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Caveolin-1 up-regulates ST6Gal-I to promote the adhesive capability of mouse hepatocarcinoma cells to fibronectin via FAK-mediated adhesion signaling

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ABSTRACT

Caveolin-1 is a major structural protein of caveolae and plays important functions in tumorigenesis and development. Hca-F and Hepa1–6 are mouse hepatocarcinoma cell lines with high and low malignant potential, respectively. Our previous studies revealed that caveolin-1 promoted cell invasion by up-regulating the glycosylation of matrix metalloproteinase inducer CD147 of Hepa1–6 and Hca-F cells. However, the roles of caveolin-1 in cell–ECM adhesion and the mechanisms involved remain unknown. This study showed that caveolin-1 overexpression in Hepa1–6 cells up-regulated sialyltransferase ST6Gal-I expression and activated FAK-mediated adhesion signaling, and down-regulation of ST6Gal-I attenuated caveolin-1-induced increase in the adhesive ability of Hepa1–6 cells to fibronectin. Conversely, caveolin-1 knockdown in Hca-F cells inhibited ST6Gal-I expression and FAK signaling-mediated cell adhesion to fibronectin. Re-expression of wild-type caveolin-1 or ST6Gal-I rescued the decreased ST6Gal-I expression and adhesion of Hca-F cells caused by caveolin-1 silencing. Further studies indicated that caveolin-1 might regulate ST6Gal-I expression through caveolin-1 scaffolding domain. Taken together, these results demonstrate for the first time that caveolin-1 can up-regulate ST6Gal-I expression and further contribute to promoting mouse hepatocarcinoma cell adhesion to fibronectin by activating FAK-mediated adhesion signaling.

1. Introduction

Tumor metastasis is a multistage process that includes the detachment of tumor cells from primary neoplasm, local invasion, intravasation, survival in the circulatory system, extravasation and colonization [1]. The extracellular matrix (ECM) is a complex mixture of matrix molecules composed of fibronectin (FN), collagen (COL), laminin (LN), proteoglycans and non-matrix proteins [2]. The adhesion of tumor cell to ECM is the key step for migration and invasive behavior during tumor metastasis [3].

Glycosylation is a post-translational modification reaction and participates in many biological processes, including cell adhesion, receptor activation and signal transduction [4]. Glycosylation modification is catalyzed by the action of glycosyltransferases [5], and aberrant alterations in glycosyltransferase expression and the corresponding glycan levels are closely correlated with tumorigenesis and development. Overexpression of *N*-acetylglucosaminyltransferase III (GnT-III) inhibited both human cervical carcinoma cell adhesion to FN and cell migration [6], and core3 *O*-glycan synthase suppressed tumor formation and metastasis in prostate carcinoma [7].

Sialyltransferases belong to glycosyltransferase family, members of which localize at the trans-Golgi apparatus. *ST6GaL-I* gene encodes the β-galactoside:α2-6-sialyltransferase 1 (ST6Gal-I), which catalyzes the transfer of sialic acid residue in a α2,6-linkage to terminal galactose of glycan chains [8]. It has been reported that enhanced expression of ST6Gal-I is positively correlated with metastatic potential and poor prognoses in colon [9], breast [10] and gastric [11] cancers.

Caveolin-1 (Cav-1) is an integral membrane protein of caveolae and is implicated in the processes of cholesterol transport, signal transduction and endocytosis [12]. *Cav-1* gene has been regarded as both a tumor suppressor gene and an oncogene according to different tumor types. It has been reported that up-regulation of Cav-1 in hepatocellular carcinogenesis (HCC) enhanced metastatic ability and led to poor prognosis [13]. Hca-F and Hepa1–6 are mouse hepatocarcinoma cell lines with high and no lymphatic metastasis potential, respectively [14]. Our previous studies showed that Cav-1 positively regulated the growth, survival and invasion of Hepa1–6 and Hca-F cells [14–16] However, the

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mechanisms by which caveolin-1 regulates cell–ECM interaction are poorly understood. We had found that Cav-1 increased the invasive capability of Hepa1–6 and Hca-F cells by up-regulating glycosylation of matrix metalloproteinase (MMP) inducer CD147 [16]. Therefore, as a tumor promoter in HCC, Cav-1 might play important roles in regulating protein glycosylation modification and cell–ECM interaction.

In this study, we found that Cav-1 overexpression in Hepa1–6 cells up-regulated the ST6Gal-I expression and FN-dependent phosphorylation of FAK. Decrease in α2,6-linked sialic acid, the catalytic product of ST6Gal-I, attenuated the adhesive capability of Hepa1–6 cells stably transfected with Cav-1. Conversely, knockdown of caveolin-1 in Hca-F cells resulted in reduced ST6Gal-I expression and adhesion of cells to FN. Reintroduction of wild-type Cav-1 or ST6Gal-I recovered the decreased adhesion of Hca-F cells caused by Cav-1 silencing. In addition, Cav-1 scaffolding domain might be involved in the regulation of ST6Gal-I expression. These results demonstrate that Cav-1 can up-regulate ST6Gal-I expression, which promotes mouse hepatocarcinoma cell adhesion to FN by activating FAK-mediated adhesion signaling pathway.

2. Materials and methods

2.1. Cell culture and mice

Mouse hepatocarcinoma cell line Hca-F was established by Department of Pathology of Dalian Medical University and Hepa1–6 was purchased from Cell Center of Peking Union Medical University (Beijing). Hca-F and Hepa1–6 cells were grown in RPMI 1640 (Gibco) and Dulbecco's modified Eagle's medium (DMEM) containing 4.5 g/L of glucose (Gibco) supplemented with 10% heat-inactivated fetal bovine serum (Gibco), respectively. The cells were incubated at 37 °C under a humidified atmosphere with 5% CO_2.

