Heliyon 10 (2024) e24671

Contents lists available at ScienceDirect

Heliyon

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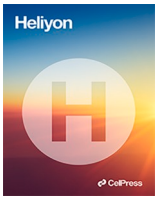

Research article

MicroRNA-124 plays an inhibitory role in cutaneous squamous cell carcinoma cells via targeting SNAI2, an immunotherapy determinant

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ARTICLE INFO

Keywords:
Cutaneous squamous cell carcinoma
microRNA-124
Snail family transcriptional repressor 2
Invasion
Immunotherapy determinant

ABSTRACT

Purpose: MicroRNAs (miRs) play multiple roles during cutaneous squamous cell carcinoma (CSCC) progression. Previous studies suggest miR-124 could inhibit cancer development in CSCC. Methods: Obtained 63 pairs of CSCC and adjacent tissues for analysis. Cultured HaCaT and two CSCC cell lines (A431 and SCL-1) in DMEM (10 % FBS). Transfected cells using Lipofectamine 2000 with various miR-124 mimics, inhibitors, or Snail family transcriptional repressor 2 (SNAI2) expression plasmid. Performed a series of assays, including real-time quantitative PCR, Western blot, CCK8, wound healing, transwell, and luciferase reporter gene assay, to examine the effects of miR-124 on CSCC cells. Results: An evident downregulation of miR-124 in CSCC tissues, which was related to advanced disease stage and nodal metastasis. Overexpressing miR-124 could reduce the proliferation, migration, and invasion abilities of CSCC cells. It was verified that miR-124 targets the SNAI2 in CSCC cells. Moreover, ectopic expression of SNAI2 rescued the suppressive effects on CSCC cells induced by miR-124 overexpression. Furthermore, miR-124 increased cell sensitivity to cisplatin. Besides, SNAI2 is a critical factor in the immune-related aspects of CSCC and its modulation may influence the response to immunotherapy. Conclusion: We demonstrate that miR-124 inhibits CSCC progression through downregulating SNAI2, and thus it may be a molecular candidate for treating CSCC in the clinic.

1. Introduction

The second most frequent type of dermal cancer is called cutaneous squamous cell carcinoma (CSCC) [1]. CSCC is more aggressive compared to other skin cancers. Although great efforts have been made in CSCC treatment, the overall survival of CSCC patients at a

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https://doi.org/10.1016/j.heliyon.2024.e24671
Received 10 November 2023; Received in revised form 21 December 2023; Accepted 11 January 2024
Available online 15 January 2024

later stage has not been improved [2–4]. Several signaling pathways regulate the progression of CSCC cells. However, proper targets in CSCC treatment are still to be identified [5,6]. Thus, exploring the molecular targets associated with CSCC and developing novel therapeutic strategies are urgently needed [7–9].

MicroRNAs (miRs) could bind to the 3′ or 5' untranslated region (UTR) of corresponding target mRNAs, resulting in translation repression [10,11]. They participate in cell proliferation, migration, and invasion [11–15]. Recently, many miRs have been dysregulated in CSCC, either promoting or suppressing cancer development [16,17]. For instance, Zhou et al. reported that miR-365 promoted CSCC progression by inhibiting the expression of nuclear factor I/B [18]. MiR-142 also functions as a tumor promoter in CSCC by inhibiting PTEN [19]. Others like MiR-203 and Mir-204 act as an inhibiting role in CSCC targeting c-myc and SHP2 respectively [20,21].

MiR-124 is a microRNA that has been extensively studied in the context of cancer. It is known to act as a tumor suppressor in several types of cancer, including breast cancer, glioblastoma, and colorectal cancer. MiR-124 regulates various cellular processes involved in cancer progression, such as cell proliferation, migration, invasion, and apoptosis. MiR-124 is also thought to be a candidate in regulating CSCC development. It is significantly downregulated in CSCC previously [22]. Moreover, Yamane et al. reported that miR-124 downregulation in CSCC increased tumor cell proliferation by activating the ERK signaling pathway [23]. According to these earlier research, miR-124 may function as a tumor suppressor in CSCC. However, the detailed mechanism of miR-124 underlying the progression of CSCC cells remains unknown. Therefore, we wish to reveal other molecular targets of miR-124 in CSCC.

2. Materials and methods

2.1. Clinical sample

The study received authorization from the Ethics Committee of Changde First People's Hospital in China, and participants provided their informed consent. Between 2014 and 2016, we gathered 63 sets of cutaneous squamous cell carcinoma (CSCC) samples and their corresponding adjacent tissues. These specimens were preserved at −80 °C following surgical removal for later analysis and experimentation. In this study, the inclusion criteria were patients who underwent surgery for CSCC without prior chemotherapy or radiotherapy. The diagnosis of CSCC was validated by histopathological studies, along with the capacity to give informed consent and sufficient organ function. Exclusion criteria included previous treatment with targeted or immunotherapy agents, presence of other malignancies, active infections, or any medical condition that could interfere with study participation or interpretation of results.

2.2. Cell culture in vitro

HaCaT is a human skin cell line. A431 and SCL-1 are CSCC cell lines (Cell Bank of the Chinese Academy of Sciences). To foster cellular development and multiplication, these cells were cultivated in Dulbecco's Modified Eagle Medium (DMEM) (supplied by ThermoFisher, Waltham, MA, USA) enriched with 10 % fetal bovine serum (FBS) (also from ThermoFisher). The incubation process took place at 37 °C with 5 % carbon dioxide.

2.3. Cell transfection

Please see the supplementary materials for the detailed methods.

2.4. Real-time quantitative PCR (RT-qPCR)

Please see the supplementary materials for the detailed methods [24].

2.5. Western blot

Please see the supplementary materials for the detailed methods.

2.5.1. CCK8 assays

CSCC cells were transfected and seeded into a 96-well plate for varying time intervals as specified. Afterward, the cells were treated with cck8 assay solution obtained from Sigma and further incubated for 3 h at 37 °C. Finally, the wells' optical density (OD) was determined with a microplate reader.

