Abstract. The increased expression of sialyl-Lewis (SLex) epitope on the surface of tumor cells has been known for decades. However, genetic manipulation of the expression of SLex and the role of SLex in cancer cell proliferation remains to be fully elucidated. The present study suggested that the monoclonal antibody of SLex (KM93) significantly inhibited the proliferation of human hepatocarcinoma (Hcc) cells. The expression levels of three sialyl-Lewis oligosaccharide antigens, SLex, SLea and dimeric SLex (SdLex), were determined on the cell surface of the MHcc97 human Hcc cell line. The expression of SLex was markedly higher in MHcc97 cells than in normal liver cells. The expression of SdLex was also relatively high, however, no significant difference was observed between normal liver cells and Hcc cells. The expression of SLea was only detected in trace quantities. Fucosyltransferase (FUT) is the key enzyme of the fucosylation step in the biosynthesis of sialyl-Lewis oligosaccharide antigens. Therefore, the present study investigated the expression of FUTs. It was found that the mRNA and protein expression levels of FUT7 were high in the MHcc97 Hcc cell line compared with levels in normal liver cells. FUT6 was also expressed at a high level, although the difference was not statistically significant between MHcc97 cells and normal liver cells. No expression of FUT3 was detected. The results were consistent with the change in sialyl-Lewis antigens. The effects of FUT7 small interfering (si)RNA transfection on the expression of FUT7, expression of SLex and MHCC97 cell proliferation were also examined. Following FUT7 siRNA transfection, the expression of FUT7 was marked downregulated, as determined by western blot and reverse transcription-quantitative polymerase chain reaction methods. The results from flow cytometry showed that the synthesis of SLex was also inhibited, which was consistent with the downregulated expression of FUT7. MHCC97 cell proliferation was also significantly inhibited following FUT7 siRNA transfection, which was correlated with suppression of the S-phase in cell cycle progression. By using inhibitors of various signaling pathways, it was found that the knockdown of FUT7 inhibited the activation of phospholipase Cγ (PLCγ) by inhibiting the translocation and phosphorylation of PLCγ. In conclusion, the results suggested that FUT7 has an important functional role in human Hcc cell proliferation by controlling cell cycle progression via the PLCγ/extracellular signal-regulated kinase signaling pathway. The inhibition of SLex and FUT7 siRNA transfection may provide a novel therapeutic methodology to treat tumors that express SLex glycoconjugates.

Introduction

The sialyl-Lewis oligosaccharide antigens (SLeα and SLeβ) are blood group related antigens which are important in inflammation by initiating the leukocyte-endothelium interaction (1,2). To date, it has been found that sialyl-Lewis antigens are involved in several molecular interactions, including differentiation, development and malignancy (3-5), and their expression is markedly enhanced in several types of carcinoma cells (6,7). It has also been reported that the expression of sialyl-Lewis oligosaccharide antigens on tumor cells is closely associated with tumor progression, and statistical relevance has been confirmed between the postoperative prognosis of tumor patients and the degree of sialyl-Lewis oligosaccharide antigen expression on tumor tissues (8,9). Therefore, sialyl-Lewis oligosaccharide antigens are widely considered as cancer markers. The present study determined the expression of three sialyl-Lewis oligosaccharide antigens, SLeα, SLeβ, and dimeric SLeβ (SdLeβ) on the cell surface of the MHCC97 human hepatocarcinoma (HCC) cell line. The expression of SLeα was higher in MHCC97 cells compared with its expression in normal liver cells. The expression of SdLeβ was also high, although did not differ significantly between the normal liver cells and MHCC97 cells. The expression of SLeβ was only detected in trace quantities. On using a monoclonal
antibody of sialyl-Lewis antigens, the results indicated that the anti-SLeα antibody KM93 significantly inhibited MHCC97 cell proliferation. The synthesis of sialyl-Lewis antigens is regulated by a set of glycosyltransferases. Fucosylation is the final step in the process of sialyl-Lewis antigen synthesis and it is catalyzed by α1,3/1,4 fucosyltransferases (α1,3/1,4 FUTs). The α1,3/1,4 FUTs catalyze the connection of fucose in a (1,3) and a (1,4) linkage to the sialylated precursors (10-15). Currently, several human α(1,3) FUT genes (αFUT-3, 4, 5, 6, 7 and 9) have been cloned, identified and characterized (15-24). Each enzyme has a unique substrate and tissue specificity (25). FUT4, FUT9, FUT7 and FUT3 efficiently fucosylate sialylated acceptors, whereas FUT4 and FUT9 preferentially fucosylate non-sialylated neutral acceptors (20,24). α1,3/1,4 FUT-3 is the only αFUT with both α1,3 and α1,4 fucosylation activity, leading to the generation of α1,3-fucosyl-containing Leα and SLeα, and its isomer α1,4-fucosyl-containing SLeβ, respectively (7). By contrast, α1,3FUT7 only catalyzes the synthesis of SLeβ (NeuAcα2/3Galβ1/4(Fucal-3)GlcNAcb1/3Galβ1/R) by adding the distal fucose residue to α2,3-sialylated lactosamine (9). FUT7 is mainly expressed in tumor cells, endothelial cells and leukocytes. There is a rigid acceptor specificity for FUT7, which can only accept the distal N-acetylglucosamine on α2,3 sialylated lactosamines to form SLeα antigen. Therefore, FUT7 is the key enzyme of SLeα synthesis (9,22,23). It has been widely reported that the expression levels of α1,3/1,4FUTs are positively correlated with tumor progression and negatively correlated with patient prognosis (8,9). For example, tumor growth was significantly increased following transfection of the FUT3 gene into PC-3 prostate cancer cells (26). Additionally, cell proliferation was significantly inhibited following gtransfection of FUT3/6 antisense RNA into colon cancer cells (27). Until now, the role of FUT7 in cancer cell proliferation and growth has not been fully elucidated.

