



## LncRNA HOXC-AS5 Affects the Proliferation, Invasion and Cell Cycle of Ameloblastoma Cells by Acting on the Target Gene HOXC13

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### ABSTRACT

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Ameloblastoma is an odontogenic tumor that occurs in the oral cavity. This tumor is a benign tumor. Ameloblastoma is very aggressive and easily causes tumor metastasis and postoperative recurrence. Studies have shown that the coding gene RNA is involved in the occurrence and development of a variety of tumors. LncRNA HOXC-AS5 as a member of the RNA family, is regarded as a marker of ameloblastoma, which can interfere with tumor cell changes by regulating and controlling the target gene HOXC13. The purpose of this article is to explore the effect of LncRNA HOXC-AS5 on the proliferation, invasion and cell cycle of ameloblastoma cells by acting on the target gene HOXC13, taking 45 patients with ameloblastoma treated in our hospital as the research object. In patients with ameloblastoma tissue, an overexpression vector was constructed by transfecting LncRNA HOXC-AS5 adenovirus and the expression of LncRNA HOXC-AS5 was reduced by the knock-down method, and the effect of LncRNA HOXC-AS5 overexpression and knock-down on the expression level of the target gene HOXC13 was detected. Using flow cytometry to detect the proliferation, invasion and cell cycle distribution of ameloblastoma cells. The research results show that overexpression of LncRNA HOXC-AS5 can reduce the expression level of the target gene HOXC13 by 18.5%. Meanwhile, the proliferation rate of ameloblastoma cells is reduced by 23.2%, and the cell invasion ability index is (145.8±10.5). Cells in G0/G1 phase account for the ratio 68.3%, which was higher than the knock-down group, and the proportion of S-phase cells was 14.6%, which was lower than the knock-down group. Therefore, it can be seen that LncRNA HOXC-AS5 has an inhibitory effect on the target gene HOXC13. When the expression level of the target gene HOXC13 is reduced, it can reduce the proliferation of ameloblastoma cells, reduce cell invasion ability, and extend the cell division cycle.

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### Introduction

Ameloblastoma (AB) is an odontogenic epithelial tumor that mainly occurs in the mouth and face. There is no difference in age and gender. AB often presents as benign tumors with strong local invasiveness and can be surgically removed. However, ameloblastoma is prone to recurrence after surgery, the recurrence rate is high, and it is easy to induce malignant transformation of the tumor, causing the ameloblastoma to metastasize to distant lymph nodes, lungs, kidneys and other organs, even to bones.

Recent studies have shown that LncRNA HOXC-AS5 is involved in epigenetic regulation, transcriptional regulation and post-transcriptional regulation (1). It is closely related to the regulation of stem cell maintenance and differentiation, embryonic development, cell cycle regulation, cell proliferation

and apoptosis, cell metabolism, and immune regulation. Hoxc13 is a member of the HOXC gene family and is located on the long arm of the chromosome (2). As a transcription factor, hoxc13 can regulate the expression of many key genes, thereby affecting the occurrence and development of cancer (3).

In order to explore the effect of LncRNA HOXC-AS5 on the proliferation, invasion and cell cycle of ameloblastoma cells by acting on the target gene HOXC13, a large number of related materials were consulted. Among them, Beppu analyzed the causes of ameloblastoma in the article and pointed out that ameloblastoma is a kind of odontogenic epithelial tumor. Although ameloblastoma is a benign tumor, it can cause facial swelling and deformation, which seriously affects the patient (4). In his article, Chen

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proposed several treatment methods for ameloblastoma, analyzed the current research status of ameloblastoma in China, and emphasized that the main methods of treatment for ameloblastoma at this stage are surgical treatment and drug-assisted treatment, and look for success (5). Jaafari found through related research that the LncRNA HOXC-AS5 gene is involved in the formation and development of ameloblastoma and introduced the mechanism of LncRNA HOXC-AS5 gene intervention in tumor cell changes, and pointed out that the research on LncRNA HOXC-AS5 will be a therapeutic success (6). Timothée found through research that HOX family member HOXC13 has a high expression level in ameloblastoma, odontogenic tumors and other tumors, the reduction of HOXC13 expression level can significantly inhibit the division of ameloblastoma cells and prolong the cell cycle (7). Lee found through clinical experiments that LncRNA HOXC-AS5 has an inhibitory effect on the target gene HOXC13. Knock-down of the target gene HOXC13 expression level can reduce the proliferation of ameloblastoma cells and reduce cell invasion. Therefore, HOXC13 may become a new treatment for ameloblastoma (8).

