GEORGIAN MEDICAL NEWS

ISSN 1512-0112

NO 2 (359) Февраль 2025

ТБИЛИСИ - NEW YORK

ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

Monthly Georgia-US joint scientific journal published both in electronic and paper formats of the Agency of Medical Information of the Georgian Association of Business Press. Published since 1994. Distributed in NIS, EU and USA.

GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman** (**Кириллица**), для текста на грузинском языке следует использовать **AcadNusx.** Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - http://www.spinesurgery.ru/files/publish.pdf и http://www.nlm.nih.gov/bsd/uniform_requirements.html
В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or compu-ter-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman** (**Cyrillic**), print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf
In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

GEORGIAN MEDICAL NEWS
No 2 (359) 2025

Содержание:

WHOLE TRANSCRIPTOME SEQUENCING AND CIRC_HSA_0001847 ON PROLIFERATION AND INVASION OF ORAL SQUAMOUS CELL CARCINOMA

Liu-Xia Shi[1,2&], Xiao-Ya Peng[1,2&], Xiao-Xu Ruan[1,2], Rui Li[1,2], Wen-Jie Wen[1,2*], Chao Deng[1,2*].

[1]School of stomatology, Wannan Medical College; 22#Wenchang West Road, Wuhu, Anhui Province, China. 241002, China.

[2]Anhui Provincial Engineering Research Center for Dental Materials and Application, Wannan Medical College, Wuhu 241002, China.

[&]These authors contributed equally to this work and should be considered co-first authors.

**Both the authors have contributed equally to the work.*

Abstract.

Background: Oral squamous cell carcinoma (OSCC) accounts for approximately 90% of malignant epithelial tumors in the oral and maxillofacial region, which is characterized by a high metastatic potential and an unfavorable prognosis. The specific process of oral mucosal carcinogenesis and progression is very complicated.

Methods: Five OSCC patients' cancer tissues and paracancerous tissues were collected for whole transcriptome sequencing analysis to screen differentially expressed mRNA, long-stranded noncoding RNA (lncRNA), and circular RNA (circRNA) and bioinformatics [gene ontology (GO) enrichment analysis with genomic database (KEGG) pathway analysis and transcript enrichment analysis, etc.]. Target molecules closely associated with oral squamous cell carcinogenesis, migration, and invasion were identified using a miRNA-circRNA association network.

Results: We screened 2310 mRNAs differentially expressed in cancer and paraneoplastic tissues, of which 1019 were upregulated and 1291 were downregulated; 14216 differentially expressed lncRNAs, of which 8829 were upregulated and 5387 were downregulated; and 167 differentially expressed circRNAs, of which 18 were upregulated and 149 were downregulated. Oral squamous cell carcinoma cells had a higher level of hsa_circ_0001847 expression than hok cells ($P<0.05$). Reduced hsa_circ_0001847 expression inhibited oral squamous cell carcinoma cell proliferation, migration, and invasiveness ($P<0.05$).

Conclusion: Oral tissues contain differentially expressed mRNAs, lncRNAs, and circRNAs, and these differentially expressed mRNAs, lncRNAs, and circRNAs may be involved in the development of OSCC.

Key words. Transcriptome sequencing, oral squamous cell carcinoma, circRNA, lncRNA, mRNA.

Introduction.

Oral squamous cell carcinoma (OSCC) accounts for approximately 90% of oral cancers [1], with approximately 300,000 new cases and 140,000 deaths per year [2]. The prognosis for oral squamous cell carcinoma is unfavorable, with a greater risk of metastasis and local recurrence [3,4]. Surgical resection, radiotherapy, and chemotherapy are the most common treatments for oral squamous cell carcinoma. Despite significant advances in surgical and radiotherapy techniques, the 5-year survival rate for patients with oral squamous cell carcinoma has remained at approximately 50% for the past 30 years [5]. The pathogenesis of OSCC is not fully understood at this time. Multiple genes and pathways [6] have been implicated in OSCC occurrence and progression. To increase the survival rate of OSCC patients, it is necessary to conduct in-depth research on the genes related to the formation and progression of OSCC. This will help identify more accurate early diagnostic indicators and corresponding prognostic treatments. Investigation of the entire transcriptome under cell-specific conditions can systematically reveal the transcriptional-level regulatory rules [7]. Scholars are currently concentrating on noncoding RNAs (ncRNAs), microRNAs (miRNAs), long noncoding RNAs (lncRNAs), and circular RNAs (circRNAs). Several studies have demonstrated that high-frequency mutant transcripts play crucial roles in cell cycle regulation, energy metabolism, and signaling pathways. These transcripts may serve as potential biomarkers for targeted therapy in clinical malignancies [8-9]. Circular RNAs (circRNAs) are a class of abundant and diverse ribonucleic acids, typically formed by selective splicing of pre-messenger RNAs [10-13]. These circular RNAs have been demonstrated to be important regulators in various biological processes, including oncogenesis, stress responses, and inflammation [14]. The dysregulation of circRNAs has been shown to be a major cause of the occurrence and development of human cancers, including oral squamous cell carcinoma (OSCC) [15-17]. Notably, due to their high stability and abundance, circRNAs are considered ideal biomarkers for human cancers [18,19].

Epithelial-mesenchymal transition (EMT) primarily refers to the biological process by which polarized epithelial cells undergo transformation into mesenchymal cell phenotypes under specific physiological or pathological conditions, thereby endowing cells with the ability to metastasize and invade [7]. The phenomenon of EMT has been identified in various human tumors, including breast cancer, liver cancer, prostate cancer, and head and neck squamous cell carcinoma. When tumor cells undergo EMT, the expression of epithelial marker molecules such as E-cadherin, zonula occluden-1 (ZO-1), and keratins is downregulated, while the expression of mesenchymal marker molecules, such as vimentin, fibronectin, and N-cadherin, is upregulated. The expression of matrix metalloproteinases-2/9 (MMP-2/9), which assist in EMT, is also upregulated, leading to a loss of cell polarity in epithelial-derived tumor cells, loosening of cell-cell junctions, and reorganization of cytoskeletal proteins [8]. Among these changes, the loss of E-cadherin at the cell membrane is a key marker of EMT in tumor cells [9]. The reduction or absence of E-cadherin is significantly associated with tumor grading, invasion, metastasis, and prognosis [10-12]. Numerous reports indicate that EMT is a critical cause of recurrence and metastasis in oral squamous cell carcinoma,

which is of great significance for the enhancement of tumor cell migration and invasion capabilities.

In this study, we aimed to determine the mechanism of OSCC proliferation and invasion at the transcriptome level, determine which specific key genes are involved in the progression of OSCC, and construct circRNA-miRNA-mRNA and lncRNA-miRNA-mRNA association analyses to further validate the biological mechanism of noncoding RNAs in OSCC to provide a more reliable theory for the clinical treatment of OSCC. This will strengthen the theoretical foundation for clinical diagnosis and treatment.

Materials and Methods.

Subject of research: A total of 10 patients aged 43-75 years old were selected from Yijishan Hospital of Wuhu City, Anhui Province, from October 2020 to July 2021. This study was approved by the Ethics Committee of Yijishan Hospital, Wuhu City, Anhui Province, and all patients or their families gave informed consent and signed informed consent (Table 1 Clinically relevant information for five patients). The clinical data and pathological characteristics of 10 patients were collected for statistical analysis, 5 of which were used for sequencing.

Sampling and sample processing:

Malignant and precancerous tissue were collected from the patient during surgery (1 cm from the edge of the cancerous tissue). The standard diameter for cancer was 0.5 cm. Tissue samples and paracancerous tissue samples were block sliced, cleaned with PBS solution, sealed in sterile lyophilised tubes, and promptly stored in a refrigerator at -80 degrees Celsius.

Construction of a library and sequencing of the transcriptome:

Shanghai Cloud Sequence Biotechnology Co. Offered high-throughput sequencing. According to the manufacturer's instructions, ribosomal RNA (rRNA) was extracted from the samples using the NEBNext rRNA Depletion Kit (New England Biolabs, Inc., Massachusetts, USA). Using the NEBNext® UltraTM II Directed RNA Library Prep Kit, sequencing libraries were produced (New England Biolabs, Inc., Massachusetts, USA). A BioAnalyzer 2100 (Agilent Technologies, USA) was used for quality control and quantification of the libraries, and the Illumina Novaseq equipment was used for 150 bp double-end sequencing.