In the present study, the effects of the knockdown of FUT7 on the expression of SLeα and human HCC cell proliferation were examined. The effects of FUT7 small interfering (si)RNAs on the expression and activation of signaling molecules were also investigated. It was found that the knockdown of FUT7 decreased the expression of SLeα, inhibited the translocation and phosphorylation of phospholipase Cγ (PLCγ), and arrested cell cycle progression, which subsequently led to the suppression of MHCC97 HCC cell proliferation. These findings indicated that SLeα and FUT7 may be important in human HCC cell proliferation. Inhibition of the SLeα oligosaccharide antigen may serve as a novel therapeutic method to treat tumors that express SLeα glycoconjugates. In addition, FUT7 maybe a potential target and FUT7 RNA interference (RNAi) may be developed as a novel therapeutic approach for SLeα-positive cancer therapy.

Materials and methods

Cell culture and inhibitors treatment. The THLE-2 normal human liver cell line and HT29 human colo-rectal adenocarcinoma cell line were purchased from the American Type Culture Collection (Manassas, VA, USA). The MHCC97 human HCC cell line was purchased from Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). The THLE-2 cell line was grown in LONZA BEGM medium (Lonza/Clonetics Corporation, Walkersville, MD, USA). The MHCC97 cell line was cultured in Dulbecco's Modified Eagle's medium (DMEM; Invitrogen, Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% FCS (Invitrogen, Thermo Fisher Scientific, Inc.). The cells were grown at 37°C in a humidified incubator under 5% CO2 and passaged when 70-80% confluent using 0.25% (w/v) trypsin solution in 0.04% (w/v) EDTA. At 1 h following passage of MHCC97 cells, PLCγ inhibitor (U73122; 10.5 mM; Merck KGaA, Darmstadt, Germany), protein kinase A (PKA) inhibitor (KT5720; 280 nM; Sigma-Aldrich; Merck KGaA), phosphoinositide 3-kinase (PI3K) inhibitor (LY294002; 16.5 mM; Sigma-Aldrich; Merck KGaA), extra-cellular-signal-regulated kinase (Erk) inhibitor (ErkI; 50 mM; Merck KGaA) and CDC25 inhibitor (CDC25 inhibitor II; 1.05 mM; Merck KGaA) were added into the culture medium and the cells were cultured for a further 24 h. Cell survival was detected through MTT assay.

