed noticeable variations in Ki-67 expression levels across the study groups. Untreated cells exhibited a Ki-67 expression of (59.99 ± 0.9235). Treatment with 20 nM miR-142-3p decreased Ki-67 expression to (41.88 ± 1.040), and using 40 nM miR-142-3p further lowered Ki-67 expression to (30.76 ± 0.8244) (Figure 5, 6).

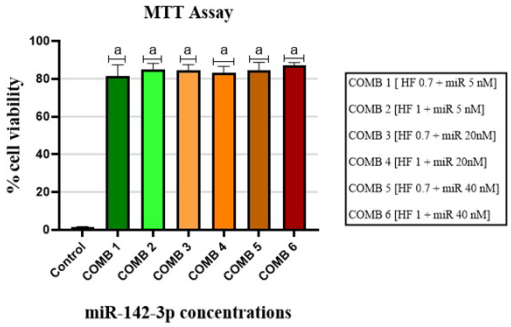

Figure 1: Column Bar graph for MTT assay showing the impact of different concentrations and combinations of miR-142-3p on cell viability following a 24-hour incubation period. Bars labeled with different letters are indicative of a significant difference. (P value < 0.05)

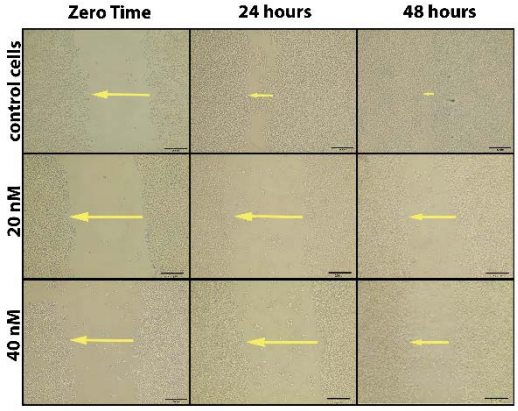

Figure 2: Migration Assay images captured at a magnification of 100 x, and a scale bar of 200 µm was used for reference. The images demonstrate the anti-migratory activity of miR- 142-3p on OSCC cells at different time intervals (0, 24, and 48 hours). Yellow arrow represents the width of the cell-free zone.

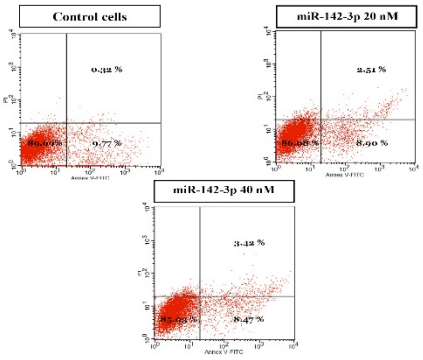

Figure 3: Scatter Plots for the flow cytometric assessment of apoptosis employing Annexin V-FITC/PI. The percentages of living cells, early apoptosis, late apoptosis, and necrosis were presented in the lower left, lower right, upper right, and upper left quadrant respectively. OSCC-4 cells subjected where subjected to different treatments where: (A) Untreated OSCC-4 cells, (B) miR-142-3p at a concentration of 20 nM, and (C) miR-142-3p at a concentration of 40 nM.

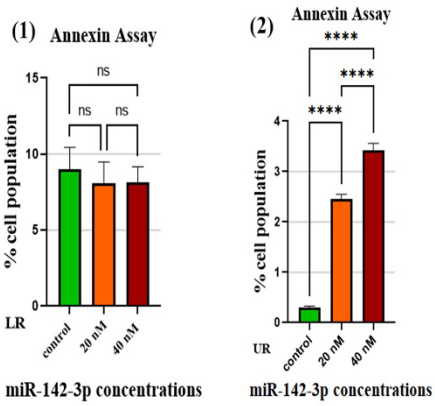

Figure 4: Column Bar graph for the quantification of the Annexin V assay Results are presented as mean ± SD Results are displayed as mean ± SD from 3 independent experiments (n=3), each performed triplicates. (B.1) LR (Early apoptosis) (B.2) UR (Late apoptosis) (***) represents significance (ns) not significant (P value < 0.05)