Principal sequencing method and content:

Following Illumina NovaSeq 6000 sequencing, double-ended reads were extracted. Q30 was utilised for quality control, and cutadapt software (v1.9.3) was used to dejoin and eliminate low-quality reads to acquire high-quality readings. Utilising cloud-based sequencing technology, distinct analyses of circRNAs, lncRNAs, and mRNAs were conducted.

circRNA: High-quality reads were aligned to the reference genome/transcriptome using STAR [2] software (v2.5.1b), and circRNA detection and identification were accomplished using DCC [3] software (v0.4.4). The discovered circular RNAs were further annotated using the circBase and Circ2Traits databases [4,5]. Data normalization and screening for differentially expressed circRNAs were performed using edgeR (v3.16.5) software [6]. GO and KEGG analyses were also performed on the genes from which the differentially expressed circRNAs originated.

LncRNA and mRNA: HISAT2 [7] software was used to align high-quality reads to the human reference genome (UCSC HG19) (v2.0.4). Using HTSeq [8] software, raw count numbers at the gene level were acquired as mRNA expression profiles, and raw count numbers at the transcript level were obtained as lncRNA expression profiles (v0.9.1). Using edgeR software (v3.16.5), differentially expressed mRNAs and lncRNAs were screened to normalize and calculate ploidy changes and p-values between the two sets of samples. Based on proximity connections, GO and KEGG pathway analyses of differential mRNAs and GO and KEGG pathway analyses of target genes were performed to determine lncRNA target genes.

Gene detection in tissue samples:

Using TRIzol (Thermo Fisher Scientific, Waltham, MA, USA) reagent, total RNA was isolated from oral squamous cell carcinoma tissues, paracancerous tissues, and transfected cells. cDNA was reverse transcribed using a reverse transcription kit, and qPCR was conducted using an SYBR qPCR kit. The primer sequences were as follows:

Cell culture and treatment:

The OSCC cell line (WSU-HN30) was routinely cultured at 37°C in DMEM containing 10% fetal bovine serum. The logarithmic OSCC cell line (WSU-HN30) was inoculated

Table 1. *Primers designed for RT-qPCR.*

Gene	**Primer sequences**	
hsa_circ_0001847	forward primer	5'-GTTCCTCACAGTCAAGCCTCAGAAG-3'
	reverse primer	5'-GAGAACAGGAGTTGACGGCAGTG-3'
hsa_circ_0001722	forward primer	5'-GCAAGGTAAATGGGAAGTTGGTAGC-3'
	reverse primer	5'-CCCTGATAGCTGTGAAAGGTGTCC-3'
hsa_circ_0083619	forward primer	5'-GGGCTTTGGTTTGAGAGCAACAAG-3'
	reverse primer	5'-ATGGCTTGAAGGATTCGGTCTTGAG-3';
hsa_circ_0007695	forward primer	5'-CATCAGGAGGAAGAAGCAAGCTCAG-3'
	reverse primer	5'-AACTGTCCTGGTTTGCTGATATGCC-3'
hsa_circ_0083619	forward primer	5'-CTTTCCTCCAACCCAACGGCTTAC-3'
	reverse primer	5'-ACTTCCTCTTCCAGTCGTCCTTCC-3'
GAPDH	forward primer	5'-ACAACTTTGGTATCGTGGAAGG-3'
	reverse primer	5'-GCCATCACGCCACAGTTTC-3'

Using the 2-Ct technique and GAPDH as the internal reference gene, the relative expression of the target gene was determined.

into a 6-well plate at a rate of 1×10^5 cells per well. The hsa_circ_0001847 knockdown plasmid (pSLenti-U6-shRNA(hsa_circ_0001847)-CMV-EGFP-F2A-Puro-WPRE) and its negative control plasmid (pSLenti-U6-shRNA(NC2)-CMV-EGFP-F2A-Puro-WPRE) were constructed and set as the sh-NC group and sh-hsa_circ_0001847 group, respectively, and were purchased from Heyuan Biological Co., LTD. Lentivirus infection was performed according to the manufacturer's instructions First, the lentiviral infection pre-experiment was conducted to determine the optimal MOI, and the amount of virus added per well was calculated (μL) = the number of cells/ titer (TU/mL) $*10^3$ at the time of infection. On the first day, the WSU-HN30 cells in good condition were counted after digestion, and the cell suspension density was adjusted to $1x10^5$cells/mL. 2mL cell suspension was added to each well and inoculated into the six-well plate. The next day, when the cells were observed to be in good condition and the cell density reached 30-40%, lentivirus was added for infection. 12 to 16 hours after virus infection, the cell status was observed and the fresh medium was replaced. Remove the medium from the orifice plate and add 2mL of complete medium to each orifice. After 72~96 hours of virus infection, the fluorescence expression efficiency of cells can be observed under fluorescence microscope, that is, the efficiency of lentivirus infection of target cells.

Wound healing assay:

For wound healing assay, 2×10^5 per well of CAL27 and WSU-HN30 cells were placed into 6-well plates after various transfections. Cells were allowed to grow until they achieved 100% confluence. Next, a scratch was made using a 200 μL pipette tip, and cells were allowed to migrate for additional 24 h. The images at 0 and 24h were recorded. Relative migratory rate was calculated by the formula: (sh-hsa_circ_0001847(wound area)0h)-(sh-hsa_circ_0001847(wound area)24h)/(sh-NC(wound area)0h)-(sh-NC(wound area)24h).

Cell-counting kit 8 (CCK-8) assay:

A CCK-8 assay (Sigma, St. Louis, MO) was used to detect cell proliferation. In brief, 1.5 × 103 cells were seeded into 96-well plates, followed by the addition of 10 μl CCK-8 after 48 h. Subsequently, an additional 2 h incubation at 37 °C and 5% CO2 was needed. The 450 nm absorbance was detected using a microtiter plate reader.

Transwell assay:

Cell invasion was analyzed with Matrigel-coated (BD Biosciences, Franklin Lakes, NJ) in 24-well Transwell chambers with 8-μm well inserts, CAL27 and WSU-HN30 cells (5×10^4 for invasion analysis) in 100ul serum-free DMEM were plated into the superior chambers, whereas the lower chamber was supplemented with 600 μL DMEM with 10% serum. 24h upon incubation, migrated or invasive cells were spotted with 0.1% crystal violet, and examined under a microscope (Nikon, Tokyo, Japan) with at least 5 random fields.

Results.

Differential mRNA analysis: As shown in Figure 1, the high-throughput sequencing analysis identified a total of 2310 differentially expressed mRNAs in cancer and paraneoplastic tissues, of which 1019 were upregulated and 1291 were downregulated (Figure 1 (a)). The top 10 upregulated genes were OASLd, MAGE6, CLG, CPX1, G1P2, CT130, HEL-N1, CRD-BP, D2S69E, and PRO940. The top 10 downregulated genes were HFE2A, CMH16, SPRYD1, COX6AH, ARPP, STARS, CK3, ASD3, CHASM, and ADH-2.

The 20 most significantly enriched KEGG pathways were chosen for information on calcium signalling pathways, adrenergic signalling in cardiac myocytes, salivary secretion, drug metabolism-cytochrome P450, metabolic effects of cytochrome P450 on xenobiotics, allograft rejection, and Th17 cell differentiation, among others. Figure 1 (b,c)

A GO enrichment analysis was conducted on 2310 genes with differential expression. The results indicated that 2091 genes were involved in biological processes, with 855 upregulated and 1236 downregulated in expression. 259 genes were involved in cellular components, with 106 upregulated and 153 downregulated in expression. 268 genes were involved in molecular functions, with 99 up regulated and 169 downregulated in expression. Figure 1 (d, e, f, g, h, i).

LncRNA differential analysis:

The sequencing results showed that there were 14216 differentially expressed lncRNAs, of which 8829 were upregulated, including ENST00000509399, ENST00000439156, ENST00000425399, ENST00000498524, ENST00000474728 and ENST00000552602, and 5387 were downregulated, including ENST00000562218, ENST00000497809, ENST00000609976, ENST00000546583, ENST00000584611, and ENST00000368527, (Figure 2 (a)).

