International Journal of Biochemistry and Cell Biology 97 (2018) 16–27

Contents lists available at ScienceDirect

International Journal of Biochemistry and Cell Biology

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

STYK1 promotes cancer cell proliferation and malignant transformation by activating PI3K-AKT pathway in gallbladder carcinoma

Yun-ping Hu[a,b,c,d,1], Zeng-bin Wu[e,1], Lin Jiang[a,b,c,1], Yun-peng Jin[a,b,c], Huai-feng Li[a,b,c], Yi-jian Zhang[a,b,c], Qiang Ma[a,b,c], Yuan-yuan Ye[a,b,c], Zheng Wang[a,b,c], Yong-chen Liu[a,b,c], Hong-zhuan Chen[d,*], Ying-bin Liu[a,b,c,*]

[a] Department of General Surgery, Xinhua Hospital, Affiliated to Shanghai Jiao Tong University School of Medicine, 1665 Kongjiang Road, Shanghai, 200092, China
[b] Shanghai Key Laboratory of Biliary Tract Disease Research, 1665 Kongjiang Road, Shanghai, 200092, China
[c] Shanghai Research Center of Biliary Tract Disease, 1665 Kongjiang Road, Shanghai, 200092, China
[d] Department of Pharmacology and Chemobiology, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China
[e] Emergency Department, Xinhua Hospital, Affiliated with Shanghai Jiao Tong University School of Medicine, No. 1665 Kongjiang Road, Shanghai 200092, China

ARTICLE INFO

Keywords:
Gallbladder carcinoma
STYK1
Proliferation
Metastasis
AKT

ABSTRACT

Gallbladder carcinoma (GBC) is the most common malignancy of the biliary tract with extremely poor prognosis. The malignant transformation of GBC is associated with cell proliferation, invasion, and epithelial-mesenchymal transition (EMT). However, the molecular mechanisms underlying GBC progression are poorly understood. We found that serine threonine tyrosine kinase 1 (STYK1) was elevated in GBC and was negatively correlated with clinical outcomes and prognosis. Overexpression of STYK1 in GBC cell lines gave rise to increased cell proliferation, colony formation, migration and invasion, thus committing cells to undergoing EMT. In contrast, silence of STYK1 led to opposite effects on cell transformation. Consistent with STYK1 gene knockdown, AKT specific inhibitor MK2206 abrogated tumor promoting action induced by STYK1, suggesting that PI3K/AKT pathway is essential for the oncogenic role of STYK1 in GBC. STYK1 shRNA in GBC cells inhibited development of xenografted tumors compared with control cells. Collectively, our findings suggest that STYK1 is a critical regulator of tumor growth and metastasis, and may serve as a potential target for GBC therapy.

1. Introduction

Gallbladder cancer (GBC) is the most commonly diagnosed malignancy of the biliary tract with an incidence of 2.5 in 100,000 individuals (Randi et al., 2006; Srivastava et al., 2011; Wu et al., 2014). It is emerging that gallstones and chronic inflammation are the most common factors to develop GBC (Wolpin and Mayer, 2010); however, the pathogenesis of the pre-cancer remains largely unknown. Given the fact that GBC lacks specific early symptoms, the clinical diagnosis is frequently delayed (Hu et al., 2014; Lazcano-Ponce et al., 2001). Therefore, most of these patients unfortunately miss therapeutic opportunities that probably restrain the disease progression, and thus rapidly succumb to the disease. Although surgical resection is known as the most common and effective treatment for GBC, high recurrence of the disease keeps static (Batra et al., 2005; Nagorney and McPherson, 1988; Tazuma and Kajiyama, 2001), indicating that GBC is currently incurable and the prognosis is very poor (Li et al., 2014). Because failure to improve patient survival is mainly ascribed to the lack of our understanding about the pathogenesis and molecular mechanisms underlying tumor malignant transformation, it is of paramount importance to identify novel factors that mediate tumor development. As anticipated, identification of such tumor-promoting factors may serve as potential biomarkers and therapeutic targets for the early diagnosis and treatment of patients with GBC.

