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CBX3 promotes proliferation and regulates glycolysis via suppressing FBP1 in pancreatic cancer

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ABSTRACT

More and more evidence has demonstrated that Chromobox protein homolog 3(CBX3) has an important role in carcinogenesis by regulating several mechanisms, such as heterochromatin formation, gene silencing, DNA replication and repair. However, its role in pancreatic cancer has seldom been discussed. In the present study, we silenced CBX3 expression in pancreatic cancer cell lines and identified the positive roles of CBX3 in cancer cell proliferation. Furthermore, we demonstrated that silencing CBX3 in pancreatic cancer cells inhibited aerobic glycolysis, the basis for providing cancer cells with building blocks for macromolecule synthesis and ATP that required. To search for the underlying molecular mechanism, we turned to examine the impact of CBX3 on the expression of FBP1, a negative regulator of aerobic glycolysis in pancreatic cancer and indicated that CBX3 negatively regulated FBP1 expression. Silencing FBP1 expression attenuated the decrease in glycolytic capacity that caused by CBX3 knockdown in pancreatic cancer cells. Taken together, these data reveal that CBX3 serves as a positive regulator of aerobic glycolysis via suppressing of the FBP1 in pancreatic cancer cells. Disrupting the CBX3-FBP1 signaling axis would be effective to treat pancreatic cancer and prevent aerobic glycolysis.

1. Introduction

Pancreatic cancer, one of the most malignant cancers, is currently ranked fourth in cancer-related mortality in developed countries and predicted to be second commonest cause within ten years [1]. Pancreatic cancer prognosis is extremely poor with five-year survival less than 7% and changed little for many decades [2]. Effective therapies for pancreatic cancer remained limited, and surgery is still the sole choice for a cure at the current stage [3]. Thus, insights into the molecular characteristics underlying pancreatic cancer pathogenesis are in urgent need to further develop novel targeted therapeutic strategies [4].

Aerobic glycolysis, which is also known as Warburg effect and a hallmark of cancer characterized by the increased conversion of glucose into lactate irrelevant to oxygen availability, is an important feature and provide massive intermediates for cell survival and new biomass building in cancer [5]. Various studies found that aerobic glycolysis play a key role in the pathogenesis of pancreatic cancer. The regulation of glycolysis-related protein, such as fructose-bisphosphatase1 (FBP1) could be epigenetically silenced and play negative roles in glycolysis [6]. These metabolism alterations and reprogramming in tumor cells contribute to the survival, proliferation and metastasis in harsh microenvironment [7]. Thus, a better understating of the underlying the regulator mechanisms of glycolysis in pancreatic cancer might aid the discovery of novel treatment opportunities which is urgently needed.

Chromobox protein homolog 3(CBX3) is a member of the heterochromatin proteins. It has been reported to be upregulated in tumors and could epigenetically regulate genes that are important for cancer development and growth [8]. It is suspected that CBX3 may be a potentially important target in cancer. Previous studies have identified CBX3 as a potential biomarker for various cancer types, such as osteosarcoma, non-small cell lung cancer, colon cancer, and prostate cancer [9]. However, the precise role and

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underlying signaling cascade of CBX3 in pancreatic cancer growth, development, and aerobic glycolysis remain unclear.

The objective of this study was to examine the regulatory role of CBX3 expression and its relationship with aerobic glycolysis in pancreatic cancer. Furthermore, we examined the impact of CBX3 on the expression of FBP1 to search for the underlying molecular mechanism.

2. Materials and methods

2.1. Cell culture

The human pancreatic cancer cell lines PANC-1, MIA PaCa-2 were obtained from ATCC and cultured according to the standard ATCC protocols. In brief, PANC-1 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM), containing fetal bovine serum (FBS) in a final concentration of 10%. MIA PaCa-2 cells were cultured in DMEM medium, with FBS concentration of 10% and horse serum in a concentration of 2.5%. The cells were cultured in a humid incubator containing 5% CO_2 at 37 °C.

2.2. RNA isolation and quantitative real-time PCR

TRIzol reagent (Invitrogen, USA) was used to extract total RNA from pancreatic cancer cells. To obtain cDNA, TaKaRa PrimeScript RT reagent Kit was used for reverse transcription. Expression status of the indicated genes and β-actin were determined by quantitative real-time PCR using an ABI 7900HT Real-Time PCR system (Applied Biosystems, USA). All reactions were run in triplicate. Primers sequences are listed below: CBX3 forward 5′-GATGCTGCTGACAAACCAAGAGG-3′, CBX3 reverse 5′-TCTTTCGCCAGCACCAAGTCTGCCT-3'. FBP1 forward 5′-CCCCAGATAATTCAGCTCCTTA-3′, FBP1 reverse 5′-GTTGCATTCGTACAGCAGTCTC-3'. β-actin forward 5′- CTACGTCGCCCTGGACTTCGAGC-3′, β-actin reverse 5′- GATGGAGCCGCCGATCCACACGG-3'.