2.6. Wound healing assays

The cells were scraped with a 100 μl pipette tip and rinsed with DMEM once they reached 95 % confluency. Cells were cultured in FBS-free DMEM for 24 h, after which the scratch width was evaluated.

2.7. Transwell assays

Please see the supplementary materials for the detailed methods.

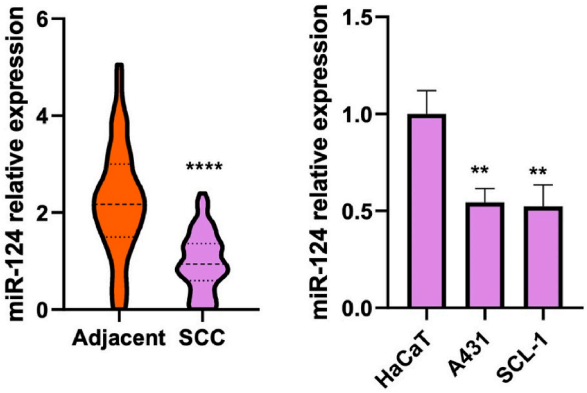

Fig. 1. Illustration of the diminished expression of MiR-124 in the context of CSCC. (A) Real-time qPCR was employed to measure MiR-124 levels in 63 paired samples, contrasting CSCC tissues with adjacent non-tumor tissues. (B) The expression of MiR-124 was also quantified in CSCC cell lines (A431 and SCL-1) and compared with that in normal human skin HaCaT cells, using real-time qPCR. In both instances, the results indicated a statistically significant downregulation of MiR-124 in CSCC tissues and cell lines (n = 63, ***P < 0.01, ****P < 0.001), underscoring its potential role in the pathophysiology of CSCC.

Table 1
Association between the MiR-124 expression and clinicopathologic characteristics in cutaneous squamous cell carcinoma.

Variables	Number (n = 63)	Low expression (n = 35)	High expression (n = 28)	P value
Age (years)				0.452
≤ 55	37	19	18	
> 55	26	16	10	
Tumor size (cm)				0.134
≤ 5	29	13	16	
> 5	34	22	12	
Differentiation				0.199
Well to moderately	51	26	25	
Poor	12	9	3	
Lymph node metastasis				0.029*
Present	18	14	4	
Absent	45	21	24	
TNM stage				0.031*
I-II	42	19	23	
III	21	16	5	

2.8. Luciferase reporter assay

Please see the supplementary materials for the detailed methods.

2.9. Tumor immuno-related analysis on SNAI2

The dataset GSE53462 was retrieved from the GEO database. KEGG pathways were subjected to GSEA focusing on SNAI2 [25]. GO terms were analyzed using GSVA, emphasizing SNAI2. Using the ESTIMATE algorithm, ESTIMATE, Immune, and Stromal scores were determined [26]. The 28 immune cell types were analyzed using the ssGSEA algorithm [27,28].

2.10. Statistical analysis

Data was shown as mean ± SD and analyzed by SPSS 21 software. Tumor immuno-related analysis on SNAI2 was conducted using R software. Data between the two groups was compared using a student t-test. Correlations were assessed using the Pearson correlation test. Data among multiple groups was compared using a one-way ANOVA. The chi-square test was used to assess correlations. P < 0.05 was considered statistically different.

3. Results

3.1. Downregulation of MiR-124 in CSCC

To elucidate MiR-124's involvement in cutaneous squamous cell carcinoma (CSCC), we assessed its expression in both CSCC lesions