Epithelial-mesenchymal transition (EMT) is a multi-step pathologic process and plays a crucial role during cancer metastasis. EMT involves a loss of epithelial properties, such as decreased expression of E-cadherin, and a gain of mesenchymal phenotypes, such as fibroblastic formation with increased expression of N-cadherin and vimentin (Lee et al., 2006; Xia et al., 2013). EMT is known to mediate a number of

Abbreviations: GBC, gallbladder carcinoma; STYK1, serinethreoninetyrosinekinase1; EMT, epithelial-mesenchymal transition; H&E, hematoxylin and eosin; qRT-PCR, quantitative real-time PCR analysis; IHC, immunohistochemicalanalysis; CCK-8, Cell Counting Kit-8; PI3K, phosphoinositide-3-kinase

* Corresponding authors at: Department of General Surgery, Xinhua Hospital, Affiliated to Shanghai Jiao Tong University School of Medicine, 1665 Kongjiang Road, Shanghai, 200092, China.
E-mail addresses: yaoli@shsmu.edu.cn (H.-z. Chen), liuyingbin@xinhuamed.com.cn (Y.-b. Liu).
[1] Equal contributors.

https://doi.org/10.1016/j.biocel.2018.01.016
Received 18 October 2017; Received in revised form 13 January 2018; Accepted 23 January 2018

cancer malignant transformation (Li et al., 2012; Wang et al., 2016). However, the molecular mechanisms that regulate EMT in GBC remain fully understood.

Serine threonine tyrosine kinase 1 (STYK1), also known as a novel oncogene with kinase domain (NOK), is comprised of a transmembrane domain, intracellular domain and kinase domain. As one member of the receptor protein tyrosine kinase like family (Liu et al., 2004; Ye et al., 2003), STYK1 has the ability of growth factor membrane receptors to promote tumor cell proliferation and tumor development (Chen et al., 2005). Aberrant expression of STYK1 has been found in a wide range of cancers, including prostate cancer, breast cancer, lung cancer, acute leukemia, and ovarian cancer (Amachika et al., 2007; Chen et al., 2014, 2005; Jackson et al., 2009; Kimbro et al., 2008; Orang et al., 2014). Recently, Wang et al. found that STYK1 is capable of promoting metastasis in human hepatocellular carcinoma through MEK/ERK and PI3K/AKT pathway (Wang et al., 2016). These data indicate that increased expression levels of STYK1 is associated with cancer progression and it may serve as a new biomarker for cancer diagnosis and prognosis. However, the pathological role of STYK1 in the development of GBC remains enigmatic. In the current study, we sought to define the relationship between expression of STYK1 and clinical outcomes, and also demonstrate its pathological and invasive activity in the GBC development. The study may yield great clinical promising for the diagnosis and the treatment of GBC.

2. Materials and methods

2.1. Tissue samples

All tissue samples were obtained from GBC patients who underwent cholecystectomy without receiving preoperative chemotherapy, radiotherapy, or androgen therapy between 2008 and 2013 at the Department of General Surgery, Xinhua Hospital, School of Medicine, Shanghai Jiao Tong University, China. Diagnosis of GBC, cholelithiasis, presence of lymph node metastases and tumor differentiation were confirmed by hematoxylin and eosin (H&E) staining. For quantitative real-time PCR analysis (qRT-PCR), fresh GBC tissues as well as the matched adjacent non-tumor tissues were frozen and stored at −80 °C within 15 min after removal. Paired non-tumor tissues were dissected at least 2 cm away from the tumor border and were confirmed to be lack of tumor cells by microscopy. For immunohistochemical staining, each tissue sample was fixed in 4% formalin immediately after removal and embedded in paraffin. This study was approved by the ethics committee of our hospital, and all patients provided written consent for the use of their tumor tissues for clinical research.

2.2. Cell culture and reagents

The human GBC cell lines GBC-SD, NOZ, OCUG-1 and EH-GB-1 were obtained from the Health Science Research Resources Bank (Osaka, Japan). The other human GBC cell line, SGC-996, was obtained from the Medical School at Tongji University (Shanghai, China). GBC-SD, OCUG-1 and EH-GB-1 were grown in high-glucose DMEM (Gibco, Grand Island, NY, USA), NOZ was cultured in Williams (Gbico) and SGC-996 was maintained in RPMI 1640 (Hyclone, Logan, TX, USA). Cells were cultured in recommended medium supplemented with 10% fetal bovine serum (Gibco, USA), 100 μg/mL streptomycin, and 100 U/mL penicillin (Hyclone). All of the cells were cultured under 5% humidified CO_2 enriched atmosphere at 37 °C.