2.2. Establishment of stable cell line

The coding region of wild-type Cav-1 was subcloned into pcDNA3.1 expression vector (Invitrogen) to generate pcDNA3.1/Cav-1. The plasmid was mixed with Lipofectamine™ 2000 (Invitrogen) according to manufacturer's instructions and added to Hepa1–6 cells; the empty vector was transfected as a control. For selection of the transfected cells, 800 μg/mL G418 was added after 48 h. After 2 months of screening, the cell lines stably expressing Cav-1 (Hepa1–6/Cav-1) and harboring empty vector pcDNA3.1 were established.

2.3. Transfection of siRNAs

The sense sequences of ST6Gal-I siRNA-1, siRNA-2 and siRNA-3 are 5′-GAAAGGGAGCGACUAUGAGtt-3′, 5′-GCCAAGGUAUUCCAGAUGCtt-3′ and 5′-GGUAUUCCAGAUGCCGAAGtt-3′. ST6Gal-I-siRNAs were mixed with Lipofectamine™ 2000 (Invitrogen) according to manufacturer's recommendation and added to Hepa1–6/Cav-1 cells. Interference efficiency was determined by Western blot assay 48 h after transfection.

2.4. Construction of RNA interference vector and transfection

Cav-1-specific small hairpin RNA (shRNA) sequences used in the construction of RNA interference (RNAi) vector were as follows: 5′-CACCGTACCTGAGTCTCCAGAAATTCAAGAGATTTCTGGAGACTCAGGTACTTTTTTG-3′ and 5′-GATCCAAAAAAGTACCTGAGTCTCCAGAAATCTCTT GAATTTCTGGAGACTCAGGTAC-3′. The oligonucleotides targeting Cav-1 or the negative control were annealed and ligated into pGPU6 vector (GenePharma), respectively. Hca-F cells were transfected with the mixture of plasmids and Lipofectamine™ 2000 (Invitrogen) according to manufacturer's recommendation. Silencing level was determined by Western blot assay 48 h after transfection.

2.5. Construction of rescue vectors and transfection

The coding region of wild-type ST6Gal-I or Cav-1 was subcloned into pcDNA3.1 expression vector (Invitrogen). The Cav-1 palmitoylation mutant (ΔC133, 143, 156A) and Cav-1 scaffolding domain (CSD) mutant (ΔCSD) were constructed by Takara Dalian. All the rescue vectors were transfected into the Cav-1 knockdown Hca-F cells, respectively. After 48 h transfection, the rescued cells were used to perform the following assays.

2.6. Real-time PCR analysis

Real-time PCR was performed with ABI PRISM 7900 detection system (Applied Biosystems) using SYBR Premix DimerEraser Kit (TaKaRa). Total RNA was extracted using TRIzol reagent (Invitrogen), and cDNA synthesis was performed using PrimeScript RT reagent Kit (TaKaRa) according to manufacturer's instructions. Specific primers for ST6Gal-I, ST3Gal-I, ST6Gal-II and GAPDH were purchased (GenePharma). Relative changes in mRNA expression were normalized with GAPDH and calculated using $2^{-\Delta\Delta CT}$ method.

2.7. Cell adhesion assay

96-Well microtiter plates (Nunc) were coated with FN (Abcam), COL (Sigma) or LN (Sigma) at different concentrations (5 nM or 10 nM). Bovine serum albumin (BSA)- and poly-L-lysine (PL)-coated wells were used as negative and positive controls, respectively. Cells (4×10^4 cells/mL) suspended in serum-free medium containing 0.1% BSA were seeded into each well and incubated for 1 h at 37 °C. The attached cells were labeled with 0.3% Crystal Violet and measured using VersaFluor Fluorometer (Bio-Rad) at 570 nm. Cell adhesion was determined from the ratio of optical density values of the experimental and positive control groups.

2.8. Western blot analysis

Protein concentration was measured with BCA assay kit (Pierce). Equal amounts of denatured proteins were subjected to 10% SDS–PAGE and blotted onto nitrocellulose membranes (Pall Corporation). Antibodies against Cav-1, ST6Gal-I, FAK, phosphorylated FAK (p-FAK), ERK1/2 and phosphorylated ERK1/2 (p-ERK1/2) (Santa Cruz Biotech Inc.) were used as the primary antibodies; GAPDH antibody (Santa Cruz Biotech Inc.) was used as a control. The detection was performed using ECL kit (Amersham Biosciences) according to manufacturer's instructions. The relative amount of protein was determined by densitometry using LabWorks software.

2.9. Lectin blot analysis

Cell lysates containing equal amounts of denatured proteins were subjected to 8% SDS–PAGE and transferred to nitrocellulose membranes. The identification was performed with 2 μg/mL biotinylated *Sambucus nigra* (SNA) lectin (Vector Laboratories, Inc.), which preferentially recognizes sialic acid attached to terminal galactose in α2,6-linkage. The blots were developed using ECL detection system (Amersham Biosciences).