In the study of the effects of LncRNA HOXC-AS5 on the proliferation, invasion and cell cycle of ameloblastoma cells by acting on the target gene HOXC13, this article summarizes and analyzes the research experience and results of a large number of predecessors. In addition, this article focuses on the research content and detection methods. Some innovations have been made. The specific innovations are as follows: First, the overexpression vector is constructed by transfecting LncRNA HOXC-AS5 adenovirus, and the expression of LncRNA HOXC-AS5 is reduced by the knock-down method, and the LncRNA HOXC-AS5 expression is analyzed from a comprehensive and objective perspective. Second, observe the effect of LncRNAHOXC-AS5 on the proliferation, invasion and cell cycle of ameloblastoma cells by acting on the target gene HOXC13 in order to lay the foundation for the later exploration of the possible mechanism of LncRNA HOXC-AS5 affecting the biological behavior of ameloblastoma. Third, this paper uses the multi-component statistical data analysis method collected by data analysis software and uses PSPY software to

collect and analyze the statistical data of the detection results of ameloblastoma cells, which greatly improves the accuracy of the research results.

## **Pathology of Ameloblastoma and the Role of HOX Family**

### **Pathological Mechanism of Ameloblastoma**

Proto-oncogenes are genes related to cell proliferation in cells. When the cell structure or function regulatory unit region of certain proto-oncogenes changes and the number of proto-oncogene binding products increases or the active factors increase, the cells will over-differentiate and proliferate. Lead to malignant tumors (9). Other members of the Jun family must combine with other members of the Fos family to form homodimers or heterodimers in order to form an AP-1 transcription-inducing factor, which is usually used to induce direct epithelial cortical properties. To indirect filling material epithelial characteristics (met) direction. Met cells are important cell morphology and their changing physiological characteristics that control the invasive tissue growth and cell metastasis of tumor cells. Through the analysis of the abnormal gene chip spectrum, the abnormal expression gene spectrum of ameloblast hemangioma showed high abnormal expression of uses series genes, reaching 6-12 times, but did not clearly describe the abnormal expression of a C-juncos gene (10). C-foes and c-Jun probe in situ hybridization experiments confirmed that the in situ positive rates of C-foeran in meroblastic tumors was 93.5%, and the in situ positive rates of c-junmRNA in meroblastic tumors were only about 36.7% (11). Therefore, it is speculated that the C-foes hormone may play a major inhibitory role in meroblastic tumors. At present, researchers tend to generally believe that the long-term regulation of c-foes of n and C-Jun of n is usually before biological, clinical symptoms have occurred, which indicates that the long-term research results of these two regulatory genes have a certain biological effect. The development potential and research value of clinical medicine application (12). The rise gene sequencing family currently has 3 main members. Some clinical scholars have found that 1p21ras is highly expressed in ameloblasts and tumor types, especially in large recurrent cell clusters. It plays a minor role in the tumor genesis of the dentin epithelium (13). These

results indicate that the change of the Raps gene plays an important role in the molecular pathology of ameloblastoma (14).

The RAF gene is an important proto-oncogene for all mammals and humans. By sequencing the paraffin-embedded specimens, BRAF mutations were found in 56% of fibroblastomas (15). Through specific PCR, 31 mutations were found in 50 fibroblast tumors. The results showed that the mutation was more likely to occur in young people, while the wild-type gene mainly occurred in the maxilla. Sanger sequencing showed that the mutation rate of meroblastic tumors is generally about 63%. In addition, the tumor mutations may also exist in meroblastic tumors and other meroblastic tumors. Studies have shown that Brave mutations are not ameloblasts, and intracellular tumor mutations are characteristic and may be related to the early occurrence and tumor development of certain dental enamel tumors (16). Smog signal genome is an important human tumor cell-related signal gene and an important signal and transducer in the process of tumor shed signal synthesis pathway (17). The shah molecular pathway is mainly involved in the entire development process, from maternal embryo reproduction to maternal organ reproduction. The smog-type genome is also the first time that it is clearly expressed in the development of dental embryos, especially at the developmental stage of the gingival bell and tooth cap shape, which has led people to speculate that SMO genes are related to dental tissue abnormalities and dental tumors (18). Since SMO gene mutations have been reported in basal cell carcinoma and meningioma, it has been suggested whether these sites are also present in ameloblastoma. It seems that the SMO gene is related to the occurrence of ameloblastoma (such as ameloblastoma) (19). However, more research is needed to determine the specific mutation site and its importance.