The KEGG pathway enrichment results indicated that the major cellular pathways involved in differential lncRNAs are the phospholipase D signalling pathway, the oxytocin signalling pathway, the Fc epsilon RI signalling pathway, the GnRH signalling pathway, choline metabolism in adrenergic signalling cancer in cardiac myocytes pathway, and other related pathways. See Figure 2 (b,c).

GO enrichment analysis of 14216 differentially expressed lncRNA target genes revealed that 1893 genes were involved in biological processes, 1372 of which were upregulated and 521 of which were downregulated. Of the 339 genes involved in cellular components, 219 genes were upregulated and 120 were downregulated; of the 327 genes involved in molecular functions, 198 were upregulated and 129 were downregulated. There were 198 upregulated genes and 129 downregulated genes. The results were shown in Figure 2 (d,e,f,g,h,i).

Variance examination of circular RNA:

Sequencing revealed 167 differentially expressed circRNA; 18 were upregulated, including hsa_circ_0017586, hsa_circ_0083619, and hsa_circ_0021570; 149 were downregulated, including hsa_circ_0005615, hsa_circ_0009061, and others. hsa_circ_0008832, etc. Figure 3 (a).

KEGG pathway enrichment results indicated that differentially expressed circRNAs were involved in cellular pathways associated with the B-cell receptor signalling pathway, MAPK signalling pathway, T-cell receptor signalling pathway, ErbB signalling pathway, natural killer cell-mediated and other related pathways. Figure 3 depicts the GO enrichment data for the shared differential circRNA Figure3 (b,c).

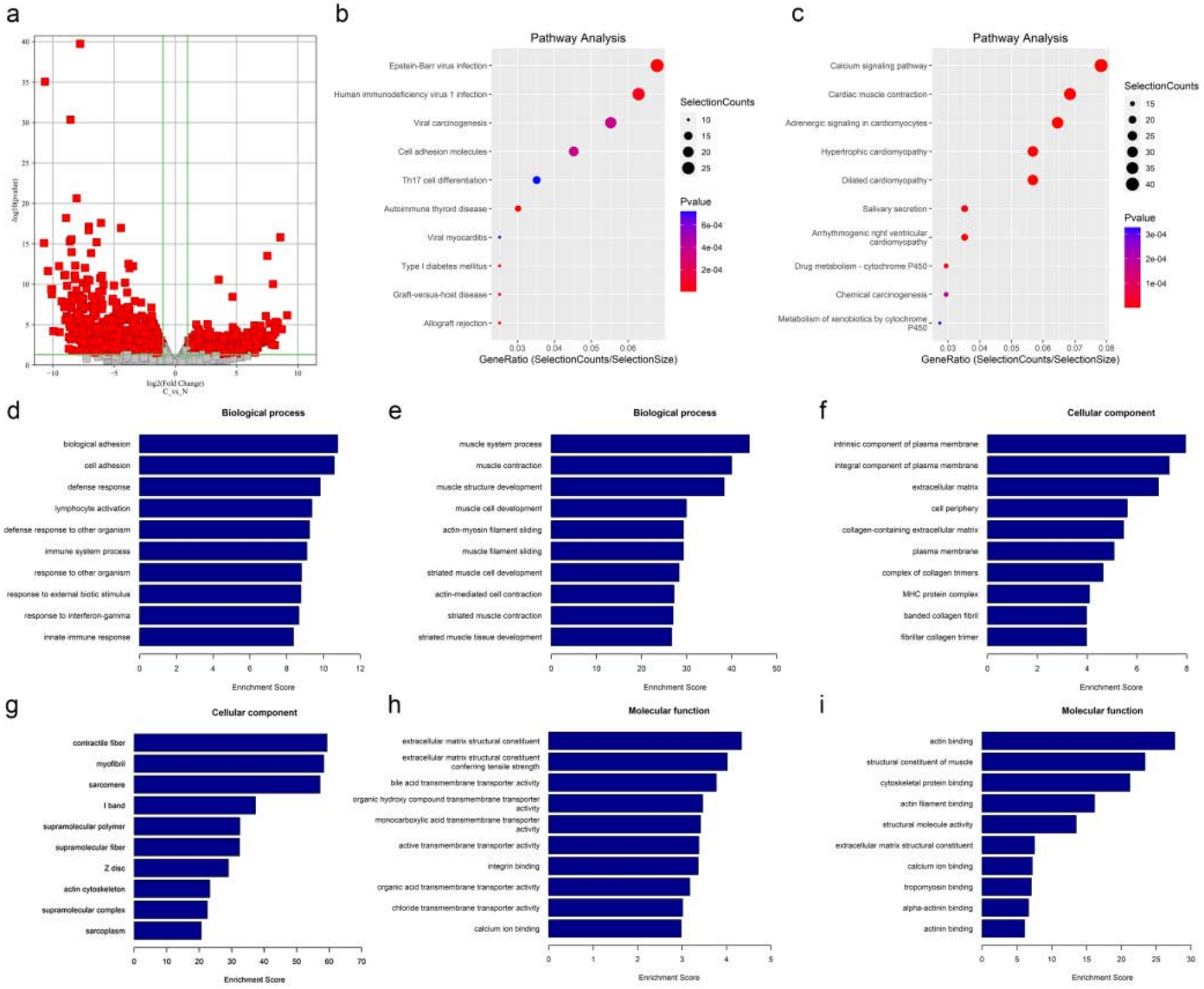

Figure 1. *mRNA differential analysis.*

(a) Volcano map of differentially expressed mRNAs.

(b) Scatter plot of KEGG pathway enrichment of differentially upregulated expressed mRNAs.

(c) Scatter plot of KEGG pathway enrichment of differentially downregulated expressed mRNAs.

(d) Graphical representation of the GO enrichment classification for upregulated expressed genes: biological processes.

(e) Graphical representation of the GO enrichment classification for down-regulated expressed genes: biological processes.

(f) A statistical plot of the GO enrichment categorization for all upregulated expressed genes: cellular fractions.

(g) A a statistical plot of the GO enrichment categorization for all downregulated expressed genes: cellular fractions.

(h) GO enrichment categorization statistics for all upregulated expressed genes: molecular function.

(i) GO enrichment categorization statistics for all upregulated expressed genes: molecular function.

