Biomedicine & Pharmacotherapy 101 (2018) 1–7

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

The clinical significance and biological function of tropomyosin 4 in colon cancer

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ARTICLE INFO

Keywords:
Tropomyosin
TMP4
Biomarker
Colon cancer

ABSTRACT

Tropomyosin 4 (TPM4) has been found to be dys-regulated, and function as oncogene or anti-oncogene in human cancers. However, there was no report on the clinical significance and biological function of TPM4 in colon cancer. This study was designed to investigate the clinical value and biological function of TPM4 in colon cancer. Thus, we detected the TPM4 expression in colon cancer clinical samples, and conducted the gain-of-function in colon cancer cell lines. In our results, TPM4 mRNA and protein expressions were reduced in colon cancer tissues and cell lines compared with normal colon tissues and colon epithelial cell line, respectively. TPM4 protein low-expression was obviously associated with clinical stage, T classification (invasion depth), N classification (lymph node metastasis), distant metastasis and differentiation. Survival analysis showed low-expression of TPM4 was an unfavorable independent prognostic factor for colon cancer patients. Moreover, the experiments in vitro suggested up-regulated TPM4 expression suppressed colon cancer cell migration, invasion and metastasis-associated gene expression including MMP-2, MMP-9 and MT1-MMP, but had no effect on cell proliferation. In conclusion, TPM4 is associated with clinical progression in colon cancer patient and acts as a tumor suppressor in colon cancer cell.

1. Introduction

Colon cancer is one of the most common types of cancer and the leading cause of cancer-associated mortality in developed countries [1,2]. Compared with western countries, the incidence of colon cancer is lower while is a substantial burden in China [3]. Genetic mutations including *adenomatous polyposis coli* (APC), *TP53*, *KRAS*, *BRAF* and *PI3KCA* have been identified to play important roles in the development and progression of colon cancer [4,5]. Despite of the development of prevention and early detection in colon cancer, the 5-year survival rate of colon cancer remains unsatisfied, particularly for those patients with advanced stage [6,7]. Therefore, it is necessary to identify novel and credible biomarkers which are associated to clinicopathological characteristics including prognosis and developed to new targets for therapeutic strategy of colon cancer.

Tropomyosin (TPM), which contains TPM1, TPM2, TPM3 and TPM4 in mammals, is a major structural component of cytoskeletal microfilament [8,9]. TPM4 is a member of the tropomyosin family and mainly involves in the contraction of skeletal and smooth muscle cells or maintaining the stability of the cytoskeleton in non-muscular cells [10,11]. In the past decade, TPM4 expression has been observed in human cancers, which has high-expression or low-expression in tumor tissues depending on tumor types [12,13]. Up to now, the expression status and biological function of TPM4 in colon cancer is still unknown. In early stage of our study, we analyzed microarray data (GSE422) which includes normal intestinal epithelia tissues, colon adenoma tissues and colon carcinoma tissues, and found TPM4 expression was significantly decreased in colon carcinoma tissues compared with normal intestinal epithelia tissues and colon adenoma tissues. In addition, we analyzed the colon cancer patient's cohort from Cancer Genome Atlas database included two hundred and ninety cases, and found patients with TPM4 low-expression had shorter survival than those with TPM4 high-expression. Therefore, we supposed that TPM4 functions as tumor suppressor in colon cancer. In order to investigate the clinical value and biological function of TPM4 in colon cancer, we detected the TPM4 expression in colon cancer clinical samples, and conducted the gain-of-function in colon cancer cell lines.

Abbreviations: TMP4, tropomyosin; PCR, quantitative reverse transcription-polymerase chain reaction; BSA, bovine serum albumin; PBS, phosphate buffered saline; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide; AJCC, American joint committee on cancer; NCCN, national Comprehensive Cancer Network

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https://doi.org/10.1016/j.biopha.2018.01.166
Received 7 January 2018; Received in revised form 31 January 2018; Accepted 31 January 2018

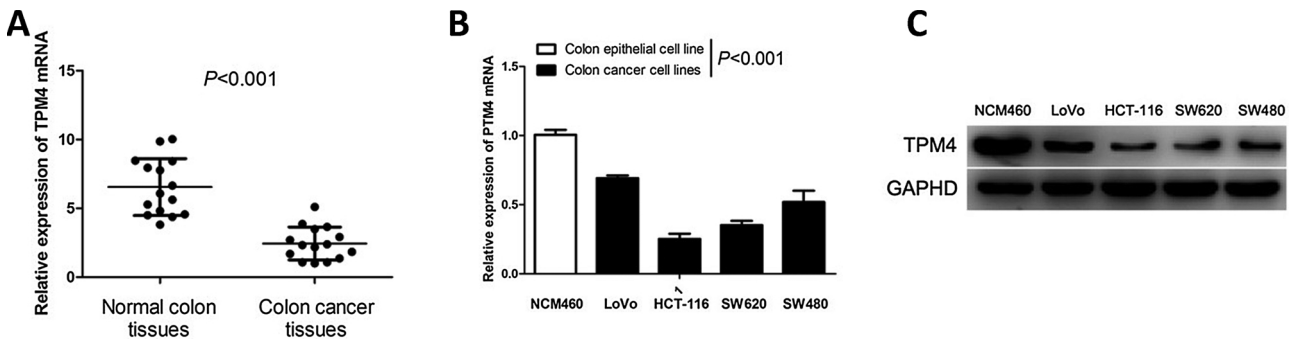

Fig. 1. Expression of TPM4 in colon cancer tissues and cell lines.
(A) Low-expression of TPM4 mRNA is observed in colon cancer tissues compared with normal colon tissues. (B) TPM4 mRNA expression is decreased in colon cancer cell lines than those in colon epithelial cell line. (C) TPM4 protein is low-expressed in colon cancer cell lines compared with colon epithelial cell line.