Tumor suppressor genes refer to genes that inhibit cell cancelation due to their existence and expression. Tumor suppressor genes play a negative regulatory role in cell growth. Tumor suppressor genes related to ameloblastoma include Rib, Rack, APC, paten, madr2 and so on. RECK gene is a new type of tumor suppressor gene discovered in recent years. It can negatively regulate matrix metalloproteinase (MMP) and inhibit the role of MMP in the degradation of

extracellular matrix and tumor angiogenesis. The expression of follicular type RECK is lower than that of plexiform type, and the expression of RECK of acanthoma type is significantly lower than other types. The expression of RECK and MMP-2 in ameloblastoma, odontogenic corneal cystic tumor and ameloblastoma were studied by RT-PCR. It was found that the low expression of RECK and the high expression of MMP-2 play a regulatory role in the invasion, recurrence and malignant behavior of ameloblastoma (20). The want signaling pathway involves three regulatory genes: APC,  $\beta$ -catenin and Axim. APC is highly expressed in tooth germs, indicating that the signal synthesis pathway in want is likely to play a serious role in inhibiting the tissue formation and cell differentiation of odontogenic bone marrow tumors. Through an in-depth study of  $\beta$ -methyl catenin and axin2-based gene expression that may play an important leading role in the want-based genetic pathway in meroblastic tumors, it can also be confirmed that they are in the prognosis and early stage of meroblastic tumors (21).

### **The Role of Hox family in Tumors**

Home box genes exist in almost all eukaryotes. Human home box genes include two types: hoi genes and Hoxie genes. Hex II genes (also called non-Hex genes) are widely distributed in the genome, and the similarity between members is very low, including the end of joint tail homology box sequence gene, the sarcomere tail homology box sequence gene and the Iroquois family tail. As a cofactor to control hex protein, it is mainly involved in the regulation of protein transcriptase (22). The front and back expression patterns of hex and the other two homologous box genes also determine the front and back (Ape) expression patterns of the same individual (23). Drosophila has a gene mutation enzyme that inhibits apex malformation. Genetic analysis of individuals shows that several tract proteins and several trig proteins involved in the oxidative expression modification of methyl groups by histones have combined to regulate the methyl expression of ahoy, and in the expression pattern of the histone demethylase. Hex is inherited in the progeny cells. PCG gene mutation can cause hex ectopic expression, while tragi gene mutation can cause hex expression down-regulation. PRC1 and PRC2 contain a series of

histone modification enzymes (24). The Zester group in PRC2 catalyzes the trimethylation of histone H3K27, which is the key to suppressing hex gene expression. Then, the CBX protein recruits PRC1 to the H3K27me<sub>3</sub> modification site to compact the chromosomes and stabilize hex gene silencing. The ATP-dependent chromosomal remodeling complex SWI/SNF can bind to the histone H3K27 acetylate CBP in the mll2-utx complex, thereby effectively coordinating the methylation and acetylation of histones and jointly resisting the gene silencing effect of PRC.

The transcriptional regulation of the Hex gene depends on its nuclear arrangement (25). Hex genes in the same cluster share different nuclear space, chromosomal structure and transcriptional regulatory elements (such as enhancers and promoters), so they belong to the two groups of transcription activation and repression. Therefore, the activation of the Hex gene depends on its position in the gene cluster. However, collinearity makes the expression of hex in different parts of the human body depend on the sequence of each hex gene in the P9 subgroup, which allows Hex genes to achieve temporal and spatial specific expression. In fact, the location information of adult cells can be determined and maintained by the Hex genes expressed at specific sites, and the differentiation direction of cells depends not only on the sequence of Hex genes expressed in the subfamily. By removing h3k27m<sub>3</sub> in the specific Hex promoter, the Hex gene was transferred from H3K27me<sub>3</sub> to h3k4me, and expression was achieved at a specific time and space. The uninterrupted integrity of the gene cluster ensures the sequential activation of the hold gene, indicating that this non-coding region is also involved in the collinear regulatory expression of hold. Histone methylation regulates hex gene expression. MOs belong to the histone acetyltransferase (HAT) family of mist. MOs recognize the histone modification of the chromosome through a tandem plant homology domain (PHD) containing a zinc finger structure, which is located at the Hex site in vivo and at the x position in vitro point catalysis plays a key role in the acetylation of histone H3K14.

