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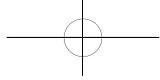
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ISSN (ONLINE): 2208-3553

ISSN (PRINT): 2208-3545

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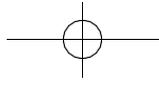
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ISSN (Online): 2208-3553

ISSN (Print): 2208-3545

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# KIF3C Promotes the Malignant Progression of Lung Cancer Cells A549

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**Abstract:** *Objective:* To investigate the role of KIF3C gene in promoting the malignant phenotype of lung cancer cells and in regulating PI3K/AKT signaling pathway. *Methods:* CCK-8 and transwell assays were used to detect the changes in cell proliferation and cell migration ability after being transfected with siKIF3C, as well as the protein expression levels of PI3K, p-PI3K, AKT, and p-AKT following the downregulation of KIF3C by Western blot. *Results:* The CCK-8 assay showed that the proliferation/viability of lung cancer cells A549 significantly reduced after being transfected with siKIF3C gene ( $P < 0.05$ ); the migration ability of lung cancer cells A549 was significantly reduced after transfected with siKIF3C gene ( $P < 0.05$ ); the levels of p-PI3K and p-AKT proteins were downregulated after KIF3C protein knockdown ( $P < 0.05$ ); however, the detection of PI3K and AKT protein levels was not statistically significant. *Conclusion:* KIF3C may promote the proliferation and migration ability of lung cancer cells A549 through PI3K/AKT signaling pathway.

**Keywords:** KIF3C; A549; EMT; Lung cancer

**Online publication:** October 18, 2022

## 1. Introduction

According to global epidemiological surveys, lung cancer is the most common malignancy with a high mortality rate. Hence, early diagnosis and screening play important roles in clinical management<sup>[1]</sup>. Kinesin family member 3C (KIF3C) is a member of the kinesin superfamily and is thought to be involved in microscopic motility, transporting upstream or inside organelles along microtubules, with microtubule binding activity and microtubule motility activity. It is also part of the kinesin complex. KIF3C is a protein-coding gene. Pathways associated with KIF3C include responses to platelet cytoplasmic calcium ion ( $Ca^{2+}$ ) elevation and Golgi to endoplasmic reticulum (ER) retrograde transport. Gene Ontology (GO) annotation indicates that KIF3C has adenosine triphosphate (ATP) hydrolytic activity and microtubule motility activity<sup>[2]</sup>. An important homolog of this gene is KIF3B, which encodes a protein that acts as a heterodimer with kinesin family member 3A to aid chromosome movement during mitosis and meiosis. Studies have shown that the overexpression of KIF3C can promote proliferation and metastasis in some tumors. However, a high expression of KIF3C protein in glioma cells is an indicator of good prognosis; clinical specimens have revealed that KIF3C expression is higher in low-grade gliomas than high-grade gliomas<sup>[3]</sup>. KIF3C also plays a role in paclitaxel resistance of breast cancer cells<sup>[4]</sup>. We have previously demonstrated that KIF3C is a tumor-promoting factor in lung cancer<sup>[4]</sup>. In this paper, we focus on the changes in the proliferation and migration ability of lung cancer cells A549 after knocking down the expression of KIF3C as well as

the activation status of PI3K/AKT signaling pathway, which may provide new concepts to the invasion and metastases of lung cancer cells A549 [5].

## **2. Materials and methods**

### **2.1. Materials**

Lung cancer cells A549 were purchased from Shanghai Cell Bank, China; fetal bovine serum (FBS) and Roswell Park Memorial Institute (RPMI)-1640 medium were purchased from Procell; transwell was purchased from Corning; KIF3C polyclonal antibody [6], PI3K, p-PI3K, AKT, p-AKT, and GAPDH antibodies were purchased from Proteintech. CCK-8 kit was purchased from Wuhan Doctor Bio [6]. TBST, radioimmunoprecipitation assay (RIPA) lysate, and enhanced chemiluminescence (ECL) kits were purchased from Shanghai Biyuntian Biotechnology Company [7].

### **2.2. Cell culture and transfection**

Human lung cancer cell line A549 was cultured and passaged according to standard conditions. Lipofectamine 3000, a liposome transfection reagent, was purchased from Invitrogen. KIF3C-siRNA and control-siRNA were purchased from Ribo Biotechnology (Guangzhou, China). A549 cells were cultured in RPMI-1640 culture medium containing 10% FBS at 37°C and 5% carbon dioxide (CO<sub>2</sub>), and transfected every 2–3 days at a ratio of 1:3. The day before siKIF3C transfection, the cells were inoculated in 6-well plate at  $0.5-2 \times 10^5$  per well, and transient transfection was performed when 30–50% of cells were passaged [8]. The transfection procedure was performed by transfecting siKIF3C and control-interfering sequence into lung cancer cells A549 according to Lipofectamine 3000 instructions [8].

### **2.3. CCK-8 experiment**

The cultured cells were divided into KIF3C-siRNA group and control-siRNA group. Single-cell suspension A549 cells were inoculated in 96-well plate at 3,000 cells/well. After the cells of both the groups were walled up and incubated, 10 µL of CCK-8 reagent was added to each well, and incubation was continued for 2 h at 37°C in an incubator to detect the growth of cells for 4 consecutive days [9]. The absorbance value of each well was detected at 450 nm by enzyme-linked immunosorbent assay (ELISA), and the growth curve of the tumor cells was plotted [10].

### **2.4. Transwell experiment**

Cell invasion assays were performed using transwell chambers with 8 µm wells. Matrix gels were made based on a 1:3 dilution of Matrigel matrix gel with RPMI-1640 medium. 50 µL of mixed gel was added to the bottom of the transwell and incubated for 2 h to form a gel. The upper chamber was inoculated with 200 µL of cell suspension without serum culture medium per well. Serum-containing medium supplemented with 10% FBS was added to the lower chamber as a chemical elicitor. After incubating for 24 h, cells from the filter were fixed with 4% paraformaldehyde and stained with hematoxylin. Five randomly selected high-magnification fields were photographed and counted; the calculated mean value was taken as the final result of the experiment [11].

### **2.5. Western blotting**

After transfection, cells from each group were collected, and the proteins were extracted by lysing the cells with RIPA. The concentration of the extracted proteins was determined by ultraviolet (UV) spectrophotometry. The proteins were electrophoresed by sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE), transferred to a membrane, closed with skimmed milk, and incubated dropwise with primary antibody overnight at 4°C; the membrane was then washed with TBST and exposed.

All primary antibody concentrations were 1:1,000, while the sheep anti-rabbit secondary antibody concentration was 1:10,000 [12].

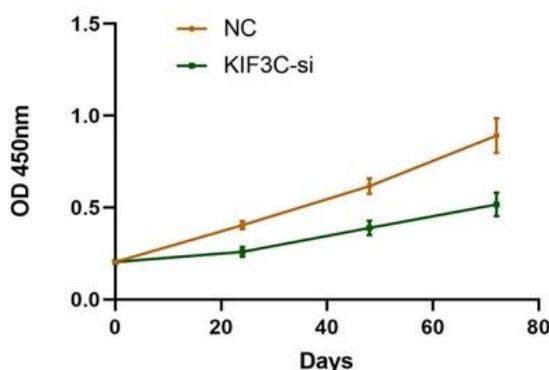
## 2.6. Statistical analysis

The experimental data were analyzed using SPSS version 26.0 and GraphPad Prism version 8.0 for statistical graphing. The experimental data were expressed as mean  $\pm$  standard deviation (mean  $\pm$  SD). The difference was considered statistically significant at  $P < 0.05$ .

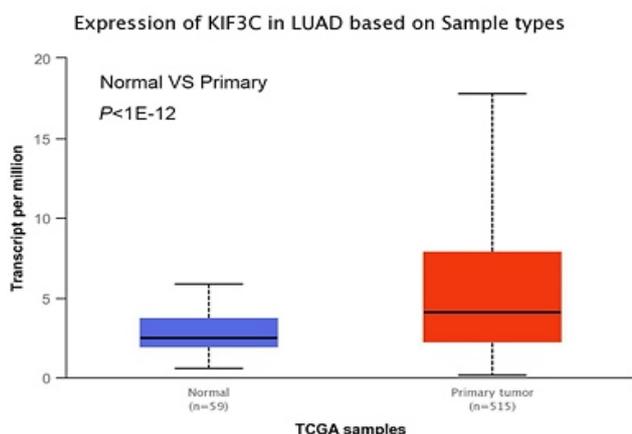
## 3. Results

### 3.1. Reduction in the viability of lung cancer cells A549 after KIF3C knockdown

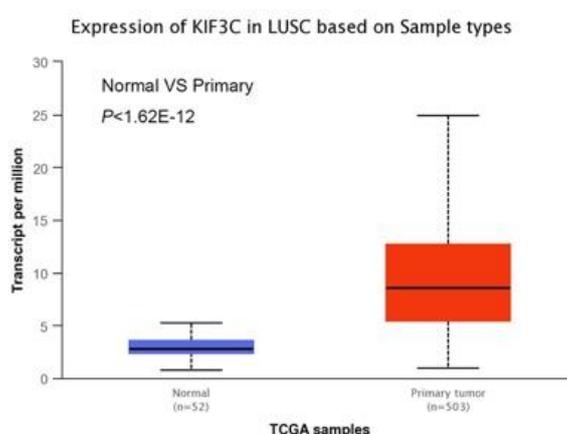
Proliferation assays revealed changes in cell proliferation after the incubation of A549 cells for 0 h, 24 h, 48 h, and 72 h with 10  $\mu$ L of CCK-8 reagent, see **Figure 1A**. CCK-8 results showed that the downregulation of KIF3C in transfected A549 cells resulted in reduced cell proliferation/viability compared to the control group. Analysis of data based on The Cancer Genome Atlas (TCGA) lung cancer tumor samples (<http://ualcan.path.uab.edu/>) revealed significantly higher mRNA levels of KIF3C in lung adenocarcinoma (LUAD) and squamous cell carcinoma (LUSC) tissues than in normal lung tissues, see **Figure 1B** and **Figure 1C** [13].



(A)



(B)

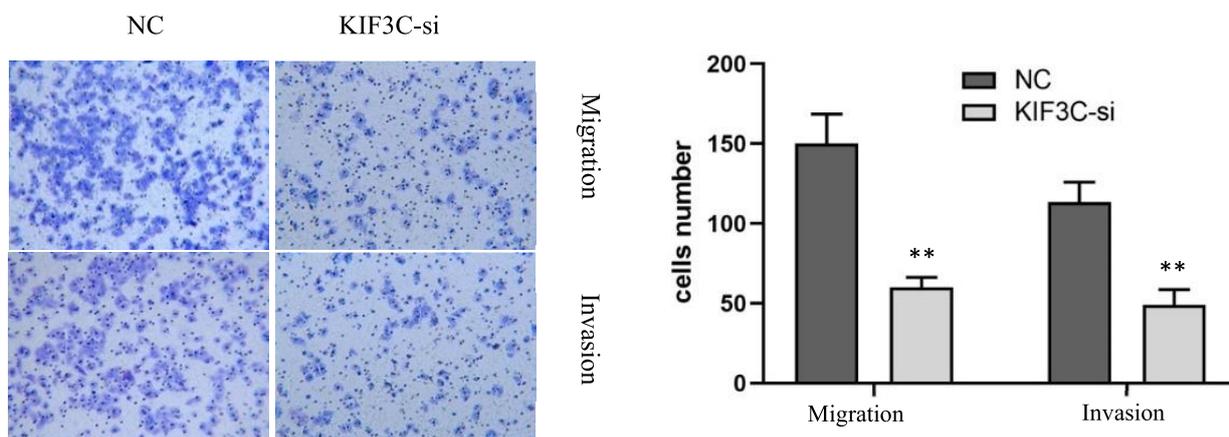


(C)

**Figure 1.** (A) CCK-8 results showing that the downregulation of KIF3C in transfected A549 cells decreased cell value-added viability. (B) The mRNA expression of KIF3C in lung adenocarcinoma tissues was higher than that in normal lung tissues. (C) The mRNA expression of KIF3C in lung squamous cell carcinoma tissues was higher than that in normal lung tissues

### 3.2. Reduction in the migration and invasion ability of A549 cells after KIF3C knockdown

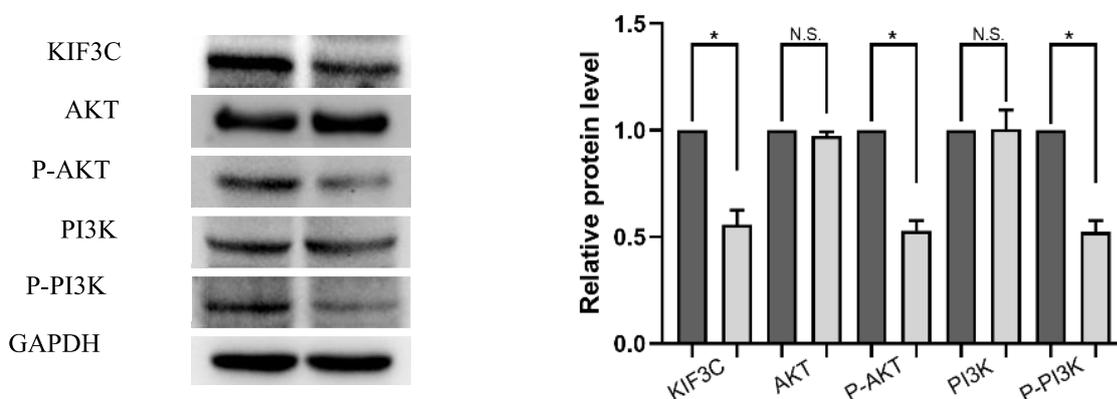
The results of the transwell experiment showed that the migration ability of A549 cells was significantly weaker than that of the control group after the A549 cells were transfected with siKIF3C for 24 h [14]. The statistical analysis of the number of cells in the field of view of the collected images showed that the number of A549 cells migrating in the knock-down group ( $60 \pm 6$ ) was significantly lower than that of the control group ( $150 \pm 19$ ) ( $P < 0.05$ ); the number of A549 cell invasion in the knockdown group ( $49 \pm 10$ ) was significantly lower than that in the control group ( $113 \pm 13$ ) ( $P < 0.05$ ), see **Figure 2**.



**Figure 2.** Changes in the migration and invasion ability of A549 cells in the KIF3C knockdown group compared to the control group (\*\* represents  $P < 0.01$ )

### 3.3. Effect of KIF3C knockdown on PI3K/AKT signaling pathway activity

Western blotting was performed to detect the expression of the key proteins in PI3K/AKT signaling pathway. The results of the study showed that the expression of p-PI3K and p-AKT was significantly downregulated in A549 cells following transfection with siKIF3C compared with the expression of the transfected control interference series ( $P < 0.05$ ); however, the detection of the total protein of PI3K and AKT was found to be statistically insignificant. This suggests that KIF3C may promote the proliferation, invasion, and metastasis of A549 cells through the regulation of PI3K/AKT signaling pathway [15].