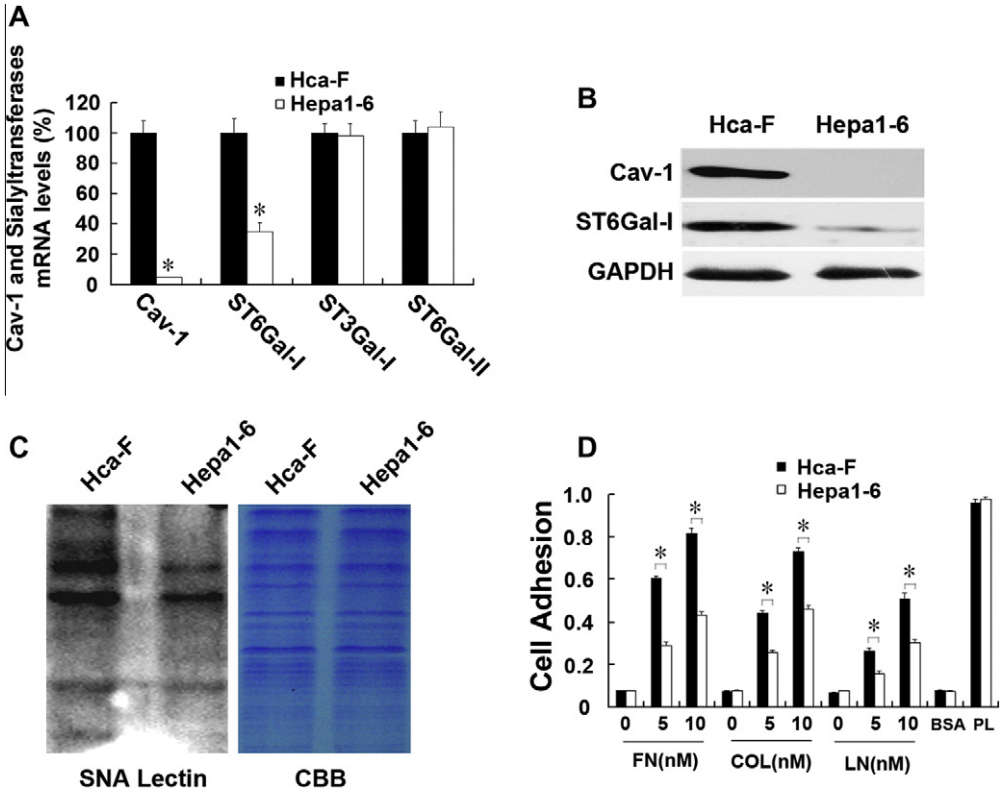

Fig. 1. Hca-F cells have higher Cav-1 and ST6Gal-I expression levels and adhesive ability to ECM than Hepa1–6 cells. (A) The mRNA levels of Cav-1 and sialytransferases were measured using real-time PCR with RNA extracted from the Hca-F and Hepa1–6 cells. (B) Western blot analysis of Cav-1 and ST6Gal-I protein levels in Hca-F and Hepa1–6 cells. GAPDH blotting served as a control. (C) Lectin blot analysis of the α2,6-linked sialic acid levels in Hca-F and Hepa1–6 cells using SNA lectin staining. Coomassie Brilliant Blue (CBB) staining of gels showed the comparable amounts of protein in each lane. (D) Cell adhesion assay of Hca-F and Hepa1–6 cells to FN, COL or LN at different concentrations (5 nM or 10 nM). BSA and poly-L-lysine (PL) were used as negative and positive controls, respectively. The data are the mean ± S.D. for three independent experiments. $^{*}P < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.10. Statistical analysis

SPSS16.0 software was used, and each assay was performed three times independently. The data were expressed as mean ± S.D. and Student's *t*-test was used to determine the significance of the differences between multiple comparisons; $^{*}P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Hca-F cells have higher Cav-1 and ST6Gal-I expression levels and adhesive ability to ECM than Hepa1–6 cells

To investigate a possible relationship between Cav-1 and sialyltransferase, the Cav-1 and three sialyltransferases (ST6Gal-I, ST3Gal-I and ST6Gal-II) mRNA expressions were analyzed using real-time PCR. The ST6Gal-I mRNA level was consistent with Cav-1 being higher in Hca-F cells than in Hepa1–6 cells, and no significant differences in ST3Gal-I and ST6Gal-II mRNA expression were observed between two cells (Fig. 1A). Western blot result showed that Cav-1 protein was detectable in Hca-F cells but absent in Hepa1–6 cells, and ST6Gal-I protein expression was higher in Hca-F cells than in Hepa1–6 cells (Fig. 1B). The ST6Gal-I catalyzes the formation of α2,6-linked sialic acid structure. As shown by SNA lectin staining (Fig. 1C), the α2,6-linked sialic acid signals in Hca-F cells were stronger than in Hepa1–6 cells. In cell adhesion assay, Hca-F cells exhibited significantly higher adhesion to ECM components in comparison to Hepa1–6 cells (Fig. 1D). These results indicate that Cav-1 may be correlated with ST6Gal-I expression, thereby influencing the cell–ECM adhesion.

3.2. Overexpression of Cav-1 promotes ST6Gal-I expression and activates FAK signaling-mediated cell adhesion to FN

To explore the effect of Cav-1 on ST6Gal-I expression and the cell adhesive ability to ECM, a Hepa1–6 cell line stably expressing Cav-1 was established. It was found that the levels of ST6Gal-I mRNA, protein and α2,6-linked sialic acid were notably increased in Cav-1 transfectants (Fig. 2A–C). Transfection of Hepa1–6 cells with Cav-1 cDNA resulted in a greatly enhanced adhesion of cells to FN compared with mock transfected cells (Fig. 2D). The levels of phosphorylated FAK were significantly up-regulated in Cav-1 transfectants compared with in mock transfectants after treatment with FN (Fig. 2E). In addition, the down-regulation of ST6Gal-I by siRNA in Cav-1 transfectants (Fig. 2A–C) inhibited Cav-1-augmented adhesion to FN and FAK phosphorylation (Fig. 2D and E). These results indicate that Cav-1 could up-regulate ST6Gal-I expression to promote cell adhesion to FN through the activation of FAK-mediated adhesion signaling.

