



## Expression of LATS1 in head and neck squamous cell carcinoma and its effect on tumor cell proliferation and invasion

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### ABSTRACT

Nowadays, the incidence and mortality of head and neck tumors are gradually increasing. Head and neck malignant tumors (such as laryngeal cancer, hypopharyngeal cancer, oral cancer, nasopharyngeal cancer, oropharyngeal cancer, and other head and neck malignancies) are more common among systemic tumors. The most common pathological head and neck tumor type is squamous cell carcinoma, accounting for about 90%. In this study, immunohistochemical methods were used to collect the normal squamous epithelial tissues of the head and neck, atypical hyperplasia tissues, and head and neck squamous cell carcinomas on a tissue chip for detection. The recombinant LATS1 overexpression plasmid was prepared and transferred into B88 cells. Western blotting, MTT, and Transwell chamber methods were used to detect the effects of LATS1 proliferation, migration, and B88 cell overexpression. The experimental results showed that in head and neck squamous cell carcinoma, the expression of LATS1 protein decreased from 59.3% to 11.3%. At the same time, this protein inhibited the proliferation, migration, and invasion of head and neck squamous epithelial cells and also inhibited epithelium-Interstitial transformation exerts its tumor suppressor effect, indicating that LATS1 may play a tumor suppressor effect as a tumor suppressor gene. An in-depth study of the role and mechanism of LATS1 protein in the occurrence of head and neck squamous cell carcinoma may provide new opportunities for the treatment of head and neck squamous cell carcinoma in the future.

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### Introduction

Combining chemotherapy (cisplatin) and targeted growth receptor therapy (cetuximab) has become the first treatment method for head and neck squamous cell carcinoma (1). Although HNSCC treatment methods have improved to increase the life quality of life, the overall survival rate of patients is not adequate. The uncontrolled growth of cancer cells is one of the main reasons HNSCC is challenging to treat.

Tumor cells have countless genetic mutations that can maintain their ability to increase and resist cell growth signals and growth inhibitors (2). Many researchers explored the clinical significance of large inhibitory tumor gene 1 (LATS1) expression in head and neck squamous cell carcinoma. They studied the effect of LATS1 on squamous cell carcinoma and B cell proliferation, migration, and invasion (3, 4).

LATS1 gene can inhibit cell proliferation and is an inhibitor. It is first phosphorylated during the initial mitotic process and then may combine with CDC2 to

form a complex. The high expression of LATS1 can not only inhibit G2-M by inhibiting the activity of CDC2 protein, but it may inhibit tumor production and lead to apoptosis (5-7).

The activation of proto-oncogenes has excellent potential application value in targeted molecular tumor therapy (8). This study aimed to evaluate the expression of LATS1 in head and neck squamous cell carcinoma and its effect on tumor cell proliferation and invasion.

### Materials and Methods

#### Experimental Design of LATS1 Expression

To preliminarily clarify the function of large tumor suppressor gene-1 (LATS1) in head and neck squamous cell carcinoma, we used immunohistochemistry to detect the expression of LATS1 protein in different squamous cell carcinoma cells and utilized RNA interference (RNAi) technology to study LATS1.

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## Test Subject

Tissue specimens of 300 patients with head and neck squamous cell carcinoma treated in a hospital from 2010 to 2020 were collected. At the same time, we collected 80 cases of normal head and neck squamous epithelial tissue and 70 cases of dysplasia to facilitate the comparison of results. Then, the above-mentioned tissues were made into paraffin specimens, and tissue chips were made and then sliced. Immunohistochemical methods detected the expression of LATS1 protein in the above-mentioned various tissues.

## Experimental Method

The clinical and pathological data of head and neck squamous cell carcinoma were collected and classified. The recombinant LATS1 overexpression plasmid was prepared and transferred into B88 cells. Western blotting, MTT, and Transwell chamber methods were used to detect the effects of LATS1 proliferation, migration, and B88 cell overexpression. Western blotting was used to detect the effect of LATS1 on epithelial stromal cells. It was related to the epithelial-mesenchymal transition-related protein  $\beta$ -catenin ( $\beta$ -catenin), E-cadherin (E-cadherin), N-cadherin (N-cadherin), matrix metalloproteinase-2 (MMP-2), and MMP-9 expression effects.