**Figure 3.** Changes in PI3K/AKT signaling pathway-related proteins after KIF3C knockdown (\* represents  $P < 0.05$ ; N.S. refers to no significance)

#### 4. Discussion

Lung cancer is one of the most common malignancies seen in clinical practice. Evidence suggests that lung cancer is a complex disease whose pathogenesis involves mutation or activation of multiple oncogenes. In addition to external environmental factors, the abnormal regulation of key genes plays a significant role in the development of lung cancer. The kinesin superfamily proteins (KIFs) are highly conserved and are microtubule-dependent kinesins that convert chemical energy released during ATP hydrolysis into mechanical energy and are involved in regulating the transport of intracellular molecules [16]. The KIF3 protein family is a subfamily member of the KIF superfamily, which includes three species, KIF3A, KIF3B, and KIF3C. The molecular motor KIF3B is a key regulator of the dendritic structure of cortical neurons. Studies have shown that KIF3B is a key determinant of cortical neuronal morphology and has an inhibitory effect on structural plasticity [8]. KIF3C plays a role in a variety of biological processes, mainly in those related to neuronal development, differentiation, and axonal transport [17]. Through bioinformatics databases and glioma patient tissues, researchers have found higher levels of KIF3C expression in low-grade gliomas than high-grade gliomas, such as glioblastomas; patients with high KIF3C expression have also been found to have longer overall survival according to a survival analysis [3]. KIF3C expression is upregulated in breast cancer tissues and may be involved in tumor recurrence and metastasis. The knockdown of KIF3C gene significantly downregulates the level of phosphorylated Smad2, thus inhibiting TGF- $\beta$  signaling pathway. Tumor metastasis is closely related to epithelial-mesenchymal transition (EMT). However, a study found that the expression of vimentin, metalloproteinase-2 (MMP2), and MMP9 was significantly upregulated by the downregulation of KIF3C expression, with a decrease in E-cad expression, thus suggesting that EMT was inhibited [18]. Through a tumor database integration analysis, we found that KIF3C mRNA levels were significantly upregulated in non-small cell lung cancer tissues, having a correlation with poor prognosis. We found that KIF3C expression was upregulated in non-small cell lung cancer cells and tissues and promoted the proliferation and metastasis of lung cancer cells. The expression of KIF3C was also found to be negatively regulated by miR-150-5p and miR-186-3p [19].

Dysregulation of PI3K/AKT signaling pathway has been demonstrated in both, tumor and non-tumor diseases. AKT only has altered phosphorylation levels after which the signaling pathway is fully activated, which in turn leads to the entry of a number of key downstream transcription factors into the nucleus, resulting in cell survival and proliferation as well as the inhibition of apoptosis. The overexpression of KIF2A promotes the malignant phenotype of A549 cells, including proliferation [20], migration, invasion, maintenance of tumor stem cell properties, and cisplatin resistance. Further mechanistic studies have revealed that the above malignant biological behaviors are acquired by KIF2A through mediating the activation of PI3K/AKT/VEGF signaling pathway [21].

There are relatively few studies on how KIF3C regulates the PI3K/AKT mechanism. The present experiment demonstrated that KIF3C could indeed affect the biological behavior of A549 cells. After interfering with KIF3C, the cell phenotype was significantly altered, including a decrease in cell proliferation/viability as well as cell invasion and migration ability. We also found similar results, in that KIF3C was able to activate PI3K/AKT signaling pathway, as evidenced by changes in the expression levels of p-PI3K and p-AKT, which may promote other intracellular mechanisms related to cellular transcriptional regulation.

In conclusion, this study reveals that KIF3C affects the malignant phenotype of cells and the regulation of signaling pathway mechanisms in non-small cell lung cancer through cellular experiments. The changes in the protein levels of PI3K/AKT and their role in the biological behavior of tumor cells have been verified at the cellular-molecular level by regulating the expression levels of KIF3C genes. Our study of the mechanisms of tumor invasion and metastasis by linking KIF3C and signaling pathway-related molecules may provide a theoretical basis for clinical gene-targeting therapy and some guidance in patient prognosis.

## Funding

This research was supported by the Medical Science Research Program of Hebei Province (20211020).

## Disclosure statement

The authors declare no conflict of interest.

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# Macrophage CCL22 Secretion Under Hypoxic Conditions Promotes the Metastasis of Triple-Negative Breast Cancer

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**Abstract:** *Objective:* To explore the mechanism by which macrophages secrete CCL22 to promote the metastasis of triple-negative breast cancer under hypoxic conditions. *Methods:* 20 ng/mL mass concentration of phorbol 12-myristate 13-acetate (PMA) cell culture medium, 4',6-diamidino-2-phenylindole (DAPI), dimethyl sulfoxide (DMSO), trypsin digestion solution, CCL18 Kit, Interleukin (IL)-10 Kit, CCL17 Kit, CCL22 Kit, TRIzol™ Reagent Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) Kit, triple-negative breast cancer cells MDA-MB-231 and BT-549, as well as other reagents were used to culture triple-negative breast cancer (TNBC) cells MDA-MB-231 and BT-549 as well as human mononuclear cells THP-1, analyze and observe the metastasis of triple-negative breast cancer cells to the lungs, the secretion of CCL22, the migration of triple-negative breast cancer, and the situation of CCR4. *Results:* Compared with normal tumor-associated macrophage (TAM), hypoxic TAM further promoted the migration of tumor cells. The number of tumor metastases in the lungs, induced by hypoxic TAM, was significantly higher than that of normoxic TAM. Hypoxia can significantly stimulate the expression of CCL22. CCL22 can significantly promote the migration of MDA-MB-231 and BT-549 cells. The expressions of CCR3, CCR4, and CCR5 in tumor tissues were significantly increased compared with normal tissues, in which CCR4 showed the most significant increase. *Conclusion:* TAM cultured under hypoxia significantly enhanced the migration ability of TNBC cells and promoted the metastasis of cancer cells to the lungs *in vivo*. The hypoxic condition induced TAM to secrete CCL22; the expression of CCL22 receptor, CCR4, in breast cancer tissues was significantly higher than that in normal tissues.

**Keywords:** Hypoxia; Macrophages; Triple-negative breast cancer; CCL22

**Online publication:** November 16, 2022

## 1. Introduction

Breast cancer is one of the malignant tumors with the highest incidence in women worldwide. There are more than 1.5 million new cases of breast cancer every year across the globe. It threatens the safety of female patients<sup>[1]</sup>. Although the mortality rate of patients has significantly dropped with the use of various comprehensive treatments, approximately 23.67% of women still die from tumor metastasis or recurrence, in which invasion and metastasis are the main causes of death<sup>[2]</sup>. The pathogenesis of breast cancer has not been fully elucidated, and its corresponding molecular mechanism also lacks strong evidence. The internal environment of a tumor is known as the tumor microenvironment (TME). A large number of studies have confirmed that TME plays a crucial role in the occurrence and development of breast cancer<sup>[3]</sup>. Macrophages are an important part of the body's innate immune response, and they are a heterogeneous cell population with high plasticity. Macrophages can be recruited around tumors under the action of

chemokines and cytokines [4]. Stimulated by cytokines in the TME, it polarizes into different types of tumor-associated macrophages (TAMs) [5]. Generally speaking, macrophages can be polarized into M1 or M2 macrophages. M2 macrophages are activated by Th2 cytokines, such as interleukin (IL)-4, IL-10, and IL-13. These alternatively activated macrophages are able to produce IL-10, CCL17, CCL22, CCL18, and IL-1 receptor antagonist (Ra)/IL-1R inducers, promote angiogenesis, local tissue reconstruction and repair, as well as promote the occurrence, development, and metastasis of cancer cells. At the same time, M2 macrophages can also inhibit inflammatory responses by regulating M1 macrophage-mediated functions and adaptive immunity, thereby indirectly promoting tumor growth [6].

## **2. Materials and methods**

### **2.1. Reagents**

20 ng/mL mass concentration of phorbol 12-myristate 13-acetate (PMA) cell culture medium, 4',6-diamidino-2-phenylindole (DAPI), dimethyl sulfoxide (DMSO), trypsin digestion solution, CCL18 Kit, IL-10 Kit, CCL17 Kit, CCL22 Kit, TRIzol™ Reagent Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) Kit, and triple negative breast cancer (TNBC) cells MDA-MB-231 and BT-549.

### **2.2. Methods**

Cell culture: TNBC cells MDA-MB-231 and BT-549 and human mononuclear cells THP-1 were cultured, and the basal medium was  $\psi = 10\%$  fetal bovine serum; they were divided into two groups, the normoxia group (37°C,  $\psi = 5\%$  carbon dioxide (CO<sub>2</sub>), and saturated humidity) and the hypoxia group (37°C, V(N<sub>2</sub>):V(CO<sub>2</sub>):V(O<sub>2</sub>) = 94:5:1 gas mixture was continuously pumped into the incubator to maintain saturated humidity).  $1 \times 10^6$  THP-1 was inoculated into a 6-well plate, and PMA was added to each well to make the final mass concentration of 20 ng/mL. After culturing for 72 h, non-adherent cells were removed; the adherent cells were M0 macrophages. Transwell method was used to detect tumor cell migration, followed by RT-PCR detection. Computed tomography (CT) was used to determine tumor metastasis, while enzyme-linked immunosorbent assay (ELISA) was used to detect the concentration of cytokines.

### **2.3. Observation indicators**

Lung metastasis, CCL22 secretion, migration of TNBC cells, and CCR4 of TNBC cells were observed and analyzed.

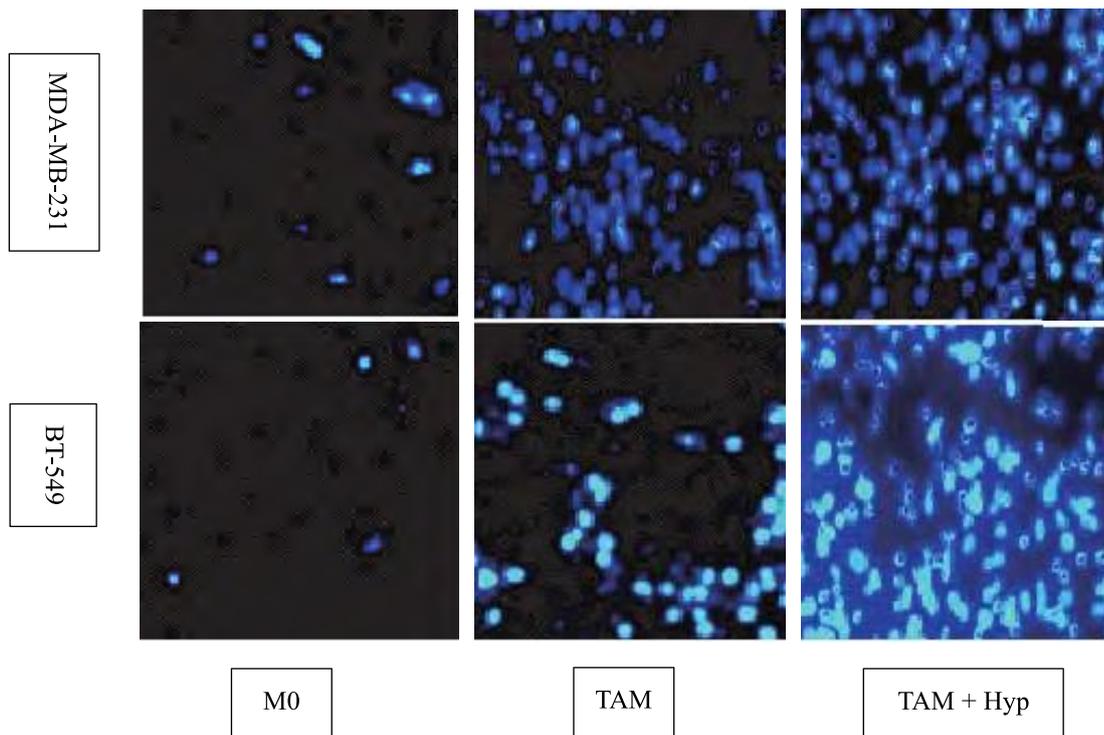
### **2.4. Statistical analysis**

SPSS 18.0 was used for statistical analysis of the experimental data. All experiments were independently repeated at least three times. The measurement data were expressed as mean  $\pm$  standard deviation ( $\bar{x} \pm s$ ), and t-test was performed for data comparison and analysis.  $P < 0.05$  indicated statistical significance.

## **3. Results**

### **3.1. Macrophages promote triple-negative breast cancer cell migration under hypoxic conditions**

Experiments were carried out according to the aforementioned methods, and after 48 h, the images were observed under the microscope as shown in **Figure 1**.



**Figure 1.** Transwell assay of triple-negative breast cancer cells treated with three conditional medias (CMs)

### **3.2. Macrophages promote lung metastasis of triple-negative breast cancer cells**

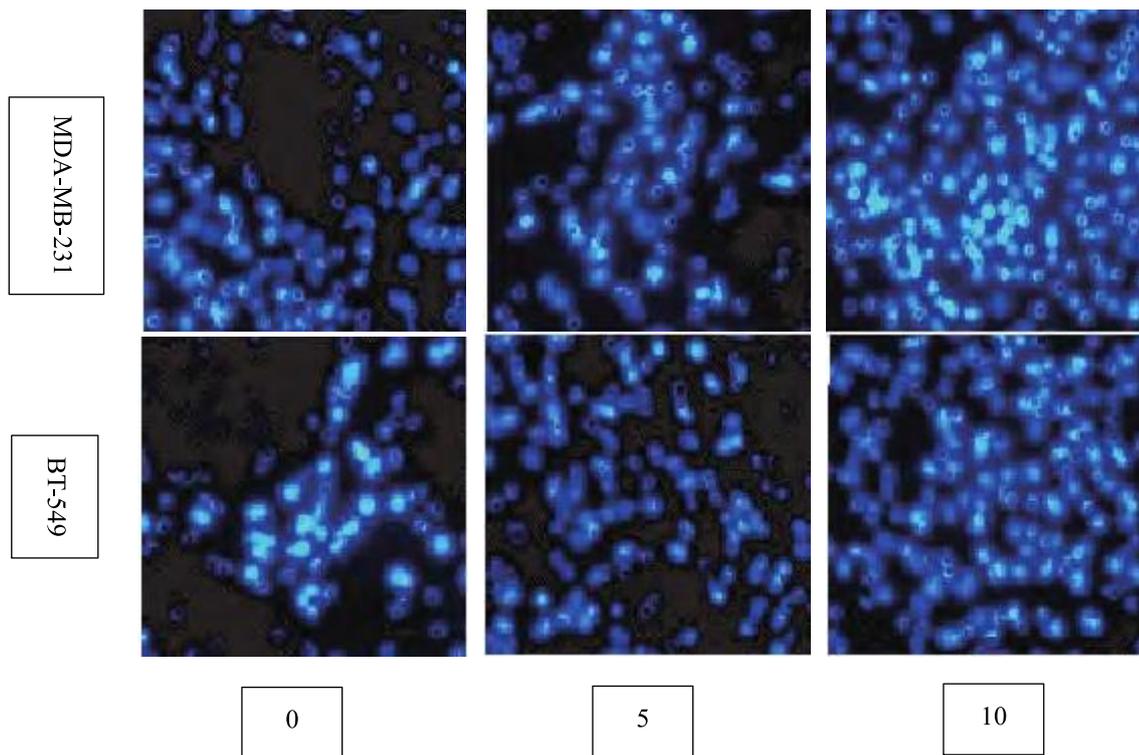
Hypoxia-treated TAMs, which were cultured *in vitro* under hypoxic conditions, significantly induced lung metastasis of MDA-MB-231 cells. The number of metastases in the lung induced by hypoxia-treated TAMs were significantly higher than those of normoxia-cultured TAMs.

### **3.3. Hypoxia induces macrophage secretion of CCL22**

CCL22 may be an important factor in the stimulation of TNBC metastasis by hypoxic TAM. In TAM, the expression of CCL18 was the highest, followed by CCL22 and IL-10 expressions, which were relatively low. Hypoxia significantly stimulated an increase in CCL22 expression, without any significant effects on the expressions of the other three cytokines.

### **3.4. CCL22 promotes lung migration of triple-negative breast cancer cells**

10 ng/mL of CCL22 can significantly promote the migration of MDA-MB-231 and BT-549 cells (**Figure 2**).