The regulation of Hex gene expression by non-coding RNA (normal) is based on the aforementioned histone modification mechanism. The normal genes

between Hex genes can be co-transcribed with Hex (26). Hex gene expression can be regulated in chis or trans to stabilize the expression pattern of adult hex genes. Some crack and microRNAs extend from the transcription of hex genes and affect the attachment of RNA polymerase II, chromosome activation and gene expression (27). In terms of trans-action, certain fold lines can delineate chromosomal silent regions for remote hex genes and regulate hex sites on different chromosomes. Hex is closely related to tumors, but it is uncertain whether hex is a key therapeutic target. From the following aspects, hex is more like the result of the tumor than the cause. Hex is not and is not the direct driving force of cell cancelation because it directly interferes with the balance between active differentiation and cell inactive differentiation and is not enough to cause more cancers. Different from other typical malignant oncogenes (or called tumor immunosuppressive cell genes), the abnormal phenotype of tumor suppressor cells is similar to the expression of multiple abnormal cells based on hex-type oncogenes in a high-dose dependence (28). Hex gene expression is usually tissue-specific, while typical non-anti-cancer genes (or cancer suppressor genes) are expressed in many types of tumor cell tissues. If hex transcription is considered to be the main cause of tumor genesis, the related research on cofactor immunomodulators and hex transcription activity will be more helpful to the research and development of anti-cancer therapeutics related to hex transcription.

## Materials and methods

### Selection of Patients and Collection of Experimental Specimens

In this experiment, 45 patients with ameloblastoma who were treated in our hospital in 2019 were selected as specimen collection objects. Among them, 28 were males with an average age of 53.6 years, and 17 were females with an average age of 56.3 years and an average length of illness. It was (3.8±1.2) years, including 33 cases of maxillary disease and 12 cases of the mandibular disease. With the consent of the patient, the lesion tissue of ameloblastoma was collected by minimally invasive surgery.

### Isolation and Culture of Ameloblastoma Cells

This experiment uses fresh ameloblastoma tissue specimens. Take the ameloblastoma tissue under aseptic conditions, and wash the AM tissue 4 times with PBS solution containing 100u/ml penicillin and 300u/ml streptomycin, each time for 5 minutes. Cut the ameloblastoma tissue into tissue pieces of 1-2mm<sup>3</sup> size, place them in the culture medium containing 1mg blood 1 collagenase, digest in a constant temperature water bath shaker at 35°C, stop digestion when the tissue pieces are flocculent, average digestion time about 10h. Cancel the mixture, centrifuge at 2000 rpm for 10 minutes to remove the supernatant, add pap's solution to wash the cells and suspend the cell pellet 3 times. After centrifugation, remove the supernatant, add k-do-sum cell culture solution, and use an electric pipette strong pipetting to disperse tyrant cells. Tyrant and pan-blue can count the staining density of the cells so that the staining density of tyrant cells is determined to be 1×10<sup>6</sup>/ml, inoculated in a 29cm<sup>2</sup> cell culture flask and placed at 35°C, 10% cultivation is carried out in an incubator with CO<sub>2</sub> saturated humidity, and the cell culture medium can be replaced once every 3 days. The used human culture fluid vase and the used culture fluid template are pre-coated with I-type human collagen.

### LncRNA HOXC-AS5 Transfection Method

Lncrnahoxc-as5 was transfected into ameloblastoma cells. One day before transfection, the ameloblastoma cells were inoculated into 300μl of the antibiotic-free medium. During transfection, the fusion degree of ameloblastoma cells should be at least 80%-85%, which can promote successful transfection. For each sample to be transfected, the operation of Lncrnahoxc-as5 and liposomes is as follows: dissolve 6u1lncrnahoxc-as5 in 80μl serum-free RPMI-1640 medium, mix it gently, and dissolve 8μl liposomes in 50 all microliter of serum-free RPMI-1640 medium, mix gently and incubate at room temperature for 5 minutes. After the eggs are incubated, the two solutions are gently mixed together. After adding the mixed solution, gently shake the 6-well plate back and forth to mix evenly. Real-time quantitative results 48 hours after transfection, PCR, cell cycle, cell invasion and other experiments were performed.