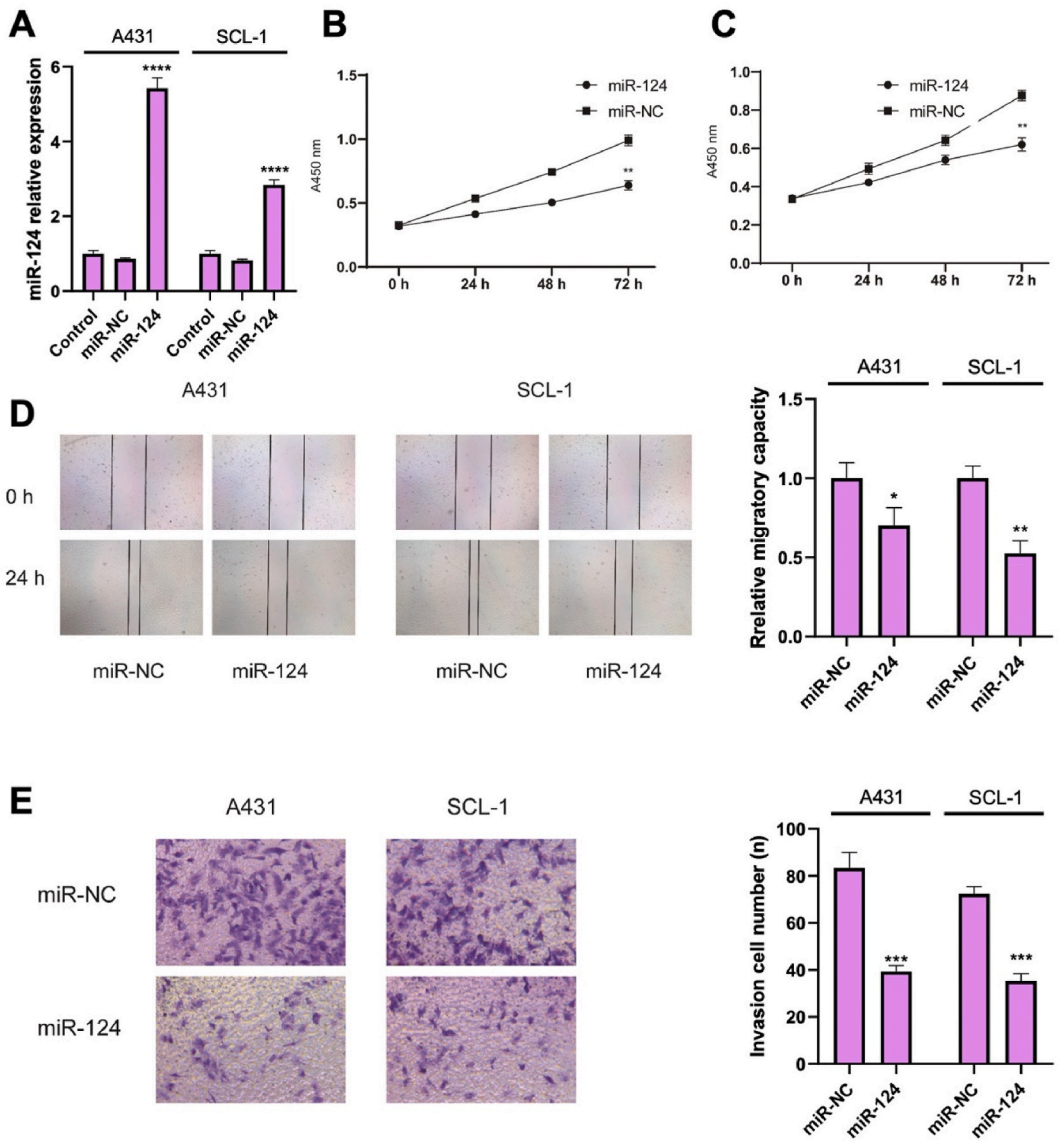

Fig. 2. Presentation of the impact of MiR-124 on the aggressive characteristics of CSCC cells. (A) The expression of MiR-124 was assessed by RT-qPCR. (B, C) Analyses of cell proliferation were conducted. (D) The assay for cell migration was performed. (E) The evaluation of cell invasion was carried out. Across these experiments, involving a sample size of n = 4, the data consistently showed that MiR-124 expression inversely affected the malignant behaviors of CSCC cells, with significant reductions in proliferation, migration, and invasion (**P < 0.01), suggesting a potential tumor-suppressive role of MiR-124 in CSCC.

and surrounding healthy tissue. Our analysis revealed a markedly reduced presence of MiR-124 in the CSCC samples compared to the non-cancerous counterparts, a disparity supported by statistical evidence (Fig. 1A). This pattern of under-expression was further confirmed in a comparative study of two CSCC cell lines against normal skin cells, with the former showing a substantial decrease in MiR-124 levels (Fig. 1B). Data from 63 patient samples, encompassing both cancerous and adjacent healthy tissues, were scrutinized. The comparative analysis of these samples demonstrated a significant correlation between MiR-124 expression and key clinical parameters, including lymph node metastasis and TNM classification (Table 1). These observations point to a potential role for MiR-124 in the development and progression of CSCC.

3.2. MiR-124 inhibits the malignant phenotypes of CSCC cells

To investigate MiR-124's function in CSCC, we initially altered its expression in vitro within cultured CSCC cells by introducing MiR-124 mimics. Post-treatment, a significant elevation in MiR-124 levels was observed (Fig. 2A). Subsequent proliferation assays (CCK-8) revealed a notable decrease in cell growth in the MiR-124 augmented group when contrasted with the miR-NC (negative

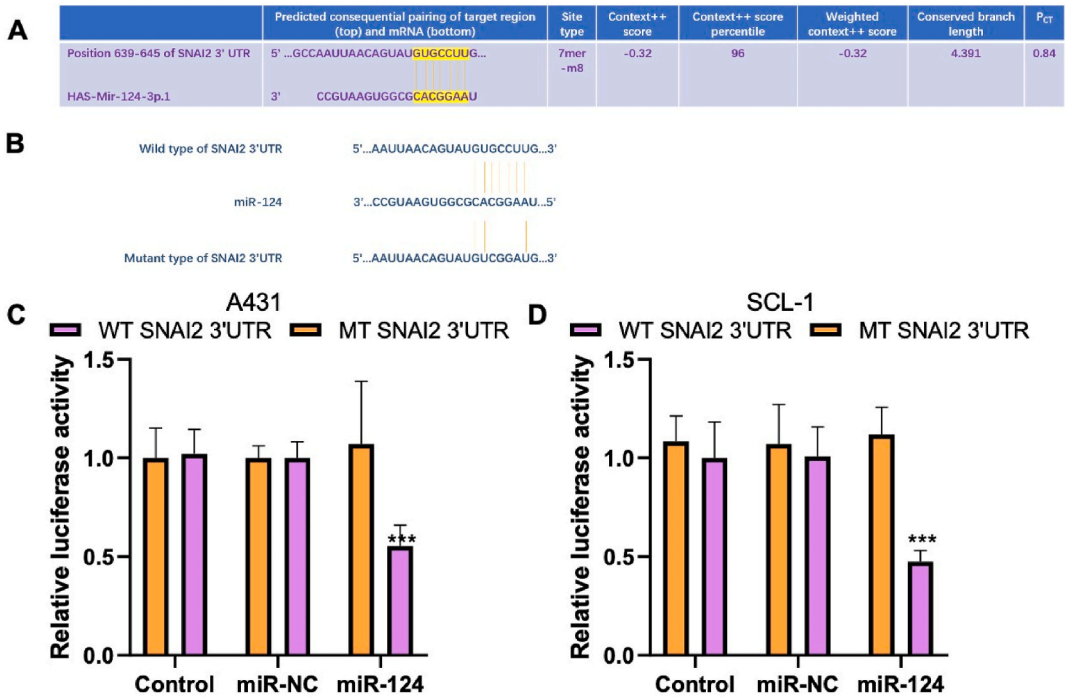

Fig. 3. Delineation of the direct targeting of SNAI2 by MiR-124 in CSCC cells. (A) It identifies the specific binding sites where MiR-124 interacts with the 3′ untranslated region (UTR) of SNAI2 mRNA. (B) The figure compares the wild type (WT) and mutant type (MT) SNAI2 3′UTR constructs. (C–D) A luciferase reporter gene assay was utilized to validate the interaction between MiR-124 and the SNAI2 3′UTR. The results, with a sample size of n = 4, demonstrated a statistically significant decrease in luciferase activity when MiR-124 was paired with the WT 3′UTR of SNAI2 (**P < 0.01), confirming that MiR-124 directly targets SNAI2 in CSCC cells.

control) group (Fig. 2B and C). Moreover, wound healing assays indicated a substantial reduction in cellular migration for the MiR-124 enhanced group versus the miR-NC group (Fig. 2D). Additionally, the invasion capabilities of the cells with MiR-124 upregulation were markedly diminished compared to the miR-NC group (Fig. 2E). These findings imply that MiR-124 plays a regulatory role in the proliferation, migration, and invasion of CSCC cells.

3.3. MiR-124 directly targets SNAI2 in CSCC cells

In our quest to decode MiR-124's mechanism within CSCC, we consulted the TargetScan database, which indicated SNAI2 as a potential target gene of MiR-124. The predicted interaction sites are depicted in Fig. 3A, highlighting the possible areas of MiR-124 attachment on SNAI2. Given that miRNAs typically function by binding to the 3′ untranslated regions (UTRs) of their target genes, we engineered mutations at these predicted sites to create both wild-type (WT) and mutant (MT) plasmids (Fig. 3B). Utilizing a dual-luciferase reporter assay for empirical validation, we observed a pronounced reduction in luciferase activity in the cohort co-expressing MiR-124 and WT SNAI2, as opposed to the mutant or control groups (Fig. 3C and D). This reduction indicates a specific binding between MiR-124 and the SNAI2 gene.

3.4. MiR-124 negatively affects SNAI2 expression in CSCC cells

Building on the predictive data suggesting MiR-124's targeting of SNAI2, we evaluated SNAI2 expression in CSCC cells under the influence of MiR-124 mimics and inhibitors to ascertain MiR-124's regulatory impact. The application of MiR-124 mimics resulted in a notable downregulation of SNAI2 at both mRNA and protein levels (Fig. 4A and B). Conversely, the introduction of MiR-124 inhibitors caused a significant suppression of MiR-124 expression relative to the control inhibitor group (Fig. 4C), which was accompanied by a marked increase in SNAI2 mRNA and protein expression (Fig. 4D and E). These outcomes reinforce the hypothesis that MiR-124 directly modulates SNAI2 expression in CSCC cells.

3.5. SNAI2 rescued the MiR-124's effects on CSCC cells

To deepen our understanding of how MiR-124's interaction with SNAI2 influences CSCC cellular behavior, we co-transfected CSCC cells with SNAI2 overexpression plasmids alongside MiR-124 mimics. This co-transfection led to a substantial increase in SNAI2's mRNA and protein levels in the MiR-124 + SNAI2 group when compared with the MiR-124 mimic control (NC) group (Fig. 5A and B).

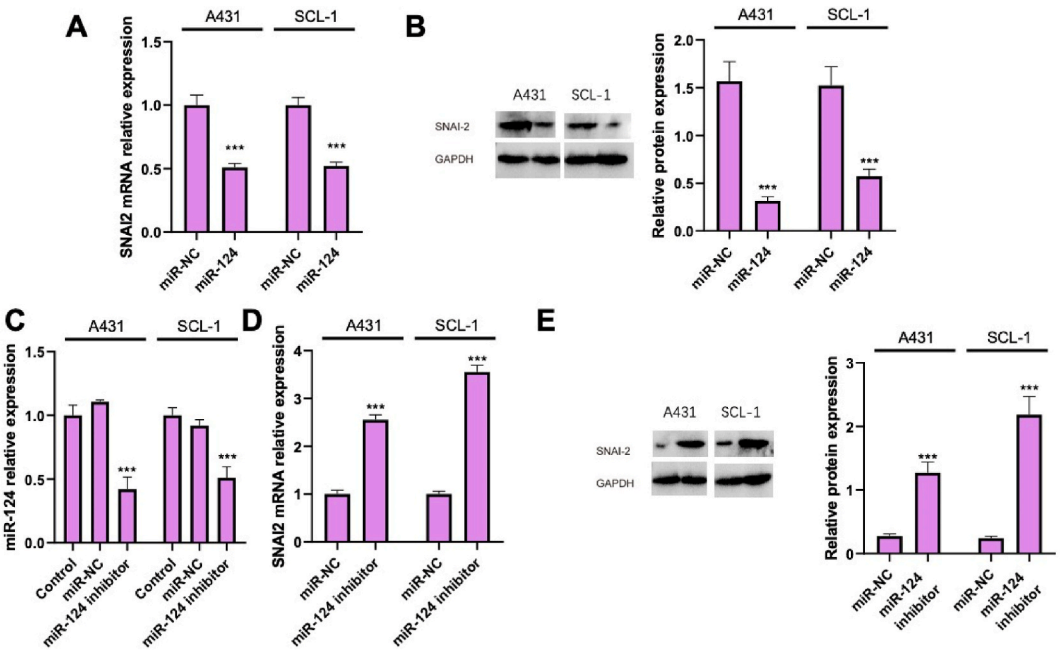

Fig. 4. Highlighting the negative influence of MiR-124 on SNAI2 expression in CSCC cells. (A–B) These panels show the quantification of SNAI2 at the mRNA and protein levels. The uncropped blots of Fig. 2B are provided in Supplementary Figs. S1 and S2. (C) The expression levels of MiR-124 were measured using RT-qPCR. (D–E) Further quantification of SNAI2 mRNA and protein levels is presented. In this set of experiments with a sample size of n = 4, the findings indicate a significant inverse relationship between MiR-124 and SNAI2 expression, with MiR-124 downregulation leading to an increase in SNAI2 levels (**P < 0.01). This suggests that MiR-124 may exert a suppressive regulatory effect on SNAI2 within CSCC cells. The uncropped blots of Fig. 2E are provided in Supplementary Figs. S3 and S4.

Further analysis using the CCK-8 assay showed a significant enhancement in cell proliferation in the MiR-124 + SNAI2 group relative to the MiR-124 NC group (Fig. 5C and D). Moreover, the wound healing assay indicated a considerable increase in migration capability in the MiR-124 + SNAI2 group versus the MiR-124 NC group (Fig. 5E). In line with these findings, the invasion assay also demonstrated a significant elevation in invasive potential in the MiR-124 + SNAI2 group compared to the MiR-124 NC group (Fig. 5F). These results imply that SNAI2 overexpression can counteract the suppressive effects of MiR-124 on the proliferation, migration, and invasion of CSCC cells.

3.6. MiR-124 increases the sensitivity of CSCC cells to cisplatin

The conducted studies have established that elevating MiR-124 levels markedly curtails the proliferation, migration, and invasion capabilities of CSCC cells. To determine if MiR-124 also heightens the responsiveness of CSCC cells to cisplatin, we administered this chemotherapeutic agent alongside transfection with MiR-124 mimics. The CCK-8 assay outcomes indicated a significant suppression of cell proliferation in the groups treated with cisplatin alone and in combination with miR-NC, compared to the control. Notably, the cisplatin + MiR-124 group exhibited the most pronounced reduction in cell proliferation among all groups tested (Fig. 6A and B). This evidence points to MiR-124's potential role in bolstering the susceptibility of CSCC cells to the cytotoxic effects of cisplatin.

3.7. Function annotation of SNAI2

To comprehensively define the pathogenic roles of SNAI2, we paid special attention to the tumor immuno-related functions of SNAI2. GSEA of KEGG pathways on SNAI2 revealed that T cell signaling, B cell signaling, and immunotherapy response were significantly associated with SNAI2 (Fig. 7A). GSVA of GO pathways on SNAI2 revealed that T cell activation, B cell activation, and immune response were significantly associated with SNAI2 (Fig. 7B).

3.8. Tumor immuno-related characteristics of SNAI2

The analysis revealed a substantial inverse relationship between SNAI2 expression and the ESTIMATE scores, which include Immune and Stromal scores, as depicted in Fig. 8A. Additionally, a significant negative correlation was also discerned between SNAI2 levels and the presence of immune cells within the same study (Fig. 8B). These correlations suggest that higher SNAI2 expression may be associated with a lower immune and stromal cell infiltration in the tumor microenvironment.

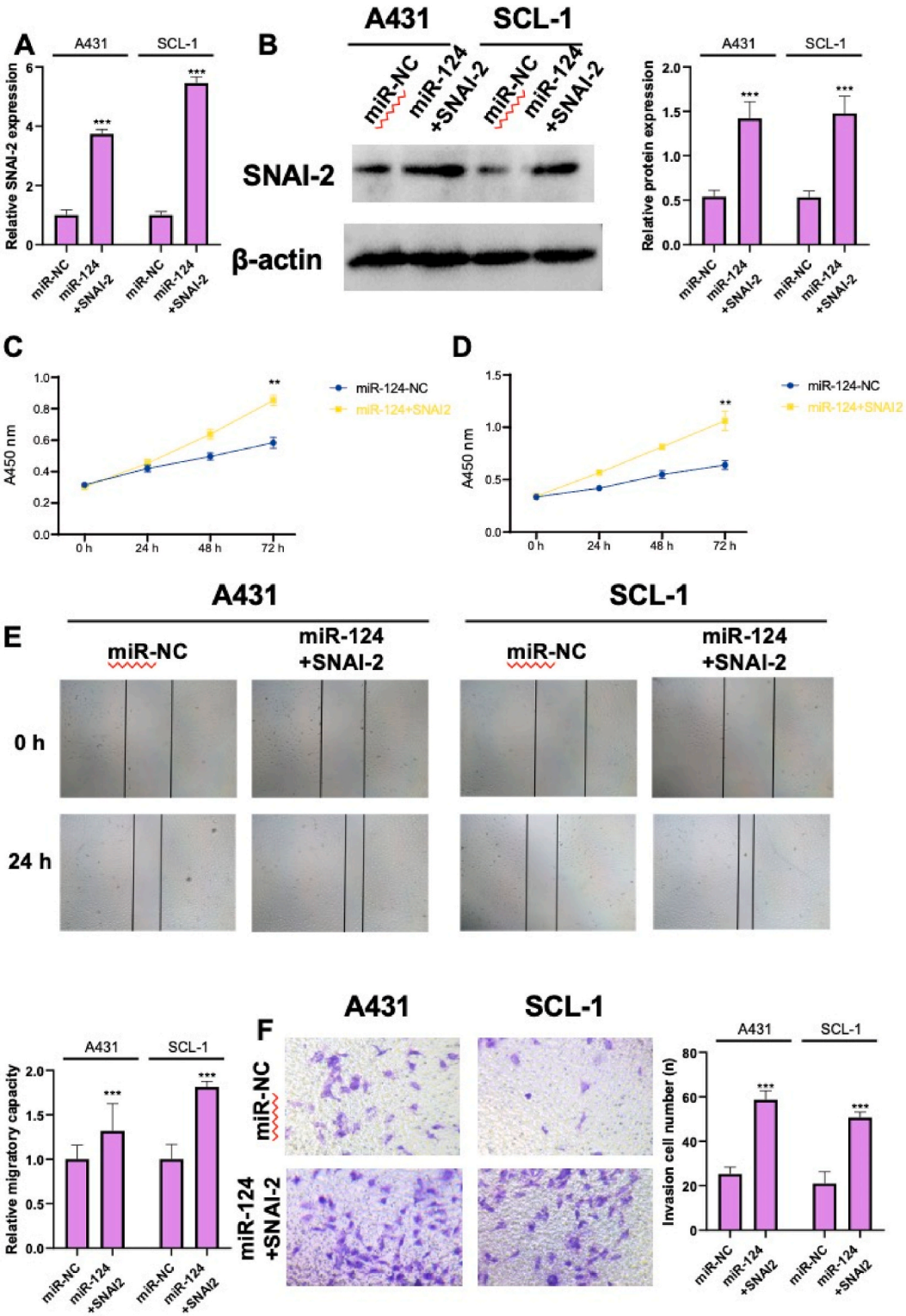

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Fig. 5. Demonstration of how SNAI2 can counteract the effects of MiR-124 on CSCC cells. (A–B) These sections detail the mRNA and protein expression levels of SNAI2. The uncropped blots of Fig. 5B are provided in Supplementary Figs. S5 and S6. (C, D) Measurements of cell proliferation are reported. (E) The assay results for cell migration are provided. (F) The data for cell invasion are depicted. With a sample size of n = 4, the results consistently show that the overexpression of SNAI2 can reverse the inhibitory effects of MiR-124 on the proliferation, migration, and invasion of CSCC cells, as evidenced by the significant changes in these cellular behaviors (**P < 0.01). This suggests that SNAI2 plays a crucial role in modulating the tumor-suppressive functions of MiR-124 in the context of CSCC.

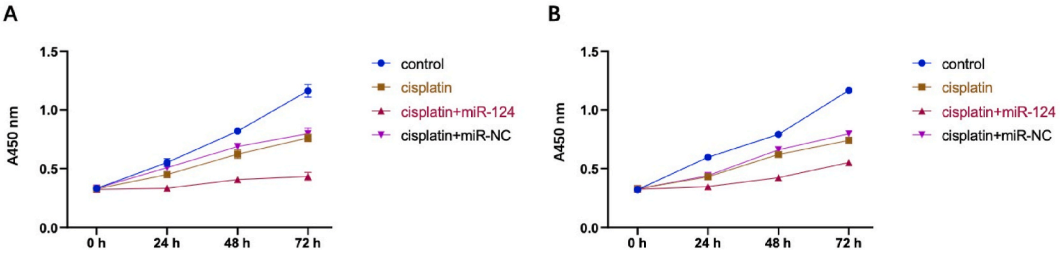

Fig. 6. Exploration of the role of MiR-124 in enhancing the susceptibility of CSCC cells to the chemotherapy drug cisplatin. (A–B) These panels present the survival rates of CSCC cells following transfection with either miR-NC (negative control) or MiR-124 and subsequent treatment with cisplatin. The cell survival was assessed using the CCK8 assay. The control group in this context refers to non-transfected cells that were not exposed to cisplatin. The experiments, conducted with a sample size of n = 4, indicate that cells transfected with MiR-124 and treated with cisplatin showed a significantly lower survival rate compared to the control and miR-NC groups (**P < 0.01). This suggests that MiR-124 transfection increases the efficacy of cisplatin in suppressing CSCC cell viability.

4. Discussion

In our study, we delved into the molecular dynamics of miR-124 and its association with the progression of CSCC. We established that miR-124 is significantly underexpressed in CSCC tissues, a phenomenon linked with advanced lymph node metastasis and higher clinical stages. The reintroduction of miR-124 curtailed the proliferation, invasion, and migration of CSCC cells. Furthermore, we identified SNAI2 as a gene directly downregulated by miR-124. Counteracting the inhibitory influence of miR-124, the overexpression of SNAI2 mitigated its suppressive effects on CSCC cell behavior. Additionally, enhancing miR-124 levels augmented the sensitivity of these cells to the chemotherapeutic agent cisplatin.

Previous research has indicated that miR-124 is frequently suppressed in various prevalent human cancers, where it typically acts as a tumor suppressor. For example, in cervical carcinoma, miR-124 exhibits downregulation and is known to impede cancer cell proliferation, invasion, migration, and the epithelial-mesenchymal transition (EMT) process by specifically targeting AEG-1 [29,30]. It also shows negative regulatory effects on bladder cancer cell proliferation and motility, as well as angiogenesis [31]. Our investigation revealed that miR-124 levels were markedly reduced in CSCC tissues relative to adjacent non-tumorous tissues, as well as in CSCC cell lines. This observation aligns with previous reports indicating a downregulation of miR-124 in CSCC A431 cells when compared to normal human epidermal keratinocytes [22]. Besides, hypermethylation of other promoter regions rather than the miR-124 promoter may be the main cause of miR-124 downregulation in CSCC [23]. We then for the first time observed that miR-124 downregulation was closely related to CSCC progression, which further suggests that miR-124 may play a key role in CSCC.

After our findings on miR-124 downregulation, we noted that its ectopic expression led to a decrease in CSCC cell proliferation, invasion, and migration. This corroborates the findings of Yamane et al. who also observed that the introduction of miR-124 into CSCC cells resulted in the inhibition of cell proliferation [23]. Considering these results alongside our own, it is suggested that miR-124 exerts a suppressive influence on the progression of CSCC. We then proceeded to identify potential targets of miR-124. Through bioinformatics analysis and luciferase reporter assays, we verified that SNAI2 is a direct target of miR-124 in A431 and SCL-1 cells. SNAI2 is known to act as a transcriptional repressor in various cellular contexts [32]. Previous studies have shown that SNAI2 could repress E-cadherin transcription, and thus promote EMT and tumor metastasis [32,33]. Moreover, the oncogenic role of SNAI2 has been reported in head and neck squamous cell carcinoma [33], tongue squamous cell carcinoma [34], and esophageal squamous cell carcinoma [35]. Invasive CSCC cells were reported to display high migratory and invasive abilities with high SNAI2 expression levels [36]. But till now, the detailed role of SNAI2 in CSCC remains unknown. The inverse relationship between miR-124 and SNAI2 protein levels in CSCC cells leads us to propose that SNAI2 may operate as a downstream mediator of miR-124's effects in these cells. Indeed, the upregulation of SNAI2 reversed the suppressive impact of miR-124 on CSCC cell proliferation, migration, and invasion, substantiating our hypothesis. In addition, the miR-124/SNAI2 signaling has also been found in NSCLC [37] and breast cancer [38].