**Figure 2.** Transwell assay of triple-negative breast cancer cells induced by different concentrations of CCL22

### 3.5. CCR4 mediates macrophages to promote triple-negative breast cancer cell migration under hypoxic conditions

The combination of CCL22 and CCR4 plays an important role in promoting breast cancer metastasis. The expressions of CCR3, CCR4, and CCR5 were significantly elevated in tumor tissues compared with normal tissues, in which the elevation of CCR4 was the most significant, as shown in Table 1.

**Table 1.** Expression ratio of CCR family genes in breast cancer tissue and normal tissue

Gene	Normal tissue expression value (N)	Tumor tissue expression value (T)	T/N
<i>CCR1</i>	280	330	1.18
<i>CCR2</i>	99	110	1.11
<i>CCR3</i>	1.8	3.4	1.89
<i>CCR4</i>	14	50	3.33
<i>CCR5</i>	150	280	1.87

## 4. Discussion

Tumor-associated macrophages have been found to be associated with increased tumor progression and metastasis. They are the emerging targets for therapeutic intervention. Intratumoral macrophages can be derived from macrophages present in the tissue or from tumor-induced myeloid cells that migrate to the tumor and differentiate into macrophages. In early tumorigenesis, resident macrophages may be part of the first response, in which they bind to other innate immune cells to initiate an inflammatory response that can coordinate adaptive immune responses and, in some cases, promote progression. In established tumors, resident macrophages or bone marrow-derived macrophages (BMDMs) tend to polarize to an M2-type or activated phenotype rather than an inflamed M1 state. This M2 wound-healing phenotype promotes tumor progression and metastasis by secreting growth and angiogenic factors, remodeling the extracellular matrix,

and suppressing immune responses. In contrast, M1-activated macrophages inhibit tumor development and tumor growth through direct effects (*e.g.*, secretion of reactive oxygen species [ROS]) or promotion of Th1 responses [7,8]. Macrophages have two functional phenotypes: classical activated macrophages, referred to as M1 macrophages, and alternative activated macrophages, referred to as M2 macrophages. In the pro-inflammatory circle, M1 macrophages are the main macrophages. The inducers of such macrophages include lipopolysaccharide (LPS), interferon gamma (IFN- $\gamma$ ), tumor necrosis factor alpha (TNF- $\alpha$ ), granulocyte-macrophage colony-stimulating factor (GM-CSF), *etc.*, which have the ability to present antigens, secrete a large amount of pro-inflammatory cytokines, such as IL-12, IL-23, and TNF- $\alpha$ , as well as other chemokines, produce nitric oxide (NO), and express inducible nitric oxide synthase, thus showing an inflammatory phenotype. Inducing factors of M2 macrophages include IL-4, IL-13, M-CSF, glucocorticoids, IL-10, *etc.* They participate in the body's balance process, such as the formation of new blood vessels, and the body's tissue remodeling and wound healing process. They highly express the anti-inflammatory cytokine IL-10 and characteristic factors, upregulate scavenger receptors and CD163, produce ornithine and polyamine, highly express Arg-1, and possess anti-inflammatory properties.

In most cases, there are two main strategies to achieve therapeutic goals by targeting TAMs. The first strategy is to reduce the density of TAMs in tumor tissue, while the second is to induce TAMs to switch from the M2 phenotype to the M1 phenotype. In breast cancer, high levels of IL-10, a marker for M2-type macrophages, have been detected *in vivo*. In addition, genetic mapping data have revealed that TAMs in breast cancer have M2-like properties. This M2-like polarization has even been observed in some cases of brain metastases from breast cancer [9], and the source of TAMs is largely dependent on the tumor type. In breast cancer models, newly recruited monocytes differentiate into TAMs, whereas in brain cancer models, TAMs originate from blood-derived monocytes and resident macrophages [10]. When TAMs are produced from monocytes recruited in the circulation, tumor cells need to secrete factors that prompt monocytes to migrate to the tumor site. In general, the phenotype of TAMs in the TME is highly plastic. In a study, human breast cancer cells were found to predispose TAMs to an anti-inflammatory phenotype by secreting M-CSF [11], whereas in an *in vivo* model of BALB/c4T1 breast cancer, tumor microenvironmental conditions promoted the differentiation of monocyte precursors into different subsets of TAMs [12-17]. These results suggest that the phenotype of TAMs differs depending on the tumor type and the activation process of TAMs is highly complex and dependent on the microenvironment in which they are located. The proportion of macrophages in tumor tissues can reach up to 50%, and the infiltration of a large number of tumor-associated macrophages is closely related to poor prognosis. Tumor-targeted therapy for macrophages has achieved excellent results in clinical trials, but its efficacy in clinical treatment is limited. At present, a variety of tumor-targeted therapy methods for tumor-associated macrophages are under investigations. The more important ones include inhibiting the recruitment of macrophages, activating the anti-tumor activity of macrophages, killing and clearing macrophages cells, as well as reversing the phenotype of macrophages.

Many cytokines promote the recruitment of tumor-associated macrophages. The CCL2/CCR2 axis plays a very important role in the recruitment of macrophages. Therefore, targeting this pathway could potentiate effective tumor therapy. In animal models, CCL2 blockers (carlumab, CNTO88) have been shown to inhibit the growth of various tumors. In addition, the chemokine CSF-1 has significant effects on the growth, differentiation, and recruitment of macrophages. Both CSF-1 (GW2580) and CSF-1R inhibitors can significantly reduce the recruitment and infiltration of M2-type cells, thereby achieving the purpose of developing specific targeted tumor therapy. Experiments have found that the activation of proto-oncogenes can increase the expression of CCL-2 and CSF-1 in mice with papillary thyroid carcinoma, leading to the accumulation of TAMs in the tumor area, thereby promoting tumor progression. Targeting the expression of chemokine receptors 2 (CCR-2) cells can reduce the number of TAMs and inhibit tumor progression.

This study showed that hypoxically cultured TAMs significantly enhanced the migration ability of TNBC cells and promoted the metastasis of cancer cells to the lung *in vivo*. TAMs were induced by hypoxic conditions to secrete CCL22. The expression of CCL22 receptor, CCR4, in breast cancer tissues was found to be significantly higher than that in normal tissues.

### Funding

This work was supported by grants from The Medical Science Research Project of Hebei Province (20220282) and the Key Laboratory of Molecular Pathology and Early Diagnosis of Tumor in Hebei Province.

### Disclosure statement

The authors declare no conflict of interest.

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# Therapeutic Effect of Bone Marrow Mesenchymal Stem Cells on Rat Bladder Cancer

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**Abstract:** *Objective:* To analyze the effect of bone marrow mesenchymal stem cell therapy on rats with bladder cancer and provide a feasible direction for the treatment of human bladder cancer. *Methods:* An animal model was constructed, and Model 1 was used as an example. Two groups of rats were injected with anti-upconversion nanoparticles (UCNPs) (experimental group) and 0.9% normal saline (control group), respectively. *In vivo* imaging was performed to determine the accuracy of the anti-UCNPs method. *Results:* There were 15 rats in the experimental group with obvious bladder swelling. Among them, 11 rats had cauliflower-like and partially brown bladder tumors, whereas the other four rats had hard, nodular-like protrusions, with indistinct borders and adhesions to the anterior wall of the rectum. Small papillary masses were observed in two rats, local mucosal thickening without tumor formation was observed in two rats, and bladder stones were observed in six rats. The bladder specimens of 15 rats in the control group were pink and shiny, without any tumors. Fourteen rats in the experimental group and 12 rats in the control group had bladder cancer lesions, accounting for 93.33% and 80%, respectively. The detection accuracy of the experimental group was significantly better than that of the control group. *Conclusion:* Multimodal nanoprobe targeting bladder cancer stem cells *in vivo* were used to image the orthotopic tumor and lymph node metastasis models of animals by anti-UCNPs imaging to observe the distribution, migration, and differentiation process of bladder cancer stem cells in model mice. It is clear that rare earth upconversion luminescent nanomaterials, modified by BCMab1 and CD44 monoclonal antibodies, can be used as probes for the detection of bladder cancer, the tracking of lymph node metastasis in bladder cancer, and the comprehensive evaluation of the overall efficacy of nanoprobe-targeted therapy for bladder cancer stem cells.

**Keywords:** Bone marrow; Stem cells; Bladder cancer

**Online publication:** November 16, 2022

## 1. Introduction

Bladder cancer (BC) is one of the most common malignant tumors with high incidence and high postoperative recurrence rate. A series of inflammatory mediators secreted by inflammatory cells, such as

interleukin (IL) and tumor necrosis factor (TNF), has an effect on tumor metastasis and invasion by causing oxidative stress damage and changes in the tumor microenvironment. This effect is mediated by neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), systemic immune-inflammation index (SII), and other blood inflammatory response markers <sup>[1,2]</sup>. PLR, NLR, *etc.* are independent factors that affect the prognosis of tumors <sup>[3]</sup>. It has been found that systemic inflammatory response (SIR) is also closely related to tumors. In this study, a rat model was used to analyze the effect of bone marrow mesenchymal stem cell therapy on rats with bladder cancer and provide a feasible direction for the treatment of human bladder cancer.

## **2. Materials and methods**

### **2.1. Experimental materials**

Normal mice and immunodeficient mice (nonobese diabetic/severe combined immunodeficiency, NOD/SCID).

### **2.2. Methods**

#### **2.2.1. Construction of animal models**

- (1) Normal mice.
- (2) Model 1: *In situ* tumor formation in immunodeficient mice (NOD/SCID): using the bladder chemical injury (1% HCl solution) and cell suspension transurethral perfusion method.
- (3) Model 2: A distant metastasis model of immunodeficient mice (NOD/SCID): using the tail vein injection method of stably transfected cell lines.
- (4) Model 3: An immunodeficient mice (NOD/SCID) subcutaneous tumorigenesis model.

#### **2.2.2. *In vivo* imaging of animals**

Taking Model 1 as an example, two groups of rats were injected with anti-upconversion nanoparticles (UCNPs) (experimental group) and 0.9% saline (control group), respectively. Then, *in vivo* imaging detection was performed on all 15 rats in each group.

The *in vivo* imaging was performed as follow: (1) reagents were injected through the tail vein of mice; (2) 980 nm wavelength infrared light was used as the excitation source, and the upconversion luminescence signal was detected at a wavelength of  $800 \pm 12$  nm; (3) signal images were detected separately at 30 min, 2 h, and 24 h after injection.

After the mice were sacrificed, tumor tissue sections were taken for histopathological examination (hematoxylin and eosin staining) and immunohistochemical experiments. The experimental results were compared to judge the correctness of the guessed mechanism and verify the accuracy of the anti-UCNPs method.

### **2.3. Observation indicators**

The accuracy of the anti-UCNPs method.

### **2.4. Statistical analysis**

SPSS 25.0 was used for data analysis. The count and measurement data were expressed as n/% and  $\bar{x} \pm s$ , respectively, and  $\chi^2$  and t tests were performed.  $P < 0.05$  was considered statistically significant.

## **3. Results**

### **3.1. Morphological observation of rat bladder**

In the experimental group, 15 rats had obvious bladder tumors, of which 11 rats had bladder tumors that

were cauliflower-like and partially brown, while the other four rats had hard nodular protrusions in their bladder mucosa with blurred borders and adhesion to the anterior wall of the rectum. Small papillary masses were observed in two rats, local mucosal thickening without tumor formation was observed in two rats, and bladder stones were seen in six rats. The bladder specimens of 15 rats in the control group were pink and shiny, without any observable tumors.

### 3.2. Accuracy of anti-UCNPs method

Through histopathological examination, it was found that 15 mice in the experimental group and the control group were all bladder cell cancer variants. Immunochemical experiments were performed on the two groups of mice, respectively. Following immunohistochemical experiments, bladder cancer cells appeared in the experimental group. There were 14 rats with bladder cancer lesions in the experimental group, accounting for 93.33%, and 12 rats with bladder cancer lesions in the control group, accounting for 80%. The accuracy of detection in the experimental group was significantly higher than that in the control group.

## 4. Discussion

Bladder cancer is one of the most common malignant tumors of the urinary system. It ranks tenth among the most common tumors in humans. It often occurs in male, in which its incidence rate is 4:1. The incidence of bladder cancer is affected by various factors, including heredity, environment, diet, genes, and other factors. At present, the clinical treatment of bladder cancer is mainly based on the combination of surgery, radiotherapy, systemic chemotherapy, and immunotherapy. However, the prognosis remains relatively poor [5,6]. In recent years, the prevention and treatment of tumors by traditional Chinese medicine has gradually attracted widespread attention. The disease name “bladder cancer” has not been clearly stated in ancient Chinese medicine books, but it can be classified according to its clinical manifestations. It belongs to the categories of “long closure,” “hematuria,” “drowned blood,” and “blood stranguria” [7-9]. The active ingredients of traditional Chinese medicine have low toxicity, less side effects, multiple targets, and multiple pathways in the treatment of tumors. In the treatment of bladder cancer, the focus should be on each molecular change involved in the tumor and the regulation of signaling pathways, which is closely related to the occurrence and development of tumors [10-12].

BCMab1+/CD44+ bladder cancer stem cells were sorted by flow cytometry and cultured *in vitro*; multimodal nanoprobe (anti-UCNPs) with different concentration gradients were added to study the interaction between multimodal nanoprobe and bladder cancer stem cells and observe the endocytosis effect of cells on the probe, the subcellular localization of the probe, as well as the effect of the probe on cell proliferation and differentiation. It is clear that the upconversion luminescence *in vivo* imaging system can be used to detect BCMab1 and CD44 monoclonal antibody-modified rare earth upconversion luminescent nanomaterials that have high luminescence efficiency and bladder cancer specificity *in vivo* [1,2].

In this study, it was found that (1) aberrantly glycosylated integrin  $\alpha 3\beta 1$  in bladder cancer cells can be specifically recognized by the monoclonal antibody BCMab1; (2) BCMab1 can significantly inhibit tumor proliferation, migration, and adhesion; (3) 1% of the total BCMab1+/CD44+ subset has strong self-renewal ability and differentiation potential; (4) GALNT1-mediated glycosylation of sonic hedgehog (SHH) protein is necessary for Hedgehog signal activation; (5) intravesical instillation of *GALNT1* siRNA and SHH-targeted inhibitors can effectively inhibit the occurrence and development of bladder tumors; (6) bladder cancer stem cells originate from bladder epithelial stem cells and bladder cancer non-stem cells. We use this feature of BCMab1 monoclonal antibody to design and prepare upconversion luminescent nanomaterials (anti-UCNPs) linked to the antibody. Using this material as a biological probe, the diagnosis of bladder cancer and the tracking and detection of lymph node metastasis can be performed non-invasively,

targetedly, and efficiently in animals by means of *in vivo* fluorescence imaging.

Some studies have found that smoking is the main pathogenic factor for bladder cancer, and about 50% of bladder cancer is caused by smoking [12]. Although the impact of smoking on the prognosis of bladder cancer is controversial, a number of studies believe that smoking increases the incidence and mortality of bladder cancer with significant gender differences; moreover, the incidence of women is lower than that of men. Previous studies have shown that when the stage of the cancer is the same, the prognosis of men is better than that of women. Several studies have shown that bladder cancer is more common in the elderly, in which most of them are over 70 years old [14,15]. For Ta~T1 bladder tumors with longer life expectancy, due to the higher risk of tumor progression, such as when the tumor is large or numerous, total cystectomy is usually recommended. Studies have reported that the long-term cancer-specific survival rate of patients undergoing immediate total cystectomy is as high as 85% to 90%. The aforementioned studies have confirmed the safety and efficacy of total cystectomy in the treatment of patients with bladder cancer. It has been reported that postoperative intravesical chemotherapy in patients with bladder cancer has a positive effect on preventing the recurrence of postoperative bladder cancer. The recurrence rate of bladder tumors within 2 years was found to be lower than those who did not receive intravesical instillation.

Bladder cancer can be divided into muscle-invasive bladder cancer (MIBC) and non-muscle-invasive bladder cancer (NMIBC) [13,14]. The primary treatment of bladder cancer is still surgery or the former in combination with other comprehensive treatments [15]. However, 70–80% of NMIBC patients relapse or progress within 5 years after surgery [16], with 10–20% of patients progressing to MIBC or distant metastatic disease [17]. Both radiotherapy and chemotherapy have significant side effects and lack selectivity. The emerging second-line treatments, such as immunotherapy and targeted therapy, have yet to be accepted due to their high cost and low patient response rate [18,19].

Nanomaterials have become one of the new directions of tumor therapy due to their strong ability to penetrate tumor tissue, low immunogenicity, and long circulation time in the blood. ROS is a general term for oxygen-containing free radicals and peroxides that easily form free radicals, which are related to oxygen metabolism in the body. Low concentrations of ROS play an important role in regulating signaling pathways, eliminating pathogens, regulating inflammation, and promoting cell proliferation. However, when ROS concentrations are high, they may damage nucleic acids, proteins, or cell membranes, thus leading to cell death. Glutathione (GSH) is one of the most important antioxidants in cells, which can protect cells from ROS damage. The level of ROS in tumor cells is often higher than that in normal cells, which leads to an adaptive increase in the level of antioxidants, such as GSH, in the body. As a result, ROS and GSH maintain a dynamic balance at high concentrations, and the cells tend to be in a state of oxidative stress.

The inherent ROS concentration in tumor cells is high. When both tumor cells and normal cells are exposed to the same amount of external ROS, the ROS level in tumor cells will easily reach the threshold of triggering cell death. On the other hand, the inherent ROS concentration in normal cells is low, and thus able to buffer a certain amount of ROS without triggering cell death.

In conclusion, multimodal nanoprobe-targeted *in vivo* tracking of bladder cancer stem cells in animal models of orthotopic tumor and lymph node metastases were used for anti-UCNPs imaging to observe the distribution, migration, and differentiation of bladder cancer stem cells in model mice. It is clear that rare earth upconversion luminescent nanomaterials, modified by BCMab1 and CD44 monoclonal antibodies, can be used as probes for the detection of bladder cancer, the tracking of lymph node metastasis in bladder cancer, and the comprehensive evaluation of the overall efficacy of nanoprobe-targeted therapy for bladder cancer stem cells.

## Funding

This research received financial support from the Key Laboratory of Molecular Pathology and Early Diagnosis of Tumor in Hebei Province, the Beijing-Tianjin-Hebei Basic Research Cooperation Special Project (2019), “Visual Stem Cell Targeted Tumor Therapy Techniques for Precise Diagnosis and Treatment of Tumors” (Project Number: 19JCZD-JC65800[Z]).

## Disclosure statement

The authors declare no conflict of interest.

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# Analysis of the Epigenetic Mechanism and Treatment of Huntington's Disease

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**Abstract:** Huntington's disease (HD) is an irreversible neurodegenerative disorder that is inherited in an autosomal dominant manner. In HD, many regions of the human brain are affected, including the striatum, thalamus, and cortex. The mechanism is by the expansion of CAG repeats, which encode glutamine (Q) in the Huntingtin gene on chromosome 4p16.3. Patients with more CAG repeats tend to have a younger age of onset and a higher risk. Mutant HTT protein, translated from mtHtt, would congregate or interact with other proteins, causing damage to the human body. Patients with HD show symptoms like chorea, which is an involuntary motor disability, cognitive deterioration, and psychiatric disturbances. Except for the genetic pathology of HD, the epigenetic mechanism of this disease has made a lot of progress in recent years. This paper primarily focuses on the alternation of deoxyribonucleic acid (DNA) methylation, histone modification, and non-coding ribonucleic acids (ncRNAs) in HD as well as the advancements of epigenetic therapy and healthcare in HD.

**Keywords:** Huntington's disease; Epigenetics; DNA methylation; ncRNAs; Healthcare

**Online publication:** November 17, 2022

## 1. Introduction

"Epigenetics" was first termed by Conrad Waddington in the 1940s whose great passion for the genetic field was evident <sup>[1]</sup>. He introduced epigenetics as the process of genotype expressed as phenotype <sup>[2]</sup>. Differing from genetics, epigenetics does not involve the change in deoxyribonucleic acid (DNA) sequence but studies the change in gene modification. The majority of epigenetics studies focus on DNA methylation, histone modification, and non-coding ribonucleic acids (RNAs) <sup>[3]</sup>. DNA methylation is a covalent modification of the DNA base, of which the most common is cytosine methylation and adenine and guanine methylation. DNA methyltransferase (DMNT) is responsible for DNA methylation that usually occurs in CpG islands <sup>[4]</sup>. Histone modification is a posttranslational modification of histone, including histone methylation, acetylation, ubiquitination, and phosphorylation. Different enzymes are expressed in the modification to regulate the structures of chromosomes and their functions <sup>[5]</sup>. Non-coding RNAs are RNAs that are unable to translate into proteins; they are found with other functions in gene expression <sup>[4]</sup>. Non-coding RNAs show alternations in different diseases. These modifications can regulate gene expression to cause diseases or symptoms in patients. Epigenetics can be changed by the environment, in which the change is known to be reversible. Therefore, epigenetic therapy has been extensively studied by scientists <sup>[4]</sup>, especially in relation to cancer, aging, cardiovascular disease, neurodegenerative diseases, and other diseases.

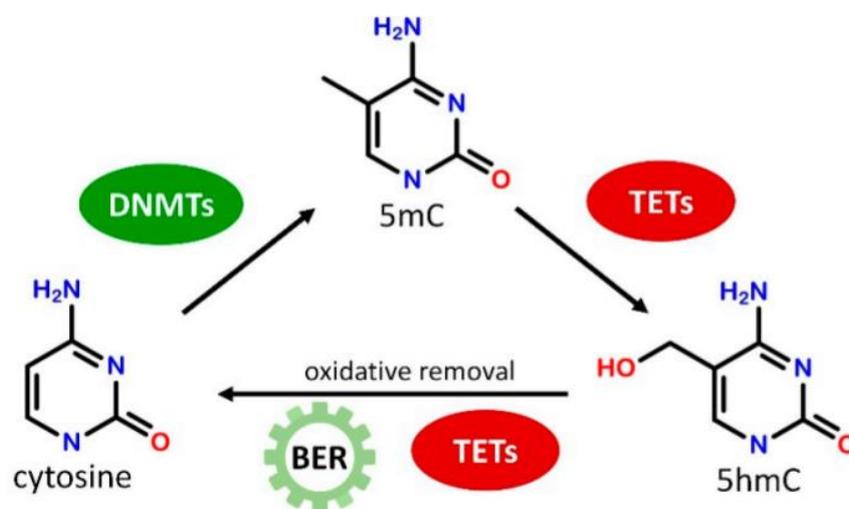
Huntington's disease (HD) is a hereditary neurodegenerative disease that occurs worldwide. HD is still known as an incurable disease today. The aim of studying the epigenetic mechanism of HD is to make advances in HD treatment, targeting at the modification and alteration of the specific gene. This paper will discuss the DNA methylation, histone modification, and alternation of ncRNAs in HD as well as the epigenetic therapy and healthcare of HD, hoping to offer some references for future research.

## 2. Analysis of the epigenetic mechanism of Huntington's disease

### 2.1. DNA methylation

DNA methylation is the most well-studied epigenetic mechanism in mammals. It plays a key role in activating and inhibiting genes [6]. DNMT is the main enzyme that transfers the methyl group from the donor S-adenosylmethionine (SAM) to 5' cytosine on the CpG island [7]. While DNMT1 takes charge of maintenance methylation in DNA replication, DNMT3a and DNMT3b are responsible for *de novo* methylation [4]. 5' methylcytosine (mC) can oxidize to 5' hydroxymethylcytosine (hmC) [8]. Generally, the inhibition of transcription is caused by adding 5mC in the promoter region; the activation of transcription is linked to a greater 5hmC [9]. DNA methylation in a promoter directly disturbs the interaction between transcription factors and the gene sequence, thus transcription is disrupted. On the other hand, the methyl group binds with methylcytosine-binding domain (MBD) to recruit repressive protein complex and histone deacetylase (HDAC), which would modify it into inactive heterochromatin to inhibit transcription [10].

As shown in **Figure 1**, methyl groups can be transferred to 5' cytosine by DNMTs to make 5mC and further modified into 5hmC by ten-eleven translocation (TET) proteins. 5hmC can also be converted back into cytosine by oxidative removal.



**Figure 1.** Methylation and hydroxymethylation of cytosine [11]

In a study, Ng *et al.* [12] used reduced representation bisulfite sequencing (RRBS) to monitor the change in DNA methylation between wildtype (STHdhQ7/Q7) and mutant HTT cell (STHdhQ111/Q111). DNA methylation can be divided into those that occur in CpG rich regions and CpG poor regions. CpG rich region often refers to promoter sequence, and in this region, the change of DNA methylation happens more frequently. Genome-wide chromatin immunoprecipitation sequencing (ChIP-Seq) was used to study the transcription level. The DNA methylation in CpG islands has a negative relationship with gene expression. It has been demonstrated that the relatively higher level of methylation in the promoter region of Ap-1, Sox 2, Pax 6, and Nes gene but lower expression in STHdhQ111/Q111 than that in wild type STHdhQ7/Q7. Meanwhile, the linkage between gene expression and the DNA methylation level in CpG poor regions

would be more complicated. The change of DNA methylation may be caused by obtaining and losing DNA-binding proteins. It has also been confirmed that HTT protein is able to bind with DNA sequence, which may directly influence the epigenetic mechanism. Some of these genes with lower expression are related to neurons and may also play a role in other neurodegenerative diseases; therefore, DNA methylation is one of the reasons to explain the cognitive deterioration in HD patients [13].

In addition, the expression of adenosine A2a receptor has been observed to be reduced in HD. Experiments have been performed to test the 5mC and 5hmC content in A2a gene in a mice model and a human brain sample. It has been shown that the reduced expression is accompanied by less 5hmC in the 5'UTR region in the R6/1 mice model, whereas 5mC was more with less 5hmC in HD patients [14].

In recent years, DNA methylation other than on 5' cytosine has also been studied. 7-methylguanine is a newly found epigenetic mechanism which might be related to the pathology of HD. Unlike 5mC, higher gene expression is shown with more methylation on guanine, and it would occur both on DNA and RNA. Through the experiment, the level of 7-methylguanine has been observed to have had changed in HD patients and mice models [15].

## 2.2. Histone modification

The role of histone proteins is to package DNA, which wraps around eight histone proteins into nucleosome, chromatin, and chromosomes. Histone modification refers to the posttranslational modification (PTM) of histone proteins, including methylation, phosphorylation, acetylation, ubiquitylation, and SUMOylation [16]. The modification of histone proteins may impact various cellular processes, such as transcription activation and repression, DNA repair, and chromosome packaging [17]. Generally, acetylation of lysine residue leads to transcription activation, while methylation of lysine and arginine residues leads to transcription repression [16]. The combination of core histone modifications creates a precise pattern that turns on or off specific genes. This is referred to as histone code [18]. Transcriptional dysregulation is a well-known pathogenic feature of HD, but its underlying epigenetic mechanism remains unclear.

Histone acetylation is a well-studied mechanism of histone modification, which have been found to be associated with numerous diseases. Histone acetylation and deacetylation are regulated by two enzymes, histone acetyltransferase (HAT) and histone deacetylase (HDAC), working in corporation with each other to regulate chromatin structure and gene transcription. HAT activity leads to a more opened chromatin structure that increases gene transcription, whereas HDAC activity condenses chromatin and leads to decreased gene transcription [19].

Recent studies have revealed that histone acetylation plays an important role in HD pathogenesis [20]. Researchers have observed reduced histone acetylation level in several HD models in the early 2000s. Furthermore, CREB-binding protein (CBP), a HAT, has been detected in Htt aggregates in HD mice models and brains from individuals with HD [18]. These findings have garnered widespread attention as they could potentially explain the cause of transcriptional dysregulation in HD.

As shown in **Figure 2**, CBP is recruited by phosphorylated CREB in normal conditions, leading to histone acetylation. In HD, abnormal Htt expression would bind to CREB and CBP, thus inhibiting the functions of HAT and reducing the acetylation level. Low acetylation level leads to a more condensed structure with less gene expression.

Further studies have provided a deeper insight into the cause of hypoacetylation seen in HD. Mutant Htt has been shown to interact with both HAT and HDAC. Due to CAG repeats in HD genes, mutant huntingtin proteins are produced, which are then cleaved by caspases to form huntingtin fragments [18]. These fragments can be transported into the nucleus of neurons and aggregated into neuronal inclusions (NIs). NIs has been shown to trap transcription factors, thus disrupting transcription of genes that are critical to cell survival. Researchers have identified CBP as one of the molecules that is associated with NIs. When

trapped by NIs, CBP will be rendered incapable of opening the chromatin structure and would fail to allow the binding of transcription factors, thereby disrupting transcription [20]. Sequestered CBP in HD affects the transcription of tumor suppressor protein p53, which leads to abnormalities in gene transcription and potential cell death.

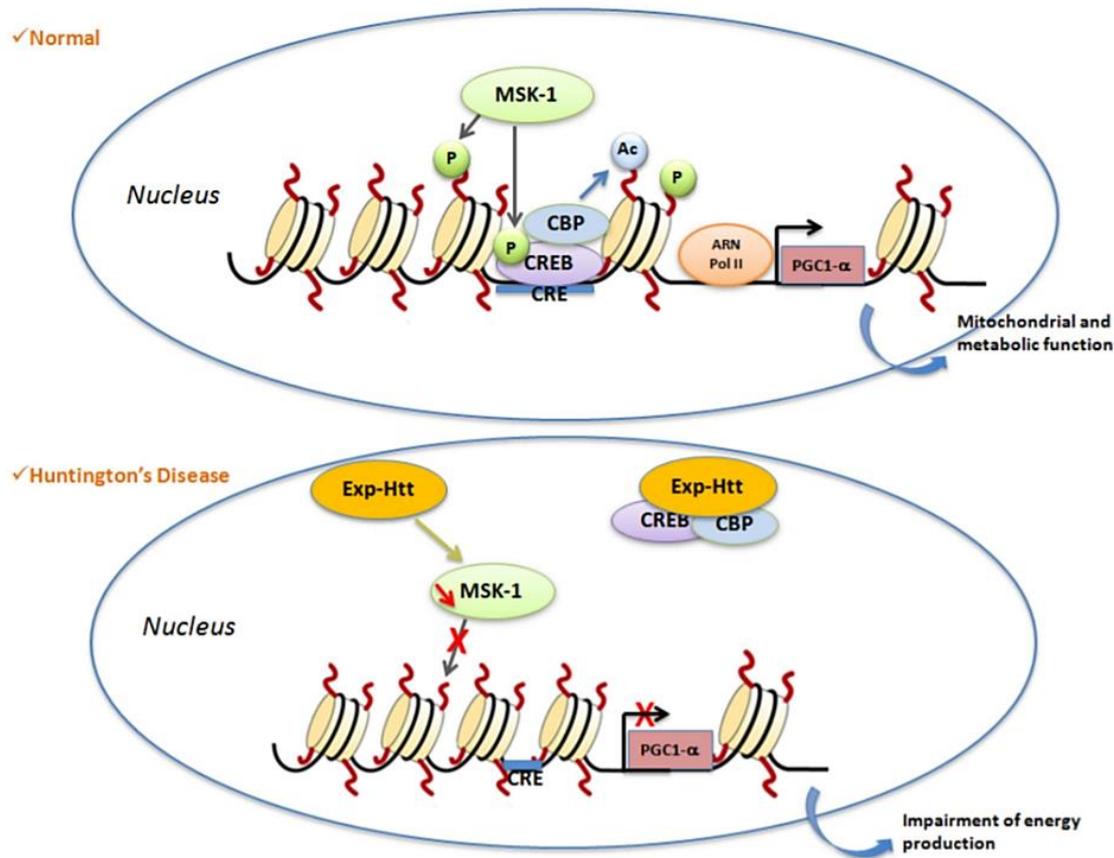


Figure 2. Histone acetylation in normal and pathologic conditions [21]

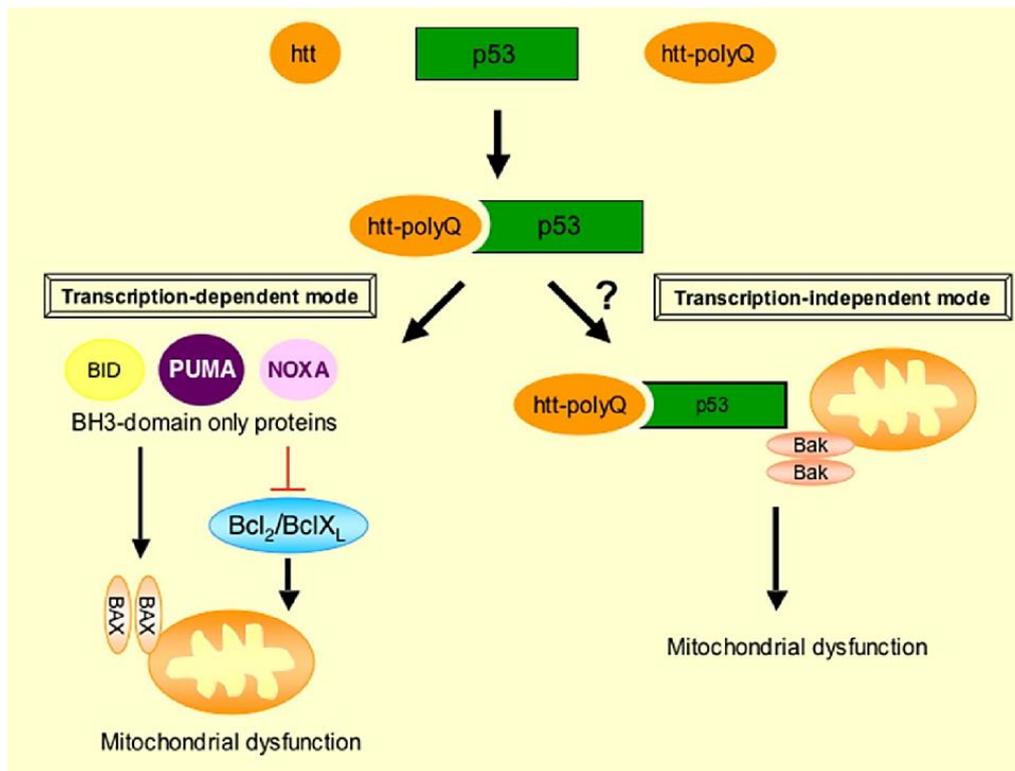
### 2.3. Alternation of non-coding RNAs

Non-coding RNAs are considered a group of RNA molecules that are not translated into proteins. They include transfer RNAs (tRNAs), ribosomal RNAs (rRNAs), long ncRNAs (lncRNA), microRNAs (miRNAs), and so on, with each having a different function. However, many of them work on gene silencing [22].

A recent study on ncRNAs in HD has demonstrated lower levels of miRNA, such as miR-22, miR-29c, miR-125b, miR-128, and others, in HD patients and models [4]. However, the impact of the regulation of each ncRNA in HD has yet to be established. The study has also shown an increase in their target mRNAs. Both miR-125b and miR-150 target p53, which increases the level of p53 in the nucleus. p53 is an important tumor suppressor gene, which is also found in the central nervous system. Therefore, p53 is able to interact with mtHtt and may cause neuronal damage in HD patients [23].

Figure 3 shows that the interaction between mutant Htt and p53 can cause mitochondrial dysfunction in both transcription-dependent and transcription-independent pathways.

The protein REST/NRSE interacts with HTT, but the expansion of repeats in mtHtt inhibits the binding, causing accumulation of REST in the nucleus. The interaction between REST and RE1 may cause damage in transcription. REST can also regulate certain miRNAs, like miR-132, which are lower in HD patients. This might explain the alternation of miRNAs [25].



**Figure 3.** Mitochondrial dysfunction as a result from the interaction between p53 and mtHtt [24]

### 3. Potential treatment

Epidemiological studies have shown that HD has much higher prevalence in western regions (10.6–13.7 individuals per 100,000) compared with that in Asia (1–7 per 1,000,000). This is attributed to genetic distinctions among populations. Data have shown that western ancestry has greater average CAG repeats with 18.4–18.7 in *HTT* genes, and less in Asian ancestry (16.9–17.4) [26].

HDAC inhibitors are considered a group of molecules that can inhibit the enzymatic activity of HDACs. The action of HDAC inhibitors causes an overall increase in histone acetylation levels, and thus activates the transcription of genes that have been previously silenced through histone deacetylation. HDAC inhibitors are commonly used in cancer therapy, as they can compensate the silencing of tumor suppressor genes [18]. These findings, together with the epigenetic mechanism of transcriptional dysregulation in HD, motivates researchers to explore the potential therapeutic effect of HDAC inhibitors in HD [27].

Several recent studies using cellular, drosophila, and mouse models have revealed some promising therapeutic effects of HDAC inhibitors on HD. Steffan *et al.* used a transgenic *Drosophila* model that expresses mutant Htt [28]. These Htt mutant fruit flies showed neuronal degeneration and reduced survival rate, of which both characteristics have also been observed in HD patients. Steffan *et al.* administered HDAC inhibitors including sodium butyrate and suberoylanilide hydroxamic acid (SAHA) to the HD flies, and they observed increased global histone acetylation level, reduced neural degeneration, and increased survival rates in HDAC-treated flies. Hockly *et al.* performed similar experiments using a HD mice model. The results showed that SAHA-treated mice had better motor coordination compared to the placebo group [28].

The therapeutic effect of SAHA has also been studied at the molecular level. Mielcarek *et al.* [28] have also performed experiments that showed a reduction in HDAC4 levels in the brain stem and cortex of HD model mice with the use of SAHA. SAHA has also been proven to reduce mutant Htt aggregation and partially restore the level of brain-derived neurotrophic factor (BDNF), a key protein for cell survival and growth, which is inhibited in HD [29].

Although HDAC inhibitors such as SAHA have shown promising therapeutic effects, significant weight loss has been found to be a potentially dangerous side effect, which hinders the development and clinical applications of SAHA-based HD treatment. Fortunately, Thomas *et al.* have demonstrated that HDAC inhibitor (HDACi) 4b shows excellent therapeutic effects when administered to HD mice [30]. HDACi 4b-treated HD mice displayed improved motor coordination with less weight loss. Furthermore, HDACi 4b also compensated for other negative effects caused by HD. Normally, the overall brain size is reduced in HD mice; however, when HD mice were treated with HDACi 4b, normal-sized brains were observed in these mice. The low toxicity of HDACi 4b makes it a promising candidate for potential HD treatment, which can be used in further phases of clinical trials.

Like most neurodegenerative diseases, HD lacks specific treatment. At present, HD is treated symptomatically. Although there is no effective drug to delay the progress of HD, reasonable drug treatment can be used to improve symptoms, such as chorea and mental disorders, to varying degrees as well as the quality of life of patients, while preventing complications. Therefore, even in the absence of effective treatment at this stage, we should pay attention to symptomatic treatment, which focuses on relieving psychological and neurological symptoms, in addition to the necessary supportive treatment, so that these patients and those who may be ill would be able to build self-confidence, assist one another, and create an optimistic family.

#### **4. Conclusion**

This paper describes the lower gene expression in HD patients with higher 5mC level and puts forward the discoveries of alternating 5hmC and 7-methylguanine, and their impact on HD mice models and human samples; the transcriptional dysregulation in HD patients caused by aggregation of CBP as HAT, and the interaction between mutant Htt fragments and neuronal inclusions; as well as the decreasing levels of ncRNAs followed with higher levels of targets like p53, which is the reason for neuronal damage, and REST protein in the nucleus, which can regulate certain miRNAs that are influenced by mtHtt. Htt protein itself interacts with several transcription factors and epigenetic regulators. Therefore, it has been hypothesized that the reversal of epigenetic marks associated with HD may restore, at least partially, the normal transcriptional program and ameliorate the pathological phenotype. Potential treatments like sodium butyrate and SAHA, focusing on HDAC inhibitors, have shown optimistic results through experiments. Using various HD models and next-generation sequencing technologies, researchers have uncovered more insights about the possible causes of transcription dysregulation in HD. Although these studies have shown strong correlation between altered epigenome and HD phenotype, our understanding of the HD epigenome is still far from satisfactory. It is still unclear to us to what extent epigenetic alteration plays a causal role in transcriptional dysregulation.

Currently, only a few epigenetic marks have been analyzed using genome-wide approaches, in which these findings highlight the complexity of HD epigenome. A comprehensive analysis of HD epigenome using genome-wide techniques is crucial to widen our understanding of HD pathology.

#### **Acknowledgements**

We would like to thank everyone who have spent their time helping us complete this review article and providing us much patience and support. Firstly, we would like to show our gratitude to Dr. Wang from John Hopkins University who taught us the basic theory of epigenetics and the relationship to diseases during the summer, as well as our teaching assistant who was with us through the whole course, helping us understand the course materials. Finally, we would also like to thank Cuihong Wang who supervised and helped us complete this article.

## Disclosure statement

The authors declare no conflict of interest.

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# Adenoid Basal Carcinoma of the Cervix with Squamous Differentiation: A Case Report and Literature Review

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**Abstract:** Adenoid basal carcinoma (ABC) of the cervix is a rare and low-incidence low-grade cervical cancer. In our practice, we encountered a case of cervical ABC with squamous differentiation and high-grade squamous intraepithelial lesion (HSIL) with gland involvement in the peripheral cervix. Reviewing relevant literature and analyzing its clinical manifestations, pathological morphology, and immunohistochemical characteristics would help deepen the understanding of this malignant tumor, so as to make a comprehensive diagnosis with differential diagnosis and prevent misdiagnosis.

**Keywords:** Adenoid basal carcinoma of cervix; Squamous differentiation; Immunohistochemistry

**Online publication:** November 17, 2022

## 1. Case study

A 64-year-old lady was admitted to a local hospital in view of postmenopausal bleeding. Internal examination revealed no abnormalities in the uterus and its appendages, including tenderness (negative), cervical atrophy, mild erosion, friability, and bleeding. Pelvic ultrasound showed postmenopausal uterus, with normal size and shape, the lining of the uterus showed strong linear echo, and the muscle layer showed uniform echo, with no obvious space-occupying lesions or abnormal echoes in the appendages. Cervical HPV showed high-risk P16 positive, ThinPrep cytologic test (TCT) showed high-grade intraepithelial lesions (HSIL) with severe inflammation. Cervical biopsy showed high-grade intraepithelial neoplasia (CIN III) at points 3, 6, 9, and 12 of the cervix. Total hysterectomy + bilateral salpingo-oophorectomy + pelvic lymph node dissection was performed.

### *Pathological examination*

#### (1) Gross examination

The excised uterus was 6 cm × 3.5 cm × 2.5 cm. The length of the cervical canal was about 1.5 cm with an outer diameter of 1.8 cm. No obvious mass was seen under the naked eye. The endometrial thickness was about 0.1 cm; the muscle wall thickness was about 1.5 cm; the size of the left ovary was 2.8 cm x

1.8 cm x 0.4 cm; the cut surface was solid, gray, and white; and the quality of the section was of medium quality. The right oviduct was about 5.8 cm long and 0.3 cm in diameter.

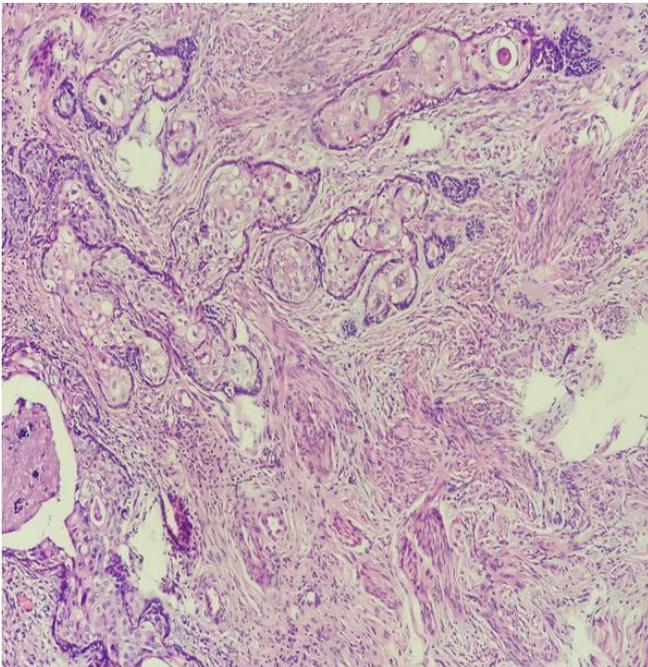
(2) Microscopic findings

Adenoid basal cell carcinoma of the cervix with squamous differentiation, and high-grade intraepithelial neoplasia (HSIL) with gland involvement in the peripheral cervix. In the underlying fibrous stroma, small nests of infiltrating tumor cell clusters were seen, some of which were solid, while some were glandular structures; some glands showed squamous cell metaplasia (**Figure 1**). Tumor cells with small cell body, little cytoplasm, round or oval hyperchromatic nuclei, inconspicuous nucleoli, and rare mitotic figures (**Figure 2**) were observed, along with surrounding HSIL areas (**Figure 3**).

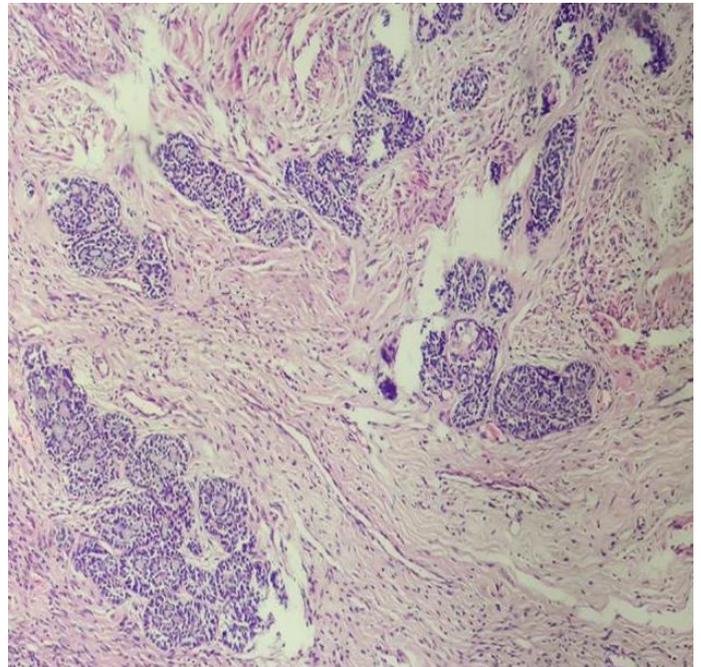
(3) Immunohistochemistry

Carcinoembryonic antigen (CEA, scaled +), Ki-67 (+ 40%), cytokeratin (CK)7 (scaled +), P63 (+), P40 (+), cluster of differentiation (CD)117 (-), and P16 (+) (**Figure 4–10**).