### Extraction of RNA from Ameloblastoma Cells

The ameloblastoma tissue of the clinical sample was taken out of liquid nitrogen and ground into a powder with a Raze-free ceramic mortar. Add the ameloblastoma tissue powder to a 5ml RNA-free centrifuge tube that has been added with 500u1 lysate, and mix evenly. If RNA is extracted from the cells, transfer the retinoblastoma cells in the culture dish directly to a 20ml sterile centrifuge tube. Transfer the supernatant to a new 5miRNase-free centrifuge tube and label it. Add iodochloroform (a ratio of 200u1 chloroform/fluorotriazole) to each centrifuge tube, shake slowly and mix with both hands, and then place the centrifuge tube at room temperature for 30 minutes. Place the sample centrifuge tube again in a centrifuge at 10°C and centrifuge at 6000 rpm for 20 minutes. Carefully take out the centrifuge tube, observe the layering of the solution in the centrifuge tube, transfer the water phase gas in the upper layer to a 3mi centrifuge tube without quality range, and mark the water phase. Add 150u1 isopropanol to the test tube each month, stir gently to mix, and then continue to stand at 40 degrees room temperature for 20-30 minutes. Put the experimental sample into a centrifuge tube and directly place it in a centrifuge with a high temperature of 10°C and centrifuge at 6000 rpm for 10 minutes. Observe the precipitation of ions running in the centrifuge tube. Discard the liquid to remove all the supernatant, add 30ml of 75% polychlorohydrin to the centrifuge tube again, shake the centrifuge tube gently, and then suspend the pellet. Place the sample centrifuge tube in a centrifuge at 10°C and 12000 rpm for 15 minutes. Remove the supernatant as much as possible, dry the supernatant at room temperature for 10-20 minutes, and add 30u1 Raze-free water to dissolve each test tube. The primer sequence during amplification is shown in Table 1.

**Table 1.** Primer sequence during amplification

Gene	Upstream primer	Downstream primer	Fragment length
LncRNAHOXC-AS5	TTTGTGGTGGT GAAGACGTG	TCCTCAAGAAT AAGGAACATA GG	120
MEOX2	TCAGTGCACATA CAGAACTTTGT	GCTGTCAACGA TACGCTACGT	112
U6	TCTGGGACCAC CTTCTTTTG	CCACCACCCTC TGTCACCTT	78
GAPDH	ACCCACTCCTC CACCTTTGAC	TGTTGCTGTAG CCAAATTCGTT	94

### Target Gene HOXC13 Expression Level Detection

The supernatant of the cell culture was collected under aseptic conditions, centrifuged at 2000 rpm to remove cell debris, and placed at  $-30^{\circ}\text{C}$  for use. Mix 10% SDS-PAGE containing 0.5% gelatin g/ml, and mix an appropriate amount of culture supernatant with sample buffer. The mixture was sampled on 20% SDS-PAGE. After electrophoresis, put the gel in an incubation solution containing 3mmol/L (sigma) and incubate overnight at  $35^{\circ}\text{C}$  to activate the zymogen HOXC13. Place it under 1.5% Gaussian 280 dye gel for 1 hour and decolorize for 2 hours at room temperature. Because hoxc13 can decompose gelatin, white bands appear in hoxc13. The expression level of hoxc13 can be calculated by the optical density analysis of UBT software.

### Detection of Cell Proliferation, Invasion and Cell Cycle of Amelocytoma

The CCK-8 kit was used to detect the changes in the proliferation of ameloblastoma cells treated with different treatments. The detailed operation steps are as follows: 6h, 12h, and 24h after LncRNAHOXC-AS5 transfection of ameloblastoma cells, the three components at different time points harvest of ameloblastoma cells. At the time point of the measurement, add 20ul of CCK-8 solution to the culture well to be measured in each well. Then the cells were cultured in a  $\text{CO}_2$  incubator for 2 hours, and finally the absorbance at 350nm was measured on the microplate reader, and the proliferation curve of the ameloblastoma cells in each different treatment group was drawn using the graph6 software, and SPSS15.0 software was used to analyze each Statistical differences in data between different treatment groups.

Use a flow cytometer to detect changes in the cell cycle of ameloblastoma. Take logarithmic growth phase ameloblastoma cells, inoculate  $1 \times 10^6$  cells/well into an 8-well plate, culture for 12 hours, trypsinize to collect the cells, fix them overnight at  $12^{\circ}\text{C}$  with absolute ethanol, add 20ug/ml PI and stain for 30 minutes, then flow up cytometer detection, using CXP flow analysis software to analyze the cell cycle.

A flow cytometer was used to detect the invasion ability of ameloblastoma cells in different treatments. The specific operation steps are as follows: prepare the ameloblastoma cells of different treatment groups

as single cell suspension (cell concentration is  $6 \times 10^5$  cells/tube), add 600ul ameloblastoma cells of different treatment groups into a 12-well plate, six the wells are placed and incubated for 24 hours. Take out the packed madrigal sub-packages from the refrigerator at  $-30^{\circ}\text{C}$  and place them in an ice bath at  $15^{\circ}\text{C}$  overnight. After taking out the Tran chamber, put it in an 18-well plate and put it on ice. Use a pre-cooled pipette tip to mix madrigal and RPMI-1640 in a ratio of 1:5, then place them on ice and take them out. The 60UL mixture was added to the bottom of the trans-chamber and incubated in  $\text{CO}_2$  at  $37^{\circ}\text{C}$  in an incubator for 0.5H. After removing the compartment, gently wipe the cells in the upper compartment with a wet cotton swab, and fix the cells with 85% ethanol for 30 minutes. After taking out the processing chamber, wipe off the fixing solution. After the cell membrane is dry, add crystal violet dye to the wells for staining, and finally, rinse with running water. Randomly count 4 high-powered fields under the microscope, and analyze the changes in cell invasiveness of different treatment methods.

### Results and discussion

#### Analysis of the Effect of LncRNA HOXC-AS5 on the Expression of Target Gene HOXC13 and the Proliferation of Ameloblastoma Cells

The study found that in the tumor area, the characteristics of ameloblastoma can still be seen. That is, the tumor cell nest has peripheral cells arranged in a columnar and fence-like nucleus away from the basement membrane. At the same time, a star-like central cell is seen, which is malignant. The main characteristics of malignant morphology of amelocytoma are abundant tumor cells, less interstitial components, increased levels of peripheral cells ( $>4$  layers) or disordered polarity, and cell atypical, and most of the stellate-reticulate cells in the central area disappear. Replaced by spindle-shaped or oval cells, tumor cell nest mitoses increased ( $>1/\text{HPF}$ ), or occasionally pathological mitosis, tumor lymphatic metastasis. Among the morphological and chemical parameters of meroblastic tumors in the nucleus, area, perimeter, isometric ellipse, isometric diameter, short diameter, average isometric diameter, roundness, and isometric axis ratio in various benign and malignant ameloblasts, sexual tumors usually have significant differences ( $P < 0.05$ ). In some ameloblastoma cells,

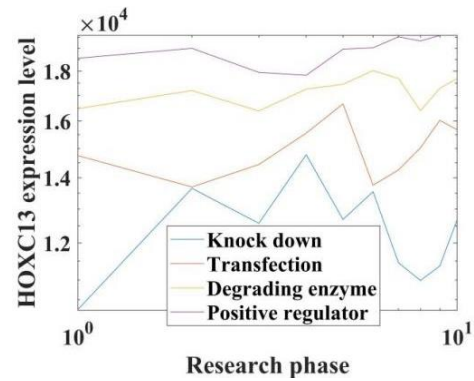
the cells have undergone a series of morphological changes, such as cytoplasmic vacillation, nuclear shrinkage, and nuclear fragmentation. The measurement results of ameloblastoma nucleus wandering parameters are shown in the label.

**Table 2.** Measurement results of nuclear-wandering parameters of ameloblastomas

Morphological parameters	Malignant ameloblastoma	Benign ameloblastomal	P value
Area	26.27±9.04	15.48±2.70	0.0245
Circumference	19.52±3.43	16.47±2.29	0.0187
Equal circle diameter	5.63±0.99	6.54±0.96	0.0332
Long Trail	7.15±1.26	2.99±0.53	0.0106
Short diameter	4.52±0.83	5.01±0.71	0.0274
Average optical density	5.97±1.04	3.65±0.86	0.0485

The research results show that when the expression of LncRNAHOXC-AS5 increases, the expression level of the target gene HOXC13 will decrease, and when the expression of LncRNAHOXC-AS5 is knocked down, the expression level of the target gene HOXC13 will increase. When LncRNAHOXC-AS5 is overexpressed, the target gene HOXC13 expression level is reduced by 18.5%. When LncRNAHOXC-AS5 expression is knocked down to the silent value, the target gene HOXC13 expression level increases by 32.6%. When LncRNAHOXC-AS5 is overexpressed and silenced, the target gene HOXC13 expression level is increased. There are statistical differences in levels ( $P < 0.05$ ). With the prolonging of the action time of LncRNAHOXC-AS5, the target gene HOXC13 expression level changes more widely and the target gene HOXC13 expression level is higher than 15h after LncRNAHOXC-AS5 overexpression intervention for 6h, which proves that LncRNAHOXC-AS5 has an inhibitory effect on the target gene HOXC13. The results of the study show that when LncRNAHOXC-AS5 is overexpressed, the expression level of the target gene HOXC13 will decrease. The relevant data are shown in Figure 1.

It can be seen from the data in Figure 1 that when LncRNAHOXC-AS5 is overexpressed, the expression level of the target gene HOXC13 for ameloblastoma will be reduced by 18.5%, and when LncRNAHOXC-AS5 is silently expressed, the expression level of the target gene HOXC13 for ameloblastoma will increase. That's 32.6%.

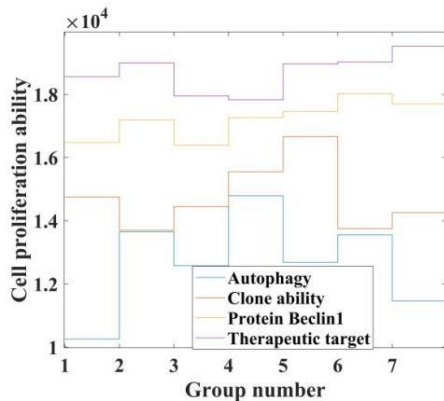


**Figure 1.** The effect of LncRNAHOXC-AS5 overexpression on the expression level of the target gene HOXC13 in ameloblastoma

The results of the study showed that the overexpression of LncRNAHOXC-AS5 decreased the expression of the target gene HOXC13, and the 12-hour apoptosis rate of ameloblastoma cells was (36.59±4.36). When the expression of LncRNAHOXC-AS5 was silenced, the expression of the target gene HOXC13 would increase. The apoptosis rate of ameloblastoma cells was (15.42±3.58)%, and there was a statistical difference between the two groups ( $P < 0.05$ ). The CCK-8 method was used to detect the effect of LncRNAHOXC-AS5 on the proliferation of ameloblastoma cells by regulating the expression of the target gene HOXC13. The results showed that the expression of the target gene HOXC13 after knocking down LncRNAHOXC-AS5 was significantly increased, and the proliferation activity of ameloblastoma cells was significantly increased ( $P < 0.05$ ), while the proliferation activity of ameloblastoma cells transfected with LncRNAHOXC-AS5 was an obvious decrease ( $P < 0.05$ ), the proliferation of ameloblastoma cells decreased. It can be seen that increasing LncRNAHOXC-AS5 can reduce the expression of the target gene HOXC13, thereby inhibiting the proliferation activity of ameloblastoma cells. The research results show that LncRNAHOXC-AS5 can reduce cell proliferation rate and increase cell apoptosis rate by acting on the target gene HOXC13. The specific data is shown in Figure 2.

From the data in Figure 2, it can be seen that LncRNAHOXC-AS5 can reduce the cell proliferation rate and vocal cell apoptosis rate by acting on the target gene HOXC13. Overexpression of LncRNA

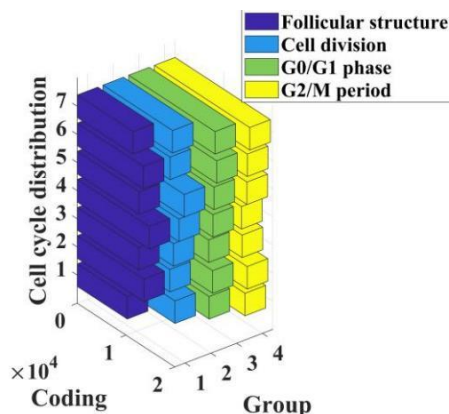
HOXC-AS5 can reduce the proliferation rate of ameloblastoma cells by 23.2%. The apoptotic rate of ameloblastoma was  $(36.59 \pm 4.36) \%$ .



**Figure 2.** The effect of LncRNAHOXC-AS5 on cell proliferation rate and cell apoptosis rate by acting on the target gene HOXC13

### Analysis of the Influence of LncRNA HOXC-AS5 on the Cell Cycle and Invasion Ability of Ameloblastoma through the Target Gene HOXC13

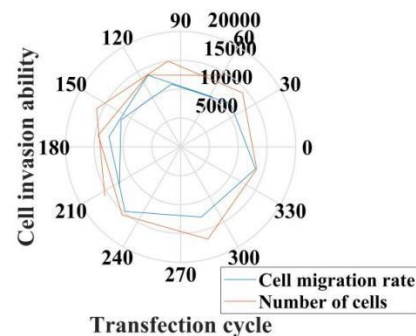
The research results show that the cell cycle of ameloblastoma is prolonged when LncRNAHOXC-AS5 is overexpressed, and the proliferation and proliferation of ameloblastoma cells mainly stagnate in G0/G1 phase. The ratio of cells in G0/G1 phase is significantly higher than that in S and G2/M phases. There was a statistically significant difference in the ratio of cells in G0/G1, S and G2/M phases ( $P < 0.05$ ). The research results show that LncRNAHOXC-AS5 can prolong the cell cycle of ameloblastoma by acting on the target gene HOXC13. The relevant data are shown in Figure 3.