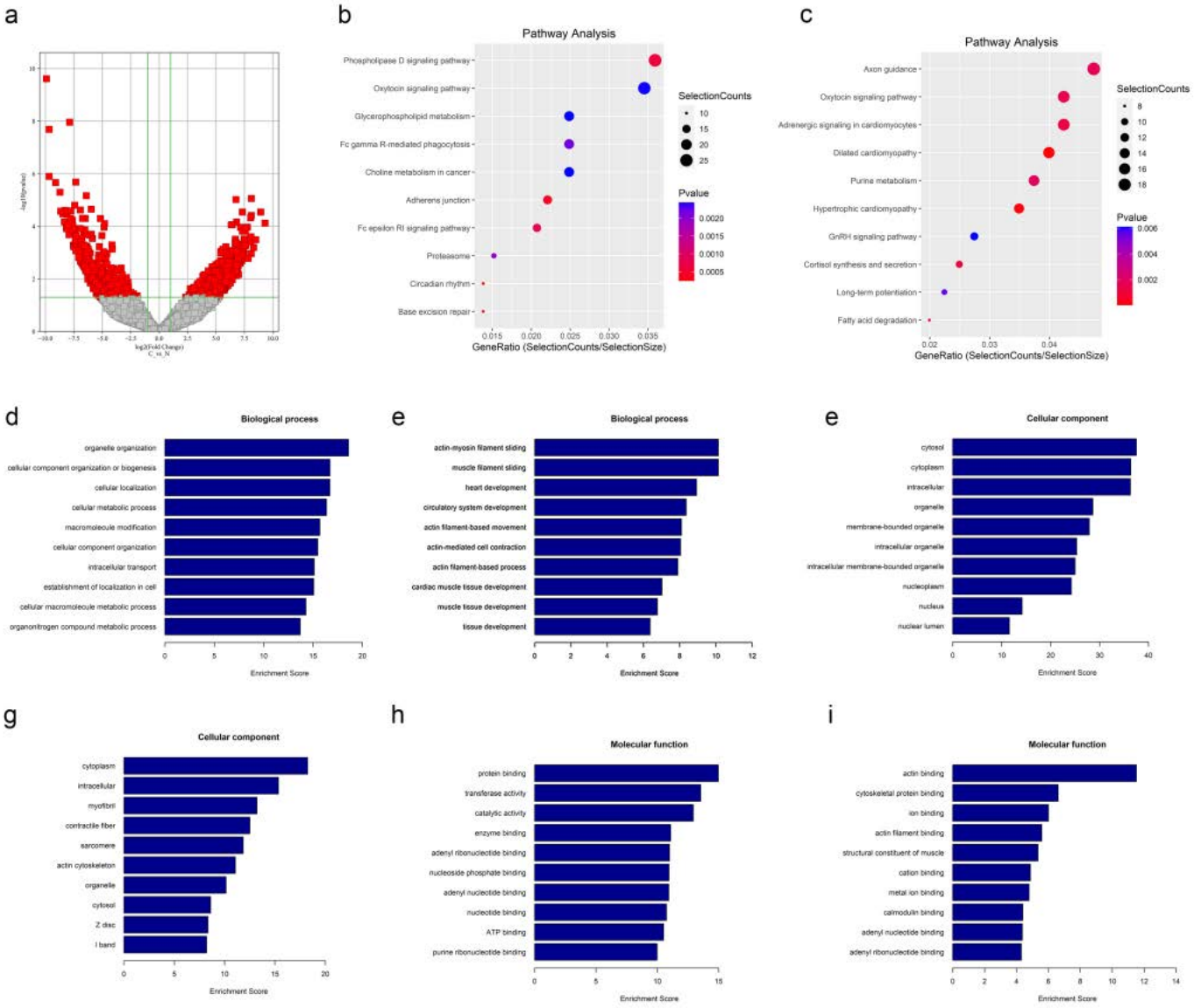

Figure 2. *LncRNA differential analysis.*

(a) Volcano map of differentially expressed long noncoding RNAs.

(b) Scatter plot of KEGG pathway enrichment of differentially upregulated expressed long noncoding RNAs scatter plot.

(c) Scatter plot of KEGG pathway enrichment of differentially downregulated expressed long noncoding RNAs scatter plot.

(d) Graphical representation of the GO enrichment classification for upregulated expressed genes: biological processes.

(e) Graphical representation of the GO enrichment classification for downregulated expressed genes: biological processes.

(f) A statistical plot of the GO enrichment categorization for all upregulated expressed genes: cellular fractions.

(g) A statistical plot of the GO enrichment categorization for all downregulated expressed genes: cellular fractions.

(h) GO enrichment categorization statistics for all upregulated expressed genes: molecular function.

(i) GO enrichment categorization statistics for all upregulated expressed genes: molecular function.

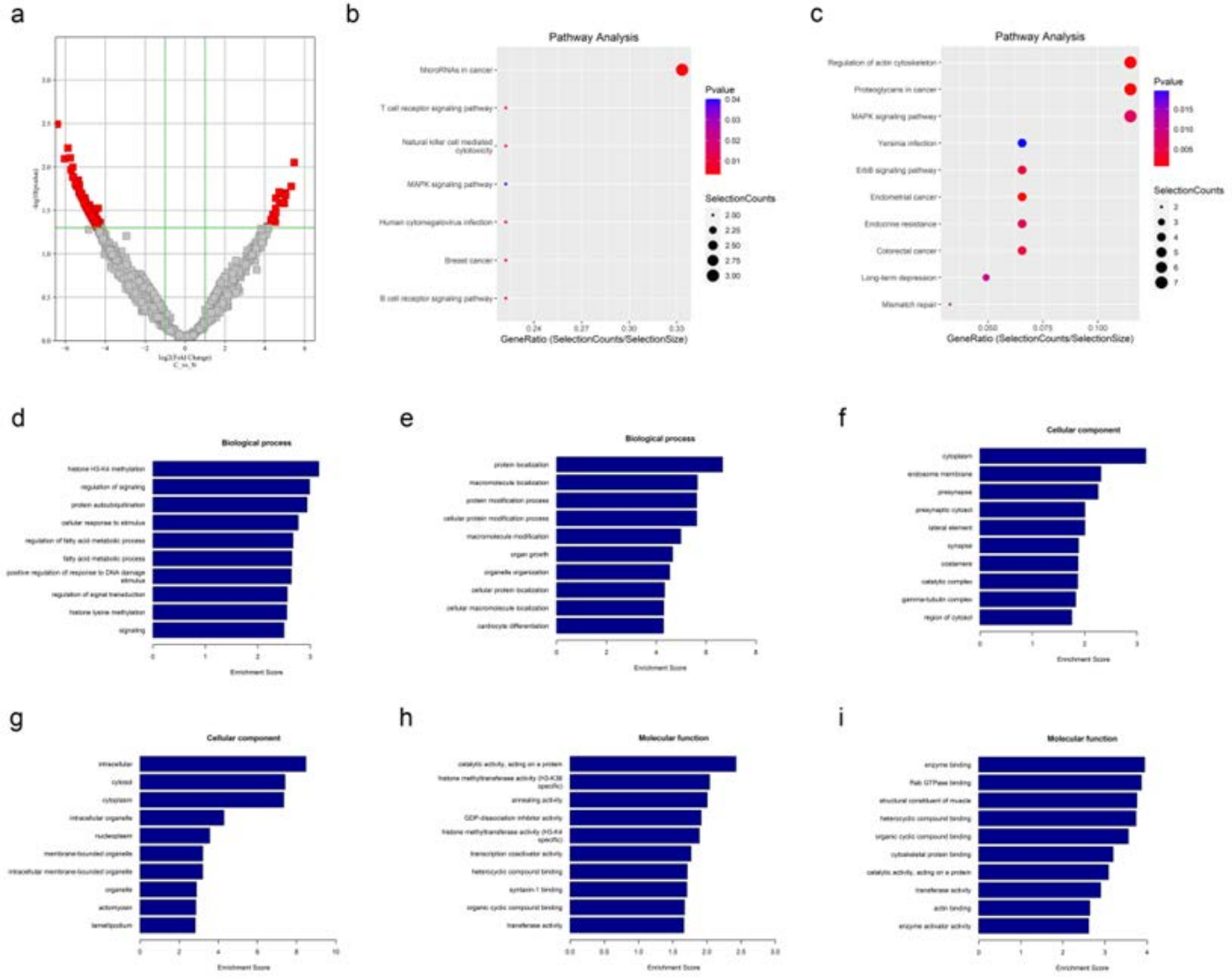

Figure 3. *circRNA differential analysis.*

(a) Volcano map of differentially expressed circular RNAs.

(b) KEGG pathway enrichment of differentially upregulated expressed circular RNA scatter plot.

(c) KEGG pathway enrichment of differentially downregulated expressed circular RNA scatter plot.

(d) Graphical representation of the GO enrichment classification for upregulated expressed genes: biological processes.

(e) Graphical representation of the GO enrichment classification for downregulated expressed genes: biological processes.

(f) A statistical plot of the GO enrichment categorization for all upregulated expressed genes: cellular fractions.

(g) A statistical plot of the GO enrichment categorization for all downregulated expressed genes: cellular fractions.

(h) GO enrichment categorization statistics for all upregulated expressed genes: molecular function.

(i) GO enrichment categorization statistics for all upregulated expressed genes: molecular function.