Immunotherapy is a critical advancement in cancer treatment, offering targeted and potentially less toxic options and reliable markers are essential for predicting patient response and personalizing therapy [39–41]. In terms of cancer immunity, SNAI2 has been found to suppress the expression of major histocompatibility complex class I (MHC-I) molecules on cancer cells [42,43]. MHC-I molecules are responsible for presenting antigens to cytotoxic T cells, which play a crucial role in immune surveillance and elimination of cancer cells [44]. By downregulating MHC-I expression, SNAI2 helps cancer cells evade immune recognition and destruction. Furthermore, SNAI2 has been implicated in the regulation of immune checkpoint molecules, such as programmed death-ligand 1

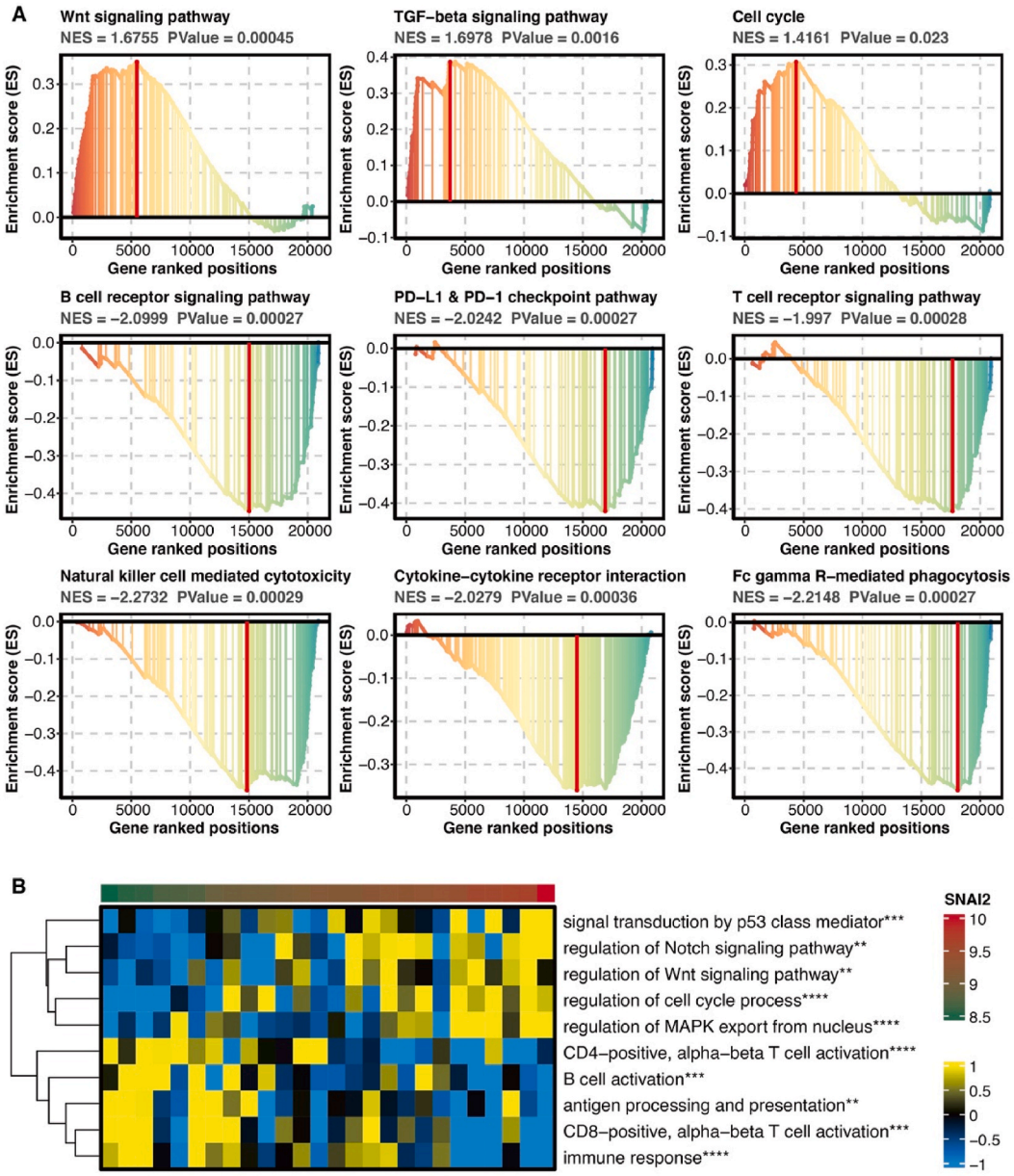

Fig. 7. Functional annotation of SNAI2. (A) GSEA of KEGG pathways on SNAI2. (B) GSVA of GO pathways on SNAI2.

(PD-L1). PD-L1 is often upregulated in cancer cells and interacts with programmed cell death protein 1 (PD-1) on T cells, leading to T cell exhaustion and immune evasion. SNAI2 has been shown to directly bind to the PD-L1 promoter and enhance its expression, thereby promoting immune evasion. In terms of immunotherapy response [45,46], SNAI2 is associated with resistance to immune checkpoint blockade therapies, such as anti-PD-1/PD-L1 antibodies. High expression of SNAI2 in tumor cells has been correlated with poor response to these therapies. This suggests that targeting SNAI2 or its downstream signaling pathways could potentially enhance the efficacy of immunotherapy. Overall, the role of SNAI2 in modulating immune responses in the context of immunotherapy against CSCC is complex and context-dependent. Depending on the specific conditions and signaling pathways involved, it can have both pro-tumorigenic and anti-tumorigenic effects. Further research is needed to fully understand the mechanisms underlying SNAI2's role in immune modulation and to explore its potential as a therapeutic target in CSCC immunotherapy.