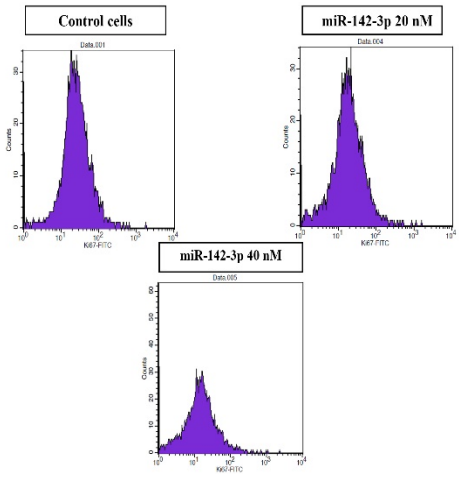

Figure 5: Histogram graph for Flow cytometric analysis of proliferation using ki-67-FITC was performed on OSCC-4 cells subjected to different treatments where: (A) Untreated cells, (B) miR-142-3p at a concentration of 20 nM, and (C) miR-142-3p at a concentration of 40 nM.

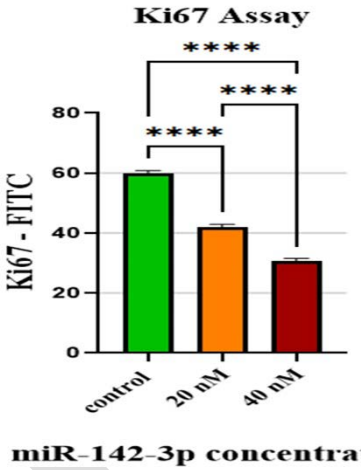

Figure 6: Column Bar graph for the quantification of the ki-67-FITC assay. Results are presented as mean ± SD Results are displayed as mean ± SD from 3 independent experiments (n=3), each performed triplicates. (***) represents significance (ns) not significant (P value < 0.05)

DISCUSSION

Despite notable advancements to comprehend the intricate involved in the development of oral cancer and progression, the development of successful treatment strategies remains pending. Challenges persist in the form of unfavorable prognosis and high recurrence rate (29).

Consequently, the formulation of efficient treatment approaches is imperative to enhance OSCC clinical outcomes. In this context, impeding pivotal molecular components in clinically relevant cancer-related pathways, such as the WNT signaling cascades which holds significance for various phases of OSCC development, is essential (30).

There has been a growing focus on potential utilization of miRNAs as prognostic indicators in different tumors (31). They have a notable impact on cancer progression through the control of oncogene or tumor suppressor gene expression (32-33). Notably, certain miRNAs with tumor suppressing properties have shown promise in mitigating cancer- associated traits like cell proliferation, migration, invasion, and resistance to chemotherapy (32-33).

Among these, miR-142-3p has attracted significant interest due to its contributions in multiple cancers (33). It actively participates in regulating oncogenic factors and holds importance as a notable miR in biomarker investigations (33).

In a study conducted by Lin et al, the possibility of miR-142-3p serving as a predictive marker for esophageal squamous cell carcinoma was highlighted (22). Nonetheless, its implications in oral squamous cell carcinoma have not been fully explored. The present study sought to investigate how miR-142-

3p impacts OSCC cell behavior, focusing on migration, proliferation, and apoptosis via its influence on the WNT pathway.

In the current investigation, miR-142-3p was tested at different concentrations (0 to 40 nM) for potential cytotoxic effect on OSCC-4 cells. No significant impact on cell viability was observed across the tested concentrations compared to untreated cells. Contrasting a study conducted by Dastmalchi et al, indicating that miR-142-3p was found to lower the cell viability in breast cancer cells (34).

This discrepancy in the effects of miR-142-3p between OSCC-4 cells and breast cancer cells emphasizes the context-specific nature of miRNA functionality. The response of cancer cells to miR-142-3p appears to be influenced by the specific cellular context, signaling pathways, and molecular makeup of the specific cancer type. These differing outcomes highlight the need for a more nuanced understanding of miRNA behavior in different cellular contexts, reinforcing the idea that miRNAs may exert different effects based on the type of cancer they interact with.

The wound healing assay demonstrated that miR-142-3p influenced cell migration, with significant reductions in cell-free area closure after 24 and 48 hours of treatment, suggesting its inhibitory effect on cell migration. In alignment with our study, Mansoori et al, demonstrated that miR-142-3p through regulating Bach-1 expression suppressed cell growth, invasion, and migration in both invasive and noninvasive breast cancer cells (35).