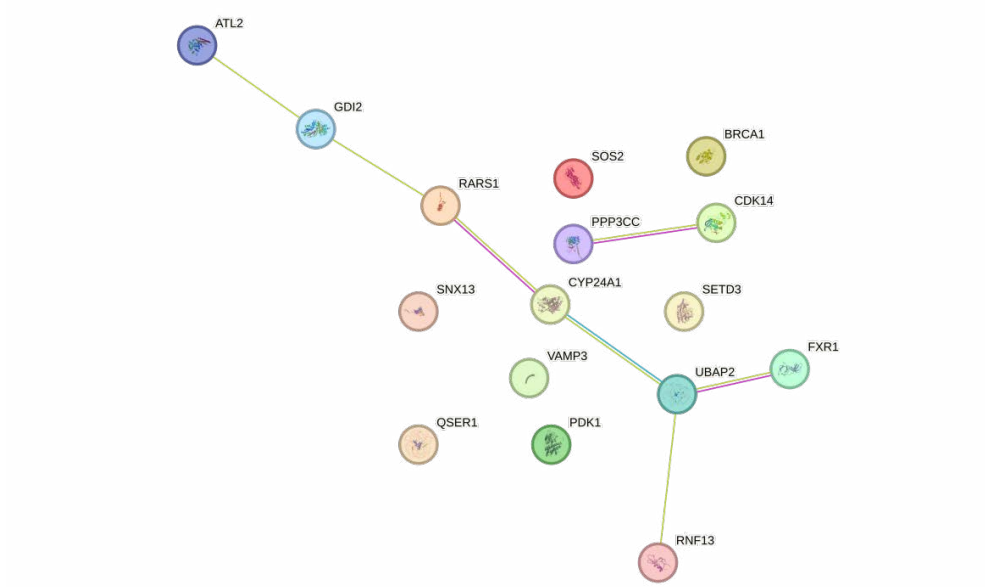

Figure 4. *(http://cn.string-db.org) for specific interaction networks of sequenced genes.*

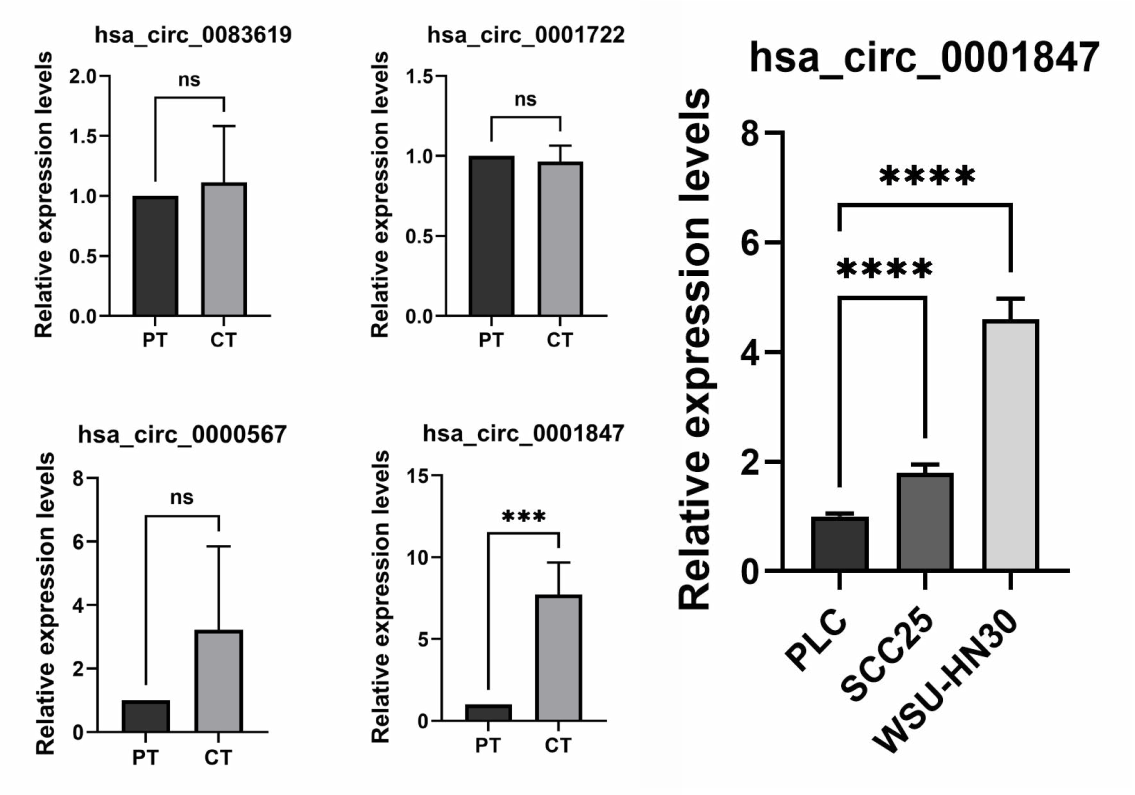

Figure 5. *Expression differences of hsa-circRNA-0001847.*

Expression of hsa_circ_0001847, hsa_circ_0001722, hsa_circ_0083619, hsa_circ_0007695, hsa_circ_0083619 in cancer tissues and adjacent tissues.

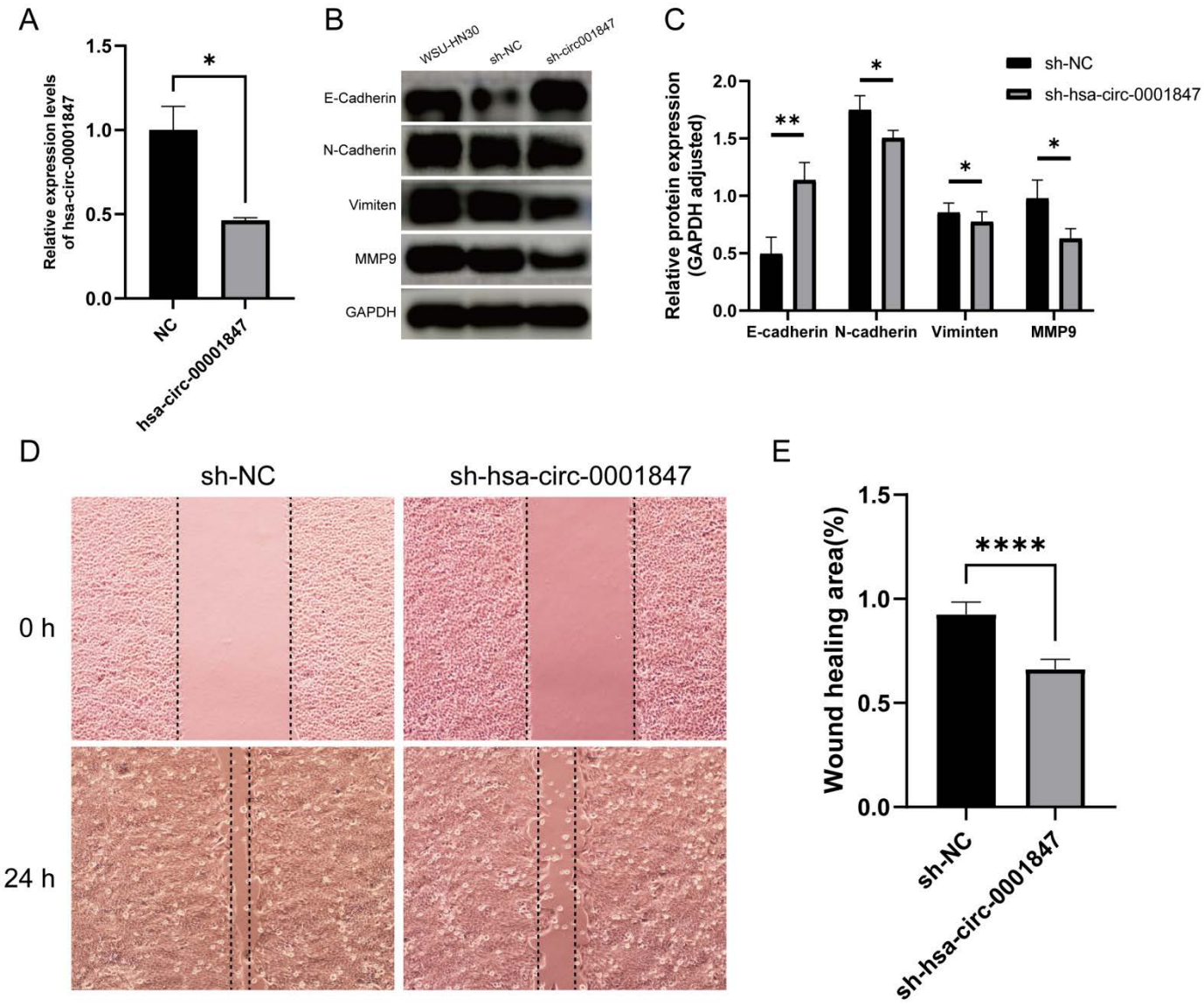

Figure 6. *hsa-circRNA-0001847 affects the EMT process.*

(A) Lentiviral transfection was used to knock down hsa-circRNA-0001847, and RT-PCR was used to verify the transfection efficiency.

