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Research Article | Araştırma Makalesi

miR-22 AND miR-30e MEDIATED REGULATION OF THE SLC2A3/NLRP3 AXIS IN ORAL SQUAMOUS CELL CARCINOMAS

ORAL SQUAMOUS HÜCRELİ KARSİNOMLARDA SLC2A3/NLRP3 AKSININ miR-22 VE miR-30e ARACILI DÜZENLENMESİ

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ABSTRACT

Objective: This study explores the regulation of SLC2A3 and NLRP3, along with the modulatory effects of specific microRNAs that target the SLC2A3/NLRP3 axis, in head and neck cancers, particularly in oral squamous cell carcinoma (OSCC), with a focus on their roles in tumor progression.

Method: SLC2A3 and NLRP3 gene expressions in HNSC were analyzed using the GEPIA database with TCGA and GTEx data. Protein-protein interactions were predicted via the STRING database, while miRNA-mRNA interactions were examined using miRDB. RT-qPCR was used to analyze gene expression in tissue samples obtained from 50 individuals with OSCC and 6 non-cancerous controls, while miRNA levels were measured using TaqMan™ Advanced miRNA Assays. Statistical analyses included t-tests, ANOVA, and Kaplan-Meier survival analysis.

Results: SLC2A3 expression level was significantly elevated in HNSC tumor samples compared to non-cancerous tissues ($p = 0.01$) and demonstrated a progressive increase with advancing tumor stages ($p = 0.0059$). NLRP3 expression level was also higher in HNSC ($p = 0.01$), although it did not show a significant association with tumor stage. In OSCC, the expression levels of both SLC2A3 and NLRP3 increased in parallel with tumor progression and exhibited a strong positive correlation ($p < 0.0001$). Notably, an elevated NLRP3 expression level was associated with reduced survival rates ($p = 0.0001$). Conversely, miR-22 and miR-30e expression levels declined as tumor stage advanced and were linked to reduced survival time ($p < 0.01$).

Conclusion: Increased SLC2A3 and NLRP3 expression levels appear to contribute to OSCC and HNSC progression, potentially through mechanisms involving the HIF-1α pathway. miR-22 and miR-30e are involved in the regulation of the SLC2A3/NLRP3 axis, and depletion of their expression levels may facilitate tumor aggressiveness, highlighting them as potential therapeutic targets in OSCC.

Keywords: Oral squamous cell carcinoma, head and neck squamous cell carcinoma, SLC2A3/NLRP3, miR-22, miR-30e

ÖZ

Amaç: Bu çalışma, baş ve boyun kanserlerinde, özellikle oral skuamöz hücreli karsinomda (OSCC), SLC2A3 ve NLRP3 genlerinin düzenlenmesini ve bu genleri hedefleyen belirli mikroRNA'ların SLC2A3/NLRP3 ekseni üzerindeki düzenleyici etkilerini incelemekte; söz konusu moleküler etkileşimlerin tümör progresyonundaki rollerine odaklanmaktadır.

Yöntem: SLC2A3 ve NLRP3 gen ekspresyonları, GEPIA veri tabanı kullanılarak, TCGA ve GTEx verileri üzerinden analiz edilmiştir. Protein-protein etkileşimleri STRING veri tabanı aracılığıyla tahmin edilmiş; miRNA-mRNA etkileşimleri ise miRDB ile değerlendirilmiştir. RT-qPCR yöntemi, OSCC'li 50 OSCC hastası ve 6 sağlıklı kontrol grubundan elde edilen doku örneklerinde gen ekspresyonlarını analiz etmek için kullanılmıştır. miRNA düzeyleri ise TaqMan™ Advanced miRNA Assay kitleriyle ölçülmüştür. İstatistiksel analizlerde t-testi, ANOVA ve Kaplan-Meier sağkalım analizleri uygulanmıştır.

Bulgular: SLC2A3 ekspresyon seviyesi, sağlıklı dokulara kıyasla HNSC tümör örneklerinde anlamlı düzeyde artmış ($p = 0.01$) ve ilerleyen tümör evreleriyle birlikte kademeli bir artış göstermiştir ($p = 0.0059$). NLRP3 ekspresyon seviyesi de HNSC'de daha yüksek bulunmuştur ($p = 0.01$), ancak tümör evresiyle anlamlı bir ilişki göstermemiştir. OSCC'de ise, SLC2A3 ve NLRP3 ekspresyon seviyeleri tümör ilerledikçe birlikte artış göstermiş ve aralarında güçlü bir pozitif korelasyon saptanmıştır ($p < 0.0001$). Özellikle, yüksek NLRP3 ekspresyon seviyesi, azalmış sağkalım oranları ile ilişkilendirilmiştir ($p = 0.0001$). Buna karşılık, miR-22 ve miR-30e ekspresyon seviyeleri tümör evresi ilerledikçe azalmış ve sağkalım süresinin azalması ile ilişkilendirilmiştir ($p < 0.01$).

Sonuç: Artmış SLC2A3 ve NLRP3 ekspresyon seviyelerinin, muhtemelen HIF-1α yolağı aracılığıyla, OSCC ve HNSC progresyonuna katkı sağladığı düşünülmektedir. miR-22 ve miR-30e, SLC2A3/NLRP3 ekseninin düzenlenmesinde rol almakta olup; bu mikroRNA'ların ekspresyon seviyelerinin azalması, tümör agresifliğine katkıda bulunabilir. Bu durum, miR-22 ve miR-30e'yi, OSCC için potansiyel terapötik hedefler olarak öne çıkarmaktadır.

Anahtar Kelimeler: Oral skuamöz hücreli kanser, baş ve boyun skuamöz hücreli kanser, SLC2A3/NLRP3, miR-22, miR-30e

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Introduction

Cancer cells rely primarily on glycolysis for energy production, unlike normal cells, and produce lactic acid even under aerobic conditions. This metabolic shift, known as the Warburg effect, is less efficient than oxidative phosphorylation but involves increased glucose uptake and utilization.[1] Similarly, inflammatory cells in the tumor microenvironment adopt a glycolytic metabolic profile when activated. In tumor-associated macrophages (TAMs), this metabolic change supports rapid ATP production and provides essential intermediates for synthesizing inflammatory proteins.[2-3] This metabolic reprogramming can enhance immune cell infiltration into tumors, potentially influencing tumor progression.[4]

One of the main proteins facilitating glucose transport to sustain energy production is Solute Carrier Family 2 Member 3 (SLC2A3), which has been shown to support rapid tumor cell proliferation.[5-8] Additionally, inflammatory cells in the tumor microenvironment express NLR Family Pyrin Domain Containing 3 (NLRP3), a protein that contributes to tumor immune evasion.[9] Although the role of NLRP3 in cancer is debated, recent studies suggest that high NLRP3 expression in TAMs promotes tumor aggressiveness. Moreover, tobacco use has been shown to suppress NLRP3 expression[10], but the impact of this on cancer progression remains unclear. This highlights the need to better understand the role of NLRP3 in head and neck squamous cell carcinoma (HNSC) including oral squamous cell carcinoma (OSCC), where tobacco exposure is common.

Studies indicate a direct link between increased glucose metabolism and cellular inflammation[11, 12] This suggests a possible interaction between SLC2A3, involved in glucose metabolism, and NLRP3, a regulator of inflammation, during cancer progression. However, the relationship between SLC2A3 and NLRP3 and their role in OSCC progression, especially considering environmental factors like tobacco that contribute to chronic inflammation, has not yet been explored.

Environmental factors influence gene regulation through epigenetic mechanisms, including changes in microRNA (miRNA) expression.[13] miRNAs regulate the expression of many genes, including SLC2A3 and NLRP3 genes.[14-16] Changes in the expression of miRNAs that bind complementarily to untranslated regions of genes involved in glucose metabolism and inflammation may contribute to the dysregulation of SLC2A3 and NLRP3 in HNSC and OSCC.[17, 18] Identifying these miRNAs could provide new opportunities for modulating SLC2A3 and NLRP3 expression through miRNA replacement therapies.[19]

Since a single miRNA can regulate multiple genes and a single gene can be controlled by multiple miRNAs[20], focusing on miRNAs that target both SLC2A3 and NLRP3 or their shared signaling pathways may be more effective for prognosis and treatment. This approach could also help develop personalized therapies targeting glucose metabolism and inflammation via miRNA modulation.

Understanding how specific signaling pathways interact with the SLC2A3/NLRP3 axis during cancer progression is crucial.[21] This is especially important in cancers like OSCC, where environmental factors play a significant role. Identifying the miRNAs that regulate SLC2A3/NLRP3 axis may reveal new molecular targets and lead to more effective, personalized treatment strategies.

In this study, we aim to investigate the interaction between SLC2A3 and NLRP3 during OSCC progression and identify the miRNAs that regulate these key molecules to better understand the molecular mechanisms driving OSCC pathogenesis. To achieve this, transcriptomic regulation of SLC2A3 and NLRP3 was analyzed in a large HNSC cohort using gene expression databases. Candidate miRNAs potentially regulating the SLC2A3/NLRP3 axis were identified through in silico prediction tools, and the association between these miRNAs and tumor aggressiveness was evaluated in a clinically homogeneous OSCC patient cohort.

Methods

Transcriptomic Profiling of SLC2A3 and NLRP3 Genes

The expression of SLC2A3 and NLRP3 in tumors from HNSC and non-tumor tissues were assessed by the Gene Expression Profiling Interactive Analysis (GEPIA) platform (http://gepia.cancer-pku.cn/; accessed on January 23, 2025). This analysis incorporated data from the Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) projects. Potential interactions between SLC2A3 and NLRP3 proteins were identified using the STRING platform V12.0 (https://string-db.org/; accessed on January 25, 2025). Additionally, the interactions between mRNAs and miRNAs were identified using the miRDB tool (https://mirdb.org/; accessed on January 25, 2025).

Clinical Samples

Formalin-fixed, paraffin-embedded (FFPE) OSCC tumor samples (n = 50) and non-tumor tissues from surgical margins (n = 6) were sourced from the pathology archives within the medical faculty of Bursa Uludağ University. Specimens were obtained from individuals who received treatment at the hospital between 2015 and 2020. Among the tumors, 11 cases (22.00%) were classified as stage 1, 19 cases (38.00%) as stage 2, 8 cases (16.00%) as stage 3, and 12 cases (24.00%) as stage 4. Perineural, vascular, or bone invasion was identified in the tumors of 27 patients (54.00%). These patients were followed clinically until 2025. Approval for the study protocols, encompassing tumor sample collection and clinical record evaluation, was granted by the Local Committee of Ethics for Clinical Research at Bursa Uludağ University (2021-7/40).

Gene Expression Analysis

FFPE tissue samples were processed to extract RNA using TRIzol reagent (Sigma, St. Louis, MO, USA).[12] RNA concentration and purity were determined by assessing the 260/280 nm absorbance ratio with an ultraviolet-

visible light spectrophotometric device (Beckman Coulter, Canada). Complementary DNA (cDNA) was generated from 100 ng of RNA utilizing the ProtoScript® II First Strand cDNA Synthesis Kit (New England Biolabs, Ipswich, MA, USA).

SLC2A3 and NLRP3 gene expression levels were measured through real-time reverse transcription PCR (RT-qPCR) with the StepOne™ Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). For NLRP3, previously designed and optimized primers were used[13], while the primer for SLC2A3 was obtained from the TaqMan™ Gene Expression Assays (Hs00359840_m1). Expression data were normalized to the housekeeping gene, actin beta (ACTB). The RT-qPCR program consisted of an initial denaturation at 95°C for 10 minutes, followed by denaturation at 95°C for 15 seconds and annealing/extension at 60°C for 60 seconds (40 cycles). The 2^−ΔΔCt method determined the expression of genes.