## Results

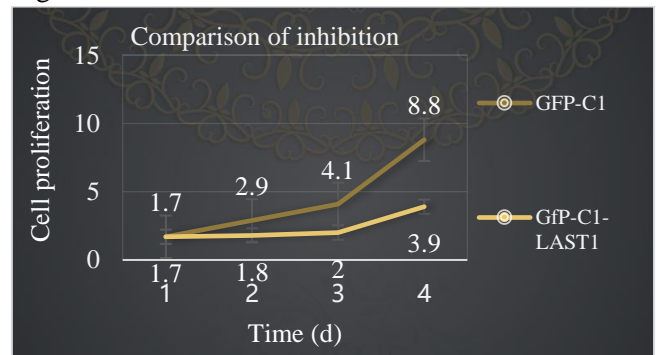
The protein expression level of LATS1 in head and neck squamous cell carcinoma tissue was significantly lower than that of normal tissues and dysplasia of head and neck tissues. In comparison, the expression level of LATS1 in the nucleus was lower than that of normal people ( $P < 0.05$ ), resulting in Cancerous. The expression of LATS1 in the cytoplasm increased sequentially ( $P < 0.05$ ). Collected clinical and pathological data of head and neck squamous cell carcinoma and found that in cancer tissues, the positive expression rate of LATS1 in the cytoplasm is related to lymph node metastasis, correlation, and positive mode ( $P < 0.05$ ), and LATS1 protein is in the nucleus or cytoplasm. There is a significant correlation between the expression and the survival and prognosis of patients with head and neck squamous cell carcinoma ( $P < 0.05$ ), as shown in Table 1.

**Table 1.** The expression levels of LATS1 protein in three groups of tissue cells

Group	n	LATS1 protein expression in the cytoplasm			Expression of LATS1 protein in the nucleus			
		-	+	PR/%	-	+	++	PR/%
Squamous epithelial cells	80	71	6	11.3%	23	31	26	71.3%
Dysplasia	70	59	6	15.8%	22	19	29	68.6%
Squamous cell carcinoma of the head and neck	300	122	70	59.3%	128	66	106	57.3%

## MTT Test Results

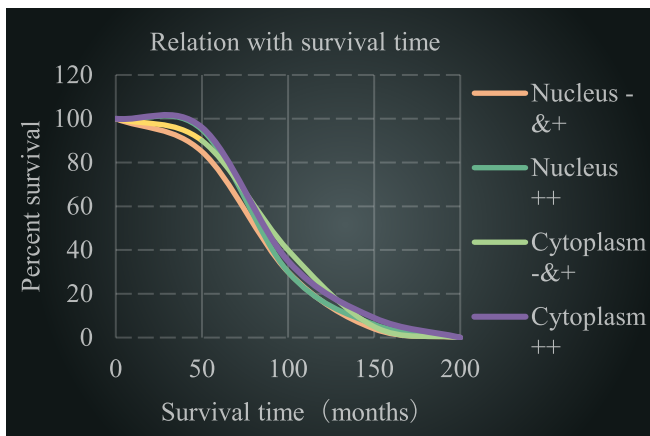
The plasmid GFP-C1-LATS1 was transferred into head and neck squamous tumor cells, and the absorbance changes of MTT were detected for 1-4 days. As shown in Figure 1, compared with the empty plasmid GFP-C1 group, the high expression LATS1 group had significantly reduced proliferation in the cells on the second, third, and fourth days. The differences were statistically significant (all  $P < 0.05$ ). We tested the effect of highly expressed protein LATS1 on the cloning ability of head and neck squamous tumor cells through cell clone formation experiments. The clone formation rate reflects two critical traits of cells, population dependence, and proliferation ability. In the head and neck squamous tumor cell clone formation experiment, the number of clones in the empty plasmid control group was about  $151 \pm 25$ , while in the cell group with high expression LATS1, the number of clones was  $57 \pm 16$ . The difference between the two was statistically significant ( $P < 0.01$ ). The increased expression protein LATS1 significantly inhibited the clonal formation of head and neck squamous tumor cells, as shown in Figure 1.



**Figure 1.** The inhibitory effect of LAST1 on tumor cell proliferation

### Relationship between LATS1 Protein Expression and Pathological Characteristics of Head and Neck Squamous Cell Carcinoma

Statistical analysis of the clinicopathological data of head and neck squamous cell carcinoma patients showed that the expression level of LATS1 was reduced in head and neck squamous cell carcinoma tissues. Still, it was widely expressed in normal tissues adjacent to cancer. This result indicates that the down-regulation of the LATS1 gene can promote the occurrence and development of head and neck squamous cell carcinoma. The survival analysis results showed that the expression of LATS1 in the nucleus and cytoplasm was not significantly correlated with the patient's survival prognosis ( $P>0.05$ ). The expression of LATS1 in the cytoplasm of cancer tissues was related to lymph node metastasis ( $P=0.004$ ). There was no significant correlation with other pathological features ( $P>0.05$ ), and the expression of nuclear LATS1 was not significantly correlated with the above factors ( $P>0.05$ ). The relationship between the expression of LATS1 protein in the nucleus and cytoplasm and survival time is shown in Figure 2.