(B) Western blot (WB) experiments revealed that the knockdown of hsa-circRNA-0001847 inhibits the EMT process.

(C) Quantitative analysis of the grayscale image from the WB experiment.

(D) After knocking down hsa-circRNA-0001847, a scratch assay was conducted to assess the migration ability of tumor cells.

(E) Quantitative bar chart of the scratch assay results.

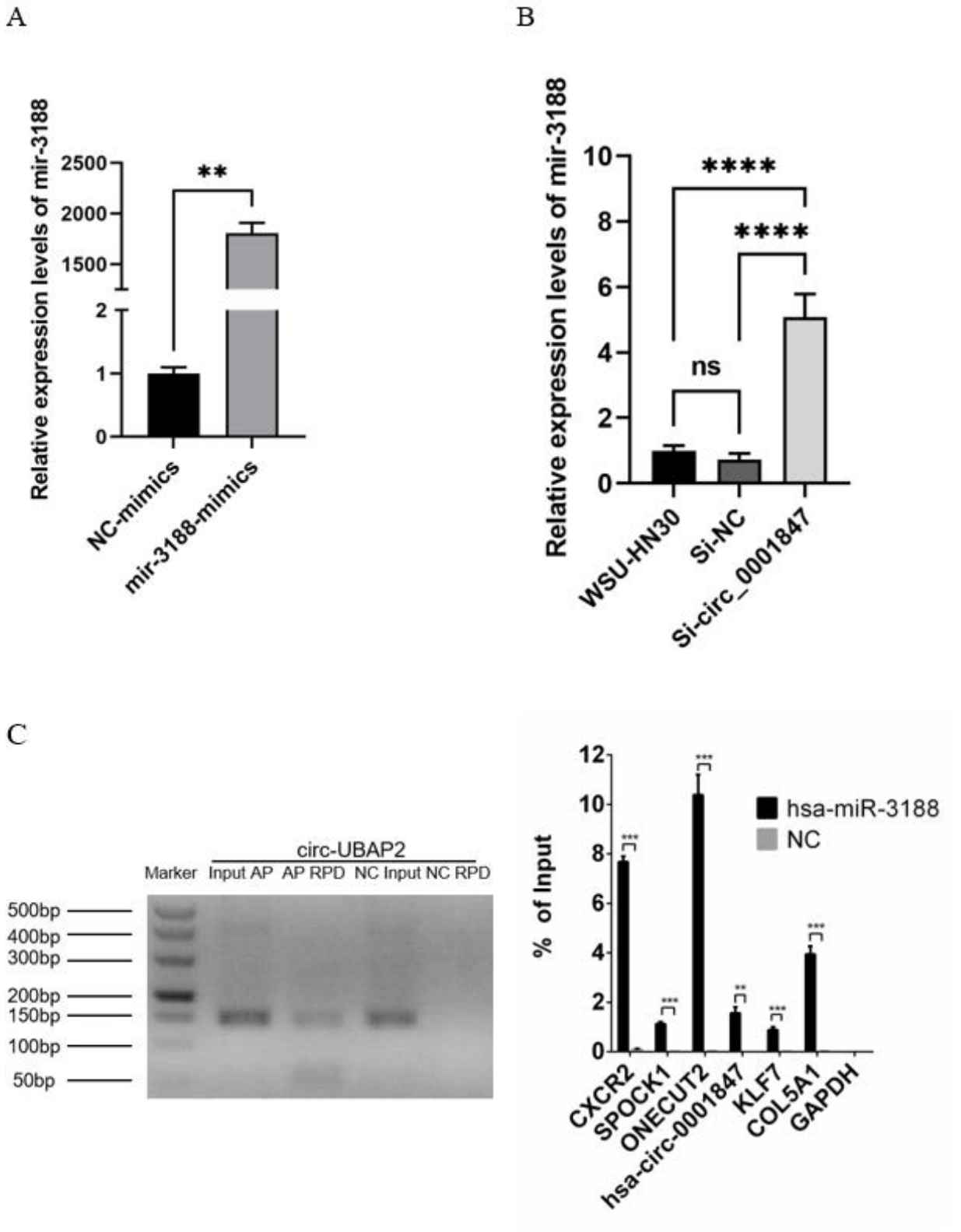

Figure 7. *hsa-circRNA-0001847 targets and binds to mir-3188.*

(A) Differential expression of mir-3188 in cancerous and normal tissues.

(B) Validation of successful transfection with mir-3188.

(C) Compared to the NC group, transient transfection with mir-3188 leads to increased expression of hsa-circRNA-0001847.

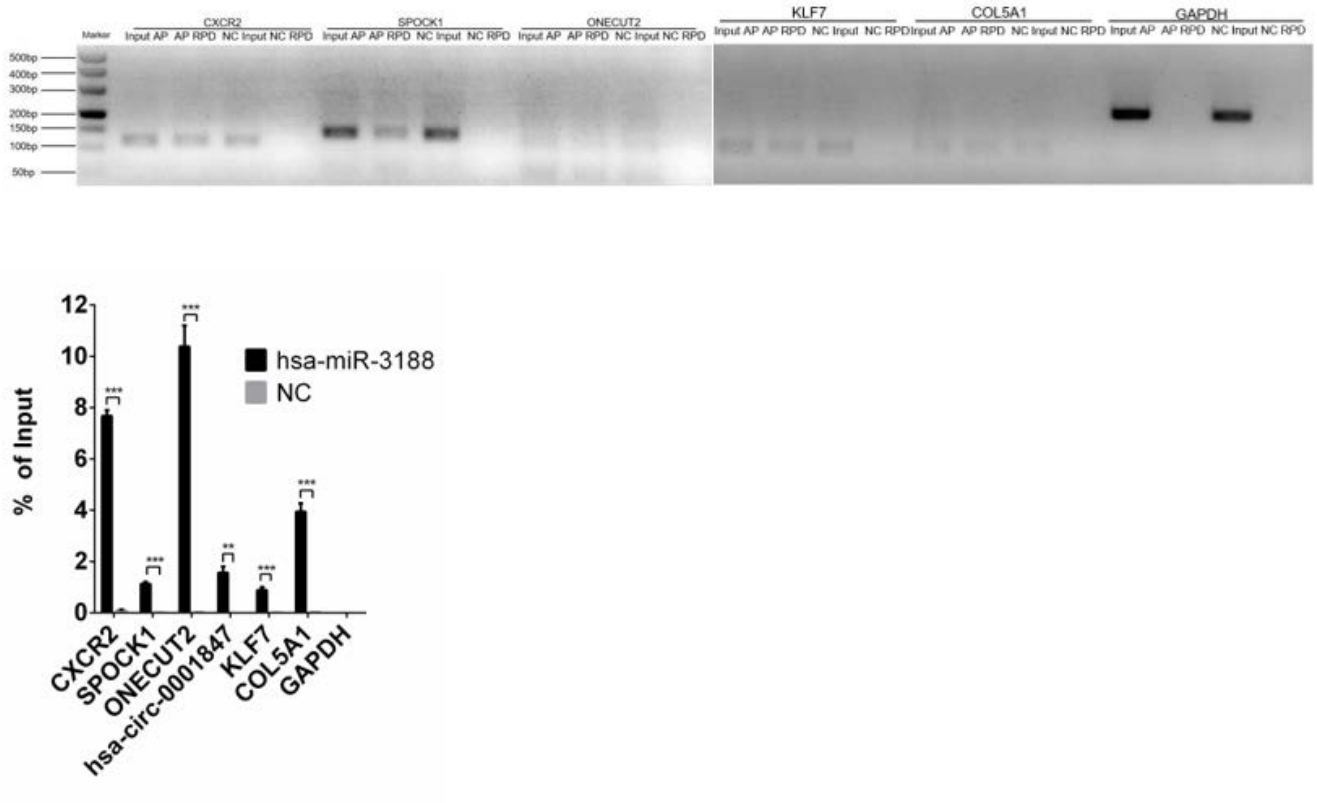

Figure 8. *Onecut2 targets and binds to miR-3188.*

(A) Electrophoresis image showing the input and pulldown of miRNA, as well as the input and pulldown of the NC (negative control).

