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Wnt/β-catenin signaling induces the transcription of cystathionine-γ-lyase, a stimgulator of tumor in colon cancer

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ABSTRACT

Cystathionine-γ-lyase (CSE) is a major endogenous enzyme producing H_2S which, as a third gasotransmitter, plays important roles in many physiological and pathological processes. The mechanism of regulating CSE gene expression is unclear and the roles of CSE/H_2S in tumor also have not got a profound understanding, especially in colon cancer. Our study demonstrated that CSE gene expression was regulated by the Wnt pathway on transcriptional level. Activating the Wnt pathway by either Wnt3a or LiCl increased CSE mRNA and protein levels, while siRNA-mediated silence of β-catenin decreased CSE mRNA and protein levels. XAV939 treatment which accelerated β-catenin degradation could reduce CSE protein level. To reveal the mechanism, two TCF/LEF binding sites were found in CSE promoter whose activity had a positive response to β-catenin overexpression in 293 T cells. Mutations of TCF/LEF binding sites led to an increase of the promoter activity. It indicated that TCF/LEF likely acted as a repressor to CSE gene transcription, and Wnt signal contributed to free β-catenin accumulation to possibly relieve the repression. Either knockdown of CSE by shRNA (shCSE) or its inhibition by PAG decreased SW480 cell proliferation, migration, and tumor xenograft growth in nude mice. In conclusion, we have demonstrated that the Wnt pathway regulates CSE gene expression on transcriptional level and CSE/H_2S plays important roles in colon cancer.

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1. Introduction

Cell signaling is pivotal for cells to communicate extensively among each other and with the environment, and dysregulation of cell signaling causes many diseases such as cancer [1]. Hydrogen sulfide (H_2S), as the third gaseous signaling molecule following nitric oxide (NO) and carbon monoxide (CO) [2], plays important roles in the cardiovascular and nervous systems [3]. Endogenous H_2S is generated in L-cysteine metabolism by two pyridoxal-5′-phosphate (PLP)-dependent enzymes, cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CSE) and another 3-mercaptopyruvate sulfurtransferase (3-MST) [2–4]. CSE, as a key H_2S-producing enzyme, catalyzes cystathionine into L-cysteine in the last step of trans-sulfuration pathway, and then L-cysteine is metabolized to yield H_2S [2,5]. CSE is prevalently expressed in many tissues, but not in the central nervous system [4]. A recent study demonstrates that vascular smooth-muscle cells (SMCs) exposed to calcium ionophore A23187, thapsigargin, or tunicamycin promotes CSE to translocate from the cytosol to mitochondria, not only in cytoplasm in accordance with common understanding [6]. CSE exists as a homotetramer [7], and belongs to the γ-family of PLP enzymes [7]. The deficiency of the activity of CSE brings about hereditary cystathioninuria (MIM 219500) [8]. Hereditary analysis shows that two nonsense mutations, namely exon 8c. 940-941delCT and exon 11c.1220delC, and two missense mutations, namely exon 2c.356C > T (T67I) and exon 7c.874C > G(Q240E), all impair the CSE's affinity for PLP and decrease enzyme activity [8,9]. Furthermore, it is also found that a single nucleotide polymorphism in exon 12, namely c.1364G > T (S403I) is linked to elevated plasma homocysteine levels, but unrelated to cystathioninuria [8–10].

Endogenous H_2S performs vital roles in many physiological processes, including vasorelaxation [11,12], angiogenesis [11,13], cellular energy production [6,14], neuromodulator [4], cytoprotection [15] and pathological processes including cardiac fibrosis [16], inflammation[4], obesity, diabetes, atherosclerosis and hypertension, etc. [17,18]. H_2S exerts its functions via multiple mechanisms including activation of signal pathways [13,19,20], activation of VEGFR2 [21], stimulation of potassium channels [3,22], increase of cellular glutathione (GSH) level [15]. Studies manifest that administering exogenous NaHS induces the proliferation of human colon cancer HCT116 cells via increase of Akt and ERK

Abbreviations: CSE, cystathionine γ-lyase; PLP, pyridoxal-5′-phosphate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; TCF, T cell factor; LEF, lymphocyte enhancer binding factor.