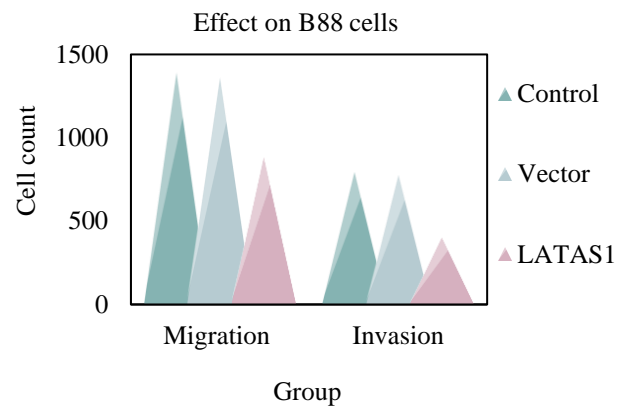


**Figure 2.** The relationship between the expression of LATS1 protein in the nucleus and cytoplasm and survival time

### Effect of LATS1 Overexpression on the Migration and Invasion of B88 Cells

The results of the Transwell cell migration experiment showed that the number of cells that migrated in the blank group, empty plasmid group, and LATS1 transfection group was  $1376.0 \pm 17.6$ ,  $1317.0 \pm 7.6$  and  $868.0 \pm 17.1$ , respectively.

Compared with the blank group and the empty plasmid group, the migration ability of B88 cells was significantly reduced after LATS1 overexpression, and the difference was statistically significant ( $P<0.01$ ). Transwell cell method invasion test results showed that the number of cells in the blank group, empty plasmid group, and LATS1 transfection group was  $785.0 \pm 8.2$ ,  $718.0 \pm 6.7$ , and  $355.0 \pm 11.9$ , respectively. Compared with the blank group and the empty plasmid group, the invasion ability of B88 cells was significantly reduced after LATS1 overexpression, and the difference was statistically significant ( $P<0.01$ ). The above results suggest that the LATS1 protein can inhibit the migration and invasion of B88 cells, as shown in Figure 3.



**Figure 3.** Effect on migration and invasion of B88 cells

### Influence of LATS1 Overexpression on Cell Cycle of Head and Neck Squamous Tumors

To explore the effect of LATS1 on the cell cycle, we tested the cell cycle of LATS1 overexpression cell lines LATS1-2 and LATS14. Experimental results found that increased expression of LATS1 can inhibit cell proliferation. This inhibition is achieved by inhibiting the kinase activity of the Cdc2/Cyclin A/B complex to block B88 transformation. Compared with control cells, these two cell lines are in the G2 phase. The number of cells increased significantly, and the number of cells in the G1 phase decreased significantly ( $P < 0.001$ ). However, there was no significant change in the cells in the S phase of the two lines. In exploring the molecular mechanism of LATS1 in inhibiting the function of head and neck squamous tumors, we found that the high expression of LATS1 in U251 cells can inhibit the expression of

cell cycle factors CCNA1. It means that LATS1 may be involved in the B88 cell cycle signaling pathway in head and neck squamous tumors, as shown in Table 2.

**Table 2.** Effects of LATS1 on the cell cycle of cell lines

Cycle	Cell line	Average DNA content
G1	PLATS 1-2	57%
	PLATS 1-4	56%
	pCtr-vector	62%
S	PLATS 1-2	26%
	PLATS 1-4	27%
	pCtr-vector	26%
G2	PLATS 1-2	17%
	PLATS 1-4	17%
	pCtr-vector	15%

## Discussion

The recurrence, metastasis, and resistance to chemotherapy of head and neck squamous cell carcinoma have become important factors that limit the improvement of patient survival (9). Therefore, improving the survival rate requires in-depth research on critical molecules in developing head and neck squamous cell carcinoma and then targeted therapy based on the molecular classification of tumor biomarkers to provide a specific theoretical and experimental basis (10). The emergence and development of head and neck squamous cell carcinoma are accompanied by genetic changes and epigenetic regulation (11).