Detection of Lewis antigen with flow cytometric analysis. Cell surface Lewis antigen expression was detected by measuring the indirect immunofluorescence intensity using the FACS Calibur system (BD Biosciences, San Jose, CA, USA). Cell Quest Pro software (version 5.1; BD Biosciences) was used for cell acquisition and analysis. The cells were detached with 2 mM EDTA, washed twice with cold PBS and then prepared as a single cell suspension. A suspension of 10⁶ cells was then incubated with mouse anti-SLeα antibody (KM93, cat. no. 11-006, 1:100, GlycoTech, Gaithersburg, MD, USA), anti-SLeβ antibody (FH6, cat. no. 11-007, 1:100, GlycoTech) and anti-SLeα antibody (CA19-9, cat. no. 11-009, 1:100, GlycoTech) at 4°C for 1 h. Chilled PBS was used to wash the cells three times to remove unbound antibodies. The cells were then incubated with R-phycocerythrin or peridinin chlorophyll protein (R-PE or Per-CP)-conjugated rabbit anti-mouse IgM (cat. no. 562033 and 553409, 1:200, BD Biosciences) at 4°C for 30 min in the dark. Chilled PBS was used to wash and remove unbound secondary antibodies. The cells were then fixed using 1% formaldehyde (Sigma; EMD Millipore, Billerica, MA, USA) for 30 min in the dark. A total of 1x10⁶ cells from each sample tube were acquired for analysis using FACS Calibur. Cells incubated with secondary antibody only and unstained cells were used as controls.

Reverse-transcription-quantitative polymerase chain reaction (RT-qPCR) analysis. Total RNA from the cells were obtained using ab RNasey Mini kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's protocol. Subsequently, 1 µg of total RNA was mixed with SYBR-Green PCR Master mix reagents of the PrimeScript RT Reagent kit (Takara Biotechnology Co., Ltd., Dalian, China) to synthesize the complementary DNA (cDNA) in the 7900HT real time PCR system (Applied Bio systems, Thermo Fisher Scientific, Inc.) according to the standard quantitative PCR protocol (25). Briefly, the RT-qPCR procedure was performed in a 20-µl reaction volume, including primers (0.25 µl, 10 pmol/l), 2X Mix SYBR-Green I (10 µl; Promega, Madison, WI, USA), template DNA (1 µl) and sterile water. The PCR steps constituted an initial denaturation step (95°C for 2 min), 50 cycles of 95°C for
Knockdown of FUT7 in MHCC97 cells by RNAi. The expression of FUT7 in MHCC97 cells was silenced using specific siRNAs (Silencer siRNA transfection, Ambion, Thermo Fisher Scientific, Inc.). Scramble siRNA (siNC; Silencer siRNA transfection, Ambion, Thermo Fisher Scientific, Inc.) was used to confirm the specificity of FUT7 siRNAs. Untransfected cells were used as control. The siRNA transfection into MHCC97 cells was performed following the manufacturer's protocol. A total of 1x10⁵ cells/ml of MHCC97 cells were re-suspended in DMEM. The transfection complexes were prepared by mixing RNAi MAX Lipofectamine transfection agent (Ambion; Thermo Fisher Scientific, Inc.) and siRNA (20 nM) in DMEM. The MHCC97 cells and transfection complexes were mixed and then incubated for 24 h at 37°C in 6-well (2x10⁵ cells/well) plates (Nalge Nunc International, Penfield, NY, USA). The cells were collected at 48 h for RNA analysis and 72 h for protein analysis. The knockdown of FUT7 was confirmed by RT-qPCR and western blot analyses.

Cell survival MTT assay. To investigate the effect of FUT7 silencing on cell proliferation and viability, MTT was added to the MHCC97 cells at 0, 24, 48, 72, 96 and 120 h following transfection with FUT7 siRNA. In detail, 5x10⁴ cells were seeded into 96-well plates and, the following day, FUT7 was knocked down as described above. MTT (20 µl, 5 mg/ml) was added into each well at different time points. Following culture for 4 h at 37°C the DMEM was gently aspirated and 100 µl of dimethyl sulfoxide was added into each well. The absorbance of formazan at a 490 nm wavelength was measured using a Bio-Rad Microplate Reader 550 (Bio-Rad Laboratories, Inc.) and a cell growth curve was plotted.

Analysis of cell cycle. Ice-cold PBS was used to wash the collected control cells and transfected cells, and 1x10⁵ cells were fixed in 70% ethanol for 24 h at -20°C. The cells were then washed twice with chilled PBS, and stained with propidium iodide (PI; 20 µg/ml, Sigma; EMD Millipore) including RNase (100 µg/ml) for 30 min at room temperature. A BD FACScan flow cytometer was used to analyze the DNA content of cell cycle phases. A total of 2x10⁴ cells per sample were collected and analyzed using BD FACS Diva™ analysis software (version 6.2; BD Biosciences).