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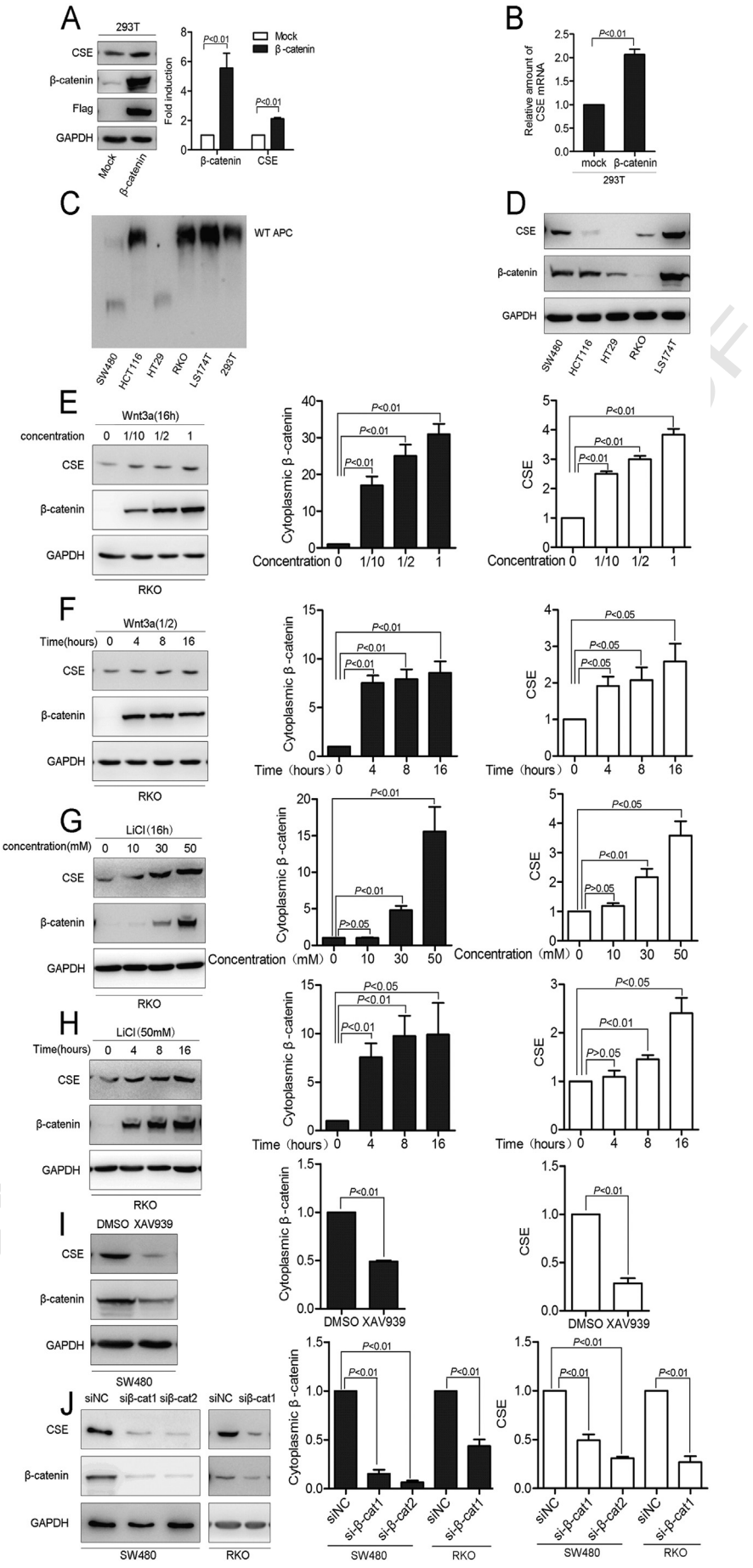
A
293T
CSE
β-catenin
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GAPDH
Mock
β-catenin
Mock
β-catenin
Fold induction
P<0.01
P<0.01
β-catenin
CSE
B
Relative amount of CSE mRNA
P<0.01
mock
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293T
C
WT APC
SW480
HCT116
HT29
RKO
LS174T
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D
CSE
β-catenin
GAPDH
SW480
HCT116
HT29
RKO
LS174T
E
Wnt3a(16h)
concentration 0 1/10 1/2 1
CSE
β-catenin
GAPDH
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Cytoplasmic β-catenin
P<0.01
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Wnt3a(1/2)
Time(hours) 0 4 8 16
CSE
β-catenin
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Cytoplasmic β-catenin
P<0.01
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Time (hours) 0 4 8 16
CSE
P<0.05
P<0.05
P<0.05
Time (hours) 0 4 8 16
G
LiCl(16h)
concentration(mM) 0 10 30 50
CSE
β-catenin
GAPDH
RKO
Cytoplasmic β-catenin
P<0.01
P<0.01
P>0.05
Concentration (mM) 0 10 30 50
CSE
P<0.05
P<0.01
P>0.05
Concentration (mM) 0 10 30 50
H
LiCl(50mM)
Time(hours) 0 4 8 16
CSE
β-catenin
GAPDH
RKO
Cytoplasmic β-catenin
P<0.05
P<0.01
P<0.01
Time (hours) 0 4 8 16
CSE
P<0.05
P<0.01
P>0.05
Time (hours) 0 4 8 16
I
DMSO XAV939
CSE
β-catenin
GAPDH
SW480
Cytoplasmic β-catenin
P<0.01
DMSO XAV939
CSE
P<0.01
DMSO XAV939
J
siNC siβ-cat1 siβ-cat2 siNC siβ-cat1
CSE
β-catenin
GAPDH
SW480 RKO
Cytoplasmic β-catenin
P<0.01
P<0.01
P<0.01
siNC
si-β-cat1
si-β-cat2
siNC
si-β-cat1
SW480 RKO
CSE
P<0.01
P<0.01
P<0.01
siNC
si-β-cat1
si-β-cat2
siNC
si-β-cat1
SW480 RKO

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