MicroRNA Expression Analysis

Total RNA was extracted using TRIzol reagent (Sigma, St. Louis, MO, USA).[12] The TaqMan™ Advanced miRNA cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA) synthesized the cDNA. The levels of hsa-miR-22 (A25576 477985_mir) and hsa-miR-30e (A25576 479235_mir) were measured using TaqMan™ Advanced miRNA Assays (Thermo Fisher Scientific, Waltham, MA, USA). Expression data for miRNAs were normalized to SNORD67 (Assay ID: 4426961 Hs03298753_s1), a reference gene.

Real-time PCR (RT-qPCR) analysis was conducted on a StepOne™ RT-PCR (Applied Biosystems, Foster City, CA, USA) under the following conditions: an initial activation at 95°C for 2 minutes, followed by denaturation at 95°C for 10 seconds and annealing/extension at 56°C for 60 seconds (40 cycles). The relative expression was calculated using the 2^−ΔΔCt method.

Statistical Analysis

The mRNA and miRNA levels in OSCC and non-tumor samples were analyzed using the independent samples t-test for comparisons between two groups and one-way ANOVA with Tukey's post hoc test for comparisons among multiple groups. Pearson correlation analysis was applied to assess the relationships between the expression levels of SLC2A3 and NLRP3, as well as their associations with miRNAs. Overall survival in OSCC patients was analyzed using the Kaplan-Meier method. Pearson's correlation analysis was conducted to examine the relationships between SLC2A3 and NLRP3 expression levels, as well as their links to miRNA expression. The Kaplan–Meier method was used to analyze overall survival in OSCC patients. All statistics were carried out using GraphPad Prism V10 (GraphPad Software, LLC, San Diego, CA, USA), with a significance threshold of $p < 0.05$, representing a 95% confidence interval, for all tests.

Results

The Regulation of SLC2A3 and NLRP3 in HNSC Progression

According to the TCGA and GTEx datasets, mRNA transcript levels of SLC2A3 were significantly elevated in HNSC tumors (n = 519) compared to non-tumor specimens (n = 44) ($p = 0.01$, Figure 1A). Furthermore, a notable increase in SLC2A3 expression was observed with advancing tumor stage ($p = 0.0059$; Figure 1B). Likewise, the transcript levels of NLRP3 were higher in HNSC tumor samples ($p = 0.01$; Figure 1C). However, it did not affect the progression in the tumor stage (Figure 1D). A positive correlation was detected between the transcript levels of SLC2A3 and NLRP3 in HNSC tumors ($p < 0.0001$; Figure 1E). Despite this, the elevated expression of SLC2A3 and NLRP3 did not have an impact on the survival of HNSC patients (Figure 1F, G). Analysis of protein interactions through the STRING database indicated that SLC2A3 and NLRP3 are connected via signaling pathways related to HIF1α (Figure 1H).

NLRP3 Plays a More Prominent Role in Tumor Aggressiveness in OSCC Compared to HNSC

A similar analysis was conducted on tumor tissues from OSCC patients who were retrospectively included in our study. The mean age of individuals with OSCC (36 males and 14 females) was 59.69 ± 13.11 years. Tumors were localized to the tongue in 33 patients, the retromolar region in 10 patients, and the floor of the mouth in 7 patients. Consistent with the HNSC cohort data from the TCGA and GTEx datasets, SLC2A3 expression in OSCC tumors increased with the advancing tumor stage (Figure 2A). In addition, an increased SLC2A3 expression was determined in tumors exhibiting invasion ($p=0.0499$, Figure 2B). However, it had no significant impact on overall survival in OSCC patients (Figure 2C).

Besides, unlike the HNSC cohort, the OSCC cohort exhibited a stage-dependent increase in NLRP3 expression level (Figure 2D). In addition, the increased level of NLRP3 expression was associated with the tumor invasion ($p=0.0006$, Figure 2E). Different from SLC2A3, the higher expression of NLRP3 was linked to poorer overall survival ($p = 0.0001$; Figure 2F).

A positive correlation ($r = 0.9061$; $p < 0.0001$) was observed between SLC2A3 and NLRP3 expression levels (Figure 2G). However, no association was observed between SLC2A3 and NLRP3 with tumor recurrence (Figure 2H, I). These findings suggest that while SLC2A3 and NLRP3 expression are closely linked and associated with tumor progression and invasion in OSCC, their expression levels may not predict tumor recurrence, indicating their role may be more relevant to the initial tumor aggressiveness rather than long-term disease relapse.

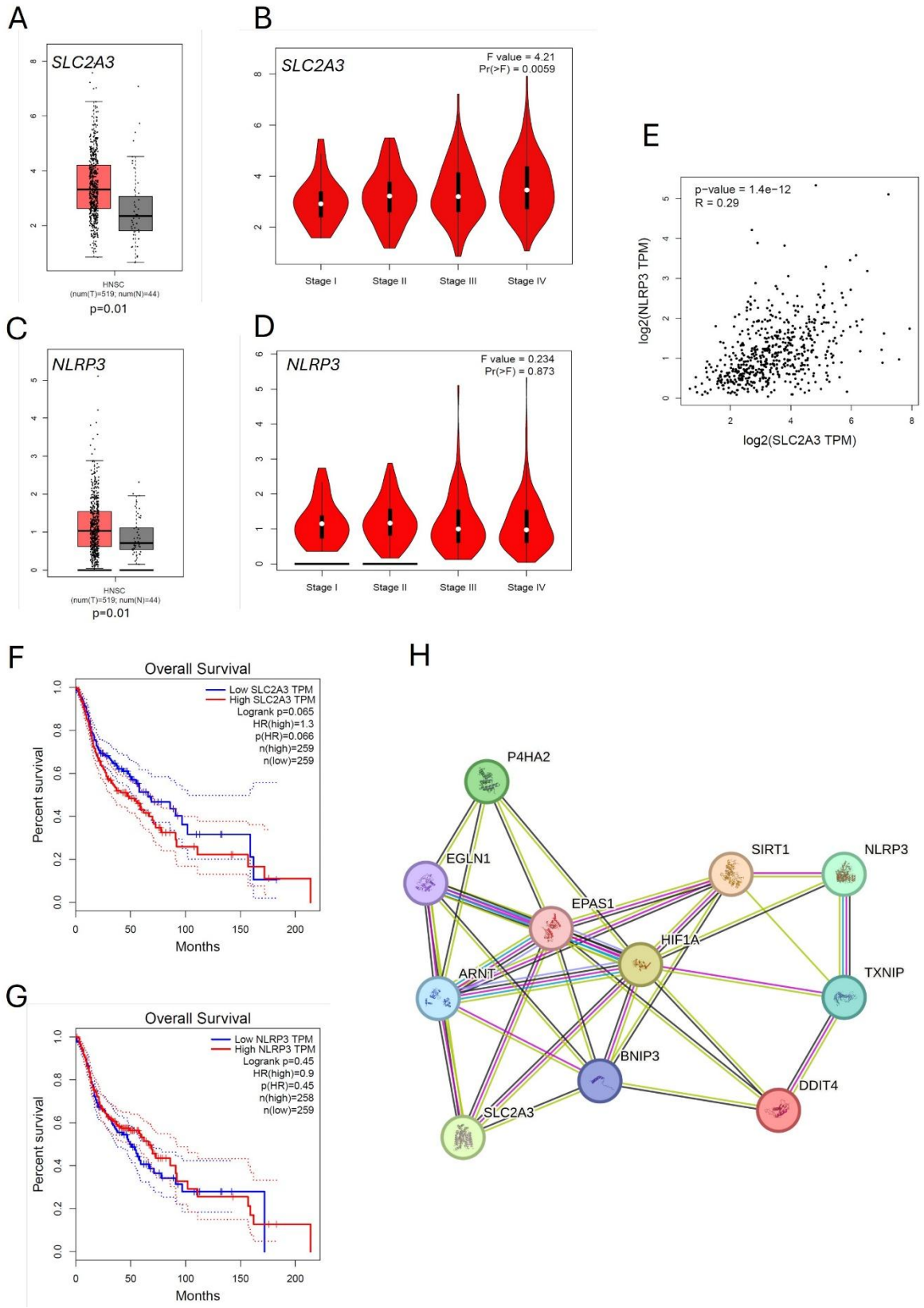

Figure 1. Regulation of SLC2A3 and NLRP3 expression in HNSC tumors analyzed via GEPIA. (a) Expression levels of SLC2A3 transcripts in HNSC tumors compared to non-tumor tissues. (b) Comparison of SLC2A3 transcript levels across different pathological stages of HNSC tumors. (c) Expression levels of NLRP3 transcripts in HNSC tumors compared to non-tumor tissues. (d) Comparison of NLRP3 transcript levels across different pathological stages of HNSC tumors. (e) Correlation analysis between SLC2A3 and NLRP3 expression in HNSC tumors. (f, g) Impact of SLC2A3 and NLRP3 on overall survival. (h) The interaction of SLC2A3 and NLRP3 based on STRING. P-values were calculated using an independent samples t-test (for a, c), one-way ANOVA followed by Tukey's post hoc test (for b, d), Pearson's correlation analysis (for e), and the Kaplan–Meier method (for f and g). N: Non-tumor tissue. TPM: Transcripts per million.