Statistical analysis. Statistical analysis was performed using SPSS 20.0 software (IBM SPSS, Armonk, NY, USA). All values are expressed as the mean ± standard error of the mean and are representative of at least three independent experiments. The data were analyzed using one-way analysis of variance. When the results were significant, post hoc testing of differences between each group was performed using Bonferroni's correction. P<0.05 was considered to indicate a statistically significant difference.

Results

Expression of sialyl-Lewis oligosaccharide antigens and FUTs in the MHCC97 human HCC cell line. The expression of three sialyl-Lewis antigens, SLε, SDLe and SLε, were determined and the results are shown in Fig. 1A and B. The expression of SLε was significantly increased in the MHCC97 human HCC cells compared with that in THLE-2 normal human liver cells. The expression of SDLe was high, however,
no significant difference was observed between the MHCC97 cells and THLE-2 cells. The expression of SLε was absent or negligible. The high expression of SLε in the HT29 cells confirmed the high affinity of the anti-SLea antibody (Fig. 1C). RT-qPCR and western blot methods were used to detect the mRNA and protein levels of FUT7 and FUT6, respectively. The mRNA and protein expression levels of FUT7 were high in the MHCC97 cells compared with expression levels in the THLE-2 normal liver cells. FUT6 was also expressed at high levels, however, no significant difference was found between the MHCC97 cells and THLE-2 cells (Fig. 1D and E). No FUT3 was detected.

Inhibition of SLε by KM93 monoclonal antibody inhibits MHCC97 cell proliferation. Different monoclonal antibodies targeting sialyl-Lewis antigens were added to inhibit the sialyl-Lewis antigens on the cell surface of MHCC97 cells and normal liver cells, and cell growth curves were plotted using the data obtained from the MTT method. It was found that the anti-SLea monoclonal antibody KM93 significantly suppressed MHCC97 cell proliferation, but not that of normal liver cells. The anti-SDLε antibody FH6 had a minimal suppression effect, which was not statistically significant. The anti-SLea antibody CA19-9 did not suppress MHCC97 cell proliferation (Fig. 1F).

FUT7 siRNAs transfection reduced the expression of FUT7 and SLε. RT-qPCR, western blot and flow cytometry methods were used to investigate the effect of FUT7 RNAi on the expression of FUT7 and SLε. The RT-qPCR and western
blot results showed that the mRNA and protein levels of FUT7 (Fig. 2A and B) were markedly lower in the FUT7 siRNA-transfected MH cc97 cells, compared with those in the untransfected wild-type cells (control). The FUT7 siRNAs effectively inhibited the expression of FUT7. The inhibitory effect of FUT7 siRNA on the expression of SLe\(^x\) was also investigated by flow cytometry. The fluorescence intensity of SLe\(^x\) in the untransfected control cells was more marked than that in the FUT7-knockdown cells (Fig. 2C and D). siNC-transfected cells were used to confirm the specificity of FUT7 siRNA. No significant difference in the expression of FUT7 or SLe\(^x\) was found between the siNC cells and untransfected control cells. The above results suggested that siRNAs of FUT7 suppressed the expression of FUT7 and markedly inhibited the synthesis of SLe\(^x\) oligosaccharide antigen.

**Knockdown of FUT7 inhibits human HCC cell proliferation.**

An MTT assay was used to investigate the impact of FUT7 knockdown in HCC cell proliferation. The number of proliferated cells was measured by detecting the absorbance at a wavelength of 490 nm. The MTT assay showed that cell proliferation of the FUT7-knockdown cells was suppressed compared with that of control cells. No significant difference was found between the cell proliferation rate of siNC cells and control cells (Fig. 3A). This indicated that the silencing of FUT7 may significantly suppress HCC cell proliferation.

