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Histone acetyltransferase 1 up regulates Bcl2L12 expression in nasopharyngeal cancer cells

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ABSTRACT

The deregulation of Bcl2L12 expression in cancer has been recognized, but the causative factors are unknown. Histone acetyltransferases (HAT) play critical roles in the regulation gene transcription. This study tests a hypothesis that the aberrant activities of HAT induce deregulation of Bcl2L12 in nasopharyngeal cancer (NPC). In this study, human NPC tissues were collected from the clinic. The expression of Bcl2L12 and HATs in NPC cells was analyzed by real time RT-PCR and Western blotting. NPC cell apoptosis was analyzed by flow cytometry. The results showed that by screening the subtypes of HAT, the levels of HAT1 were uniquely higher in NPC as compared with non-cancer nasopharyngeal tissue. The levels of Bcl2L12 in NPC cells were positively correlated with HAT1. HAT1 involved in the STAT5 binding to the Bcl2L12 promoter. HAT1 increased the expression of Bcl2L12. Bcl2L12 mediated the effects of HAT1 on suppressing NPC cell apoptosis. Absorption of the HAT1 shRNA plasmid-carrying liposomes induced NPC cell apoptosis. In conclusion, inhibition of HAT1 can induce NPC cell apoptosis via increasing Bcl2L12 expression, which can be a potential therapy for NPC treatment.

1. Introduction

Nasopharyngeal cancer (NPC) is an endemic disease. Although the prevalence of NPC is only 0.2 to 0.5 per 100,000 people in some countries such as the United States and Europe, it is about 25–50 per 100,000 people in Southeast Asia, the Mediterranean Basin and the Arctic [1,2]. In the cases of NPC at early stages, the results of treatment with radiotherapy usually are satisfied [3]. However, promote metastasis can be found in NPC patients in advanced stages, which can be refractory to be radically removed from the body [4].

Cumulative reports indicate that the infection of Epstein–Barr virus (EBV) is associated with the pathogenesis of NPC [5]. In general, EBV can naturally infect the epithelial cells in the nasopharyngeal mucosa; but the virus is naturally lytic in the cells. However, EBV does establish stable infection in the nasopharyngeal epithelial cells via a mechanism of harboring a p16 deletion or overexpressing cyclin D1/Bmi-1 [6], which is regarded as the premalignant infection. Yet, the entry mechanism by which EBV infects nasopharyngeal epithelial cells has not been fully defined. The pathogenesis of NPC remains to be further investigated.

The deregulation of apoptosis is proposed playing a critical role in the pathogenesis of cancer, including NPC [7,8]. Apoptosis is also called the programme cell death. Fas and p53 are proposed playing very important role in the induction of apoptosis [9,10]. Apoptosis is a physiological process in eliminating damaged or un-repairable cells, or senescent cells. If the apoptotic machinery is compromised, the cells may grow out control and may develop into cancer cells [11]. Apoptosis can be regulated by multiple factors. A large number of substances or molecules have been tested to regulate apoptosis in cancer cells [12]. Yet, the detail mechanism to induce cancer cell apoptosis needs to be further elucidated.

Recent research indicates that the Bcl2 like protein-12 (Bcl2L12) plays a critical role in suppressing p53 expression in glioma cells [13]. Bcl2L12 is a member in the Bcl2 family. Overexpression of Bcl2L12 can induce resistance to apoptosis. Bcl2L12 also neutralizes caspase-7 and caspase-3 [14]. Signal transducer and activator of transcription 5 (STAT5) is the transcription factor of Bcl2L12 [15]. Yet, the factors regulating the expression of Bcl2L12 has not been fully understood.

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Since the histone acetyltransferases (HAT) play critical roles in the gene transcription, we screened the expression of HAT family in NPC tissue extracts and found high expression of HAT1 in NPC as well as Bcl2L12. HAT1 belongs to type B HAT, is responsible for the acetylation of newly synthesized histones H3 and H4. Therefore, we hypothesize that HAT1 up regulates Bcl2L12 in NPC cells to interfere with NPC cell apoptosis. Indeed, via molecule biological approaches, we found that HAT1 up regulated the expression of Bcl2L12 in NPC cells that suppressed NPC cell apoptosis. To knock down the HAT1 gene induced NPC cell apoptosis.

2. Materials and methods

2.1. Reagents

The shRNA kits of Bcl2L12 (sc-141674) and HAT1 (sc-145898), antibodies of HAT1 (B-10), STAT5 (A-9), acetylated (ac) H3 (AH3-120), acH4 (AE-4) and RNA polymerase II (Pol II) (B8-1) were purchased from Santa Cruz Biotech (Santa Cruz, CA). The Bcl2L12 antibody (ab108346) was purchased from abcam (Cambridge, MA), The reagent kit of chromatin immunoprecipitation, DC-Chol, DOPE, Doxorubicin (Dox), liposome preparation, Annexin V kit and propidium iodide (PI) were purchased from Sigma Aldrich (St. Louis., MO). The reagents for RT-qPCR, Western blotting, gene transcription and molecular cloning were purchased from Invitrogen (Carlsbad, CA).