2.3. Protein extraction and western blot analysis

The detailed protocol for Western blot assay was described previously [10]. In brief, equal amounts of protein from each sample were subjected to electrophoresis in SDS-polyacrylamide gel, and analyzed by Western blot. CBX3, FBP1 antibodies were purchased from Abcam, and antibodies against β-actin were produced by Proteintech.

2.4. Lentivirus production and stable cell line selection

To silence CBX3 expression, pLKO.1 TRC cloning vector (Addgene plamid: 10878) was used [11]. 21bp targets against CBX3 were 5′-CTGGCGAAAGAGGCAAATATG-3′ and 5′-GCGTTTCTTAACTCTCAGAAA-3′ respectively. 21bp targets against FBP1 were 5′-CGACCTGGTTATGAACATGTT-3'. Lentiviral particles were produced by co-transfection of pLKO.1-shCBX3 or pLKO.1-shFBP1 expressing constructs with packaging vectors psPAX2 and pMD2.G into HEK-293T cells in a ratio of 4:3:1. Stable shRNA expressing cell lines were obtained by infection of PANC-1 and MIA PaCa-2 dells with lentiviral particles that followed by puromycin selection.

2.5. CCK-8 proliferation assay

Cell proliferation assay was performed by using CCK-8 reagents (Dojindo, Japan) and performed according to the manufacturer's protocol.

2.6. Colony-formation assay

PANC-1 and MIA PaCa-2 cells (5×10^2) stably expressing shRNA targets against CBX3 and its relative control cells were seeded into 6-well plates. After cultivating for 14 days, 4% paraformaldehyde was used to fix the cells followed by staining with 1% crystal violet. After staining, the colonies were counted by using bright field microscopy.

2.7. Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR)

Seahorse' Bioscience XF96 Extracellular Flux Analyzer was used to measure cellular glycolysis capacity and cellular mitochondrial respiration, all according to the manufacturer's instructions of seahorse XF Cell Mito stress test kit or Glycolysis Stress Test Kit [12].

2.8. Dual-luciferase assay

The promoter region of FBP1 covering from −2000 to +200 was amplified from PANC-1 genomic DNA and cloned into pGL3-basic vector to generate pGL3-FBP1 construct. The impact of CBX3 on FBP1 promoter activity was assessed by transfecting of pGL3-FBP1 into PANC-1 and MIA PaCa-2 cells with empty vector or CBX3 expression vector. The luciferase activities were assessed by using Promega's Dual luciferase assay kit.

2.9. Chromatin immunoprecipitation (ChIP)

To check the occupancy status of CBX3 on FBP1 promoter, we performed ChIP assay with Millipore's EZ-ChIP kit. The primer sequences to test CBX3 occupied region were forward: 5′- GACAGAAGGGCCAGGTGA-3′ and reverse: 5′- GCCAGAGAGAAAGCT ATGACTG -3'. ChIP assay was carried out according to procedures that described previously [13].

2.10. Statistical analyses

Statistical analyses were performed by SPSS software (version 17.0, IBM Corp., Armonk, NY, USA) using independent t tests (for continuous variables) and Pearson's χ^2 tests (for categorical variables). Statistical significance was based on two-sided p values of <0.05.

3. Results

3.1. Impairment of cell proliferation and colony formation ability by CBX3 knockdown

Two different shRNAs specifically targeting human CBX3 gene were designed and their knockdown efficiency was examined in two human pancreatic cancer cell lines PANC-1 and MIA PaCa-2. It was confirmed that both shRNAs inhibited CBX3 expression significantly at mRNA level and protein level in these two cell lines (Fig. 1A and B). We then examined the impact of CBX3 knockdown on cell proliferation in human pancreatic cancer cell lines. The results revealed that two shRNAs led to significant cell proliferation impairment in both cell lines as reflected by CCK-8 proliferation assay (Figue.1C and 1D). In addition to cell proliferation status, colony formation ability in both human pancreatic cancer cell lines treated with either one of the two CBX3 shRNAs was analyzed and colony formation ability was significantly impaired in both cell lines infected with either CBX3 shRNA as compared to those treated with scrambled shRNA (Fig. 1E and F).

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