**FUT7 knockdown decreases HCC cell distribution into the S-phase.** To further investigate the suppression of cell proliferation in FUT7-knockdown cells, cell cycle distribution was analyzed by flow cytometry. As shown in Fig. 3B and C, flow cytometry with PI-stained cells showed that untransfected control cells and siNC cells were presented in the G0/G1 (58.78±6.45 and 60.52±6.17%), S (25.20±2.17 and 22.18±2.87%) and G2/M (2.97±0.60 and 3.10±0.77%) phases. In the FUT7 siRNA-transfected MHcc97 cells, the S-phase fraction was lower (16.40±2.10% for FUT7 siRNA-1 and 13.27±2.75% for FUT7 siRNA-2 cells, P<0.05) and the G0/G1 fraction was higher (69.62±6.84% for FUT7 siRNA-1 and 67.72±6.53% for FUT7 siRNA-2 cells, respectively, P<0.05). This indicated that the fractions of cells in the S-phase of cell cycle, which represents a population of dividing cells, decreased following the knockdown of FUT7. A concomitant increase was observed in the number of cells at the G0/G1 phases. These results showed that FUT7 knockdown suppressed cell proliferation by inhibiting cell
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Figure 3. Cell proliferation and cell cycle assay after knockdown of FUT7. MHCC97 cells were transfected with FUT7 siRNAs and siNC. Cell proliferation was analyzed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H tetrazolium bromide assay and cell cycle was analyzed by flow cytometry. (A) Cell proliferation assay following knockdown of FUT7. (B) Cell cycle assay following knockdown of FUT7. (C) Flow cytometry profiles of MHCC97 cells following transfection. *P<0.05 (n=3), vs. untransfected control cells. FUT, fucosyltransferase; SLex, sialyl-Lewis X; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; siRNA, small interfering RNA; siNC, scramble-siRNA transfected cells; Control, untransfected cells.

Figure 4. Knocking down FUT7 affects cell survival via various intracellular signaling mediators. MHCC97 cells were transfected with FUT7 siRNAs. After 1 h, inhibitors of signaling molecules were added to the culture medium, and the cells were cultured for a further 48 h for RNA analysis and 72 h for protein analysis. (A) mRNA levels of FUT7 were quantified by reverse transcription-quantitative polymerase chain reaction following treatment with inhibitors. (B) Following treatment with the inhibitors, cell survival was quantified using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H tetrazolium bromide assay in the THLE-2 normal liver cells and MHCC97 hepatocarcinoma cells. (C) Cells were permeabilized and centrifuged to isolate the cytosolic proteins and particulate-conjugated proteins. Results of western blot analysis of the target proteins are shown with profiles. (D) Quantitative analysis of relative protein levels. *P<0.05 (n=3), vs. Control; **P<0.01 (n=3), vs. Control. FUT, fucosyltransferase; Ctrl, control (no inhibitor); ErkI, extracellular signal-regulated kinase inhibitor; siRNA, small interfering RNA; siNC, scramble-siRNA transfected cells; PLCγ, phospholipase Cγ; PLCγ1-C, PLCγ1-M, PLCγ1 in cytoplasm; p-PLCγ1, phosphorylated PLCγ1 conjugated with plasma membrane; p-PLCγ1, phosphorylated PLCγ1 conjugated with plasma membrane; Control, untransfected cells.
cycle progression into the S-phase. These results further emphasized the importance of the expression of FUT7 on cell proliferation.

**Effects of inhibiting PKA, PI3K/AKT and PLCγ/Erk signaling pathways on the expression of FUT and MHCC97 cell proliferation.** To understand the mechanisms involved, the present study examined the effects of inhibitors of signaling pathways on the mRNA levels of FUT7. The RT-qPCR results showed that, in MHCC97 cells, PLCγ inhibitor, PKA inhibitor and PI3K inhibitor significantly reduced the mRNA levels of FUT7. By contrast, the Erk inhibitor and CDC25 inhibitor had no effect on the mRNA level of FUT7 (Fig. 4A). The MTT results showed that inhibitors of PLCγ and Erk reduced cell survival in normal liver cells, CDC25, PI3K, PLCγ and Erk inhibitors decreased the proliferation of MHCC97 cells, with the most marked effect observed with PLCγ inhibitor (Fig. 4B). These results indicated that the PLCγ inhibitor significantly reduced the mRNA level of FUT7, and inhibited MHCC97 HCC cell proliferation and survival.

To observe the effect of FUT7 siRNA transfection on the activation of PLCγ, the proteins in the cytoplasm were isolated from particulate-conjugated proteins. The contents of PLCγ1 and phosphorylated PLCγ1 were detected by western blot analysis. The results (Fig. 4C and D) showed that FUT7 siRNA transfection decreased the content of PLCγ1 in the cytoplasm, and decreased the content of PLCγ1 and phosphorylated PLCγ1 conjugated on the plasma membrane. The results demonstrated that FUT7 siRNA transfection inhibited the translocation and phosphorylation of PLCγ1, and thus inhibited the activation of PLCγ1. Therefore, the aforementioned results indicated that FUT7 may affect cell proliferation through the activation of PLCγ. As Erk is the downstream signaling molecule of PLCγ (30), the results indicated that FUT7 modulated cell proliferation via the PLCγ/Erk signaling pathway in MHCC97 cells.

**Discussion**

Cell surface glycoconjugates have important functional roles in cancer cell proliferation, growth, metastasis and invasiveness. It has been reported that the changing expression and components of glycoconjugates are correlated with carcinogenesis in several types of cancer (3-9). Abnormal elevation in the expression of sialyl-Lewis oligosaccharide antigens (SLeα and SLex) has been correlated with the malignancy of a wide range of tumors (6-9). Several antibodies targeting the sialyl-Lewis oligosaccharide have been developed as potential therapeutic approaches for the treatment of sialyl-Lewis oligosaccharide-positive tumors (31-33). However, there has been limited investigation of methods to effectively inhibit the synthesis of sialyl-Lewis oligosaccharide and its effects on tumor cell proliferation and growth.

It has been reported that the SLeα oligosaccharide is mainly expressed in tumor cells derived from digestive organs, including the pancreas, rectum, biliary tract and colon (34). In addition, it has been reported that the SLeα oligosaccharide is mainly expressed in ovarian, breast and pulmonary tumor cells (35). It has also been reported that the level of SLeα oligosaccharide was significantly higher in serum of patients with HCC than in serum of normal controls and patients with liver cirrhosis (36). It has also been shown that the expression of SLeα oligosaccharide is closely associated with the growth of HCC (37-39). The results of the present study also indicated that the expression of SLeα in HCC tissues was higher than in adjacent normal liver tissues. In addition, the findings indicated that the expression of SLeα was significantly increased in MHCC97 human HCC tumor cells compared with that in normal liver cells. The expression of SLeα was unchanged and the expression of SLeα was only detected in trace quantities.

FUT7 is the key enzyme for the synthesis of the SLeα oligosaccharide, therefore, the present study investigated the expression of FUT7 in HCC. The results indicated that the expression of FUT7 was markedly increased in MHCC97 cells compared with normal liver cells. Following transfection with FUT7 siRNAs, the expression of SLeα was significantly decreased in the MHCC97 cells. It has been reported that FUT7 has high substrate specificity and that FUT7 is the only FUT responsible for the synthesis of SLeα Lewis oligosaccharide antigen (22,23). FUT3/6 is responsible for the synthesis of SLeα and SLeα, and FUT6 is the major enzyme which is responsible for the synthesis of SLeα from SLeα (20). The results of the present study indicated that the expression of FUT6 was also high, however, no statistically significant difference was found between the MHCC97 cells and normal liver cells. The mRNA and protein levels of FUT7 and FUT6 were correlated with the expression of sialyl-Lewis antigens (SLeα and SLeα) in the MHCC97 cells. The expression of FUT7 has been reported to be altered in several types of tumor (26,27). It has also been documented that the expression of glycans-related gene in human HCC cells, which is responsible for the synthesis of N-glycan and glycolipids, particularly the sialyl-Lewis antigen, was significantly increased (40). However, the mechanism of FUT7 in cancer cell growth has not been investigated fully in human HCC cells.

The results of the present study showed that FUT7 is not only responsible for SLeα synthesis, but is also involved in cell proliferation, siRNAs of FUT7 suppressed the expression of FUT7 and markedly inhibited the synthesis of SLeα oligosaccharide antigen and MHCC97 HCC cell proliferation. The results also indicated that anti-SLeα monoclonal antibody KM93 significantly suppressed MHCC97 HCC cell proliferation. Therefore, the suppression of cell proliferation following FUT7 RNAi may be due to the decreased synthesis of SLeα. The detailed functional role of SLeα is being investigated in subsequent investigations.

Following the binding of hepatocyte growth factor (HGF) ligand to the HGF receptor (receptor tyrosine kinase (MET)) on the liver cell surface, the kinase catalytic activity of MET is activated, which triggers trans-phosphorylation of the tyrosines of MET, including Tyr 1234 and Tyr 1235, Tyr 1349 and Tyr 1356. These tyrosines recruit and engage a number of signaling transducers, including PI3K, Src homology 2-containing phosphotyrosine phosphatase2, and PLCγ, therefore initiating a whole spectrum of biological activities, including cell survival, proliferation, migration and invasion. The PLCγ/Erk signal transduction pathway is one of the most important signaling pathways in regulating cell proliferation, growth and differentiation (30). PLCγ can be divided into three subclasses: PLCγ1, PLCγ and PLCδ, according to their different structures (41).
PLCγ is one of the direct downstream signal transducers of growth factor receptors and has an important functional role in regulating cell growth and proliferation. PLCγ1 is widely distributed in various tissues and cells, whereas PLCγ2 is mainly distributed in the lungs, spleen and thymus. Therefore, PLCγ1 was selected as the main signaling target for the present study. The activation of PLCγ1 involves a series of molecular events (42). First, ligands conjugate with growth factor receptors that have receptor protein tyrosine kinase activity. The receptors are then dimerized and autophosphorylated. The intracellular domain of receptors is autophosphorylated to form the phosphorylated tyrosine residues, and these residues of the receptors form the binding site of the SH2 domain of PLCγ1. Subsequently, PLCγ1 is phosphorylated at certain tyrosine residues and is activated by receptor kinase. The activated PLCγ1 then separates from its receptors and translocates to the plasma membrane by binding phospholipid phosphatidylinositol 3,4,5 triphosphate (PIP3) through its PH structure. Subsequently, PLCγ1 can hydrolyze phospholipid phosphatidylinositol 3,4,5 bisphosphate (PIP2) to produce diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3). The phosphorylation and translocation of PLCγ1 from the cytosol to the plasma membrane are necessary for its activation and function. Therefore, the activation of PLCγ1 can be detected by its phosphorylation and translocation. The experimental results showed that the proliferation of MHCC97 cells was suppressed following FUT7 siRNA transfection. The relative content of PLCγ1 conjugated with the plasma membrane of MHCC97 cells transfected with FUT7 siRNAs was also lower than that of the control cells, and the phosphorylated PLCγ1 conjugated with the plasma membrane was also decreased. This indicates that FUT7 knockdown inhibited the phosphorylation and translocation of PLCγ1 from the cytosol to the plasma membrane, and thus, inhibited the activation of PLCγ1. In addition, siRNA targeting FUT7 reduced cell proliferation and survival. The PLCγ1 inhibitor U73122 significantly reduced the mRNA levels of FUT7 and inhibited the proliferation and survival of the MHCC97 cells. The experiment using PLCγ1 inhibitor supported the hypothesis that the expression level of FUT7 is important to sustain MHCC97 HCC cell proliferation and survival. The mechanistic mechanism of PLCγ/ERK pathway regulating the mRNA expression of FUT7 remains to be fully elucidated. It was hypothesized that activated PLCγ1 cleaves the PIP2 into DAG and IP3. DAG and calcium work together to activate protein kinase C. This leads to a series of phosphorylation events downstream in the mitogen-activated protein kinase (MAPK) cascade. First, the activated PKC activates RAF, and RAF kinase phosphorylates and activates MAPK kinase. This ultimately results in the phosphorylation and activation of ERKs. ERK regulates the activities of several transcription factors, including c-Jun, activating transcription factor 2, ELK1 and heat shock factor 1. By altering the levels and activities of transcription factors, ERK alters the transcription of genes that are involved in regulation of cell proliferation and cell cycle, including FUT7. The detailed signaling mechanism and the factors involved in PLCγ1 regulating the gene transcription of FUT7 remain fully elucidated.

In conclusion, the findings of the present study revealed that the suppression of PLCγ/Erk signaling pathway inhibited human HCC cell proliferation, which was, at least partially, mediated by the decreased expression of FUT7 and its product, SLEx. The results demonstrated that FUT7 siRNAs downregulated SLEx synthesis, inhibited PLCγ activation, arrested cell cycle progression and effectively reduced MHCC97 HCC cell proliferation. These results suggest that FUT7 siRNAs maybe a potential therapeutic agent to treat human SLEx-positive cancer. Therefore, targeting FUT7 for inactivation with RNAi technology is an attractive approach for controlling HCC cell proliferation.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors’ contributions

DL and HL conceived and designed the experiments; DL, HS, ZB and GB performed the experiments; WW, MI and JL analyzed the data; DL and HL wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethical Committee of China Medical University (Shenyang, Liaoning, China). Written informed consent was obtained from each patient recruited.

Patient consent for publication

Written informed consent was obtained from each patient.

Competing interests

The authors declare that they have no competing interests.

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