2.2. Ethic statement

Patients with NPC or hypertrophic adenoids were recruited into this study. An informed written consent was signed by each patient or the immediate relatives of child patients. The using human tissue in the present study was approved by the Ethics Committee at Shenzhen University.

2.3. Patients

Patients with NPC were recruited at the clinic of our hospital. The diagnosis and management were performed by our surgeons and pathologists. The demographic of the NPC patients are presented in Table 1. Patients with one of the following conditions were excluded from the present study: multiple cancers; severe organ diseases; received specific cancer therapies; other immune diseases.

2.4. Collection of NPC tissue and control tissue

The biopsies of NPC were obtained from NPC patients following the routine procedures established in our hospital. The surgically removed adenoid gland tissues were obtained from patients suffered from hypertrophic adenoids.

Table 1
Demographic data of human subject.

Characteristics	NPC patients	Controls
Male	12	9
Female	10	7
Age	38–55	8–10
Biopsy	22	5.8
Total stage		
I	15	5.8
II	6	15.0
III	1	43.3
IV	0	35.8

Controls: Patients with hypertrophic adenoids.

2.5. Preparation of protein extracts

Protein extracts were prepared from NPC biopsies, surgically removed adenoid glands and cultured cells with our established procedures [16]. Briefly, cells were lysed by a lysing buffer (10 mM HEPES, pH 7.4, 10 mM NaCl, 1.5 mM MgCl2, 0.5 mM DTT, 0.2% Nonidet P-40, and 0.2 mM PMSF); human tissues were homogenized with a homogenizer. The lysates were centrifuged for 10 min at 13,000 rpm. The supernatant was collected as the cytosolic protein extracts. The pellets were lysed with a nuclear lysing buffer (20 mM HEPES-KOH, pH 7.9, 25% glycerol, 420 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 0.5 mM DTT, 0.2 mM PMSF, and 1 × protease inhibitor cocktail) for 30 min and centrifuged for 10 min at 13,000 rpm. The supernatant was collected as the nuclear protein extracts. The protein was quantified by the BCA method. All the procedures were performed under 4 °C.

2.6. Cell culture

The BEAS-2B cells (a normal human lung epithelial cell line) and HEK293 cells were purchased from ATCC (Manassas, VA); the NPC cell lines (HNE2 cells and CNE2 cells) were obtained from the Cancer Center, Sun Yat-sen University (Guangzhou, China). The cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 0.1 mg/ml streptomycin, 100 U/ml penicillin and 2 mM glutamine. The medium was changed in 1–2 days. The cell viability was greater than 99% as assessed by Trypan blue exclusion assay.

2.7. Real time quantitative RT-PCR (RT-qPCR)

The total RNA was extracted from the cultured cells or human tissue with the TRIzol reagents. The cDNA was synthesized with a reverse transcription kit following the manufacturer's instructions. The samples were amplified in a qPCR device with the SYBR Green Master Mix and the primers listed in Table 2. The results are presented as fold change against the internal control, the housekeeping gene β-actin.

2.8. Western blotting

The total proteins were extracted as described above. The proteins were fractioned with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a PVDF membrane. The membrane was incubated with 5% skim milk for 30 min to block the non-specific binding, incubated with antibodies of interest or isotype IgG (control) overnight at 4 °C, washed with TBST (Tris-buffered saline Tween 20) for 3 times, incubated with the second antibodies (labeled with peroxidase) for 1 h at room temperature, washed with TBST for 3 times again. The immune blots on the membrane were developed by the

Table 2
Primers used in the present study.

Molecule	Forward	Reverse
GCN5	tccccattgacctgaagacc	cctccctccttgagcttgaa
PCAF	cctcttctggacttgaggca	tggtctctggtccaagcatt
Tip60	ctacaatgtggcctgcatcc	ttgatggtgatctgtggcct
MOZ	tgcccaatcctttctaagcc	acactacttactgagcgccg
MORF	cttaatggactgtggcgtcg	ctgctctcctgggtcaaaga
HBO1	ccacgaactcctcctccatt	agtcagggattgtggtgagg
P300	ttgtgaagagccccatggat	gctttgcatcactgggtcaa
SRC-1	ggtaaaaggtgcaggtgtgg	acaaagtacggggaaggagg
HAT1	gtgtacccagacaaaacccg	ccgggaaaaacagggcaaat
TIF-2	gttttgccagccacttacca	aacacactggcgtttttctcc
SRC-3	acatgggagtcctggtcttg	ctggaaaagctgtggttccc
TAF-1	aagttcctgggcctaactcc	ctcttccggatgctgctttc
CLOCK	gtgttacatcgcgccatcat	cattaggagggctgagaggg
Bcl2L12	cttctatgctttggtggccc	tacagaacagctccaccagg