Table 1
Expression of TPM4 protein between colon.

Group	cases	TPM4		*p*
		Low expression (%)	High expression (%)	
Colon cancer tissues	114	65(57.0)	49(43.0)	P = .003
Normal colon tissues	38	11(28.9)	27(71.1)	

2. Materials and methods

2.1. Patients and samples

Fifteen pairs of fresh colon cancer tissue and adjacent normal tissue, one hundred and fourteen paraffin-embedded colon cancer samples and thirty-eight paraffin-embedded normal colon samples were collected, and the pathological information (age, gender, histological type, clinical stage, tumor depth, lymph node metastasis, distant metastasis, differentiation and family history) was retrieved from the archives of the Pathology Department of Fifth Hospital of Wuhan. Fresh tissue samples were immediately frozen in liquid nitrogen and kept at −80 °C. The histopathological diagnosis was respectively diagnosed by two pathologists. Clinical staging and system treatment were performed according to the 7th edition of the AJCC Cancer Staging Manual and NCCN guideline, respectively. The Research Ethics Committees of Fifth Hospital of Wuhan approved this protocol and written informed consents were obtained from each patient. The entire study was performed based on the Declaration of Helsinki.

2.2. Real-time PCR

RNA isolation and TPM4 mRNA expression determination were carried out according to previous description [14]. The sequence-specific forward and reverse primers sequences for TPM4 were 5′-ACGGT TGCAAAACTGGAAAA-3′ and 5′-TTGGCTCTGGATGGAAAATC-3′, respectively. Forward and reverse primers sequences for GAPDH mRNA were 5′-ATGGGGAAGGTGAAGGTCG-3′ and 5′-GGGGTCATTGATGGCA ACAATA-3′, respectively.

2.3. Immunohistochemistry

Immunohistochemical analysis was performed to measure TPM4 protein expression in one hundred and fourteen paraffin-embedded colon cancer samples and thirty-eight paraffin-embedded normal colon samples. In brief, paraffin-embedded sections were deparaffinized in xylenes for 20 min and rehydrated in graded concentrations of ethanol (100%, 95%, 90%, 80% and 70%). The sections were submerged in EDTA antigenic retrieval buffer and microwaved for antigen retrieval. They were then treated with 3% hydrogen peroxide in methanol to quench endogenous peroxidase activity, followed by incubation with 5% bovine serum albumin to block nonspecific binding. Sections were incubated with anti-TPM4 (1:200 dilution, Abcam) overnight at 4 °C. After washing, tissue sections were treated with secondary antibody, followed by incubation with conjugated horseradish peroxidase streptavidin. Tissue sections were then counterstained with hematoxylin, dehydrated, and mounted. The tissue sections stained immunohistochemically for TPM4 were reviewed, and scored separately by two pathologists blinded to the clinical parameters. Any disagreements were arbitrated by the third pathologists. For TPM4 assessment, staining intensity was scored as 0 (negative); 1 (weak); 2 (moderate); or 3 (strong), and staining extent was scored as 0 (0%); 1 (1%–25%); 2 (26%–50%); 3 (51%–75%); or 4 (70%–100%) depending on the percentage of positively stained cells [15]. The sum of staining intensity and the staining extent scores was used as final staining score. Low expression of TPM4 was defined as 0–4 scores; high expression of TPM4 was defined as more than 4 scores.

2.4. Western blot

Total protein was extracted using RIPA (Thermo Scientific, USA) for Western blot. Equal amounts of protein were denatured and then separated by 10% SDS-PAGE. The target proteins were incubated with the following primary antibodies: TPM4 antibody (Abcam, USA), MMP-2 (Cell Signaling Technology, USA), MMP-9 (Cell Signaling Technology, USA), MT1-MMP (Cell Signaling Technology, USA), or GADPH antibody (CWBIO, China). Then the proteins were incubated with homologous secondary antibodies (CWBIO, China). For HRP detection, an ECL chemiluminescence kit (CWBIO, China) was used. Intensity of blots was performed by Quantity One Software (Bio-Rad, USA).

2.5. Database analysis

The Cancer Genome Atlas database was used to analyze the prognostic significance of TPM4 in colon cancer patients. The colon cancer patient's cohort from Cancer Genome Atlas database included two hundred and ninety cases.

2.6. Cell lines and cell cultures

Four human colon cancer cell lines (LoVo, HCT-116, SW620 and SW480) and a human colon epithelial cell line (HCM460) were obtained from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences. They were cultured as suggested by the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences.