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Hepatocellular Carcinoma-associated Protein TD26 Interacts and Enhances SREBP1 Activity to Promote Tumor Cell Proliferation and Growth

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Abstract
Hepatocellular carcinoma (HCC) is a leading cause of cancer-related death in worldwide.

\[ \text{TD26} = \text{tumor size} \]
Abstract

was highly expressed in HCC tumor

increased lipogenesis in HCC cells

promoted HCC cell proliferation and tumor growth
Introduction
Introduction

Angiopoietin-like protein 8 (ANGPTL8)

C19orf80
lipasin
RIFL
TD26

Gm6484 in mice
expressed:

- in liver
- white adipose
- brown adipose tissues
is a novel but atypical ANGPTL family member
promotes ANGPTL3 cleavage
Hepatocellular Carcinoma (HCC)

- the most common primary malignancy of liver cancer
- sixth most common cancer
- second leading cause of cancer-related death worldwide due to its poor prognosis
Introduction

- Sorafenib is the first-line treatment for advanced HCC
- Sorafenib targets VEGFR, PDGFR and Raf
- In HCC patients the responsive rate to sorafenib remains low.
- Oncogenic pathways: PI3K-AKT, JAK-STAT and hypoxia
- Aberrant lipogenesis cause to cancer
Increased lipid biosynthesis has been reported to promote HCC.

**SREBP1** = sterol regulatory element-binding protein 1

Transcriptional master regulator

Promoting cancer cell growth and metastasis
Introduction

SREBPs

SREBP1

helix-loop-helix–leucine zipper

SREBP2

expression genes involved in biosynthesis of lipid and cholesterol.
Introduction

SREBPs

inactive

S1P

S2P

mature SREBPs
Introduction

SREBP1

synthesis

cholesterol, FA, triglycerides

SREBP2

synthesis

cholesterol

FASN
SCD1
ACC1
ACLY
cell proliferation in varieties of human cancers

activated by the oncogenic AKT-mTORC1 signaling pathway

positively correlate with tumor size and tumor-node metastasis
Introduction

C-terminus (aa from 121 to 198) TD26

nuclear SREBP1

AMPK

Lipogenesis

tumor cell proliferation

tumor progression
Material and methods
Material and methods

samples 56 of primary HCC tumor tissues were 56 of matched normal tissues collected 96 HCC tumor

Renji Hospital School of Medicine, Shanghai Jiaotong University.
Material and methods

56 of primary HCC tumor tissues
56 of matched normal tissues

96 HCC tumor

qPCR and western blot
tissue microarray
Material and methods

Samples were collected:
- 56 of primary HCC tumor tissues
- 56 of matched normal tissues
- 96 HCC tumor samples
- 163 HCC tumor samples

Shanghai Eastern Hepatobiliary Surgery Hospital
Cell lines and cell culture

SMMC-7721
HepG2
Huh-7
SUN-449
SUN-387
MHCC-97L
MHCC-97H
Hep3B,
HEK 293T

DMEM with 10% FBS
Xenograft studies in nude mice

BALB/c (nu/nu) mice

6-week

1.0×10^6 tumor cells

The harvested tumors were:

- IHC
- Western blot with antibodies against TD26 (Sigma)
- PCNA (Cell Signaling Tech)
- Ki67 (Abcam)
Total RNA was extracted using Trizol kit (Invitrogen)
cDNA with a cDNA Synthesis kit (Takara, Japan)
Quantitative PCR

TD26 (F: 5' - CTTAAAGGCTCACGCTGACAAG-3'; R: 5' - TGGAGTCTCCTCCTGGATCTGTC-3')

SREBP1 (F: 5' - GCTGCTGACCGACATCGAA-3'; R: 5' - CCAGCATAGGGTGAGGTCAA-3')

FASN (F: 5' - TATGAAGCCATCGTTGGACGG-3'; R: 5' - CATGCTGTAGCCCACGAGT-3')