In developing malignant tumors, metastasis and invasion are critical biological characteristics. Epithelial-mesenchymal transition is a reversible process in which epithelial cells gain a functional mesenchymal phenotype by losing their binding and polarity and promoting cell invasion and metastasis (12). The epithelial-mesenchymal transition has a common cytological mechanism, mainly manifested by the decrease in intracellular adhesion proteins ( $\beta$ -catenin protein, E-cadherin protein, etc.) and phenotypic mesenchymal markers (stromal markers), the expression of N- Katerini protein increases and at the same time, cytoskeleton changes. Also, MMP-2 and MMP-9 can transform epithelial-mesenchymal cells, degrade cell-matrix, and change the microbial environment of cells. More transcription factors, including ZEB and TWIST, can regulate epithelial-mesenchymal transition by starting their operations in turn. Eventually, the cell adhesion ability is reduced,

which leads to an increase in its migration ability and an increase in affinity with the distal layer so that epithelial cancer cells invade and metastasize (13).

Large tumor suppressor gene 1 (LATS1) is a crucial growth suppressor in the Hippo pathway that controls organ size and tumorigenesis and is a member of dbf2-related nuclear protein kinase-related nuclear protein (14). Through cell-to-cell contact, LATS1 is activated to control cell proliferation speed and organ volume.

LATS1 plays a vital role in regulating the cell cycle and apoptosis. LATS1 can control the levels of CydlinA and CyclinB, disrupt the cell cycle in the G2M phase, and inhibit cell proliferation and growth. At the same time, it has been found that LATS1 causes apoptosis in certain cell lines. The death and upregulation of pro-apoptotic proteins (such as Bax, p53) respond to extensive DNA damage, ultraviolet radiation, chemotherapy, oncogene activation, and growth factor withdrawal (15-17). Recently, because mitosis has been found in the centrosome, it is inseparable from the formation of the shaft, which is the reason for the involvement of LATS1.

For this reason, the loss of control of various such cells can lead to irregular chromosomal abnormalities and cells. LATS1 plays a significant role in the management and stability of cell homeostasis (15). There are astrocytoma, breast cancer, head, and neck squamous cell carcinoma, colon cancer, cervical cancer, gastric cancer, and breast cancer, and it reduces the occurrence of tumors. The loss of LATS1 can cause soft tissue sarcoma and other tumors (16).

LATS1 inhibits the proliferation and differentiation of cancer cells. It induces apoptosis of cancer cells, all achieved by reducing the essential YAP target genes of the Hippo signaling pathway. Therefore, LATS1 is considered a tumor suppressor gene (18). Studies have shown that, according to various conditions, the stress of active cells on LATS1 can promote the apoptosis of apoptotic cells (19-21). The human protein LATS1 and Drosophila WTS protein markers are widely conserved in terms of protein structure and function, reflecting their highly profound significance in the growth and development of individuals (16).

In head and neck squamous cell carcinoma, the expression of LATS1 protein is significantly lower than that of normal squamous epithelial tissue (17). The transfer of LATS1 from the nucleus to the

cytoplasm may occur, and the expression of LATS1 in the cytoplasm is related to lymph node metastasis (20). There is no apparent correlation between the expression of LATS1 in the nucleus or cytoplasm and the survival and prognosis of patients with head and neck squamous cell carcinoma (21). Compared with genomics and proteomics, the determination of metabolites has the advantages of a higher degree of automation and a lower cost of sample collection and processing. In addition, measuring changes in the concentration of metabolites is more sensitive than measuring changes in the rate of biochemical reactions and can more directly reflect the disease process. Like many other researchers (20), we believe that metabolite pattern analysis can be used to understand the malignant process of head and neck squamous cell carcinoma and then find characteristic metabolites for early diagnosis or prognostic monitoring of malignant head and neck squamous cell carcinoma.

The pathogenesis of squamous cell carcinoma of the head and neck is complex (1). Current studies generally believe that the onset of head and neck squamous cell carcinoma is a multi-factor and multi-stage process, but the exact pathogenesis is still unclear (22). The research on HNSCC sensitivity factors has made significant progress, but many issues still need further exploration. Epidemiological studies have shown that smoking, drinking, HPV infection, SNP, methylation, and miRNA targeted regulation are all susceptible factors for HNSCC, and its pathogenic mechanism will gradually be confirmed. Understanding the sensitivity factors and mechanisms of HNSCC can provide a potential basis for the clinical treatment of HNSCC and improve the level of treatment, thereby improving the quality of life of patients and prolonging their survival time (23).

At present, metabolic research on the malignant process of head and neck squamous cell carcinoma is still in its infancy. In the past, due to individual differences in samples or heterogeneity of tumor tissues, the traditional selection of clinical specimens for metabolic studies would affect the identification of metabolites (24). With the development of cell culture technology, metabolic tumor cell lines enriched in vitro can overcome the above shortcomings and have the advantages of solid control, high stability, and good repeatability.

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