This study has showcased that in terms of early apoptosis, miR-142-3p at both 20 nM and 40 nM concentrations did not significantly impact the percentage of cells undergoing apoptosis in comparison to untreated cells. However, for late apoptosis, treating cells with miR-142-3p at 20 nM and 40 nM concentrations resulted in significant increases in apoptotic cell percentages. Consistently, Shen et al, in their study established that miR-142-3p amplifies colon cancer cells responsiveness to drug therapy and triggers apoptotic processes (36). Conversely, Zhu et al, showed that miR-142- serves as an oncogenic promoter in gastric cancer through its interaction with FOXO4, leading to elevated rates of cell proliferation and suppressed apoptosis (37).

In the broader context of the study's objective to assess the impact of miR-142-3p through the WNT pathway on OSCC 4, the findings suggest that the WNT pathway, when modulated by miR-142-3p, may play a more significant role in influencing late apoptotic events in OSCC 4 cells. The minimal effect on early apoptosis suggests that, at these concentrations, miR-142-3p may not play a prominent role in triggering the early apoptotic events in OSCC 4, including the early morphological and biochemical changes typically associated with apoptotic initiation cells (38). The WNT pathway's involvement may not be as pronounced during the early stages of apoptosis. While miR-142-3p concentration-dependent impact on late apoptosis suggests that, miR-142-3p, may exert a more noticeable influence on the later stages of apoptosis. Implying that the modulation of the WNT pathway by miR-142-3p becomes more apparent during the execution phase of programmed cell death.

The Ki-67 assay results showed notable differences in Ki-67 expression levels among the groups. Untreated cells had high Ki-67 expression, which decreased with miR-142-3p treatment suggesting a decline in cellular division. In consistence with our findings, several studies (35-36,39) have demonstrated that increased levels of miR-142-3p result in a notable decrease in cell proliferation in various cancer types like breast, pancreatic, and colon cancers. In contrast, the research conducted by Qi et al. demonstrated that miR-142-3p was elevated in nasopharyngeal carcinoma (NPC) and that suppressing miR-142-3p led to a decrease in NPC cell proliferation. This suggests that miR-142-3p plays an oncogenic role in NPC, promoting cellular proliferation, progression, and causing cell cycle arrest (40).

In summary, the anti-proliferative potential of miR-142-3p was demonstrated through a significant reduction in both cellular proliferation and migration in OSCC-4 cells. It does not exert significant cytotoxic effects on OSCC-4 cells at various concentrations. While miR-142-3p does not impact early apoptosis, it promotes late apoptosis in a dose-dependent manner, with higher concentrations having similar effects to concentrations.

While our findings indicated promising anti-proliferative effects, however, the study focused on a single cell line, a limited range of miR-142-3p concentrations and comparison of miR-142-3p effect against its antagonist was not employed.

Therefore, it is imperative to carry out additional in vitro and in vivo investigations to validate our findings and identify potential therapeutic targets for managing OSCC clinically. Given the acknowledged limitations of this study, the null hypothesis was refuted.

CONCLUSION

MicroRNA-142-3p demonstrates significant potential as a viable therapeutic option for treating OSCCs. It effectively reduces cell proliferation and migration while exhibiting no notable cytotoxicity. Although further research is required to delve into its apoptotic effects, the outcomes of our study collectively indicate that miR-142-3p holds promise as a valuable therapeutic agent against OSCCs.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

FUNDING STATEMENT
Financial support for this study was not provided by any public, commercial, or nonprofit entities.

REFERENCES

1. Nagata M, Kurita H, Uematsu K, Ogawa S, Takahashi K, Hoshina H, et al. Diagnostic value of cyclin dependent kinase/cyclin dependent kinase inhibitor expression ratios as biomarkers of locoregional and hematogenous dissemination risks in oral squamous cell carcinoma. Mol Clin Oncol. 2015;3:1007-13.
2. Blatt S, Krüger M, Ziebart T, Sagheb K, Schiegnitz E, Goetze E, et al. Biomarkers in diagnosis and therapy of oral squamous cell carcinoma: A review of the literature. J Craniomaxillofac Surg. 2017;45:722-30.
3. Yang Y, Zhou H, Zhang G, Xue X. Targeting the canonical Wnt/β-catenin pathway in cancer radioresistance: Updates on the molecular mechanisms. J Cancer Res Ther. 2019;15:272-7.
4. Savagner P. Leaving the neighborhood: molecular mechanisms involved during epithelial-mesenchymal transition. Bioessays. 2001;23:912-23.
5. Batlle E, Clevers H. Cancer stem cells revisited. Nat Med. 2017;23:1124-34.
6. Jayanthi P, Varun BR, Selvaraj J. Epithelial–mesenchymal transition in oral squamous cell carcinoma: An insight into molecular mechanisms and clinical implications. J Oral Maxillofac Pathol. 2020;24:189.
7. Moyret-Lalle C, Ruiz E, Puisieux A. Epithelial-mesenchymal transition transcription factors and miRNAs: “Plastic surgeons” of breast cancer. World J Clin Oncol. 2014;5:311-22.
8. Zhao X, Lu Y, Nie Y, Fan D. MicroRNAs as critical regulators involved in regulating epithelial-mesenchymal transition. Curr Cancer Drug Targets. 2013;13:935-44.
9. Chen K, Rajewsky N. The evolution of gene regulation by transcription factors and microRNAs. Nat Rev Genet. 2007;8:93-103.
10. Wang J, Chen J, Sen S. MicroRNA as Biomarkers and Diagnostics. J Cell Physiol. 2016;231:25-30.
11. Brennecke J, Hipfner DR, Stark A, Russell RB, Cohen SM. bantam Encodes a Developmentally Regulated microRNA that Controls Cell Proliferation and Regulates the Proapoptotic Gene hid in Drosophila. Cell. 2003;113:25-36.
12. Bartel DP. MicroRNAs: Genomics, Biogenesis, Mechanism, and Function. Cell. 2004;116:281-97.
13. Chen CZ, Li L, Lodish HF, Bartel DP. MicroRNAs Modulate Hematopoietic Lineage Differentiation. Science. 2004;303:83-6.
14. Moghadasi M, Alivand M, Fardi M, Moghadam KS, Solali S. Emerging molecular functions of microRNA-124: cancer pathology and therapeutic implications. Pathol Res Pract. 2020;216:152827.
15. Hayes J, Peruzzi PP, Lawler S. MicroRNAs in cancer: biomarkers, functions and therapy. Trends Mol Med. 2014;20:460-9.
16. Dickman CTD, Lawson J, Jabalee J, MacLellan SA, LePard NE, Bennewith KL, et al. Selective extracellular vesicle exclusion of miR-142-3p by oral cancer cells promotes both internal and extracellular malignant phenotypes. Oncotarget. 2017;8:15252-66.
17. Fernandez C, Bellosillo B, Ferraro M, Seoane A, Sanchez-Gonzalez B, Pairet S, et al. MicroRNAs 142-3p, miR-155 and miR-203 are deregulated in gastric MALT lymphomas compared to chronic gastritis. Cancer Genomics Proteomics. 2017;14:75-82.
18. Wu L, Cai C, Wang X, Liu M, Li X, Tang H. MicroRNA-142-3p, a new regulator of RAC1, suppresses the migration and invasion of hepatocellular carcinoma cells. FEBS Lett. 2011;585:1322-30.
19. Deng B, Zhang YI, Zhang S, Wen F, Miao Y, Guo K. MicroRNA-142-3p inhibits cell proliferation and invasion of cervical cancer cells by targeting FZD7. Tumour Biol. 2015;36:8065-73.
20. Lei Z, Xu G, Wang L, Yang H, Liu X, Zhao J, et al. MiR-142-3p represses TGF-β-induced growth inhibition through repression of TGFβR1 in non-small cell lung cancer. FASEB J. 2014;28:2696-704.
21. Li Y, Chen D, Jin LU, Liu J, Li Y, Su Z, et al. Oncogenic microRNA-142-3p is associated with cellular migration, proliferation and apoptosis in renal cell carcinoma. Oncol Lett. 2016;11:1235-41.
22. Lin RJ, Xiao DW, Liao LD, Chen T, Xie ZF, Huang WZ, et al. MiR-142-3p as a potential prognostic biomarker for esophageal squamous cell carcinoma. J Surg Oncol. 2012;105:175-82.
23. Isobe T, Hisamori S, Hogan DJ, Zabala M, Hendrickson DG, Dalerba P, et al. miR-142 regulates the tumorigenicity of human breast cancer stem cells through the canonical WNT signaling pathway. Elife. 2014;3:e01977.
24. Hu T, Phiwpan K, Guo J, Zhang W, Guo J, Zhang Z, et al. MicroRNA-142-3p Negatively Regulates Canonical Wnt Signaling Pathway. PLoS One. 2016;11:e0158432.
25. Tolosa L, Donato MT, Gómez-Lechón MJ. General Cytotoxicity Assessment by Means of the

MTT Assay. Methods Mol Biol. 2015;1250:333-48.
26. Cory G. Scratch-wound assay. Methods Mol Biol. 2011;769:25-30.
27. Wilkins RC, Kutzner BC, Truong M, Sanchez-Dardon J, McLean JR. Analysis of radiation-induced apoptosis in human lymphocytes: flow cytometry using Annexin V and propidium iodide versus the neutral comet assay. Cytometry. 2002 May 1;48(1):14-9.
28. Kim KH, Sederstrom JM. Assaying Cell Cycle Status Using Flow Cytometry. Curr Protoc Mol Biol. 2015 Jul 1;111:28.6.1-28.6.11.
29. Bai Y, Sha J, Kanno T. The role of carcinogenesis-related biomarkers in the Wnt pathway and their effects on epithelial–mesenchymal transition (EMT) in oral squamous cell carcinoma. Cancers (Basel). 2020;12:555.
30. Mahomed F, Altini M, Meer S. Altered E-cadherin/β-catenin expression in oral squamous carcinoma with and without nodal metastasis. Oral diseases. 2007;13:386-92.
31. Li P, Dong J, Zhou X, Sun W, Huang H, Chen T, et al. Expression patterns of microRNA-329 and its clinical performance in diagnosis and prognosis of breast cancer. Onco Targets Ther. 2017:10:5711-8.
32. Yang M, Zhai X, Ge T, Yang C, Lou G. miR-181a-5p promotes proliferation and invasion and inhibits apoptosis of cervical cancer cells via regulating inositol polyphosphate-5-phosphatase A (INPP5A). Oncol Res. 2018;26:703-12.
33. Colamaio M, Puca F, Ragozzino E, Gemei M, Decaussin-Petrucci M, Aiello C, et al. miR-142–3p down-regulation contributes to thyroid follicular tumorigenesis by targeting ASH1L and MLL1. J Clin Endocrinol Metab. 2015;100:E59-69.
34. Dastmalchi N, Safaralizadeh R, Khojasteh SM, Shadbad MA, Hosseinpourfeizi MA, Azarbarzin S, et al. The combined restoration of miR-424-5p and miR-142-3p effectively inhibits MCF-7 breast cancer cell line via modulating apoptosis, proliferation, colony formation, cell cycle and autophagy. Mol Biol Rep. 2022;49:8325-35.
35. Mansoori B, Mohammadi A, Ghasabi M, Shirjang S, Dehghan R, Montazeri V, et al. miR-142-3p as tumor suppressor miR in the regulation of tumorigenicity, invasion and migration of human breast cancer by targeting Bach-1 expression. J Cell Physiol. 2019;234:9816-25.
36. Shen WW, Zeng Z, Zhu WX, Fu GH. MiR-142-3p functions as a tumor suppressor by targeting CD133, ABCG2, and Lgr5 in colon cancer cells. J Mol Med (Berl). 2013;91:989-1000.
37. Zhu J, Tian X, Zhang Y, Bai Y, Pei K. MiR-142-3p is a tumor promoter by directly targeting FOXO4 to promote gastric cancer cell growth and suppress cell apoptosis in gastric cancer. Int J Clin Exp Pathol. 2016;9:12079-87.
38. Patel VA, Longacre A, Hsiao K, Fan H, Meng F, Mitchell JE, et al. Apoptotic cells, at all stages of the death process, trigger characteristic signaling events that are divergent from and dominant over those triggered by necrotic cells: Implications for the delayed clearance model of autoimmunity. J Biol Chem. 2006 Feb 24;281(8):4663-70.
39. MacKenzie TN, Mujumdar N, Banerjee S, Sangwan V, Sarver A, Vickers S, et al. Triptolide Induces the Expression of miR-142-3p: A Negative Regulator of Heat Shock Protein 70 and Pancreatic Cancer Cell Proliferation Triptolide Induces miR-142-3p in PDAC. Mol Cancer Ther. 2013;12:1266-75.
40. Qi X, Li J, Zhou C, Lv C, Tian M. MiR-142-3p suppresses SOCS6 expression and promotes cell proliferation in nasopharyngeal carcinoma. Cell Physiol Biochem. 2015;36:1743-52.