(B) Analysis chart indicating that the enrichment of onecut2 with miR-3188 is the most significant

A total of 659 genes were involved in biological processes, with 210 being upregulated and 449 being downregulated; 84 genes were involved in cellular components, with 29 being upregulated and 55 being downregulated; and 206 genes were involved in molecular functions, with 26 being upregulated and 180 being downregulated. There were 206 molecular function-related genes, of which 26 were upregulated and 180 were downregulated. Figure 3 (d, e, f, g, h, i).

Investigation of the relationships between circRNA and microRNA:

circRNA is known to have a highly stable ring structure and is often used as a miRNA sponge regulatory gene expression. We interpreted the sequencing files and inputted the 18 differentially expressed circRNAs from the sequencing files into the inquiry website (http://cn.string-db.org). One of them, hsa_circ_0001847 (circ-UBAP2), was found to have the most connections with other circRNA in this specific interaction network, which attracted our attention. In addition, in order to narrow the research scope, we screened 5 circRNAs with high cross occurrence rates of BP, CC and MF in GO functional analysis in sequencing files. They are hsa_circ_0083619 (circ-PPP3CC), hsa_circ_0001722(circ-CDK14), hsa_circ_0007695(circ-SOS2), hsa_circ_0000567(circ-SETD3), hsa_circ_0001847(circ-UBAP2), which verified its differential expression in cancerous and para-cancerous tissues, found that, in contrast, The expression of hsa_circ_0001847(circ-UBAP2) was relatively stable (Figure 4), so the target circRNA of this experimental study was determined to be hsa_circ_0001847. Through sequencing files, we learned that circRNA has a strong correlation with microRNA in cancer. Figure 3 (b), such a significant difference has aroused our attention, and subsequent experiments are carried out based on this as a breakthrough. We selected the top 5 microRNA with the strongest correlation with hsa_circ_0001847 in the sequencing file and constructed the circRNA-microRNA interaction regulatory network diagram. The data show that circRNAs and microRNA have complex regulatory linkages, and circRNAs correspond to several microRNA and microRNA interact with many circRNAs. circRNAs is known to have a highly stable ring structure and is often used as a miRNA sponge regulatory gene expression. We interpreted the sequencing files and input the 18 differentially expressed circRNAs in the sequencing files into the inquiry website (http://cn.string-db.org) (Figure 4). One of them, hsa_circ_0001847 (hsa_circUBAP2_057), was found to have the most connections with other circRNAs in this specific interaction network, which attracted our attention. In addition, in order to narrow the research scope, we screened 5 circRNAs with high cross occurrence rates of BP, CC and MF in GO functional analysis in sequencing files. They are hsa_circ_0083619 (circ-PPP3CC), hsa_circ_0001722(circ-CDK14), hsa_circ_0007695(circ-SOS2), hsa_circ_0000567(circ-SETD3), hsa_circ_0001847(circ-UBAP2), which verified its differential expression in cancerous and para-cancerous tissues. In contrast, the expression of hsa_circ_0001847(circ-UBAP2)

was relatively stable. Therefore, hsa_circ_0001847 was chosen as the gene of interest for the further study (Figure 4).

Elevated Expression of hsa-circRNA-0001847 in OSCC:

To investigate the correlation between the expression of hsa-circRNA-0001847 and the occurrence of OSCC, we employed RT-PCR to assess the expression levels of hsa-circRNA-0001847 in both normal and OSCC cells. Our findings revealed a significant upregulation of hsa-circRNA-0001847 in OSCC tissues and cell lines, with particularly high expression observed in the WSU-HN30 cell line (Figure 5).

Knocking down hsa-circRNA-0001847 restricts the migratory and invasive capabilities of OSCC:

By using lentiviral transfection to knock down hsa-circRNA-0001847, we verified the transfection efficiency with RT-PCR (Figure 6A) and then conducted cellular function assays and WB experiments to explore the impact of hsa-circRNA-0001847 on the proliferative, migratory, invasive, and EMT capabilities of OSCC. The WB experiments showed that compared to the sh-NC group, the expression of E-cadherin increased, while the expression of N-cadherin, Vimentin, and MMP9 decreased, indicating that (Figure 6B-C). The scratch assay revealed that the migration rate of the sh-hsa-circRNA-0001847 group was lower compared to the sh-NC group (Figure 6D-E). In summary, our results demonstrate that the knockdown of hsa-circRNA-0001847 inhibits the migratory and invasive capabilities of OSCC.

hsa-circRNA-0001847 targets mir-3188:

The miRNA sponge is one of the major functions of circRNAs. Utilizing circBank [25] and RNAhybrid, we identified that hsa-miR-3188 is a potential target of hsa-circ-0001847. To explore the relationship between hsa-circRNA-0001847 and miR-3188, we first verified the transfection success after transfecting miR-3188 (Figure 7A). Subsequently, we examined the expression of hsa-circRNA-0001847 following the transfection of miR-3188 and found that the expression of hsa-circRNA-0001847 was upregulated upon the addition of miR-3188 mimics (Figure 7B), indicating a positive correlation between the two. In addition to this, we conducted miRNA-pulldown assays, and the results also confirmed that hsa-circRNA-0001847 can function by adsorbing miR-3188 (Figure 7C).

hsa-circRNA-0001847 regulates the expression of onecut2 by competitively binding to mir-3188:

CircRNAs are known to function as miRNA sponges, and we utilized circBank and RNAhybrid to identify potential targets of hsa-circ-0001847, discovering that hsa-miR-3188 could be one such target. To further investigate the relationship between hsa-circRNA-0001847 and miR-3188, we performed a miRNA pulldown assay, which confirmed that hsa-circRNA-0001847 can interact with miR-3188 by adsorption (Figure 8). Moreover, we conducted experiments with spock1, cxcr2, klf7, and col5a1, predicted target genes of miR-3188, and found that miR-3188 does indeed have a targeting relationship with these genes.

Discussion.

Noncoding RNAs have a crucial regulatory role in many biological processes. In this study, based on whole-transcriptome sequencing, the differentially expressed mRNAs, lncRNAs, and circRNA in cancer and paracancerous tissues of five OSCC patients were simultaneously analyzed. Two-by-two association analysis was used to investigate the related transcriptional regulatory mechanisms. Hsa-circRNA-0001847 is significantly overexpressed in OSCC, suggesting its potential research value in the initiation and progression of OSCC, and it may emerge as a potential diagnostic and therapeutic target for OSCC. Knockdown of hsa-circRNA-0001847 inhibits the proliferation, migration, invasion, and EMT of WSU-HN30 and CAL27 cells. Furthermore, we utilized PCR and miRNA pulldown assays to confirm the targeting relationship between hsa-circRNA-0001847 and miR-3188, as well as the targeting relationship between miR-3188 and onecut2. This study reveals that hsa-circRNA-0001847 influences the invasion and metastasis of oral squamous cell carcinoma by regulating the miR-3188/onecut2 axis.

The intricate regulatory interactions between mRNA, lncRNA, and circRNA are strongly linked to the onset and progression of OSCC. After creating the OSCC miRNA-circRNA regulatory network and examining its topology, hsa_circ_0001847 was identified as a crucial node in this study. The functional enrichment and survival partitioning of these critical nodes and their associated RNAs were further analysed. According to the findings of this study, hsa_circ_0001847 may influence tumour growth by binding to miRNAs and influencing downstream genes, according to the findings of this study. By the synergistic activation of SHP2/PLK1 signalling, the hsa_circ_0001971/miR-186 and hsa_circ_0001847/miR-296 signalling pathways boost oral squamous cell proliferation. [10] In addition, hsa-miR-571, hsa-miR-3188, hsa-miR-548s, hsa-miR-4511, hsa-miR-22-3p, hsa-miR-4649-3p, hsa-miR-4425, hsa-miR-3611, hsa-miR-3173-5p hsa-miR-3928-3p, hsa-miR-370-3p, hsa-miR-3182, hsa-miR-4445-5p, hsa-miR-4715-5p, hsa-miR-6799-3p, hsa-miR-4708-3p, hsa-miR-516b-5p, hsa- miR-6769a-3p, hsa-miR-6777-3p, hsa-miR-6815-3p, hsa-miR-8081, hsa-miR-3926, hsa-miR-455-3p, and hsa-miR-943 for a total of 21 microRNAs, were also mentioned in hsa_circ_0001847-related interaction network, where hsa-miR-3188 was associated with tumour cell proliferation, apoptosis, migration and the MAPK pathway [11].