**Figure 3.** The effect of LncRNAHOXC-AS5 on the cell cycle distribution of ameloblastoma by acting on the target gene HOXC13

It can be seen from the data in Figure 3 that LncRNAHOXC-AS5 can prolong the ameloblastoma cell cycle by acting on the target gene HOXC13. Overexpression of LncRNAHOXC-AS5 will make the G0/G1 phase cells account for 68.3%, which is higher than the knock-down group. The proportion of S-phase cells was 14.6%, which was lower than the knock-down group.

The experimental results in this paper show that the overexpression of LncRNAHOXC-AS5 significantly affects the migration of ameloblastoma cells. The migration rate of ameloblastoma cells transfected with LncRNAHOXC-AS5 decreases by 43.8%, and the expression of LncRNAHOXC-AS5 are silenced. Analysis of statistical data found that the number of cells that passed through the tran chamber when LncRNAHOXC-AS5 was overexpressed was  $(268.5 \pm 24.6)$ , and the number of ameloblastoma cells that passed through the trans chamber after silencing LncRNAHOXC-AS5 was  $(583.2 \pm 39.5)$ , indicating that LncRNAHOXC-AS5 paired. The invasion ability of ameloblastoma cells has an inhibitory effect. The research results show that LncRNAHOXC-AS5 can reduce the migration and invasion ability of ameloblastoma cells by acting on the target gene HOXC13. The specific data are shown in Figure 4.



**Figure 4.** The effect of LncRNAHOXC-AS5 overexpression on the invasion and migration rate of ameloblastoma cells

It can be seen from Figure 4 that LncRNAHOXC-AS5 can reduce the migration ability and invasion ability of ameloblastoma cells by acting on the target gene HOXC13. Overexpression of LncRNAHOXC-AS5 reduces the invasion ability index of ameloblastoma cells to  $(145.8 \pm 10.5)$ . The migration rate of cell tumor cells decreased by 43.8% after transfection of LncRNAHOXC-AS5.



## Conclusions

Ameloblastoma is an odontogenic tumor that occurs in the oral cavity. This tumor is a benign tumor. Ameloblastoma is very aggressive and easily causes tumor metastasis and postoperative recurrence. Studies have shown that the coding gene RNA is involved in the occurrence and development of a variety of tumors. Lucerne HOXC-AS5 as a member of the RNA family is regarded as a marker of ameloblastoma, which can interfere with tumor cell changes by regulating the target gene HOXC13.

The results show that when LncRNAHOXC-AS5 is overexpressed, the expression level of the target gene HOXC13 in ameloblastoma will be reduced by 18.5%. When LncRNAHOXC-AS5 is silently expressed, the expression level of the target gene in ameloblastoma HOXC13 will increase by 32.6%. LncRNAHOXC-AS5 can reduce cell proliferation rate and promote cell apoptosis rate by acting on the target gene HOXC13. Overexpression of LncRNA HOXC-AS5 can reduce the proliferation rate of ameloblastoma cells by 23.2%, and the apoptosis rate of ameloblastoma cells. It is  $(36.59\pm 4.36)$  %. HOXC13 may become a new target for treatment of ameloblastoma.

The study found that LncRNAHOXC-AS5 can prolong the cell cycle of ameloblastoma by acting on the target gene HOXC13. Overexpression of LncRNAHOXC-AS5 will make G0/G1 phase cells account for 68.3%, which is higher than the knock-down group, and the S phase cells accounted for 14.6%, lower than the knock-down group. LncRNAHOXC-AS5 overexpression reduced the invasion ability index of ameloblastoma cells to  $(145.8\pm 10.5)$ , and the migration rate of ameloblastoma cells transfected with LncRNAHOXC-AS5 decreased by 43.8%. Therefore, it can be seen that LncRNA HOXC-AS5 has an inhibitory effect on the target gene HOXC13. When the expression level of the target gene HOXC13 is reduced, it can reduce the proliferation of ameloblastoma cells, reduce cell invasion ability, and extend the cell division cycle.

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## Interest conflict

The authors declare that they have no conflict of interest.

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