MK2206 (Cat. No.431050) was purchased from MedChem Express (New Jersey, USA), and incubated with NOZ and SGC-996 for 20 μM (10 h).

2.3. Vector construction and lentivirus-mediated RNA interference

The full-length STYK1 cDNA (GenBank accession number NM_018423) was amplified and subcloned to into pCDNA3.0-N-myc expressing vector using the primers listed in Table S1. Then the STYK1 expressing plasmid was transfected into SGC-996 and NOZ cells with empty vector as a control. The expression level of STYK1 was then examined by western blot.

The target siRNA sequence to STYK1 was 5′-GGTGGTACCTGAACT GTAT-3′ as reported previously (Chen et al., 2016), and the negative control sequence was 5′-TTCTCCGAACGTGTCACGT-3′. STYK1 siRNA and negative control RNA were synthesized and inserted into the lentivirus core vector containing a GFP reporter and puromycin resistance. Recombinant lentiviruses expressing STYK1-shRNA or negative control shRNA were provided by Genechem (Shanghai, China). Cells were transfected with lentivirus at an MOI (multiplicity of infection) of 40 for 48 h and then selected with puromycin (1 μg/mL) for 3 days. The expression level of STYK1 in the infected cells was validated by qRT-PCR analysis and western blot assays.

2.4. Antibodies and western blot analysis

The primary antibodies against E-cadherin, N-cadherin, *p*-AKT (Thr308), AKT, anti-vimentin, anti-caspase 3, anti-cleaved caspase 3, anti-Bcl-2, *p*-ERK1/2 (Thr202/Tyr204), ERK1/2 and anti-Bax were purchased from Cell Signaling Technology (Danvers, MA, USA). STYK1 antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-myc antibody and the internal control anti-GAPDH (1:5000) antibody were obtained from Abways technology (Shanghai, China). Briefly, GBC cells were washed twice with ice-cold PBS and collected cells were lysed in lysis buffer (Beyotime, Shanghai, China) supplemented with Complete Mini Protease Inhibitor Cocktail and Phsopshostop (Roche, Switzerland). The protein concentration in the cell extracts was measured using the bicinchoninic acid assay system (Beyotime, Shanghai, China) with BSA as a standard. Equal amount of protein was separated by SDS-PAGE with pre-stained protein molecular weight marker (Fermentas, Burlington, Canada) as a standard, which was then transferred to a polyvinylidene fluoride (PVDF) membrane (Millipore, Darmstadt, Germany). Membranes were blocked with 5% skim milk and then incubated with the following respective primary antibodies including anti-STYK1 (1:200), anti-N-cadherin (1:1000), anti-E-cadherin (1:1000), anti-vimentin (1:1000), anti-caspase 3 (1:1000), anti-cleaved caspase 3 (1:1000), anti-Bcl-2 (1:1000), anti-*p*-AKT (Thr308) (1:1000), anti-AKT (1:1000), anti-Bax (1:1000), anti-myc (1:1000), *p*-ERK (1:1000), ERK (1:1000) and internal control anti-GAPDH (1:5000) antibodies at 4 °C overnight. After washing 3 times with TBST buffer, the membranes were incubated with a goat anti-rabbit/anti-mouse-peroxidase-conjugated second antibody (1:5000, Abcam, Cambridge, UK), and the bands were visualized using an enhanced chemiluminescent detection reagent from Pierce (Rockford, IL, USA). Photographs were obtained and the bands were scanned using the Gel Doc 2000 (BioRad, USA).

2.5. Immunohistochemical staining and scoring

Immunohistochemical staining was performed using a standard immunoperoxidase staining procedure. In brief, the endogenous peroxidase activity was blocked with 3% H_2O_2 for 30 min. The sections were incubated with an anti-STYK1 antibody (Abcam, ab97451) at room temperature for 2 h after blocking. Then the sections were incubated with a goat anti-rabbit-peroxidase-conjugated second antibody (Santa Cruz). Finally, DAB (3,3-diaminodbenzidine) substrate (Dako, Carpinteria, CA, USA) was added and the slides were counterstained with hematoxylin. Paraffin-embedded mouse tumor samples were processed similarly as described above for staining of N-cadherin and PCNA (Cell Signaling Technology).

STYK1 expression in GBC specimens was analyzed in a blinded manner without prior knowledge of the clinical data. Sections were semi-quantitatively scored for the percentage of positive staining cells