3.3. The reintroduction of Cav-1 in Cav-1 knockdown Hca-F cells restores the ST6Gal-I expression and intracellular FAK adhesion signaling

To further verify the effect of Cav-1 on ST6Gal-I expression and the role of ST6Gal-I in Cav-1-regulated cell adhesion, the wild-type Cav-1 or ST6Gal-I was transfected into Cav-1 knockdown Hca-F cells. The ST6Gal-I expression levels were reduced in Cav-1 shRNA transfectants compared with control transfectants, and the re-expression of wild-type Cav-1 or ST6Gal-I in Cav-1 knockdown cells significantly restored the ST6Gal-I expression and α2,6-linked

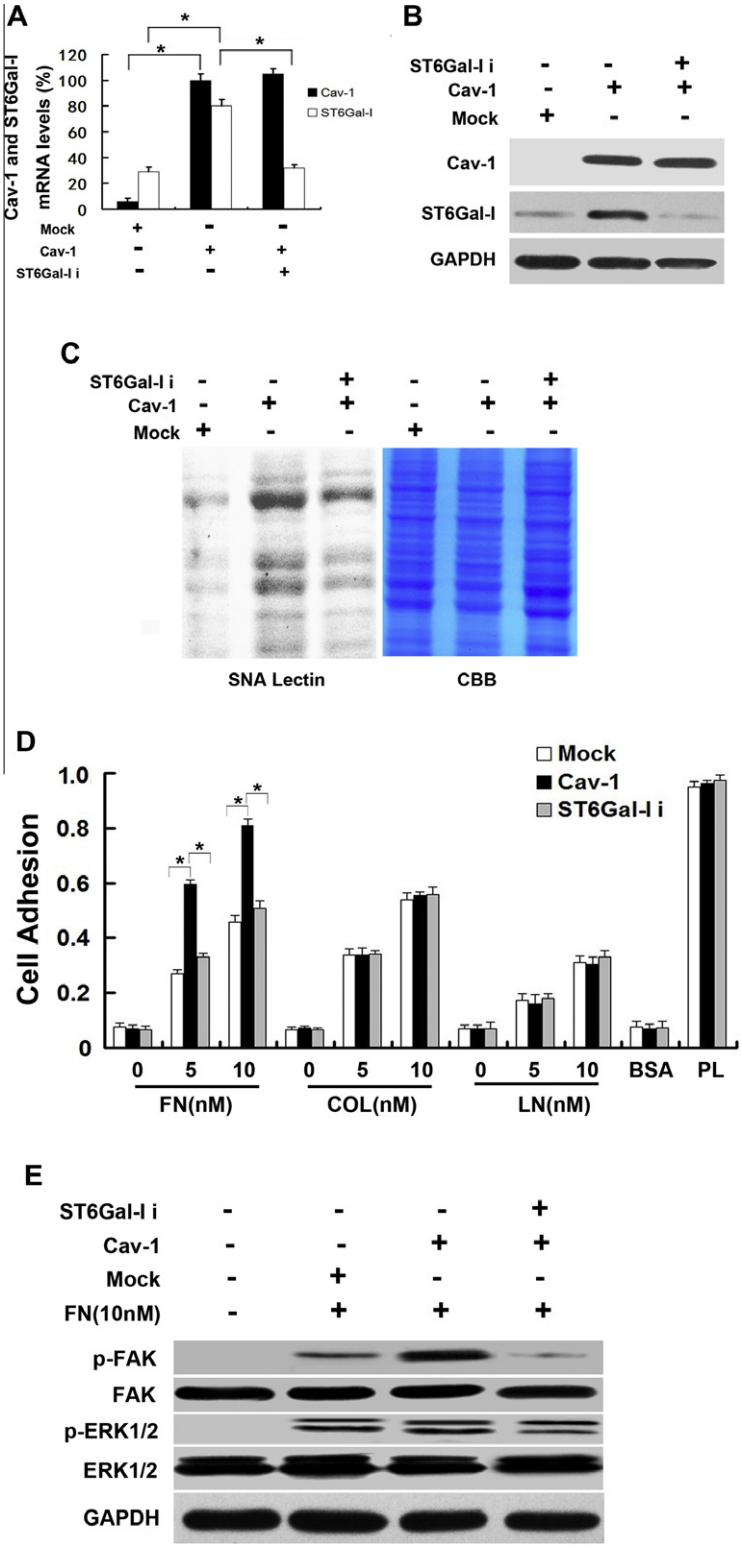

Fig. 2. Cav-1 overexpression up-regulates ST6Gal-I expression and activates FAK signaling-mediated cell adhesion to FN. (A) Cav-1 and ST6Gal-I mRNA levels were measured using real-time PCR with RNA extracted from the Hepa1–6 cells harboring empty vector (Mock), Hepa1–6/Cav-1 cells (Cav-1) and ST6Gal-I siRNAs transfected Hepa1–6/Cav-1 cells (ST6Gal-I i). (B) Western blot analysis of Cav-1 and ST6Gal-I protein expression in Mock, Cav-1 and ST6Gal-I i cells. (C) α2,6-Linked sialic acid levels were detected using Lectin blot assay with SNA lectin staining in Mock, Cav-1 and ST6Gal-I i cells. (D) Cell adhesion assay of Mock, Cav-1 and ST6Gal-I i cells to FN, COL or LN at different concentrations (5 nM or 10 nM). (E) Western blot analysis of phosphorylated FAK and ERK1/2 levels in Mock, Cav-1 and ST6Gal-I i cells treated with FN (10 nM). The data are the mean ± S.D. for three independent experiments. $^{*}P < 0.05$. Serum-starved cells were harvested and resuspended in serum-free DMEM with 0.1% BSA and seeded into dishes coated with FN (10 nM). The cell lysates were identified with either anti-p-FAK or anti-p-ERK1/2 antibody to verify the phosphorylation levels of FAK and ERK1/2 or with anti-FAK and anti-ERK1/2 antibody to detect total amount of FAK and ERK1/2.

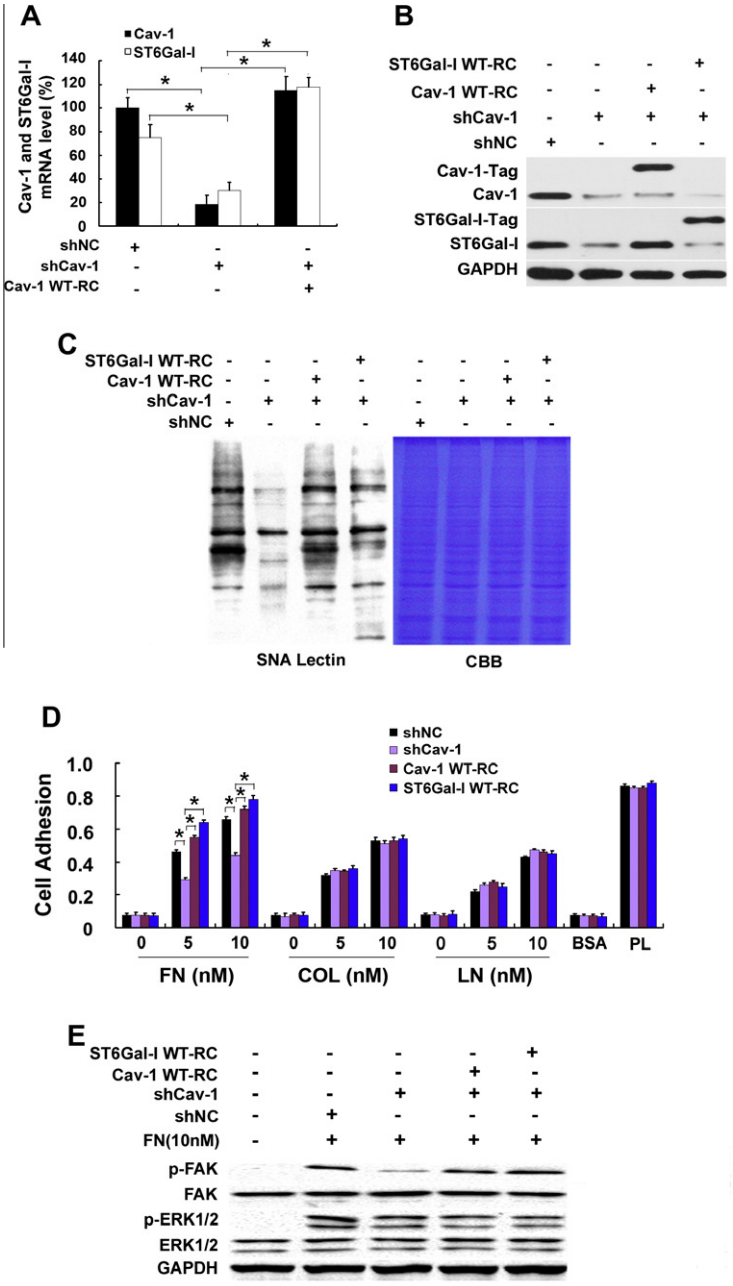

Fig. 3. The reintroduction of Cav-1 in Cav-1 knockdown Hca-F cells restores the ST6Gal-I expression and intracellular FAK adhesion signaling. (A) Cav-1 and ST6Gal-I mRNA levels were measured using real-time PCR with RNA extracted from the negative control transfected Hca-F cells (shNC), the Cav-1 knockdown Hca-F cells (shCav-1) and the wild-type Cav-1 rescued knockdown cells (Cav-1 WT-RC). (B) Western blot analysis of Cav-1 and ST6Gal-I protein levels in shNC, shCav-1, Cav-1 WT-RC and the wild-type ST6Gal-I rescued Cav-1 knockdown cells (ST6Gal-I WT-RC). (C) Lectin blot assay analyzed the α2,6-linked sialic acid levels in shNC, shCav-1, Cav-1 WT-RC and ST6Gal-I WT-RC cells by SNA lectin staining. (D) Cell adhesion assay of shNC, shCav-1, Cav-1 WT-RC and ST6Gal-I WT-RC cells to FN, COL or LN at different concentrations (5 nM or 10 nM). (E) Western blot analysis of phosphorylated FAK and ERK1/2 levels in shNC, shCav-1, Cav-1 WT-RC and ST6Gal-I WT-RC cells treated with FN (10 nM).

sialic acid levels (Fig. 3A–C). Cell adhesion assay showed that the reduced adhesion of cells to FN, resulted by Cav-1 silencing, was rescued by the transfection of wild-type Cav-1 or ST6Gal-I into Cav-1 shRNA transfectants (Fig. 3D). The phosphorylation levels of FAK were remarkably recovered in Cav-1- or ST6Gal-I-rescued cells compared with in Cav-1 knockdown cells (Fig. 3E). These results suggest that Cav-1 knockdown in Hca-F cells decreases ST6Gal-I expression and attenuates the cell adhesive capability to FN, which can be resistant by the reintroduction of wild-type Cav-1 or ST6Gal-I.

3.4. Cav-1 scaffolding domain up-regulates ST6Gal-I expression in Cav-1 knockdown Hca-F cells

To determine which domain of Cav-1 regulates ST6Gal-I expression, the Cav-1 palmitoylation mutant or Cav-1 scaffolding domain (CSD) mutant was transfected into Cav-1 knockdown Hca-F cells. The ST6Gal-I mRNA level was significantly increased in palmitoylation mutant but not CSD mutant transfected Cav-1 knockdown cells compared with un-transfected knockdown cells (Fig. 4A). The ST6Gal-I protein and α2,6-linked sialic acid levels were restored in

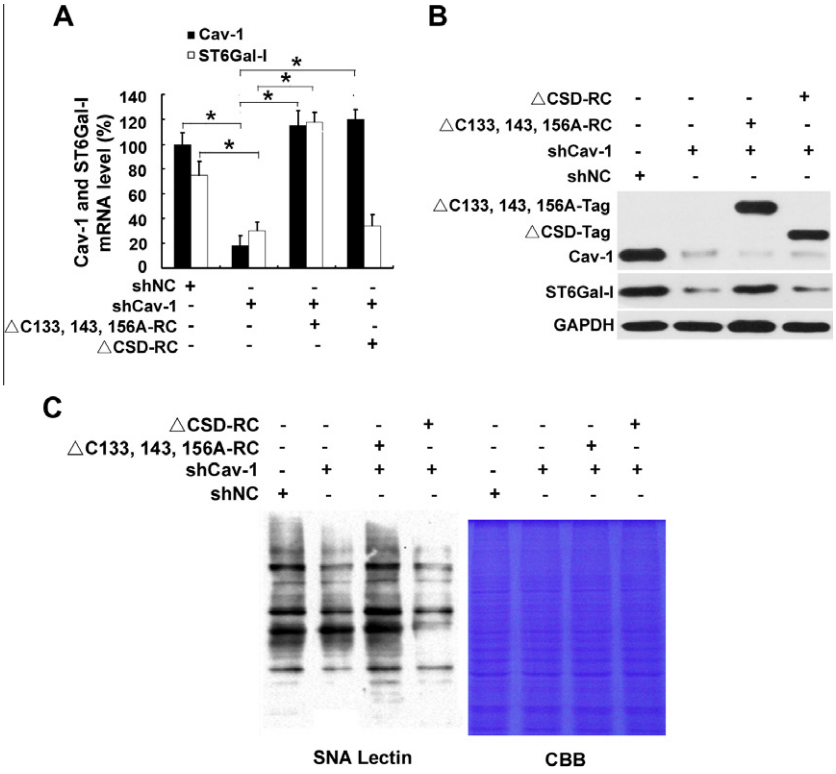

Fig. 4. Cav-1 scaffolding domain up-regulates ST6Gal-I expression in Cav-1 knockdown Hca-F cells. (A) Cav-1 and ST6Gal-I mRNA levels were measured using real-time PCR with RNA extracted from shNC, shCav-1, the palmitoylation mutant rescued Cav-1 knockdown cells (△C133, 143, 156A-RC) and the CSD mutant rescued Cav-1 knockdown cells (ΔCSD-RC). (B) Western blot analysis of Cav-1 and ST6Gal-I protein levels in shNC, shCav-1, ΔC133, 143, 156A-RC and ΔCSD-RC cells. (C) α2,6-linked sialic acid levels were detected using Lectin blot analysis with SNA lectin staining in shNC, shCav-1, ΔC133, 143, 156A-RC and ΔCSD-RC cells.

palmitoylation mutant but not CSD mutant rescued knockdown cells (Fig. 4B and C). These results indicate that Cav-1 via CSD up-regulates ST6Gal-I expression in Cav-1 knockdown Hca-F cells.

4. Discussion

The current studies suggest that Cav-1 positively regulates migration and invasion in HCC metastasis. Atabey et al. reported that Cav-1 expression was gradually increased in neoplastic stage with respect to pre-neoplastic stage during HCC and promoted hepatocellular motility and invasiveness [17]. Our previous studies also showed that curcumin inhibited the migration and invasion of Hca-F cells by down-regulating Cav-1 expression [18]. These findings indicate that Cav-1 may act as oncogene rather than tumor suppressor in HCC.

Cell–ECM interaction plays essential roles in the metastatic behavior of tumor cells. However, the roles of Cav-1 in the adhesion of HCC cells to ECM remain poorly understood. In this study, we overexpressed Cav-1 in Cav-1-negative Hepa1–6 cells and knocked down Cav-1 in Cav-1-positive Hca-F cells, respectively. The results indicated that Cav-1 positively regulated the adhesion of mouse hepatocarcinoma cells to ECM. Cell–ECM interaction can lead to intracellular phosphorylation, and focal adhesion kinase (FAK) activity and tyrosine phosphorylation will be preferentially up-regulated in response to cell-matrix contact [19]. In agreement with this notion, we confirmed that Cav-1 promoted the FN-dependent phosphorylation of FAK in mouse hepatocarcinoma Hepa1–6 and Hca-F cells. These results suggest that Cav-1 can up-regulate the FN-mediated cell adhesion through activating FAK signaling. However, the roles of Cav-1 in mediating cell adhesion of different tumors to ECM still deserve further study.

There is accumulating evidence that Cav-1 may play important roles in regulating the modification of glycosylation. Taniguchi et al. reported that subcompartmental localization of *N*-acetylglucosaminyltransferase III was regulated by Cav-1 within the Golgi apparatus [20]. Cav-1 could activate Wnt/beta-catenin signaling pathway [21], and activated Wnt/beta-catenin signaling induced glycosyltransferase gene *DPAGT1* expression via the binding of beta-catenin/Tcf complex to *DPAGT1* promoter [22]. Tang and Hemler et al. found that Cav-1 suppressed the *N*-glycan levels of CD147 in human embryonic kidney cell line HEK293 and rhabdomyosarcoma cell line RD [23,24]. We had proven that Cav-1 increased the invasive capability of mouse hepatocarcinoma cells by up-regulating CD147 glycosylation [16]. The disparity between our results and those of Tang et al. may be due to the different cell lines used.

The sialyltransferase ST6Gal-I is the key enzyme to catalyze the synthesis of terminal sialic acid attached to galactose with an α2,6 linkage. In present study, our data showed that the expression of ST6Gal-I was consistent with Cav-1 expression being reduced in Cav-1 knockdown Hca-F cells or increased in Hepa1–6 cells stably expressing Cav-1. The wild-type Cav-1 could restore the ST6Gal-I expression and the levels of its catalytic product (α2,6-linked sialic acid) in Cav-1 knockdown Hca-F cells. In addition, we found that the overexpression of Cav-1 in Hepa1–6 cells had no effect on ST3Gal-I and ST6Gal-II expression by real-time PCR (Fig. S1). These results suggest that Cav-1 can up-regulate ST6Gal-I expression and α2,6-linked sialic acid levels in mouse hepatocarcinoma cells. We also found that Cav-1 overexpression in Hepa1–6 cells up-regulated the levels of β1,6 branching in *N*-glycan and *N*-acetyllactosamine in complex and hybrid *N*-glycan by flow cytometry analysis (data not shown). Thus, Cav-1 might be involved in the *N*-glycan expression of mouse hepatocarcinoma cell surface.

Some groups reported that elevated levels of ST6Gal-I and α2,6-linked sialic acid had been observed in carcinomas of the colon, cervix and brain [25–27]. Varki et al. demonstrated that ST6Gal-I promoted tumor growth and inhibited differentiation of spontaneous mammary cancers in mice [28]. It has been reported that α2,6-linked sialic acid on platelet endothelial cell adhesion molecule (PECAM) was necessary for its homophilic adhesion and antiapoptotic function [29]. Our previous studies on the antiapoptotic role of Cav-1 in mouse hepatocarcinoma cell lines [15] may be due to Cav-1-induced increase in α2,6-linked sialic acid levels. Since ST6Gal-I plays an important role in different tumor behaviors, we hypothesize that ST6Gal-I may be involved in the effect of Cav-1 on the mouse hepatocarcinoma cellular adhesion to ECM. In our studies, overexpression of both wild-type Cav-1 and ST6Gal-I could rescue the decreased adhesion of Hca-F cells caused by Cav-1 silencing. The down-regulation of ST6Gal-I directly inhibited Cav-1-induced increase in the adhesive ability and FAK phosphorylation of Hepa1–6 cells. These results indicate that Cav-1 modulates the adhesion phenotype of mouse hepatocarcinoma cells through affecting the ST6Gal-I expression.

Cav-1 is an integral membrane protein associated with various membranous structures, including endoplasmic reticulum (ER), Golgi, and plasma membranes. Cav-1 scaffolding domain (CSD), an important domain located in the NH_2-terminal region, is required for caveolin dimerization and critical in controlling the interactions between Cav-1 and numerous signaling proteins. The COOH-terminal region of Cav-1 contains three palmitoylated cysteine residues, which are relevant to oligomerization [30]. Han et al. have reported that COOH-terminal palmitoylation is crucial for Cav-1 to attach the plasma membrane [31]. Here, we found that CSD might be involved in the regulation of ST6Gal-I expression in mouse hepatocarcinoma cells. However, a detailed study to elucidate how CSD modulates ST6Gal-I transcription is a challenging issue for the future.

In conclusion, this study demonstrates for the first time that Cav-1 can up-regulate the expression of glycosyltransferase ST6Gal-I via Cav-1 scaffolding domain, and the increase in ST6Gal-I expression directly promotes tumor cell adhesion to FN through FAK-mediated adhesion signaling. These findings suggest that the sequential regulation of Cav-1, ST6Gal-I and cell adhesion to FN might provide a new insight into revealing the molecular mechanism of tumor metastasis.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.09.086.

References

[1] D.X. Nguyen, P.D. Bos, J. Massague, Metastasis: from dissemination to organ-specific colonization, Nat. Rev. Cancer 9 (2009) 274–284.
[2] A.L. Berrier, K.M. Yamada, Cell–matrix adhesion, J. Cell Physiol. 213 (2007) 565–573.
[3] C.M. Bagi, Cancer cell metastases, J. Musculoskelet. Neuronal Interact. 2 (2002) 565–566.
[4] K. Ohtsubo, J.D. Marth, Glycosylation in cellular mechanisms of health and disease, Cell 126 (2006) 855–867.
[5] J. Gu, N. Taniguchi, Potential of N-glycan in cell adhesion and migration as either a positive or negative regulator, Cell Adh. Migr. 2 (2008) 243–245.
[6] T. Isaji, J. Gu, R. Nishiuchi, Y. Zhao, M. Takahashi, E. Miyoshi, K. Honke, K. Sekiguchi, N. Taniguchi, Introduction of bisecting GlcNAc into integrin alpha5beta1 reduces ligand binding and down-regulates cell adhesion and cell migration, J. Biol. Chem. 279 (2004) 19747–19754.
[7] S.H. Lee, S. Hatakeyama, S.Y. Yu, X. Bao, C. Ohyama, K.H. Khoo, M.N. Fukuda, M. Fukuda, Core3 O-glycan synthase suppresses tumor formation and metastasis of prostate carcinoma PC3 and LNCaP cells through down-regulation of alpha2beta1 integrin complex, J. Biol. Chem. 284 (2009) 17157–17169.
[8] A. Harduin-Lepers, V. Vallejo-Ruiz, M.A. Krzewinski-Recchi, B. Samyn-Petit, S. Julien, P. Delannoy, The human sialyltransferase family, Biochimie 83 (2001) 727–737.
[9] T. Petretti, W. Kemmner, B. Schulze, P.M. Schlag, Altered mRNA expression of glycosyltransferases in human colorectal carcinomas and liver metastases, Gut 46 (2000) 359–366.
[10] M.A. Recchi, M. Hebbar, L. Hornez, A. Harduin-Lepers, J.P. Peyrat, P. Delannoy, Multiplex reverse transcription polymerase chain reaction assessment of sialyltransferase expression in human breast cancer, Cancer Res. 58 (1998) 4066–4070.
[11] S. Gretschel, W. Haensch, P.M. Schlag, W. Kemmner, Clinical relevance of sialyltransferases ST6GAL-I and ST3GAL-III in gastric cancer, Oncology 65 (2003) 139–145.
[12] T. Fujimoto, H. Kogo, R. Nomura, T. Une, Isoforms of caveolin-1 and caveolar structure, J. Cell Sci. 113 (Pt. 19) (2000) 3509–3517.
[13] Z.B. Zhang, L. Cai, S.G. Zheng, Y. Xiong, J.H. Dong, Overexpression of caveolin-1 in hepatocellular carcinoma with metastasis and worse prognosis: correlation with vascular endothelial growth factor, microvessel density and unpaired artery, Pathol. Oncol. Res. 15 (2009) 495–502.
[14] H. Zhou, L. Jia, S. Wang, H. Wang, H. Chu, Y. Hu, J. Cao, J. Zhang, Divergent expression and roles for caveolin-1 in mouse hepatocarcinoma cell lines with varying invasive ability, Biochem. Biophys. Res. Commun. 345 (2006) 486–494.
[15] S. Wang, L. Jia, H. Zhou, X. Wang, J. Zhang, Caveolin-1 promotes the transformation and anti-apoptotic ability of mouse hepatoma cells, IUBMB Life 60 (2008) 693–699.
[16] L. Jia, S. Wang, H. Zhou, J. Cao, Y. Hu, J. Zhang, Caveolin-1 up-regulates CD147 glycosylation and the invasive capability of murine hepatocarcinoma cell lines, Int. J. Biochem. Cell Biol. 38 (2006) 1584–1593.
[17] M. Cokakli, E. Erdal, D. Nart, F. Yilmaz, O. Sagol, M. Kilic, S. Karademir, N. Atabey, Differential expression of Caveolin-1 in hepatocellular carcinoma: correlation with differentiation state, motility and invasion, BMC Cancer 9 (2009) 65.
[18] S. Wang, S. Yu, W. Shi, L. Ge, X. Yu, J. Fan, J. Zhang, Curcumin inhibits the migration and invasion of mouse hepatoma Hca-F cells through down-regulating caveolin-1 expression and epidermal growth factor receptor signaling, IUBMB Life 63 (2011) 775–782.
[19] M.D. Schaller, Cellular functions of FAK kinases: insight into molecular mechanisms and novel functions, J. Cell Sci. 123 (2010) 1007–1013.
[20] K. Sasai, Y. Ikeda, H. Ihara, K. Honke, N. Taniguchi, Caveolin-1 regulates the functional localization of N-acetylglucosaminyltransferase III within the golgi apparatus, J. Biol. Chem. 278 (2003) 25295–25301.
[21] H. Yamamoto, H. Komekado, A. Kikuchi, Caveolin is necessary for Wnt-3a-dependent internalization of LRP6 and accumulation of beta-catenin, Dev. Cell 11 (2006) 213–223.
[22] P.K. Sengupta, M.P. Bouchie, M.A. Kukuruzinska, N-glycosylation gene DPAGT1 is a target of the Wnt/beta-catenin signaling pathway, J. Biol. Chem. 285 (2010) 31164–31173.
[23] W. Tang, S.B. Chang, M.E. Hemler, Links between CD147 function, glycosylation, and caveolin-1, Mol. Biol. Cell 15 (2004) 4043–4050.
[24] W. Tang, M.E. Hemler, Caveolin-1 regulates matrix metalloproteinases-1 induction and CD147/EMMPRIN cell surface clustering, J. Biol. Chem. 279 (2004) 11112–11118.
[25] Y. Zhu, U. Srivatana, A. Ullah, H. Gagneja, C.S. Berenson, P. Lance, Suppression of a sialyltransferase by antisense DNA reduces invasiveness of human colon cancer cells in vitro, Biochim. Biophys. Acta 1536 (2001) 148–160.
[26] P.H. Wang, W.L. Lee, Y.R. Lee, C.M. Juang, Y.J. Chen, H.T. Chao, Y.C. Tsai, C.C. Yuan, Enhanced expression of alpha 2,6-sialyltransferase ST6Gal I in cervical squamous cell carcinoma, Gynecol. Oncol. 89 (2003) 395–401.
[27] Y. Kaneko, H. Yamamoto, D.S. Kersey, K.J. Colley, J.E. Leestma, J.R. Moskal, The expression of Gal beta 1,4GlcNAc alpha 2,6 sialyltransferase and alpha 2,6-linked sialoglycoconjugates in human brain tumors, Acta Neuropathol. 91 (1996) 284–292.
[28] M. Hedlund, E. Ng, A. Varki, N.M. Varki, Alpha 2–6-Linked sialic acids on N-glycans modulate carcinoma differentiation in vivo, Cancer Res. 68 (2008) 388–394.
[29] S. Kitazume, R. Imamaki, K. Ogawa, Y. Komi, S. Futakawa, S. Kojima, Y. Hashimoto, J.D. Marth, J.C. Paulson, N. Taniguchi, Alpha2,6-sialic acid on platelet endothelial cell adhesion molecule (PECAM) regulates its homophilic interactions and downstream antiapoptotic signaling, J. Biol. Chem. 285 (2010) 6515–6521.
[30] C.M. Thomas, E.J. Smart, Caveolae structure and function, J. Cell Mol. Med. 12 (2008) 796–809.
[31] J.M. Han, Y. Kim, J.S. Lee, C.S. Lee, B.D. Lee, M. Ohba, T. Kuroki, P.G. Suh, S.H. Ryu, Localization of phospholipase D1 to caveolin-enriched membrane via palmitoylation: implications for epidermal growth factor signaling, Mol. Biol. Cell 13 (2002) 3976–3988.