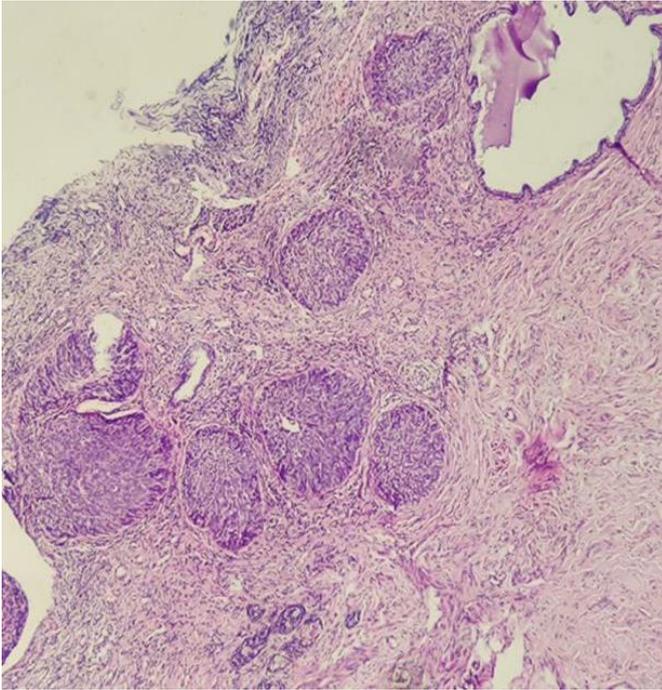
Pathological diagnosis: adenoid basal cell carcinoma of the cervix with squamous differentiation, and high-grade intraepithelial neoplasia (HSIL) with gland involvement in the surrounding cervix.



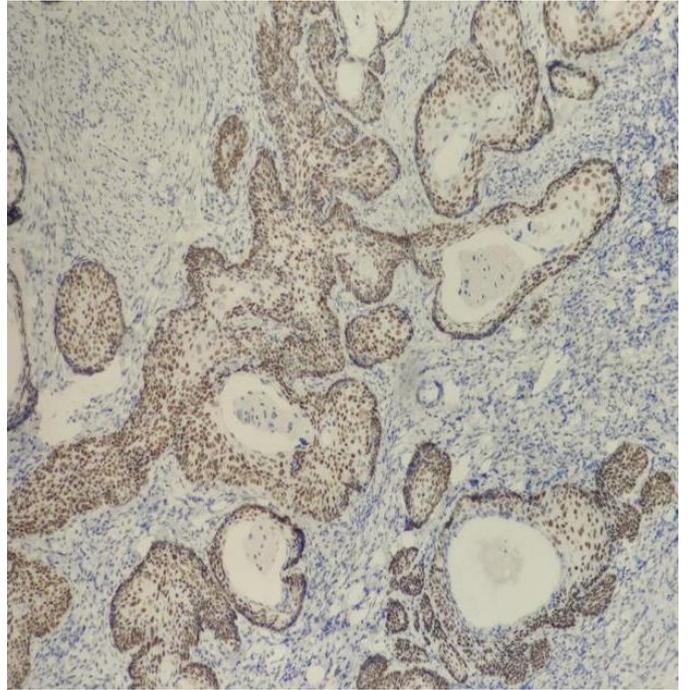
**Figure 1.** Part of the tumor cell mass having gland-like structures, with squamous metaplasia seen in some glands



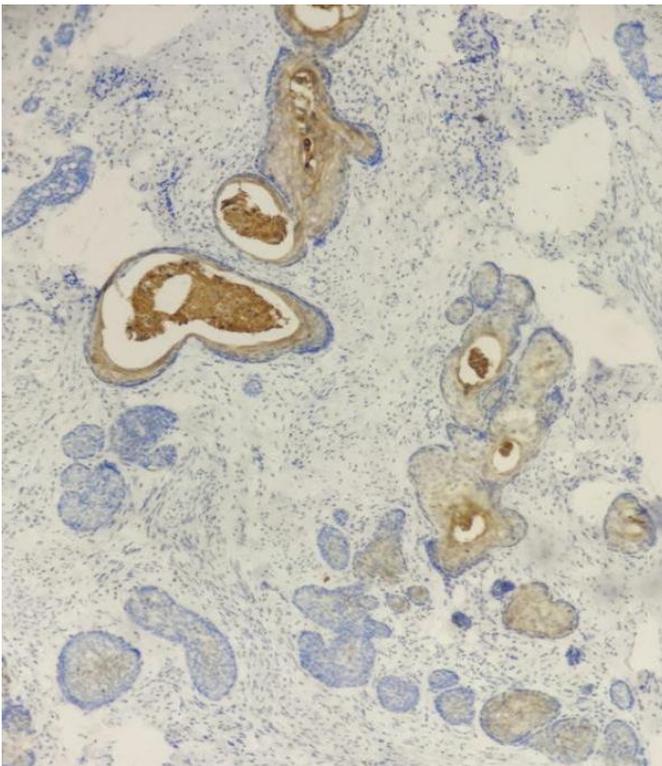
**Figure 2.** Tumor cells with small cell body, little cytoplasm, round or oval hyperchromatic nuclei, inconspicuous nucleoli, and rare mitotic figures



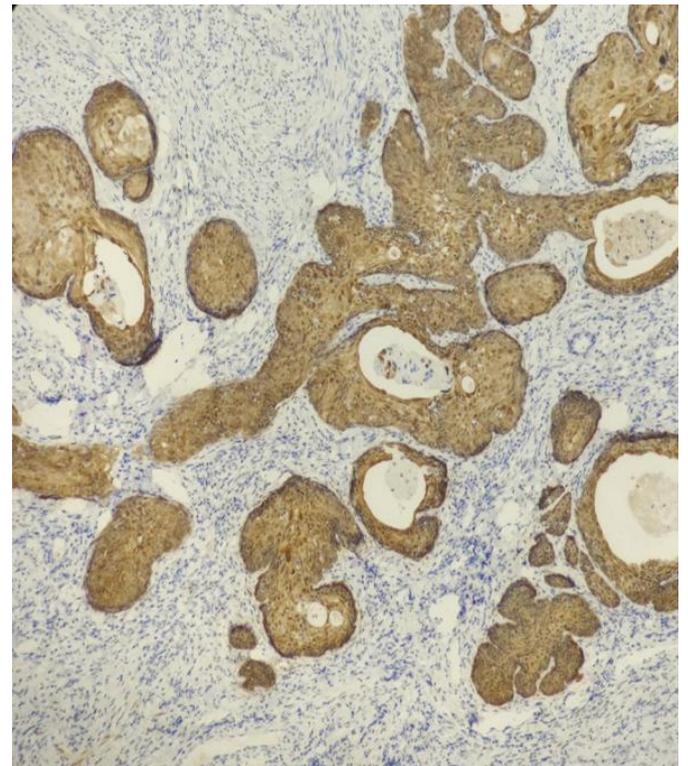
**Figure 3.** Visible surrounding HSIL area



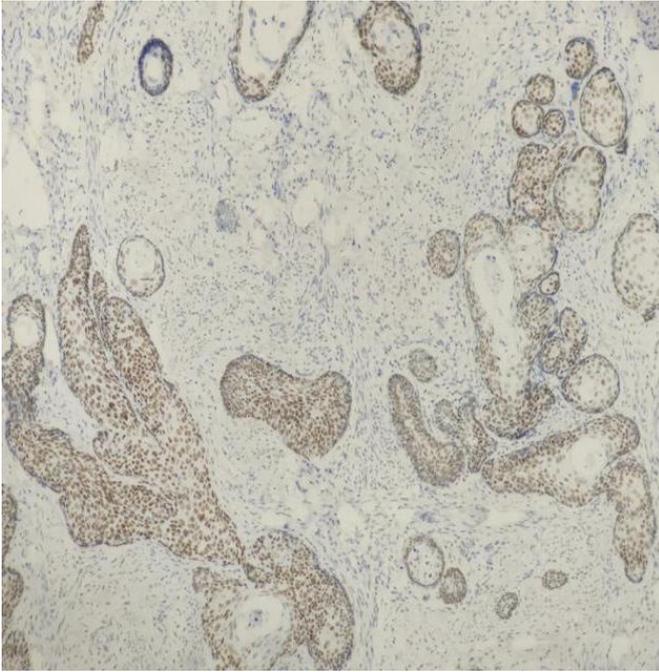
**Figure 4.** P63 positive  $\times 100$  on immunohistochemistry



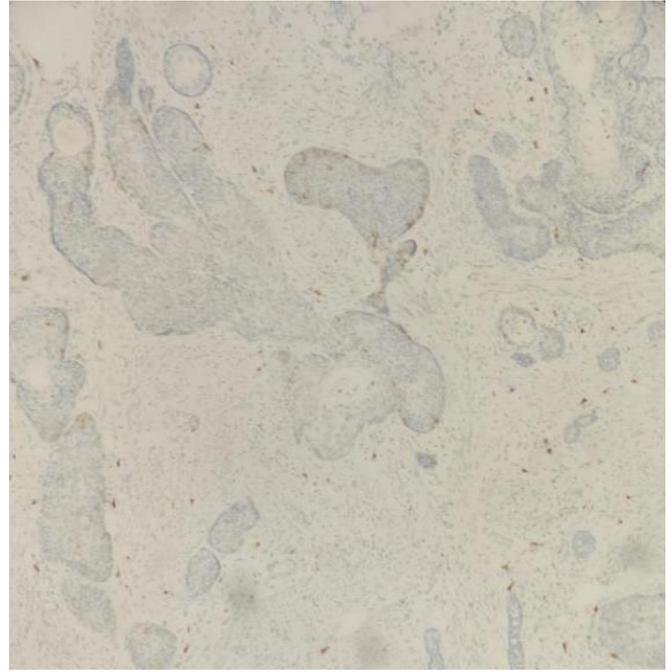
**Figure 5.** CEA sporadic positive  $\times 100$  on immunohistochemistry



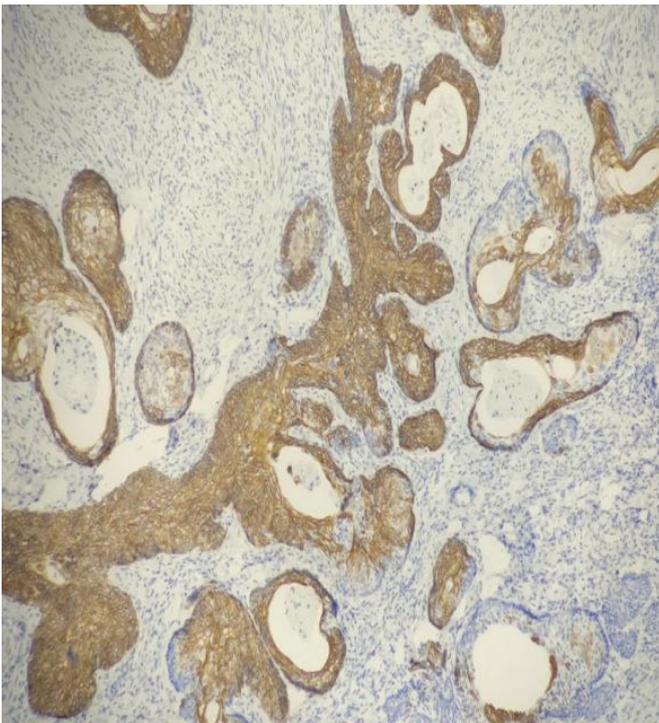
**Figure 6.** P16 positive  $\times 100$  on immunohistochemistry



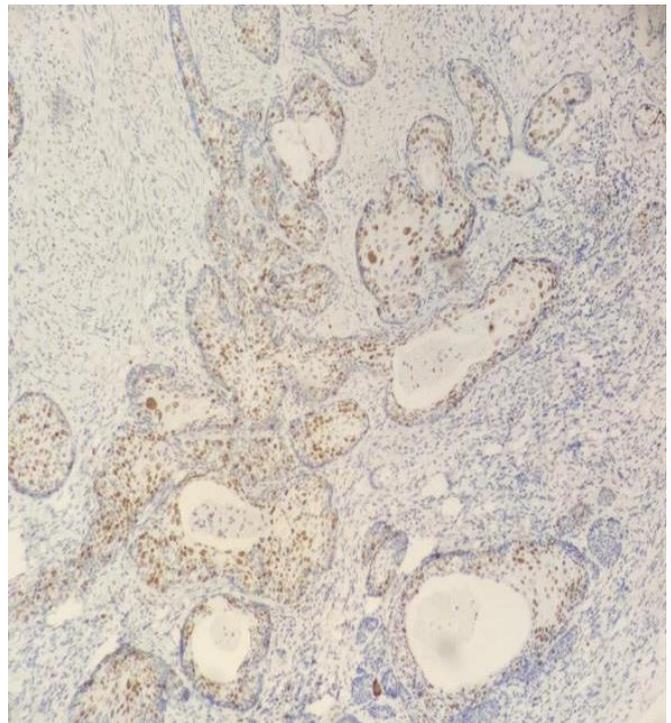
**Figure 7.** P40 positive  $\times 100$  on immunohistochemistry



**Figure 8.** CD117 negative  $\times 100$  on immunohistochemistry



**Figure 9.** CK7 sporadic positive  $\times 100$  on immunohistochemistry



**Figure 10.** Ki67 (40% positive)  $\times 100$  on immunohistochemistry

## 2. Discussion and literature review

Cervical adenoid basal carcinoma (ABC) is a rare cancer with a low incidence. This disease was first reported by Baggish<sup>[1]</sup> in 1966. In 1985, van Dinh and Woodruff<sup>[2]</sup> proposed ABC as an independent tumor. Although the biological behavior of ABC is mostly inert, it often shows invasive growth<sup>[3]</sup>. This cancer commonly occurs in postmenopausal women. With the deepening of research, it has been found that its incidence is gradually increasing in young women<sup>[4]</sup>. The majority of patients with ABC have no specific

clinical manifestations. Some patients present with postmenopausal vaginal bleeding, while others with lower abdominal pain. In the majority of these cases, no obvious mass is observed in the cervix, and there are no specific abnormalities seen in gynecological examination. Some patients visit the doctors because of high-risk HPV infection and/or abnormal cytology screening [5]. In this case, the patient was referred for postmenopausal bleeding, and high-risk HPV infection and abnormal cytology were found upon examination.

With regard to its microscopic features, ABC is often arranged in small nests or cords, and the cell body of the cancer cells is small and round- or spindle-shaped, similar to basal cells, with little cytoplasm, round and oval nuclei, inconspicuous nucleoli, and partial nucleoli. The cells are transparent, and the peripheral cells are arranged in a palisade-like manner; the cells are densely arranged, mostly in a solid mass, with a cavity in the center, similar to a gland-like structure, which can form a cavity or cribriform structure, and occasionally squamous epithelial differentiation [6]. In this case, there was squamous differentiation. ABC alone is rare; in most cases, high-grade squamous intraepithelial lesions co-exist with ABC, with some cases having malignant tumors, such as squamous cell carcinoma, small cell carcinoma, and adenoid cystic carcinoma.

Immunohistochemistry shows that ABC is often positive for BCL-2, CK5/6, and P63, negative for CK7, and strongly positive for P16. According to literature, P16-positive patients are often accompanied by HPV16 infection [7]. In this case, the patient was high-risk P16-positive. On the other hand, P53 is often weakly positive in ABC, but there are a few cases that show strongly positive P53. The immunophenotype of this case is generally consistent with that reported in literature.

In terms of differential diagnosis, cervical ABC needs to be differentiated from several diseases.

#### (1) Basaloid squamous cell carcinoma

Basaloid squamous cell carcinoma is a special subtype of squamous cell carcinoma, with very few primary cervical cases [8]. Under the microscope, it is mainly observed as solid nests or islands; additionally, comedo-like necrotic material can be seen in the center of the cancer nests, with the interstitium showing obvious fibrous connective tissue reaction; cell atypia and mitotic figures are evident, with the peripheral cells of the cancer nest arranged in a palisade [9]. ABC has adenoid-like structures, with cavities and a cribriform arrangement, small and uniform cells, with little atypia, rare mitotic figures, and rarely seen desmoplastic connective tissue.

#### (2) Adenoid cystic carcinoma

Adenoid cystic carcinoma is more common in salivary glands and has a high degree of malignancy [10]. It rarely occurs in the cervix. Two types of tumor cells can be seen under the microscope: glandular epithelial cells and variant myoepithelial cells. The glandular epithelial cells are arranged in adenoids, and variant myoepithelial cells are distributed around them. Pale eosinophilic secretions can be seen in the gland cavity. Some cells are solid and have cribriform arrangement but some may be in tubular arrangement [11]. In terms of immunohistochemistry, glandular epithelial cells express CD117, whereas variant myoepithelial cells express P63 and S-100; adenoid cystic carcinoma is characterized by t(6;9)(q22-23;p23-24) gene translocation, resulting in *MYB-NFIB* fusion gene, expressed as MYB protein overexpression [12]. Cervical ABC differs from adenoid cystic carcinoma in terms of the tumor cells, immunohistochemistry, and molecular detection.

#### (3) Neuroendocrine tumors (NTCs)

NTCs are relatively rare, accounting for about 5% of all cervical malignancies [13]. They have certain characteristics, including strong invasiveness, easy recurrence, and easy metastasis. They are similar to small cell carcinoma of the lung and have poor prognosis [14]. Studies have shown that NTCs are related to HPV infection [15], especially HPV18 [16]. Although carcinoid syndrome and Cushing's syndrome are occasionally seen [15], some studies have reported that cervical NTCs may originate from the reserve

cells under the cervical columnar epithelium <sup>[17]</sup>, mainly manifested as insular, trabecular, organoid, or diffuse flaky distribution, accompanied by necrosis, apoptosis, and a large number of mitotic figures (> 20/10 HPF); small cells with few cytoplasm, hyperchromatic nucleoli, inconspicuous nucleoli, and large cells often with organoid differentiation; as well as medium or large tumor cells, with vacuolar nuclei and large nucleoli. Immunohistochemical expressions of neuroendocrine markers CgA, SSTR-2, Syn, and CD56 are absent in ABC.

The pathological morphology of this case shows typical features of ABC with squamous epithelial differentiation. In this context, attention should be paid to the possibility of well-differentiated squamous cell carcinoma since this patient also has high-grade intraepithelial lesion (HSIL). However, well-differentiated squamous cell carcinoma usually forms papillary or nest-like structures, the cancer cells are arranged in a tiled pattern, keratinized beads and single cell keratinization can be seen, and necrosis can also be seen in the center of the cancer nest, which is not seen in this case.

Simple ABC develops slowly and has good prognosis as lymph node metastasis and invasive growth are usually rare. Therefore, for patients with ABC alone or combined with CIN, the treatment should be determined according to the age of the patient. For women in childbearing age and those who still wish to be pregnant, cervical conization should be performed. For postmenopausal women, total hysterectomy with follow-ups should be considered. Other than that, ABC is known to be associated with other malignant tumors; thus, the treatment and prognosis also depend on the histological type and clinical stage of the associated tumors.

#### **Disclosure statement**

The authors declare no conflict of interest.

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# Influence of Painless Delivery Techniques on the Psychology of Primipara in Obstetrics Clinic

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**Abstract:** *Objective:* To explore the influence of painless childbirth technique on the psychology of primipara. *Methods:* From July 2020 to June 2022, 108 parturients who received analgesic during delivery in Shaanxi Provincial People's Hospital were selected as the research subjects (painless delivery group), and 92 parturients who gave birth naturally during the same period were selected as controls (natural delivery group). Psychological tests were performed on the patients. *Results:* The depression and anxiety scores of parturients in the natural delivery group were significantly higher than those in the painless delivery group ( $P < 0.05$ ); 9.0% of the patients had coexisting anxiety and depression. *Conclusion:* The application of painless delivery techniques in obstetrics can improve the negative emotions experienced by primipara, improve their self-efficacy, and relieve their psychological pressure.

**Keywords:** Obstetrics clinic; Painless childbirth; Maternal psychology

**Online publication:** November 29, 2022

## 1. Introduction

In 1885, an American surgeon named Giam Capa stated in an interview with the British medical journal *New England Journal of Medicine* that the pain experienced during childbirth makes the mother unwilling to give birth again. Since then, the treatment of pain during childbirth began to receive attention and concern from the medical circle and the society. In the mid-1990s, with the development of anesthesia technology and the continuous advancements in painless delivery techniques, painless delivery has become a new trend, in which an increasing number of women are opting for painless delivery. At present, most hospitals in our country are supportive of painless delivery techniques, but there are still some hospitals that are unwilling or unable to accept painless delivery due to limited conditions, the pregnant women's own conditions, and a misunderstanding of anesthesia.

Painless delivery techniques are analgesic methods that help relieve labor pain through local anesthesia. Clinical methods of painless childbirth can be divided into pharmacological and non-pharmacological methods. Pharmacological methods include combined spinal-epidural anesthesia and epidural anesthesia [1-6]. The general principle is as follows: local anesthetics are injected into the subarachnoid space and/or epidural space to block the spinal nerve root and temporarily paralyze the innervated area so as to relieve labor pain [7,8]. The time of intrathecal puncture for labor analgesia is short and well tolerated by patients. The commonly used drugs include ropivacaine and sufentanil or the combination of fentanyl. Its side effects include anesthetic accidents with a small incidence rate, puncture failure, and insignificant analgesic effect. Once the anesthetic drug has taken effect, some pregnant women may experience lower limb weakness and urinary retention, while fetal hypoxia may occur as a result of transient hypotension caused by drugs in a

very small proportion of women. In clinical work, some patients have concerns because of these side effects, but the vast majority of pregnant women are very satisfied with the pain-relieving effect of anesthetic drugs, leading to the maturity and extensive use of painless delivery techniques. In clinical practice, higher requirements have been put forward for anesthesiologists. It is necessary to master anesthesia methods and safe operation specifications, along with the selection of appropriate anesthetic dosage to complete the anesthesia process. A reasonable control of anesthesia dosage not only allows for optimum labor pain effect, but also prevents adverse effects on the fetus.

## 2. Materials and methods

### 2.1. General information

From July 2020 to June 2022, 108 parturients who received painless delivery in Shaanxi Provincial People's Hospital were selected as the research subjects (painless delivery group), and 92 parturients who gave birth normally during the same period were selected as controls (natural delivery group). Psychological tests were performed on the patients.

### 2.2. Method

The parturients in the painless delivery group adopted painless delivery techniques. The spinal anesthesia used for painless delivery included epidural anesthesia and combined spinal-epidural anesthesia, among which the latter was the most commonly used. The commonly used drugs were ketamine, ropivacaine, fentanyl, and so on. The decision to perform the operation was made by the attending doctor following a comprehensive assessment based on various factors, including the pregnant woman's physical condition, complications, labor duration, and uterine contraction pain.

### 2.3. Observation indicators

The psychological state of the primipara.

### 2.4. Statistical analysis

SPSS 22.0 was used for data analysis. The measurement data were expressed as mean  $\pm$  standard deviation ( $\bar{x} \pm s$ ), and t test was used to compare the data between groups and within groups;  $\chi^2$  test was used to compare the count data, expressed as n (%);  $P < 0.05$  indicated that the difference was statistically significant.

## 3. Results

### 3.1. Comparison of anxiety and depression scores between the two groups

The anxiety and depression scores of the painless delivery group were significantly lower than those of the natural delivery group ( $P < 0.05$ ), as shown in **Table 1**.

**Table 1.** Comparison of anxiety and depression scores between the two groups

Group	Number of cases	Depression	Anxiety
Painless delivery group	108	6.23 $\pm$ 3.05	6.17 $\pm$ 2.96
Natural delivery group	92	7.71 $\pm$ 3.53	7.85 $\pm$ 3.41
t		3.1810	3.7298
P		0.0017	0.0002

### 3.2. Occurrence of anxiety and depression symptoms

Coexisting anxiety and depression was observed in 9.0% of the patients. See **Table 2** for details.

**Table 2.** Occurrence of anxiety and depression symptoms (n/%)

Group	None	May be present	Present
Depression symptoms	122 (61.0)	46 (23.0)	32 (16.0)
Anxiety symptoms	92 (46.0)	87 (43.5)	21 (10.5)
Coexisting anxiety and depression symptoms	133 (66.5)	49 (24.5)	18 (9.0)

## 4. Discussion

Painless delivery techniques are special analgesic methods for labor. Analgesic effects can be achieved through the action of local anesthetic drugs on the subarachnoid space or epidural space, without adverse effects on both the mother and baby. First of all, painless delivery techniques can effectively reduce the pain of the parturient, shorten the duration of pain, reduce the increased catecholamine secretion caused by pain stimulation during labor, and increase the safety of both mother and baby to the threat of uterine vasoconstriction. Secondly, painless delivery can reduce maternal fear of vaginal delivery, reduce the rate of cesarean section, and is conducive to maternal and child safety.

### 4.1. Analgesic effects of painless delivery techniques

Painless delivery techniques allow analgesic effects to be achieved through pharmacological and non-pharmacological methods. In this paper, we mainly discuss the pharmacological method. Analgesic effects are achieved through epidural anesthesia or combined spinal-epidural anesthesia. With the advancements in anesthesia techniques and drugs, the application of painless delivery techniques in obstetrics has matured with more ideal management measures. Painless delivery is now widely recognized and used for parturients [9,10].

### 4.2. Technical considerations for painless delivery

Painless delivery has certain benefits for pregnant women, but the following points should be paid attention to in the specific implementation process: (1) there are high requirements for anesthesia technology; (2) anesthesia indications must be strictly controlled; (3) whether surgical methods and doses are selected according to maternal requirements; (4) whether bleeding points need to be monitored during the operation; (5) a series of measures should be taken, such as preventing infection, prenatal assessment of pregnant women to select a suitable delivery method, and choosing the correct type, dosage, and administration method of analgesic drug (such as amphetamine) to prevent the inhibition of spontaneous breathing and circulation in the mother caused by drugs, which may result in adverse effects, leading to fetal death or serious injury to the mother. If the parturient has underlying cardiovascular disease, the scope and dosage of anesthetic drugs should be strictly limited. For platelets less than  $70 \times 10^9/L$ , the use of labor analgesia is prohibited. If certain analgesic drugs cannot be used due to special reasons, the clinician should be notified, or consent should be taken before administration, so as to prevent complications that may threaten the life of these women [11-15].

### 4.3. Prenatal adaptation

Prenatal adaptation refers to a period of preparation for pregnant women who are about to enter vaginal delivery, mitigating their resistance to painless delivery techniques and providing psychological counseling to reduce their fear. As labor progresses, pregnant women will develop a fear response to childbirth, which

will lead to psychological problems, such as anxiety and depression. This may also lead to postpartum depression in severe cases. It is important to pay attention to the psychological changes of the parturient during pregnancy, alleviate her fear of childbirth, be patient, give encouragement, and provide professional consultation when necessary. Appropriate exercise during pregnancy can regulate nervous tension, promote the secretion of endorphins, relieve anxiety and depression, and benefit fetal development. Exercise can also be used to distract attention and in slow progress of labor. For pain and discomfort symptoms, such as shortness of breath, emergency measures should be taken in time when the symptoms become apparent, and an anesthesiologist should be contacted for assistance in diagnosis and treatment. Through this, women would be able to better understand the pain during uterine contractions. Pregnant women in the prenatal adaptation period should actively communicate with their doctors to understand the possible situations that could occur during childbirth and take certain countermeasures for them. It is also important to provide psychological counseling to pregnant women as they should maintain a good mood, eat reasonable diets, reduce exercise to ensure adequate sleep, maintain a good attitude during labor, as well as avoid anxiety and tension. Psychological counseling can effectively alleviate various discomfort symptoms in pregnant women, especially during childbirth, and reduce the fear of labor. However, there remain a small number of pregnant women who have had an impact on their psychology due to changes in blood pressure and changes in the labor process brought about by intrapartum anesthesia, which increases the risk of postpartum depression. Therefore, good communication between pregnant women and their family members is of importance in the prenatal adaptation stage to reduce their fear, build their confidence in childbirth, and prepare them psychologically to adapt to various situations that could occur during the delivery process. In addition to psychological counseling, it also provides a good environment for postpartum recovery. In that way, there will be better cooperation with delivery work; delivery rates and obstetric clinical quality will improve; cesarean section rate, risk of maternal and child adverse events, and the incidence of puerperal psychological disorders will reduce; and the quality of life of both mother and child will improve, along with the mother's postpartum physical and psychological quality. There will be a profound impact on the entire family if this period is navigated well as an expectant mother.

#### **4.4. Psychological counseling after childbirth**

Painless delivery techniques in obstetrics clinics are mainly used in the delivery process. From the preparation period before delivery to the delivery process, and to the period after delivery, primiparous women should fully understand the delivery process and methods. In addition, it is important to pay attention to the postpartum recovery methods and precautions for guidance. In order to ensure that mothers are able to recognize the advantages of painless delivery as a delivery method, prenatal and various stages of the labor process can be publicized. By understanding the entire process, it reduces the mental stimulation of pain to the parturient and prevents the deterioration of postpartum depression symptoms caused by pain during childbirth. Other than that, it is necessary to pay attention to the physical condition of the parturient after childbirth. Postpartum women should avoid overworking and be well-rested. In addition, they should maintain a positive attitude and cheerful mood, while avoiding excessive mood swings. It is also important to observe the psychological aspects of these women, as some may experience adverse psychological conditions, such as anxiety. In such an event, a doctor should be contacted for assistance as soon as possible. If we can create a good family environment, through timely psychological counseling and medication, postpartum women would be able to go through puerperium smoothly.

In conclusion, the application of painless delivery techniques in obstetrics can improve the negative emotions of primipara, improve their self-efficacy, and relieve their psychological pressure.

## Disclosure statement

The authors declare no conflict of interest.

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# The Mechanism of Long Non-Coding RNA *SNHG7* in Cholangiocarcinoma Cell Proliferation, Migration, and Epithelial-Mesenchymal Transition

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**Abstract:** *Objective:* To investigate the mechanism of long non-coding RNA *SNHG7* and its regulatory effect on the proliferation, migration, and epithelial-mesenchymal transition of cholangiocarcinoma cells. *Methods:* A total of 20 pairs of cholangiocarcinoma and adjacent non-tumor bile duct tissues were collected from patients with cholangiocarcinoma who underwent surgery in the Affiliated Hospital of Hebei University (Hebei, China). Cholangiocarcinoma cell lines CCLP-1, QBC939, RBE, and HCC-9810 as well as normal human biliary epithelial cell line HIBEC were purchased for cell culture. We performed cell transfection, quantitative real-time polymerase chain reaction (qRT-PCR) to detect gene expression, Cell Counting Kit-8 (CCK-8) experiment to determine cell proliferation ability, scratch test to determine cell migration ability, and Transwell test to detect cell invasion ability. *Results:* The expression of lncRNA *SNHG7* in cholangiocarcinoma cell lines CCLP-1, QBC939, RBE, and HCC-9810 was  $3.21 \pm 1.01$ ,  $3.03 \pm 1.02$ ,  $2.98 \pm 1.21$ , and  $3.12 \pm 1.14$ , respectively, while its expression in normal cell line HIBEC was  $3.21 \pm 1.21$ ; the expression of lncRNA *SNHG7* in CCLP-1 was the highest; compared with HIBEC, the p values were all less than 0.05, indicating that the difference was statistically significant. The expression of miR-520f-3p in CCLP-1, QBC939, RBE, and HCC-9810 was  $1.45 \pm 0.75$ ,  $1.55 \pm 0.71$ ,  $1.54 \pm 0.73$ , and  $1.61 \pm 0.72$ , respectively, while its expression in normal cell line HIBEC was  $3.21 \pm 1.21$ ; the expression of miR-520f-3p in CCLP-1 was the lowest, and compared with HIBEC, the p values were all less than 0.05, indicating that the difference was statistically significant. In qRT-PCR, the expression of lncRNA *SNHG7* of si-NC ( $3.21 \pm 1.11$ ) was significantly higher than that of si-*SNHG7* ( $1.14 \pm 0.76$ ), and the p value was less than 0.05, indicating that the difference was statistically significant. In the CCK-8 experiment, the proliferation ability of CCLP-1 cells of the si-NC group at 24 h, 48 h, and 72 h was  $0.61 \pm 0.59$ ,  $0.75 \pm 0.68$ , and  $1.36 \pm 0.71$ , respectively; the proliferation ability of CCLP-1 cells of the si-*SNHG7* group at 24 h, 48 h, and 72 h was  $0.51 \pm 0.64$ ,  $0.59 \pm 0.59$ , and  $0.63 \pm 0.61$ , respectively; there was a significant decrease in the proliferation ability, and the p value was less than 0.05, indicating a statistically significant difference. After 24 h of scratch treatment, compared with the si-NC group, the migration ability of CCLP-1 cells of the si-*SNHG7* group was reduced ( $t = 6.356$ ,  $P = 0.026$ ). The results of Transwell test showed that the cell invasion ability of CCLP-1 in the si-*SNHG7* group was significantly reduced compared with the si-NC group ( $t = 7.845$ ,  $P = 0.032$ ). *Conclusion:* Exploring the gene expression mechanism in relation to the occurrence and development of cholangiocarcinoma is beneficial to future clinical work in terms of diagnosis, treatment, and prognosis. The knockdown of lncRNA *SNHG7* can effectively inhibit the proliferation, migration, and invasion of cholangiocarcinoma.