Because of their stable structure, high expression abundance, and tissue-specific and highly conserved features, circRNAs, a subclass of circular RNAs, are becoming the latest hotspot for research in the field of noncoding RNAs [12,13]. Among the differentially expressed circRNAs screened in this investigation, hsa_circ_0083619[14], hsa_circ_0001722 [15], and hsa_circ_0001847 [16] have been demonstrated to be closely associated with the development of oral squamous cell carcinoma. In our screening process, due to the higher stability and differential expression of hsa_circ_0001847, hsa_circ_0001847 was chosen for in-depth analysis in this work, and the results indicated that the level of hsa_circ_0001847 was higher in cancer tissues than in paracancerous tissues ($P<0.05$), which was consistent with the literature [16]. Wang et al. [16] discovered that the expression of hsa_circ_0001847 was higher in glioma tissues than in normal tissues ($P<0.05$) and that the decreased level of hsa_circ_0001847 inhibited cell proliferation,

migration, and invasion, increased apoptosis, and slowed tumour growth in vivo. Hence, hsa_circ_0001847 possesses the potential to serve as a pharmacological target. In addition, some circRNAs, such as circRNA UBAP2 [17], and others, such as circRNA MAPK1 [18], have been demonstrated to exert oncogenic effects in OSCC. Many previously unknown differentially expressed circRNAs were also found in our study, and their regulation mechanisms for OSCC require additional validation.

The results of the whole transcriptome multiplex association analysis in this study and the current investigation suggested that hsa_circ_0001847 may be regulated by associated miRNAs, altering the expression of downstream target genes and playing a role in the process of cancer proliferation and invasion. In this study, the relative expression of hsa_circ_0001847 in cancer and paracancerous tissues of three OSCC patients was also evaluated. The results indicated that the level of hsa_circ_0001847 in cancer tissues of these three patients was substantially higher than that in paracancerous tissues ($P<0.05$).

Using second-generation sequencing technology, the whole transcriptome of tumour and paracancerous tissues from five patients with OSCC was sequenced in this work. An in-depth investigation led to the selection of hsa_circ_0001847, a critical gene that may be strongly associated with OSCC proliferation, invasion, and migration. Additional topological analysis of the miRNA-circRNA multiple association network demonstrated that hsa_circ_0001847 may be regulated by related miRNAs, hence affecting the expression of downstream target genes and the incidence of OSCC.

Conclusion.

Irisin is a multifunctional myokine secreted by adipocytes, cardiac myocytes, and skeletal muscle, possibly mediating a wide range of metabolic processes including insulin resistance, muscle endurance, endothelial function, inflammatory and immune reactions and bone osteoblast activity [9].

Declarations.

Acknowledgements.

Not applicable.

Funding.

1. Anhui University scientific research project, KJ2021ZD0103.
2. Key Research Foundation of Wannan Medical College, WK2021Z01.
3. Wuhu City science and technology plan key research and development project 2022yf62.
4. Program for Excellent Sci-tech Innovation Teams of Universities in Anhui Province, 2023AH010073.

Availability of data and materials.

All data generated or analyzed during this study are included in this published article.

Authors' contributions.

Conceived and designed the experiments: SLX, PXY, RXX, LR, WWJ, DC; performed the experiments: RXX, PXY; wrote the paper: SLX, WWJ, LR, RXX, PXY, DC. SLX and WWJ contributed equally. All authors read and approved the final manuscript.

Competing interests.

The authors declare that they have no competing interests, and all authors should confirm its accuracy.

Ethics approval.

Written informed consents were obtained from all participants, and this study was permitted by the Medical Ethics Committee of Wannan Medical College.

Patient consent for publication.

Not applicable.

REFERENCES

1. Chi AC, Day TA, Neville BW. Oral cavity and oropharyngeal squamous cell carcinoma--an update. CA Cancer J Clin. 2015;65:401-421.
2. Torre LA, Bray F, Siegel RL, et al. Global cancer statistics, 2012. CA Cancer J Clin. 2015;65:87-108.
3. GGonzález-Ramírez I, Soto-Reyes E, Sánchez-Pérez Y, et al. Histones and long non-coding RNAs: the new insights of epigenetic deregulation involved in oral cancer. Oral Oncol. 2014;50:691-695.
4. Takahashi H, Yanamoto S, Yamada S, et al. Effects of postoperative chemotherapy and radiotherapy on patients with squamous cell carcinoma of the oral cavity and multiple regional lymph node metastases. Int J Oral Maxillofac Surg. 2014;43:680-685.
5. Kim JW, Park Y, Roh JL, et al. Prognostic value of glucosylceramide synthase and P-glycoprotein expression in oral cavity cancer. Int J Clin Oncol. 2016;21:883-889.
6. Ali J, Sabiha B, Jan HU, et al. Genetic etiology of oral cancer. Oral Oncol. 2017;70:23-28.
7. Chai AWY, Lim KP, Cheong SC. Translational genomics and recent advances in oral squamous cell carcinoma. Semin Cancer Biol. 2020;61:71-83.
8. Chen JB, Li LC, Lee WC, et al. Effect of clinical factors on trajectory of functional performance in patients undergoing hemodialysis. Ren Fail. 2021;43:90-96.
9. Obeng E. Apoptosis (programmed cell death) and its signals - A review. Braz J Biol. 2021;81:1133-1143.
10. Jun W, Shaobo O, Xianhua Z, et al. Deregulation of hsa_circ_0001971/miR-186 and hsa_circ_0001874/miR-296 signaling pathways promotes the proliferation of oral squamous carcinoma cells by synergistically activating SHP2/PLK1 signals. Sci Rep. 2021;11:20561.
11. Chen X, Chen J. miR-3188 Regulates Cell Proliferation, Apoptosis, and Migration in Breast Cancer by Targeting TUSC5 and Regulating the p38 MAPK Signaling Pathway. Oncol Res. 2018;26:363-372.
12. He AT, Liu J, Li F, et al. Targeting circular RNAs as a therapeutic approach: current strategies and challenges. Signal Transduct Target Ther. 2021;6:185.
13. Limb C, Liu DSK, Veno MT, et al. The Role of Circular RNAs in Pancreatic Ductal Adenocarcinoma and Biliary-Tract Cancers. Cancers (Basel). 2020;12:3250.
14. Zhang H, Zhang B, Chen Y, et al. Downregulated hsa_circ_0036988 promotes proliferation and metastasis in oral squamous cell carcinoma. Cancer Biomark. 2021;31:375-383.

15. Gong S, Zhang Y, Pang L, et al. A novel CircRNA Circ_0001722 regulates proliferation and invasion of osteosarcoma cells through targeting miR-204-5p/RUNX2 axis. J Cancer Res Clin Oncol. 2023.
16. Wang J, Li T, Wang B. hsa_circ_0001847 functions as sponges of miR-1205 and miR-382 to promote glioma progression by modulating STC1 expression. Cancer Med. 2021;10:1815-1828.
17. Jun W, Shaobo O, Xianhua Z, et al. Deregulation of hsa_circ_0001971/miR-186 and hsa_circ_0001874/miR-296 signaling pathways promotes the proliferation of oral squamous carcinoma cells by synergistically activating SHP2/PLK1 signals. Sci Rep. 2021;11:20561.
18. Dai Y, Zhu Y, Xu H. circ_0004872 inhibits proliferation, invasion, and glycolysis of oral squamous cell carcinoma by sponged miR-424-5p. J Clin Lab Anal. 2022;36:e24486.