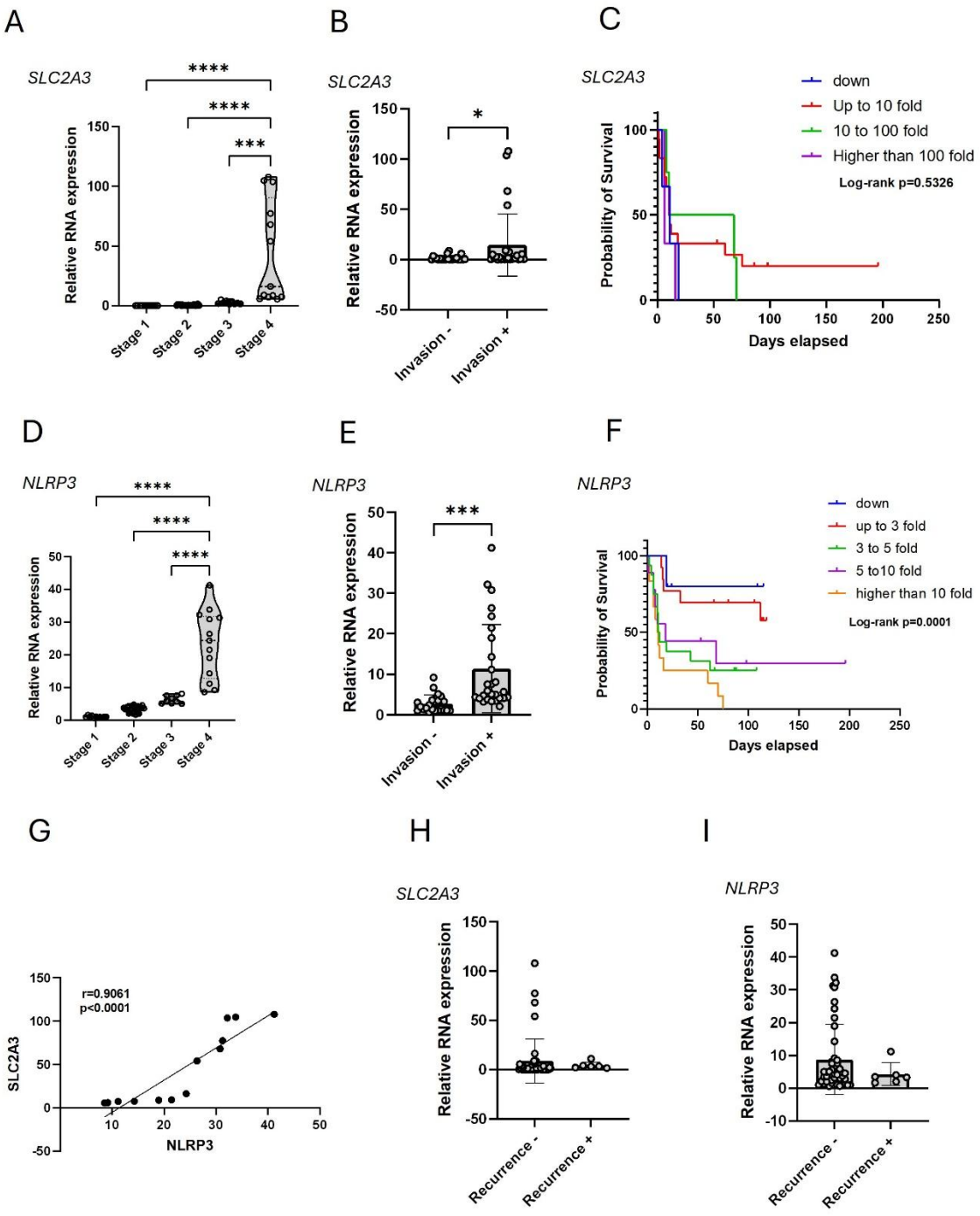

Figure 2. Regulation of SLC2A3 and NLRP3 expression in OSCC tumors. (a) RNA levels of SLC2A3 across various pathological stages of OSCC tumors. (b) Impact of SLC2A3 expression on the invasive potential of tumors. (c) Association between SLC2A3 expression and overall survival in OSCC patients. (d) RNA levels of NLRP3 at different pathological stages of OSCC tumors. (e) Influence of NLRP3 expression on tumor invasiveness. (f) Prognostic significance of NLRP3 expression in relation to overall survival in OSCC. (g) Correlation between SLC2A3 and NLRP3 RNA expression in OSCC tumor tissues. (h, ı) Evaluation of SLC2A3 and NLRP3 expression with respect to tumor recurrence in OSCC. P-values were determined by one-way ANOVA followed by Tukey's post hoc test (for a and d), independent samples t-test (for b, e, h, and ı), Kaplan–Meier survival analysis (for c and f), and Pearson's correlation test (for g).

The Role of miRNAs in the SLC2A3/NLRP3 Axis

The miRDB database predicted that miR-22 and miR-30e may regulate the mRNAs of genes encoding proteins involved in the HIF1A signaling pathway, which is part of the SLC2A3/NLRP3 axis, as shown in Figure 1H. Therefore, the impact of miR-22 and miR-30e on tumor aggressiveness in OSCC tumors was investigated. Table 1 lists the mRNAs predicted to interact with miR-22 and miR-30e, along with their binding scores. Furthermore, the interactions between the proteins encoded by these mRNAs and SLC2A3/NLRP3 are illustrated in Figure 3A.

In OSCC tumor tissues, the expression of both miRNAs was found to decrease as the tumor stage progressed (Figure 3B, C, $p < 0.0001$). Additionally, the expression levels of these miRNAs were positively correlated in OSCC tumors (Figure 3D, $r = 0.9589$, $p < 0.0001$). Consistent with miRDB's prediction, NLRP3 and miR-22 were negatively correlated ($r = -0.2859$, $p = 0.0210$). However,

while miR-22 exhibited a negative correlation with SLC2A3, this relationship was not found to be statistically significant (Figure 3E, F). The expression of miR-30e showed an inverse trend with both NLRP3 and SLC2A3, though the negative correlation did not reach statistical significance (Figure 3G, H). These findings suggest that the downregulation of miR-22 and miR-30e may contribute to the upregulation of components within the SLC2A3/NLRP3 axis, potentially promoting tumor progression in OSCC. Although the correlations were not uniformly strong or statistically significant, especially for miR-30e, the overall expression patterns support a potential regulatory role of these miRNAs in modulating tumor aggressiveness through post-transcriptional repression mechanisms.