**Keywords:** RNA *SNHG7*; Cholangiocarcinoma; Cell proliferation; Migration

**Online publication:** November 29, 2022

## 1. Introduction

Cholangiocarcinoma (CCA) is a highly malignant tumor originating from bile duct epithelial cells. The incidence and mortality of CCA have been rising globally in recent years. Early invasion and metastasis are the distinguishing features of CCA, and the average survival time after diagnosis is less than 24 months. Although chemotherapy has improved the treatment status of CCA to a certain extent, the 5-year survival rate of CCA patients is only about 30%. Therefore, there is an urgent need to understand the molecular mechanisms of CCA occurrence and metastasis and seek new diagnostic and therapeutic approaches. Long non-coding RNAs (lncRNAs) are a group of transcripts longer than 200 nucleotides with little or no protein-coding capacity [1,2]. It has been reported that lncRNA plays an important role in many biological functions, such as regulating DNA transcription, regulating gene activity, and remodeling chromosome structure [3]. *SNHG7* is a conserved lncRNA that has important biological functions in different cancers. For example, the knockdown of lncRNA *SNHG7* inhibits the proliferation and migration of cholangiocarcinoma cells by activating the Wnt/ $\beta$ -catenin pathway, and lncRNA *SNHG7* sponges miR-449a to promote the progression of pituitary adenoma [4-7]. At present, there are several studies on cholangiocarcinoma cells. This study mainly investigates the role of lncRNA *SNHG7* in the proliferation and migration of cholangiocarcinoma cells.

## 2. Materials and methods

### 2.1. General information

A total of 20 pairs of cholangiocarcinoma and adjacent non-tumor bile duct tissues were collected from patients with cholangiocarcinoma who underwent surgery in the Affiliated Hospital of Hebei University (Hebei, China) with approval from the Ethics Committee of Hebei University People's Hospital and informed consent from the patients' families. The cholangiocarcinoma cell lines CCLP-1, QBC939, RBE, and HCCC-9810 as well as the normal human biliary epithelial cell line HIBEC were purchased.

### 2.2. Methods

#### 2.2.1. Cell Culture

CCLP-1, QBC939, RBE, HCCC-9810, and HIBEC cells were all cultured in Dulbecco's Modified Eagle Medium. All mediums were supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin double antibody. They were then placed in an incubator at 37°C with 5% volume fraction carbon dioxide (CO<sub>2</sub>).

#### 2.2.2. Cell transfection

The CCLP-1 cells were inoculated in 6-well plates before transfection. When the cell confluency reached 40–50%, *si-SNHG7*, *si-NC*, *miR-520f-3p* mimics, *miR-NC*, *miR-520f-3p* inhibitor, and *inh-NC* were transfected into CCLP-1 cells according to the instructions on LipofectamineTM3000 transfection reagent. After transfection, the cells were cultured for 24 h, and the cells in the logarithmic growth phase were taken for subsequent experiments.

#### 2.2.3. qRT-PCR detection of gene expression

TRIzol Kit was used, and the RNA was reverse transcribed into cDNA using a reverse transcription kit. SYBR Green Mastermix Kit and C1000 thermal cycler were used for qRT-PCR experiments, and *GAPDH* and *U6* were selected as internal controls for *SNHG7* and *miR-520f-3p*, respectively.

#### 2.2.4. CCK-8 experiment to detect cell proliferation ability

The cells in the logarithmic growth phase following transfection were digested with trypsin and seeded in

96-well plates at 1,000/well, with 5 multiple wells in each group. 10  $\mu\text{L}$  of CCK-8 solution was added to each well at 0 h, 24 h, 48 h, and 72 h, respectively, and it was cultured for 4 h. Then, the absorbance (A) value at a wavelength of 450 nm was detected by a microplate analyzer. The proliferation ability of the cells was evaluated.

### 2.2.5. Scratch test to detect cell migration ability

$5 \times 10^5$  CCLP-1 cells were transfected with the corresponding vector into a 6-well plate. After the cell confluence reached 90%, a ruler and a pipette tip were used to draw a line in the plate. Subsequently, phosphate-buffered saline (PBS) was used to wash three times, and serum-free culture medium was added. The scratch distance was observed with a fluorescent inverted microscope at 0 h and 24 h.

### 2.2.6. Transwell test to detect cell invasion ability

A layer of Matrigel matrix membrane was spread on the upper layer of the Transwell chamber, 200  $\mu\text{L}$  of cell suspension ( $1 \times 10^5/\text{mL}$ ) after transfection was seeded into the upper chamber of the Transwell chamber, and 800  $\mu\text{L}$  of medium containing 10% serum was added into the lower chamber. The cells were cultured for another 24 h; those remaining in the upper chamber were gently wiped off with a cotton swab. The cells were fixed with formaldehyde for 30 min and stained with 0.1% crystal violet for another 30 min. The invaded cells were observed with an inverted microscope ( $\times 100$ ).

## 2.3. Observation indicators

lncRNA *SNHG7* gene expression and *miR-520f-3p* expression.

## 2.4. Statistical analysis

All experiments were repeated at least three times. The results were reported as mean  $\pm$  standard deviation (SD) based on at least three replicates. GraphPad Prism 8.4.0 and SPSS 24.0 were used to perform the statistical analysis. Independent sample t-test was used to compare the means of two independent samples. The measurement data were expressed as mean  $\pm$  standard deviation ( $\bar{x} \pm s$ ). Paired t-tests were performed to assess the significant differences between the groups. The differences were statistically significant when the p value was less than 0.05.

## 3. Results

### 3.1. Expression of lncRNA *SNHG7* and *miR-520f-3p* in cell lines

The expression of lncRNA *SNHG7* in cholangiocarcinoma cell lines CCLP-1, QBC939, RBE, and HCCC-9810 was  $3.21 \pm 1.01$ ,  $3.03 \pm 1.02$ ,  $2.98 \pm 1.21$ , and  $3.12 \pm 1.14$ , respectively, while its expression in normal cell line HIBEC was  $3.21 \pm 1.21$ . The expression of lncRNA *SNHG7* in CCLP-1 was the highest, and compared with HIBEC, the p values were all less than 0.05, indicating that the difference was statistically significant. The expression of *miR-520f-3p* in cholangiocarcinoma cell lines CCLP-1, QBC939, RBE, and HCCC-9810 was  $1.45 \pm 0.75$ ,  $1.55 \pm 0.71$ ,  $1.54 \pm 0.73$ , and  $1.61 \pm 0.72$ , respectively, while its expression in normal cell line HIBEC was  $3.21 \pm 1.21$ . The expression of *Mir-520F-3p* in CCLP-1 was the lowest, and compared with HIBEC, the p values were all less than 0.05, indicating that the difference was statistically significant (Table 1).

**Table 1.** Expression of lncRNA *SNHG7* and *miR-520f-3p* in cell lines

Items (n=20)	lncRNA <i>SNHG7</i>	<i>miR-520f-3p</i>	t	P
HIBEC	1.54 ± 0.83	3.21 ± 1.21	5.0899	< 0.05
CCLP-1	3.21 ± 1.01	1.45 ± 0.75	6.2567	< 0.05
QBC939	3.03 ± 1.02	1.55 ± 0.71	5.3258	< 0.05
RBE	2.98 ± 1.21	1.54 ± 0.73	4.5571	< 0.05
HCCC-9810	3.12 ± 1.14	1.61 ± 0.72	5.0084	< 0.05

P: compared with HIBEC

### 3.2. Knockdown of *SNHG7* can inhibit the proliferation of cholangiocarcinoma CCLP-1 cells

In qRT-PCR, the lncRNA *SNHG7* expression of si-NC ( $3.21 \pm 1.11$ ) was significantly higher than that of si-*SNHG7* ( $1.14 \pm 0.76$ ), and the p value was less than 0.05, indicating that the difference was statistically significant. In the CCK-8 experiment, the proliferation ability of CCLP-1 cells in the si-NC group at 24 h, 48 h, and 72 h was  $0.61 \pm 0.59$ ,  $0.75 \pm 0.68$ , and  $1.36 \pm 0.71$ , respectively, while that of the si-*SNHG7* group was  $0.51 \pm 0.64$ ,  $0.59 \pm 0.59$ , and  $0.63 \pm 0.61$ , respectively. There was a significant reduction in the proliferation ability, and the p value was less than 0.05, thus indicating that the difference was statistically significant (Table 2).

**Table 2.** Analysis of the proliferation ability of CCLP-1 cells

Group (n = 20)	qRT-PCR detection		CCK-8 experiment	
	lncRNA <i>SNHG7</i>	24 h	48 h	72 h
si-NC	$3.21 \pm 1.11$	$0.61 \pm 0.59$	$0.75 \pm 0.28$	$1.36 \pm 0.71$
si- <i>SNHG7</i>	$1.14 \pm 0.76$	$0.51 \pm 0.64$	$0.59 \pm 0.19$	$0.63 \pm 0.61$
t	6.8812	0.0007	2.1146	3.4877
P	< 0.05	> 0.05	> 0.05	< 0.05

### 3.3. Knockdown of *SNHG7* can inhibit the migration and invasion of cholangiocarcinoma CCLP-1 cells

After 24 h of scratch treatment, the migration ability of CCLP-1 cells in the si-*SNHG7* group was reduced compared with the si-NC group ( $t = 6.356$ ,  $P = 0.026$ ). The results of Transwell experiment showed that the invasive ability of CCLP-1 cells in the si-*SNHG7* group was significantly reduced compared with the si-NC group ( $t = 7.845$ ,  $P = 0.032$ ).

## 4. Discussion

CCA is a malignant tumor originating from the epithelial cells in intrahepatic and extrahepatic bile ducts. CCA, which is the second most common primary hepatocellular carcinoma malignancy, is the most common biliary tract malignancy worldwide. CCA is resistant to conventional chemotherapy and radiotherapy; in addition, there is a lack of available methods for early diagnosis and treatment of CCA. The majority of patients who are diagnosed with CCA are in advanced stages. Radical surgery is recommended only for patients in an early stage.

Its poor sensitivity to chemotherapy and radiotherapy is an important reason for the poor prognosis of patients [8-10]. Although many scholars have made huge efforts in improving the treatment of CCA, cholangiocarcinoma is still one of the deadliest diseases. Therefore, it is important to identify effective biomarkers and therapeutic targets for early diagnosis and treatment so as to improve the therapeutic effect.

The occurrence and development of CCA are associated with abnormal cell cycle regulation and imbalance of cell proliferation and apoptosis, resulting in the indefinite proliferation of tumor cells. Excessive cell proliferation is not only a characteristic of tumors, but also the impetus for various malignant biological behaviors of tumor cells. Apoptosis is a key process initiated in cancer cells, but it involves various complex pathways and regulators that cancer cells use to escape cell death. The abnormal proliferation and apoptosis of tumor cells, which result from the joint action of multiple factors and the abnormal expression of multiple signaling pathways, are known to be associated with the abnormal expression of multiple genes [11-14].

Tumors develop as a result of the joint action of multiple factors, genes, and signaling pathways. The balance between cell proliferation and apoptosis is disrupted, there is abnormal cell cycle regulation, and cells begin to proliferate indefinitely. Excessive cell proliferation is not only a characteristic of tumors, but also an impetus for carcinogenesis. Studies have shown abnormally high expression of *SNHG7* in various tumor tissues, and downregulating or inhibiting its expression can promote apoptosis and inhibit tumor cell proliferation. In bladder cancer, the expression of lncRNA *SNHG7* in cancer tissues was significantly higher than that in adjacent normal tissues, and the high expression of *SNHG7* in vitro could significantly promote the proliferation of cholangiocarcinoma cells. Further gain-of-function and loss-of-function experiments have confirmed that the high expression of *SNHG7* promotes the proliferation of cancer cells by upregulating the expression of inhibitor of DNA binding/differentiation 2 (*ID2*).

Tumor invasion and metastasis are the hallmarks of malignant tumors. They involve the shedding of tumor cells from the primary lesion, followed by the infiltration of these cells into tissues, forming new metastases in adjacent or distant tissues and organs that are consistent with the nature of the primary tumor through tissue infiltration or circulating blood and lymph. *H19* was originally proposed as a tumor suppressor gene. It plays an inhibitory role in tumor invasion and metastasis. In hepatocellular carcinoma, the downregulation of lncRNA *SNHG7* expression can promote the invasion and metastasis of tumor cells. This process is accompanied by the upregulation of Akt and cell division cycle (*Cdc25*) as well as the downregulation of glycogen synthase kinase-3 beta (*GSK-3β*). Studies have found that a low expression of lncRNA *SNHG7* activates P13K/AKT signaling, which in turn inhibits the expression of the downstream target gene *GSK-3β* and the phosphorylation of *Cdc25A* by *GSK-3β*, and upregulates the expression of cell division cycle 25a (*Cdc25a*) in the cell cycle pathway. The disorder in the regulation of cell cycle promotes the invasion and metastasis of liver cancer cells [15-17].

This research explores the gene expression mechanism in the occurrence and development of cholangiocarcinoma. The results from this research may provide reference for future clinical work in terms of diagnosis, treatment, and prognosis. The knockdown of lncRNA *SNHG7* can effectively inhibit the proliferation, migration, and invasion of cholangiocarcinoma.

## Disclosure statement

The authors declare no conflict of interest.

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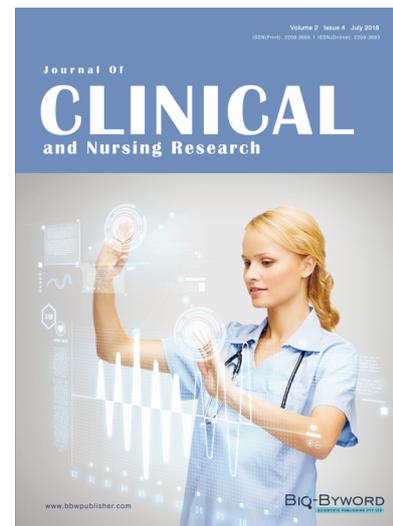
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