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Inhibitory mechanism of KIF3A gene on nasopharyngeal carcinoma stem cells

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ABSTRACT

To investigate the inhibitory mechanism of the KIF3A gene on nasopharyngeal carcinoma (NPC) tumor stem cells. Set up a blank control group (BCG), NPC group, and KIF3A silence (si-KIF3A) group. The BCG cells were nasopharyngeal normal epithelial cell line NP69, without any treatment, and were cultured routinely; The NPC group cells are human NPC cell line CNE2 cells, which are not subjected to any treatment and are cultured routinely; si-KIF3A group cells were cultured in the offspring of human NPC cell line CNE2 infected with Lentivirus knockdown KIF3A gene. CCK8 was used to detect the cell proliferation ability, Western blot and qPCR were used to detect protein and related mRNA expression, while cell migration and invasion were detected using Transwell. The KIF3A protein and mRNA in the NPC group were higher than those in the BCG (P<0.05), while those in the si-KIF3A group cells were lower compared to BCG cells (P<0.05). The cell proliferation, migration and invasion activity in the si-KIF3A group were reduced than those in BCG (P<0.05). Si-KIF3A group cells HIF-1, NF-κB was reduced than that of BCG (P<0.05). The expression level of HIF-1, NF-κB protein in si-KIF3A group cells was reduced compared to BCG cells (P<0.05). Knocking down the KIF3A gene can reduce the vitality of NPC stem cells, and inhibit the malignant phenotype of NPC stem cells, via inhibiting HIF-1 and NF-κB expression.

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Introduction

Nasopharyngeal carcinoma (NPC), is the most common head and neck malignant tumor in our country. The pathogenesis is not completely clear. The pathogenesis of it may be related to many factors, such as chemical carcinogens, Epstein-Barr virus infection, and genetic and epigenetic changes (1). 98% of NPC belong to low-differentiated squamous cell carcinoma and are moderately sensitive to radiation, so the clinical treatment of NPC is mainly based on radiotherapy. However, according to clinical studies, about 55% of NPC patients have recurred and metastasized within 5 years after radiotherapy, resulting in the failure of radiotherapy for NPC patients, which may be related to the radiotherapy resistance and the ineffectiveness of conventional radiation dose (2). According to the tumor stem cell theory, nasopharyngeal cancer stem cells are the root cause of distant metastasis and in situ recurrence of nasopharyngeal cancer, because nasopharyngeal cancer stem cells are resistant to radiotherapy (3). Nuclear transcription factor (NF-κB) and hypoxia-inducing factor (HIF-1), which are highly activated in oxygen-poor environments, are the reasons for the resistance of NPC stem cells to radiotherapy (4). Driver family member 3A (KIF3A) is a class of microtubule dynamin. As a subunit of heterotrimer driver 2 that regulates microtubule function and transport, KIF3A is involved in a variety of cellular processes. It is associated with the progression of prostate cancer, oral squamous cell carcinoma, and cutaneous squamous cell carcinoma (5). However, the effects of KIF3A on NPC and NPC stem cells are even not clear. Based on this, this study explored the inhibitory mechanism of the KIF3A gene on NPC stem cells, providing potential therapeutic targets for the clinical treatment of NPC.

Materials and Methods

Materials and reagents

Human NPC cell line CNE2 and nasopharyngeal normal epithelial cell line NP69 were purchased from ATCC cell bank in the United States.KIF3A, HIF-1, NF-κBmRNA primers and β-actin primers (Sigma Corporation); apoptosis kit by qPCR, apoptosis kit by immunohistochemistry, Apoptosis kit by CCK-8, Annexin VFITC/PI Kit (Shanghai Biyantian Company); Transwell (Corning Corporation); Matrigel was purchased from BD Company.KIF3A, HIF-1, NF-κB-antibody (Abcam Biotechnology Limited). This study required cell knockdown of the KIF3A gene lentivirus (Shanghai Jikai Gene Co., LTD.).

Cell culture and treatment

Resuscitated human NPC cell line CNE2 was inoculated in a T25 culture bottle with RPMI-1640 medium, and nasopharyngeal normal epithelial cell line NP69 was inoculated in T25 culture bottle with RPMI-1640 medium without serum and cultured in a constant temperature

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incubator (37℃, 5%CO2). Pancreatic enzyme digestion, passage and cryopreservation were performed according to cell growth. Blank control group (BCG), NPC group and KIF3A silence (si-KIF3A) group were set. The cells of the BCG were normal nasopharyngeal epithelial cell line NP69, which was cultured conventionally without any treatment. CNE2 cells in the NPC group were routinely cultured without any treatment. KIF3A silenced cells of CNE2 cells were infected with KIF3A gene knockdown lentivirus and cultured in the progeny. Each experiment was repeated six times.

Western blot

After the solution was centrifuged, the supernatant was taken for test, protein concentration was determined by BCA method, gel preparation, electrophoresis for 90min, gel cutting, film transfer for 90min, milk sealing, after cleaning, it was incubated with primary and secondary antibodies successively, and then developed. The results were analyzed by Bio-Rad image laboratory software.

qPCR

The cell density was adjusted to 1×106 cells/well, which were inoculated in 6-well plates and cultured in a constant temperature incubator (37℃, 5%CO2). After the stable passage of the si-KIF3A group of CNE2 cells infected with the KIF3A gene knockdown lectin virus, cells in each group were extracted by RNA extraction kit. Using the One Step Prime Script miRNA cDNA kit will be microRNAs reverse transcription for cDNA synthesis, using microRNAs carries on the q-PCR detection kits, according to the kit instruction to complete the cycle. After the reaction was completed, mRNA relative expression was calculated in the software.

Detection of cell proliferation by CCK8

Each group of cells was cultured with 10μlCCK8 solution per well for 4h in a constant temperature incubator (37℃, 5%CO2). The proliferative ability of cells in each group was detected by enzyme label (OD450nm).

Detection of cell migration and Invasion ability by Transwell Method

After the stable passage of human NPC cell line CNE2 with knockdown KIF3A gene lentivirus infection, the cells in the KIF3A silent group were inoculated with 5 × 105 cells per well in the Transwell upper chamber. The low and high-dose groups were treated with propofol for 48 hours, while the B CG was given the same amount of DMEM culture medium, fixed and stained, and the purple-stained transmembrane cells were counted under the microscope. Calculate the ability of cell migration. In order to detect the invasive ability of cells, Matrigel was used to cover the upper chamber of the Transwell chamber in the ultra-clean worktable, and the following steps were the same as cell migration.

Statistical Methods

SPSS22.0 was used for data processing and analysis, measurement data was represented by ($\bar{x}$±s). F-variance analysis was used for multi-group comparison, and the LSD-t test was used for pairwise comparison between multiple groups.

Results

KIF3A Expression in NPC cells

The KIF3A protein and mRNA in the NPC group were raised compared to BCG (P<0.05) (Table 1).

Establishment of NPC stem cell lines silencing KIF3A gene

The KIF3A protein and mRNA in the si-KIF3A group were reduced compared to BCG (P<0.05) (Table 2).

Effects of silencing KIF3A gene on NPC stem cells

Cell proliferation activity, migration and invasion in the si-KIF3A group were reduced compared to BCG (P<0.05) (Table 3).

Table 1. KIF3Aexpression in NPC cells.

Group	n	KIF3A protein	KIF3A mRNA
BCG	6	0.40±0.14	1.05±0.26
NPC	6	0.98±0.24	2.12±0.35
t		-8.085	-6.011
P		0.000	0.000

Table 2. KIF3Aproteinand mRNA expression.

Group	n	KIF3A protein	KIF3A mRNA
BCG	6	0.94±0.25	3.36±1.03
Si-KIF3A	6	0.41±0.13	1.59±0.57
t		4.607	3.683
P		0.001	0.004

Table 3. Effects of silencing KIF3A gene on NPC cells.

Group	n	proliferation activity	Number of migrated cells	Number of invasive cells
BCG	6	0.28±0.04	142.74±24.34	121.05±20.26
Si-KIF3A	6	0.18±0.03	106.48±17.44	93.12±16.35
t		4.899	2.966	2.628
P		0.001	0.014	0.025

Table 4. HIF-1 and NF-κB mRNA relative expression levels in NPC stem cells.

Group	n	HIF-1 mRNA	NF-κB mRNA
BCG	6	2.71±0.72	2.34±0.76
Si-KIF3A	6	1.63±0.41	1.28±0.34
t		3.193	3.119
P		0.010	0.011

Table 5. Effect of silencing KIF3A gene on HIF-1 and NF-κB protein expression in NPC stem cells.

Group	n	HIF-1	NF-κB
BCG	6	1.25±0.37	0.93±0.35
Si-KIF3A	6	0.84±0.21	0.51±0.17
t		2.361	2.644
P		0.040	0.025

Effect of silencing KIF3A gene on HIF-1 and NF-κB mRNA relative expression levels in NPC stem cells

The HIF-1 and NF-κBmRNA levels in the si-KIF3A group were reduced than those in BCG (P<0.05) (Table 4).

Effect of silencing KIF3A gene on HIF-1 and NF-κB protein expression in NPC stem cells

The HIF-1 and NF-κB protein in the si-KIF3A group were reduced than those in BCG (P<0.05) (Table 5).

Discussion

NPC is originating from the mucosal epithelium of the nasopharynx, often occurring in the top anterior wall of the nasopharynx and the recesses of the pharynx (6). Clinical epidemiological studies of NPC have found that the incidence of NPC has an obvious ethnic and regional tendency, and tends to occur in southern China and Southeast Asia, with about 50 cases in every 100,000 people (7). The pathogenesis and etiology of NPC have not been fully explained. Currently, it is believed that it is related to three factors, namely Epstein-Barr virus infection factor, family genetic factor, environment and diet factor (8). Image-guided intensity modulated radiation therapy is currently the main clinical treatment for NPC, but there are still 20%-30% of NPC patients who have treatment failure due to distant metastasis, local recurrence, and uncontrolled local lesions, and the quality of life of patients is still not ideal (9). Therefore, an in-depth study of the pathogenesis and etiology of NPC, the determination of the molecular mechanism of the proliferation, invasion and migration of NPC cells, and the search for new therapeutic targets are of great clinical significance for slowing down the pathological progression of NPC, extending the survival time of patients, and improving the patients survival.

Tumor stem cells are special cell subgroups with self-renewal ability, invasion and metastasis ability, multidirectional differentiation function, high chemoradiotherapy resistance, and biological functions similar to embryonic stem cells (10). Current studies believe that tumor stem cells exist in many solid tumors, and the existence of tumor stem cells is closely related to distant metastasis, local recurrence and treatment resistance of tumors, and is the main cause of tumor recurrence and chemoradiotherapy resistance (11). In recent years, tumor stem cells from different tissue sources have been isolated successively (12). In addition, many studies have found that there are cells with stem cell characteristics in NPC, which play a critical role in the recurrence and metastasis of NPC and the resistance to radiotherapy and chemotherapy (13). Based on this, this study explored the inhibitory mechanism of the KIF3A gene on NPC stem cells, aimed to provide potential therapeutic targets for NPC treatment.

KIF3A is a subunit of heterotrimer drive protein 2 that regulates microtubule function and transport. Its head has sites that link microtubules and bind catalytic ATPase, and uses energy generated by hydrolysis of ATP to move along microtubules, thus playing a role in cell division, and intracellular macromolecule transport (14). KIF3A is associated with the progression of many kinds of cancers (15). Scholars have found that KIF3A is related to the malignancy and prognosis of tumors, and the higher the expression level, the worse the prognosis of cancer patients (16). This study showed that the KIF3A protein and mRNA levels in NPC were raised than those in the BCG, meanwhile, in the si-KIF3A group those were reduced than those in BCG. Cell proliferation activity, cell migration and cell invasion in the si-KIF3A group were reduced than those in BCG. It is suggested that the knockdown of the KIF3A gene can reduce the cell viability of NPC stem cells, and inhibit the proliferation, migration and invasion of NPC stem cells.

The hypoxic microenvironment is common in solid tumors and is an important factor affecting tumor radiotherapy sensitivity. Studies have found that HIF-1 is highly expressed in an aerobic environment, and HIF-1α, as the oxygen function regulation subunit of HIF-1, is also increased (17). HIF-1α is a dimer transcription factor widely found in humans and mammals. It is a critical cellular regulatory factor involved in the regulation of intracellular oxygen metabolism. Hif-1α-regulated genes are mainly used in some key links of tumor occurrence and development, such as cell invasion and migration, extracellular matrix remodeling, DNA damage and repair, tumor metabolism, apoptosis and autophagy, angiogenesis, and tumor proliferation under hypoxia conditions (18). In addition, the signaling pathway is the focus of research on endocrine-related diseases, osteoarthritis and other diseases. NF-κB is a nuclear transcription factor widely distributed in cells of various systems throughout the body with various functions, regulating the transcription of adhesion factors, complement, cytokines, chemokines and other cytokines in the human body (19). NF-κB is widely distribu-

ted in inflammatory cells such as lymphocytes, neutrophils, macrophages and chondrocytes. In the resting state, NF-κB has no biological activity and exists in the cytoplasm in the form of inactive trimers. When the body is under stressful conditions such as ischemia and hypoxia, mechanical injury, viral infection, chronic strain and activation of extracellular matrix degradation products, NF-κB is activated, thus initiating transcription of related target genes and participating in pathophysiological processes such as immune response, inflammatory response and apoptosis, thus aggravating inflammatory response in the body and producing a series of adverse consequences (20). Here, the HIF-1 and NF-κBmRNAs and proteins levels in si-KIF3A cells were reduced than those in BCG cells It is suggested that KIF3A can inhibit the expression of HIF-1 and NF-κB in NPC stem cells. It has been confirmed that NPC CNE-2 stem cells can amplify the electrical biological signals of NPC tissues by inducing the HIF-1/NF-κB signaling pathway, so as to promote the proliferation, migration and invasion of NPC, resist the damage of NPC cells caused by external radiotherapy.

In conclusion, knockdown of the KIF3A gene can reduce the viability of NPC stem cells, and inhibit the proliferation, migration and invasion of NPC stem cells, which may be related to the inhibition of HIF-1 and NF-κB expression by KIF3A.

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