SCD1 (F: 5' - CACTTGGGAGCCCTGTATG-3'; R: 5' - TGAGCTCCTGCTGTTATGCC-3')

ACC1 (F: 5' - CTTGAGGGCTAGGTTCTCTTG-3'; R: 5' - CTGGTTCAGCTCCAGAGGTT-3')

ACLY (F: 5' - CAGTCCCAAGTGCCAGATCCC-3'; R: 5' - GTCTCGGGAGCAGACATAGT-3')

β-actin (F: 5' - TCACCCACACTGTGCCCATCTACGA-3'; R: 5' - CAGCGGAACCGCTCATGGCC AATGG-3').
Mass Spectrometry using an HPLC system and mass spectrometer.

- Tumor cells
  - 1.5 ml chl & meth (2/1)
  - Vortex 1 min
  - 3000 rpm
  - 10 min
- Supernatant
  - 800 ul
  - 200 ul isopropanol / methanol
  - Supernatant

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Intracellular triglyceride levels were measured using a triglyceride assay kit (Nanjing Jiancheng Bioengineering Institute, China).

Intracellular cholesterols were detected using a cholesterol assay kit (Nanjing Jiancheng Bioengineering Institute, China).
Luciferase reporter assays

SREBP1 reporter vector
[luciferase P]

24h
cell lysates were analyzed using the Dual-luciferase reporter assay kit(Promega,USA)
Coimmunoprecipitation

Cells harvested

Cells lysed in IP lysis buffer (Thermo Fisher, Inc)
protease inhibitor cocktail (Merck Millipore, Germany)

30 min at 4°C centrifuge at full speed

Extracts of proteins

Incubated for 24h at 4°C

Antibodies

Protein A/G (Pierce)

Pr + Ab

4°C
Coimmunoprecipitation

Pr+Ab+  
Pr A/G

5 wash  
with IP lysis buffer  
(15 min/time)

SDS-PAGE  
&  
Western Blot

Primary antibodies:  
✓ anti-TD26 (Biolegend)  
✓ anti-SREBP1 (Santa Cruz)  
✓ anti-AMPK (Santa Cruz),  
✓ anti-IgG (Abcam)
Results
Results

TD26 is upregulated in HCC tissues and is a poor prognostic marker in HCC

TD26 promotes HCC cell proliferation in vitro

qPCR and western blot assays showed TD26 expression:
in HepG2 & Huh7
in SMMC-7721 & MHCC-97L cells
Results

TD26 promotes HCC tumor growth *in vivo*

TD26 positively correlates with lipogenesis in HCC cells and tissues

TD26 enhances SREBP1 transactivity by increasing the nuclear form of SREBP1(nSREBP1)
TD26 interacts with nSREBP1 to block AMPK

TD26 interacting with nSREBP1 is essential for TD26 mediated tumor progression in HCC cells
Discussion

TD26 is highly expressed in HCC tumor tissues

TD26 is positively correlated with tumor size

TD26 increases HCC cell proliferation and tumor growth by enhancing SREBP1-dependent lipogenesis
Discussion

TD26 can negatively regulate NF-kB

Western blot and ELISA assays showed that secretory signal peptide is not required for TD26 expression but is essential for secretion of TD26

These findings indicate that TD26 can function intracellularly independent of its secretory feature.
Several studies have reported increased TD26 levels in obesity and/or diabetes patients. A recent study indicates increased TD26 levels in cirrhosis patients. Diabetes, obesity, and cirrhosis are risk factors for HCC, it is conceivable that TD26 may play a role in HCC development. SREBP1 should be a promising therapeutic target of cancer.
we demonstrate that TD26 is a novel positive regulator of SREBP1 in HCC by interacting with SREBP1 to compete AMPK.

C-terminal TD26 may play a major role in modulating TD26-mediated increase of lipogenesis and cell proliferation in HCC.
Thank you for attention