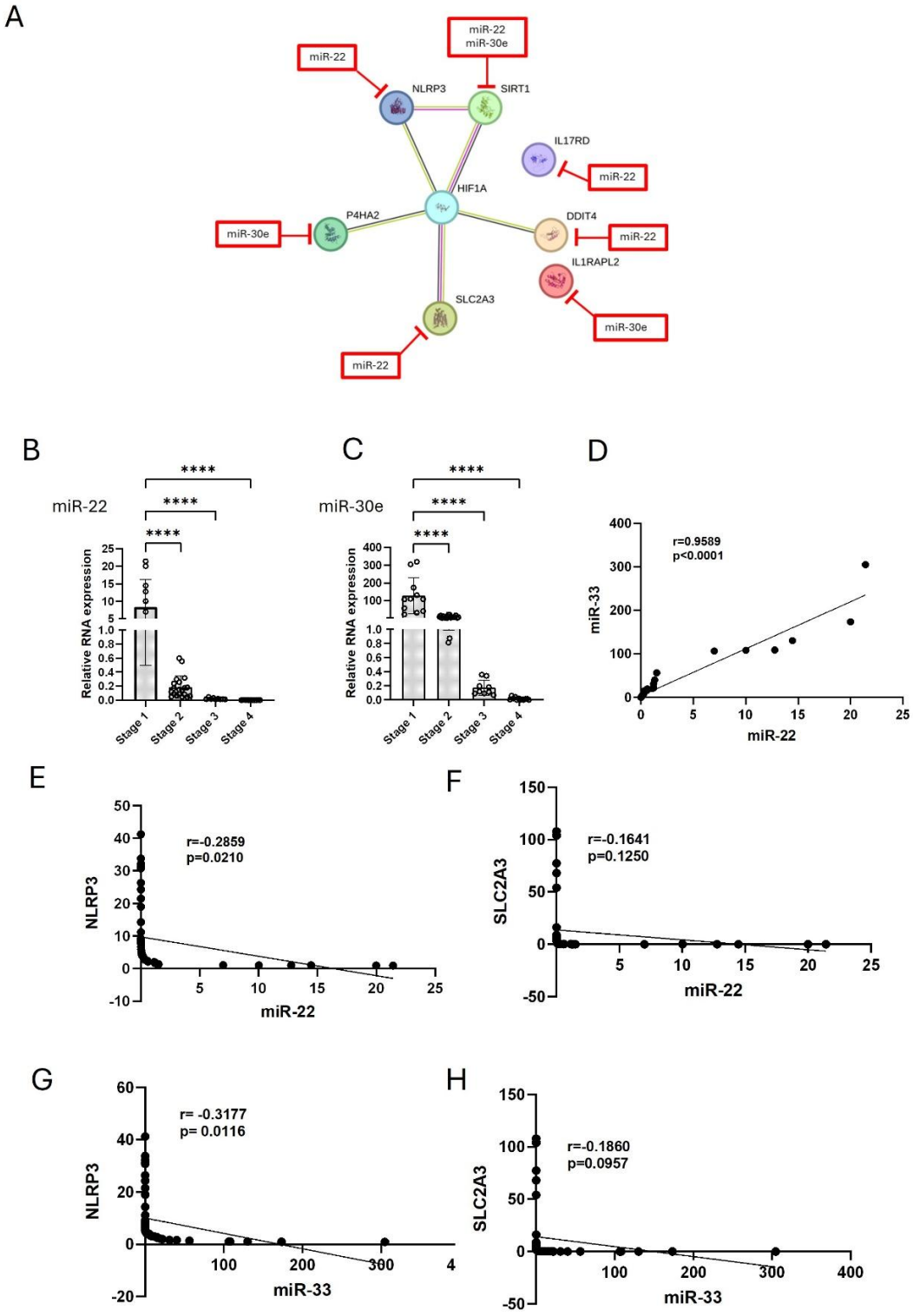

Figure 3. miR-22 and miR-30e influence the expression of SLC2A3 and NLRP3 in OSCC tumors. (a) Predicted interactions of miRNA with genes involved in the SLC2A3/NLRP3 axis. (b, c) Comparison of miRNA expression levels across different pathological stages of OSCC tumors. (d–h) Correlation analysis between miR-22 and miR-30e expression and the RNA expression of SLC2A3 and NLRP3 in OSCC tumors. P-values were determined using one-way ANOVA followed by Tukey's post hoc analysis (for b and c), and Pearson's correlation analysis (for d–h).

Table 1. Predicted miRNAs targeting genes involved in the SLC2A3/NLRP3 axis according to the miRDB database.

miRNA name	miRNA sequence	Target gene	Target score	Seed location	Prediction of targeted sequence in the gene 3'UTR
hsa-miR-22	AAGCUGCCAGUUGAAGAACUGU	NLRP3	94	457	421-agaactagtt gactatatat tatgttgaaa ttttatggca gctatttatt tatttaaatt
		SIRT1	94	530	481-tgattcttta acacaattat ttttaaacac tggcattttc caaaactgtg gcagctaact
		DDIT4	96	524, 616	481-tatcttgttt ttctgatcgg agcatcacta ctgacctgtt gtaggcagct atcttacaga 601-tgaaaaatta cacctggcag ctgcgtttaa gccttccccc atcgtgtact gcagagttga
		IL17RD	96	1484, 1491, 5161	1441 gtgtatattt ctgatgcaaa tgtcatactc actgctctgt aaaggcagct ggcagctttt 5161 ggcagctacc cctccctccc aattttagat cctattttta cacatctcta tagatatcac
hsa-miR-30e	UGUAAACAUCCUUGACUGGAAG	P4HA2	94	320	301-aaccaaagtt ctgatacctt gtttacatgt ttgtttttat ggcatttcta tctattgtgg
		IL1RAPL2	94	143	121-ggcttttata cttaaatatt tttgtttaca tttccagaat agtggggg
		SIRT1	63	67	61 aatgaatgtt tacttgtgaa ctcgatagag caaggaaacc agaaaggtgt aatatttata

miR-22 and miR-30e Promote OSCC Tumor Progression

The analysis of miR-22 and miR-30e expressions in relation to tumor progression revealed a significant reduction in miR-22 expression in tumors exhibiting invasion (Figure 4A, p = 0.0038). However, no substantial connection was observed between reduced miR-22 levels and tumor recurrence (Figure 4B). On the other hand, lower miR-22 expression was linked to poorer overall survival in patients (Figure 4C, p = 0.0075). Similarly, miR-30e was diminished in tumor samples of patients with perineural, vascular, or bone invasion compared to those without invasion (Figure 4D, p = 0.0062). While no direct correlation between miR-30e and tumor recurrence was found (Figure 4E), reduced miR-30e was linked to shorter survival in patients (Figure 4F, p = 0.0079). These findings showed that the decreased expression of miR-22 and miR-30e in OSCC tumors is associated with advanced tumor stage, invasive features, and poorer overall survival, supporting their potential role in regulating tumor aggressiveness.[22, 23]

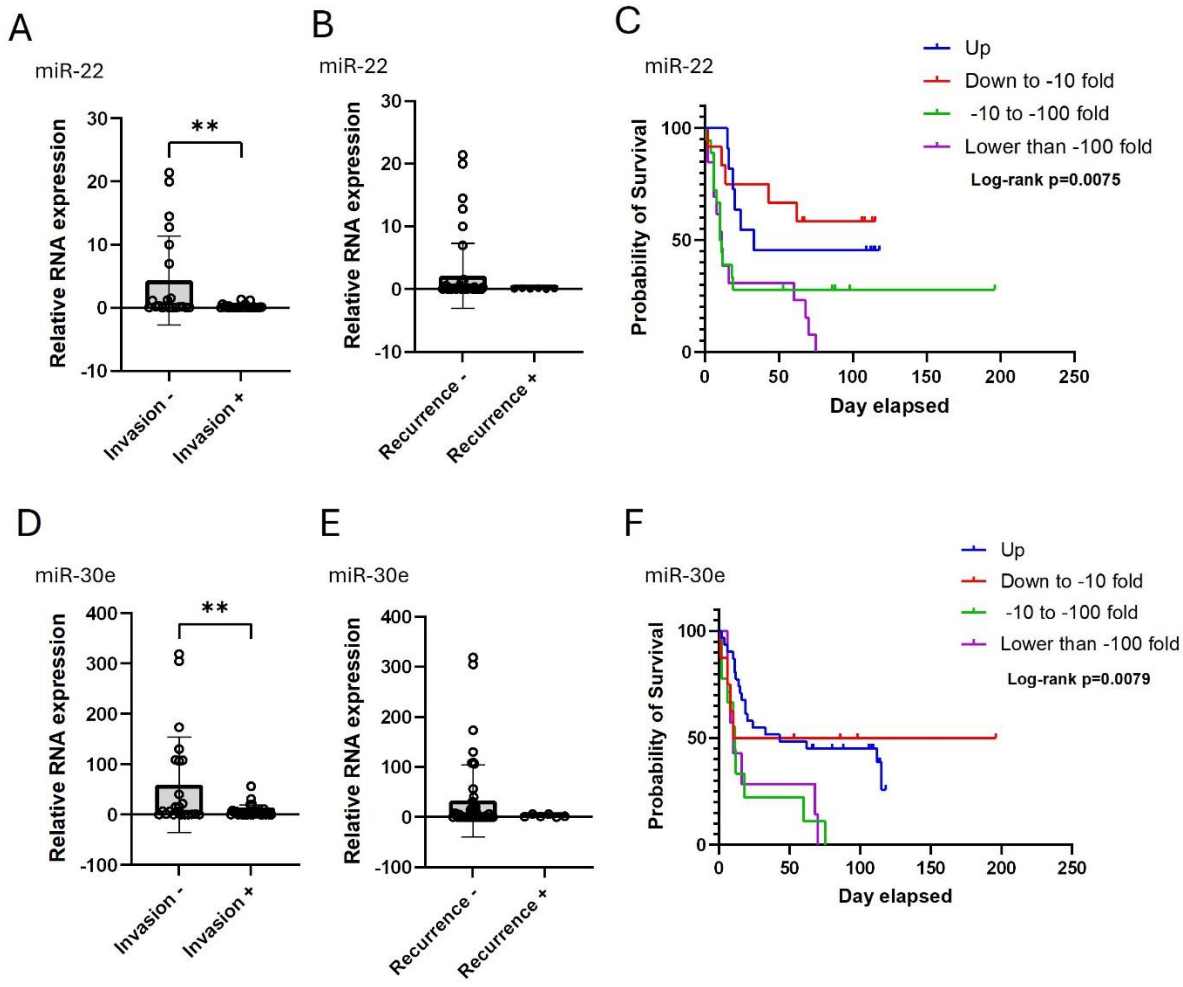

Figure 4. The role of miR-22 and miR-30e in OSCC tumor progression. (a, b) Effect of miR-22 on tumor invasion and recurrence in OSCC. (c) Impact of miR-22 on overall survival of OSCC patients. (d, e) Effect of miR-30e on tumor invasion and recurrence in OSCC. (f) Impact of miR-30e on the survival of OSCC patients. P-values were calculated using an independent samples t-test (for a, b, d, and e), and the Kaplan–Meier method (for c, f).

Discussion

In this study, we focused on uncovering how SLC2A3 and NLRP3 contribute to OSCC progression and examined the involvement of miRNA-mediated regulation within this axis to better understand the shared molecular drivers underlying these two interconnected pathways in OSCC.

Based on our findings, no significant change in NLRP3 expression level was observed based on tumor stage in HNSC cases, while in OSCC, NLRP3 expression increased with tumor stage. Most of the head and neck region cancers arise from the mucosal epithelium of the oral cavity, making OSCC a major cancer type among HNSC cases.[24] However, HNSC encompasses a wider spectrum of cancers, including laryngeal cancers. While OSCC is primarily influenced by factors such as tobacco use and alcohol consumption, pharyngeal cancers are mainly caused by human papillomavirus infection.[24] Therefore, the genes involved in carcinogenesis in HNSC may not necessarily exhibit the same alteration patterns as those involved in the carcinogenesis of more homogeneous tumor types like OSCC. Based on our findings, while NLRP3 expression is not affected by tumor stage in all HNSC cases, it seems to contribute to the progression of OSCC. These findings suggest that different initiating factors that induce inflammation in cancer may lead to variations in the regulation of NLRP3.

In contrast, SLC2A3 expression level increased with tumor stage in both HNSC and OSCC cases. This suggests that disruptions in glucose metabolism may be a key mechanism driving tumor aggression across all HNSC tumors. Although SLC2A3 alone did not influence survival in OSCC cases, its expression was found to correlate with both SLC2A3 and NLRP3 expression levels as tumor progression advanced, indicating a potential link between glucose metabolism and inflammasome formation in the tumor.

Gene-gene interaction analysis suggests that the interplay between SLC2A3 and NLRP3 may be mediated through HIF-1α signaling. Previous research in PC12 neuronal cells has demonstrated that SLC2A3 expression, regulated by the HIF-1α transcription factor, can activate oncogenic signaling pathways such as PI3K and mTOR.[25] Moreover, accumulating data indicates that HIF-1α is crucial for the activation of the NLRP3 inflammasome.[26] Supporting this, our analysis of signaling pathway interactions indicates that the SLC2A3/NLRP3 axis is connected via HIF-1α through key intermediates, including Prolyl 4-Hydroxylase Subunit Alpha 2 (P4HA2), Sirtuin 1 (SIRT1), and DNA Damage-Inducible Transcript 4 (DDIT4/REDD1). These proteins are involved in the PI3K/AKT/mTOR pathway, a critical signaling cascade known to drive cancer cell proliferation, angiogenesis, epithelial-to-mesenchymal transition (EMT), and chemoresistance when aberrantly expressed .[27-30]

Furthermore, our microRNA prediction analysis suggests that those proteins may be regulated by miR-22 and miR-30e. P4HA2 is involved in the remodeling of the extracellular matrix under hypoxic conditions in cancer, facilitating EMT and cell migration.[31, 32] Additionally, silencing of miR-30e has been shown to lead to overexpression of P4HA2, which contributes to liver fibrosis through chronic inflammation-mediated fibroblast activation.[33] Furthermore, hypoxia-induced overexpression of P4HA1 has been reported to enhance endothelial glycolysis, promoting angiogenesis.[34] SIRT1, regulates inflammation and cancer by deacetylating key targets such as NFκB p65 and p53.[35] Moreover, interactions between SIRT1 and NLRP3 have been shown to trigger inflammatory signaling pathways in conditions such as traumatic brain injury, neuroinflammation, depression, and liver damage.[36] SIRT1 is also known to influence glucose and lipid metabolism through its deacetylase activity,[37] with a reported positive correlation with SLC2A3.[38] DDIT4/REDD1, a protein activated under stress conditions, also contributes to the activation of NFκB, which is crucial for transcribing proteins necessary for the formation of the NLRP3 inflammasome complex.[39] Additionally, the activation of DDIT4/REDD1 has been linked to atypical NF-κB activation, which can contribute to metabolic dysfunction.[40] In our study, we observed a stage-dependent downregulation of miR-30e in OSCC tumors, particularly in association with tumor invasion. Notably, miR-30e expression was inversely correlated with NLRP3 levels. These findings suggest that the silencing of miR-30e in OSCC may promote EMT and influence NLRP3 expression via NFκB-mediated mechanisms.

Moreover, our miRNA prediction analysis suggests that NLRP3 may be regulated by miR-22, and that RNA transcripts encoding pro-inflammatory cytokine receptors such as IL1RAPL2 and IL17RD could also be miR-22 targets. We observed that miR-22 expression decreases with advancing tumor stage and shows a negative correlation with NLRP3 levels. These findings indicate that miR-22 may be involved in the regulation of inflammatory signaling pathways. In line with this, a previous study in hepatocellular carcinoma demonstrated that miR-22 regulates HIF-1α, and that reduced miR-22 expression is associated with increased tumor aggressiveness and chemoresistance.[41] Although the negative correlation between miR-22 and SLC2A3 in our dataset did not reach statistical significance, the direction of the correlation suggests a potential regulatory relationship. Taken together, these findings suggest that the reduced expression of miR-22 observed in OSCC may contribute to the dysregulation of proteins involved in both glucose metabolism and inflammation, particularly those interacting with HIF-1α, thereby weakening the control of key pathways that influence tumor progression.

Considering the expression of miR-30e and miR-22 was positively correlated in our OSCC cohort, it appears that depletion of miR-22 and miR-30e may enhance cellular inflammation and tumor aggressiveness in OSCC through interactions with NLRP3. In addition, these findings support the idea that miR-22 and miR-30e may influence tumor progression by regulating both NLRP3 and glucose metabolism, including the indirect dysregulation of SLC2A3.

To summarize, our research emphasizes the critical involvement of NLRP3 and SLC2A3 in the progression of HNSC, especially OSCC. While NLRP3 expression did not show a consistent pattern across all HNSC cases, it was found to increase with tumor stage in OSCC, suggesting its involvement in tumor progression. In contrast, SLC2A3 expression was upregulated in both HNSC and OSCC cases as the tumor stage advanced, indicating a potential link between disrupted glucose metabolism and tumor aggressiveness across various HNSC tumors. Our gene-gene interaction analysis suggests that the relationship between SLC2A3 and NLRP3 may be mediated through HIF-1α signaling, with potential involvement of the PI3K/AKT/mTOR pathway. Specifically, the depletion of miR-22 and miR-30e appears to enhance tumor aggressiveness in OSCC, suggesting that these miRNAs play critical roles in regulating NLRP3 and glucose metabolism. It should be emphasized that this study was based on a patient cohort, in which inter-individual differences such as genetic background and environmental exposures may have influenced the observed molecular patterns. Therefore, further in vitro and in vivo studies are warranted to validate the functional roles of miR-22 and miR-30e in regulating glucose metabolism and NLRP3-mediated inflammatory responses in OSCC and to evaluate their potential as therapeutic targets. Nonetheless, these findings contribute to a better understanding of the complex molecular mechanisms underlying OSCC and highlight the potential importance of miR-22 and miR-30e as regulatory molecules involved in both glucose metabolism and NLRP3-mediated inflammatory responses. Targeting these miRNAs could offer new avenues for therapeutic strategies aimed at mitigating tumor growth driven by enhanced glucose uptake and chronic inflammation in OSCC.

Compliance with Ethical Standards

The research protocol was approved by the Clinical Research Ethics Committee of Bursa Uludağ University (2021-7/40).

Conflict of Interest

The authors of this study declare no conflict of interest.

Author Contributions

GT, BT, MA: Design; MA, HC, HCI, AAP, IK, AD, ÖS: Data collection and processing; GT, MA, ÖS, CT, ME, BT: Analysis and interpretation; GT, MA: Literature review; GT, MA: Writing.

GT and MA contributed equally to this work.

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