To sum up, the downregulation of miR-124 in CSCC suggests that it may play a significant role in the malignant progression of this cancer type. This finding highlights the potential importance of miR-124 as a therapeutic target in CSCC. Furthermore, the study suggests that miR-124 can inhibit CSCC in vitro by targeting SNAI2. This indicates that miR-124 may have a tumor-suppressive effect by regulating the expression of SNAI2. Additionally, the increased cell sensitivity to cisplatin, a commonly used chemotherapy drug, further supports the potential therapeutic value of miR-124 in CSCC treatment. Considering these findings, miR-124 holds promise as a

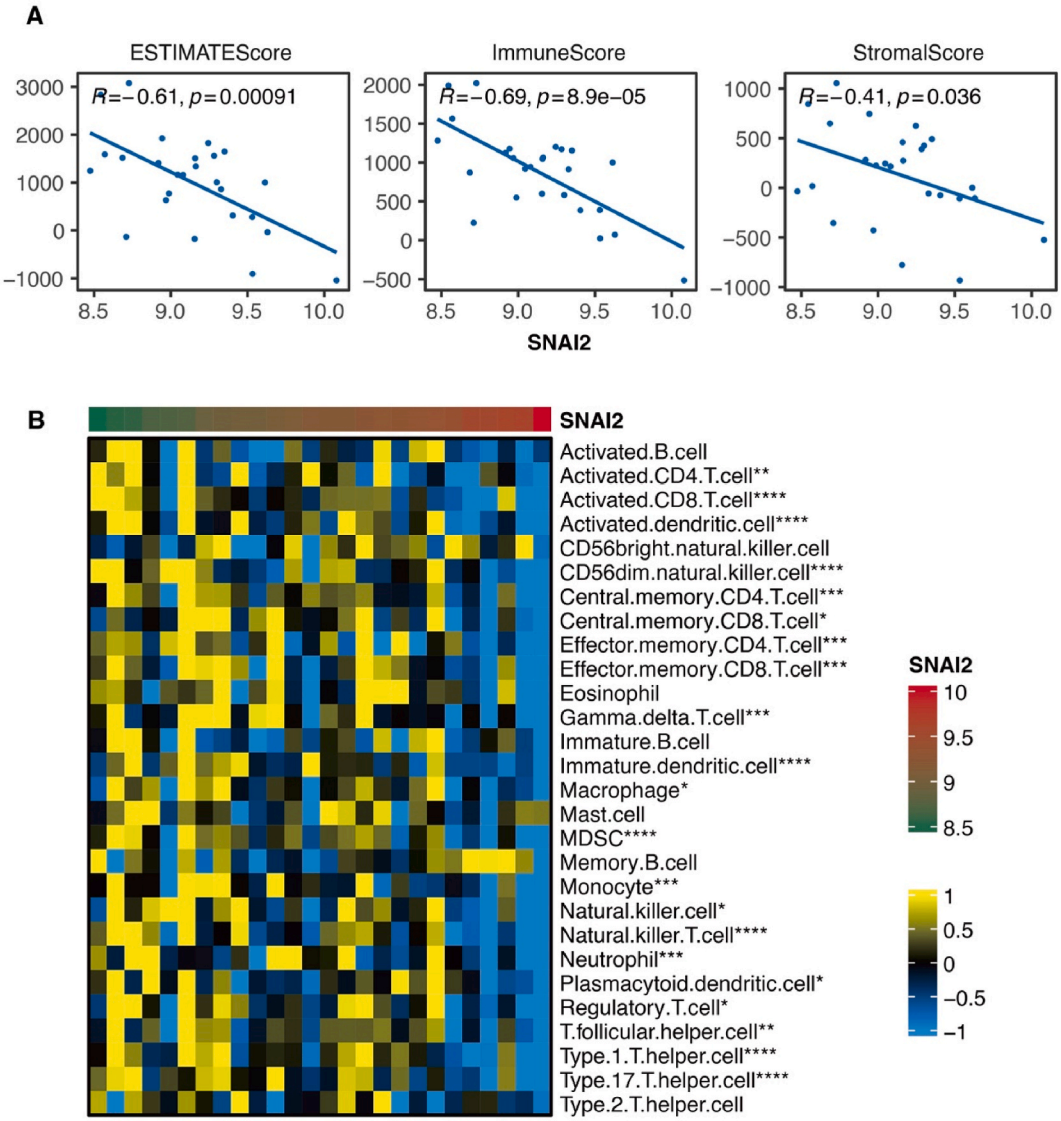

Fig. 8. Tumor immuno-related characteristics of SNAI2. (A) The correlation between SNAI2 and ESTIMATE, Immune, and Stromal scores. (B) The correlation between SNAI2 and immune cells.

therapeutic candidate for CSCC. Further research and clinical trials are needed to explore its efficacy and safety in human patients. If proven effective, miR-124 could potentially be used as a targeted therapy for CSCC, either alone or in combination with existing treatment modalities. Moreover, the study also suggests that SNAI2 may serve as an immunotherapy determinant. This implies that targeting SNAI2 in immunotherapy strategies against CSCC could potentially enhance the effectiveness of these treatments. Further investigations are required to fully understand the underlying mechanisms and potential clinical implications of SNAI2 in immunotherapy for CSCC. In conclusion, the findings of this study provide valuable insights into the role of miR-124 and SNAI2 in CSCC. The downregulation of miR-124 and the potential therapeutic effects of targeting SNAI2 highlight the importance of further research in this area. These results pave the way for the development of novel therapeutic approaches, including the potential use of miR-124 as a therapeutic candidate and the exploration of SNAI2 as an immunotherapy determinant in CSCC.

There are also some limitations of our study. Firstly, in vivo validation is further needed to confirm the pathogenic roles of SNAI2 and miR-124. Secondly, the specific mechanisms of how SNAI2 is connected to the immune activity and immunotherapy response in CSCC are expected to be revealed by more in vitro and in vivo experiments. Thirdly, more datasets could be included for performing the external validation of SNAI2.

Ethical statement

The study was conducted by the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of Changde First People's Hospital in China (Approval No. YX-2020-081-01). Written informed consent was taken from all the patients.

Data sharing statement

The original data could be obtained from the corresponding author.

Data availability statement

Not applicable.

CRediT authorship contribution statement

Hao Feng: Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Xing Hu:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Renli Yan:** Writing – review & editing, Validation, Software. **Xiaomin Jia:** Writing – review & editing, Validation, Software, Methodology. **Hao Feng:** Writing – review & editing, Validation, Software, Methodology, Investigation. **Nan Zhang:** Writing – review & editing, Validation, Software, Methodology. **Xiao Chen:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Supported by Hunan Provincial Natural Science Foundation 2020JJ8001, 2021JJ30399, 2023JJ30344, Hunan Provincial Health Commission Foundation 202104122479, Lhasa Health Technology Program Foundation LSKJ202104.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e24671.

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