Glycoconj J (2008) 25:843–850
DOI 10.1007/s10719-008-9144-6

E-cadherin core fucosylation regulates nuclear β-catenin accumulation in lung cancer cells

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Received: 22 February 2008 / Revised: 8 May 2008 / Accepted: 9 May 2008 / Published online: 15 June 2008

Abstract E-cadherin expressed highly in 95C and lowly in 95D lung cancer cells which were from the same patient, but core-fucosylated E-cadherin highly expressed in 95D cells. Therefore, Fut8 and Fut8-RNAi constructs were transfected into 95C and 95D cells, respectively. In Fut8-transfectants, reduction of nuclear β-catenin was noted when E-cadherin was core-fucosylated, while accumulation of nuclear β-catenin was observed in Fut8-RNAi transfectants. In E-cadherin-negative MDA-MB-231 cells either Fut8 or Fut8-RNAi transfection couldn't affect nuclear β-catenin. However, cotransfection of E-cadherin with Fut8 caused nuclear β-catenin reduction. Furthermore, enhanced binding of E-cadheirn with β-catenin as well as α-catenin were observed in Fut8-transfectants, and reduction of tyrosine 654 phosphorylation on β-catenin and its transcriptional activity were also noted at the same time. Overall, the current results suggested that core-fucosylated E-cadherin regulated nuclear β-catenin accumulation in lung cancer cells.

Keywords Core fucosyltransferase · E-cadherin · β-catenin

Abbreviation

LCA	lens culinaris agglutinin
Fut8	α1,6-fucosyltransferase
siFut8	Fut RNAi
IP	Immunoprecipitation
TCF	T-cell factor
95D cells	giant cell carcinoma of lung with highly metastatic potential
95C cells	giant cell carcinoma of lung with low metastatic potential

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Introduction

Core fucosyltransferase (Fut8) catalyzes the transfer of a fucose residue from GDP-fucose to the innermost GlcNAc residue of N-linked oligosaccharides on glycoproteins via α1-6 linkage. Core-fucosylated *N*-glycan in protein modification can usually be altered in the case of malignancies such as the tumorigenesis of liver, lung and stomach. Serum α-fetoprotein was reported to be core-fucosylated in patients with hepatoma and has thus been employed as an early implication of diagnosis [1].

E-cadherin is the key component for adherens junctions between epithelial cells. It is a single transmembrane protein mediating cell–cell adhesion via homotypic calcium-dependent interaction, which plays an important role in cell migration and is thus involved in tumor metastasis. Cytoplasmic molecules called catenins bind the intracellular domain of E-cadherin to the actin cytoskeletons. Addition of bisecting *N*-acetylglucosamine residues to E-cadherin enhanced cell–cell binding to suppress metastasis due to enhanced E-cadherin expression or down-regulated tyrosine phosphorylation level of E-cadherin [2]. The role of core fucose in the molecule had not been known yet. Recently, there were some reports showing that core fucosylation of TGF-β1 and EGF receptors can regulate

the receptor activation in downstream signaling of the cells [3, 4].Our previous study showed fucosylation was associated with cell migration [5] and core fucosylation of E-cadherin had a strong negative effect on the cell adhesion mediated by E-cadherin due to the modulation of E-cadherin's conformation [6].

Thus, we further investigated, whether the core fucosylation of E-cadherin has regulatory effects on the intracellular target of E-cadherin and its downstream signaling molecule β-catenin. In this study, we are going to observe the effects of core fucosylation regulation of E-cadherin on its binding affinity of catenins and tyrosine phosphorylation.

Experimental procedures

Cell cultures Human lowly and highly metastatic giant lung cancer lines (95C, 95D) were from the General Hospital of PLA (Beijing, China) [7]. Human breast cancer cell lines MDA-MB-231, MDA-MB-468 and MCF-7 were derived from American Type Culture Collection (ATCC). 95C and 95D cells were cultured in RPMI 1640 and MCF-7 in DMEM, while MDA-MB-231and MDA-MB-468 cells were grown in Leibovitz's L-15 medium. The medium was supplemented with 10% heat-inactivated (56°C, 30 min) FBS. All cell lines grew under standard incubator conditions (37°C, 5% CO_2).

Transfection Fut8 cDNA expression vector (Fut8-pcDNA 3.1c) that expressed Fut8 and si-Fut8 vector (si-Fut8-pSuper with neomycin resistance) that produces specific siRNA(si-Fut 8) were used as previously described [6]. Two sequences, (1) TCCGACACCGATACCGACA, (2) AGGCTGTGGCTATGGCTGT, were used in the experiment, but sequence (1) was selected for further study for its stronger silence effect. The vector with the scrambled sequence was used as control. E-cadherin full length cDNA was constructed in the pcDNA-3.1c plasmid. The day before transfection, about 1×10^6 cells were seeded in the media onto 60 mm dish and incubated for 24 h. The next day, cells were transfected with Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. The transfection efficiency was evaluated by green fluorescent protein (GFP) expression. The transfected cells were collected for the following experiments after a 48-h incubation. Stable transfection was performed using G418 screening.

Preparation of total cellular and nuclear extracts To obtain the total cellular extract, cells were washed with ice-cold PBS for three times and lysed in pre-chilled lysis buffer (50 mM Tris–HCl, pH 6.8, 2% SDS, 10% glycerol) containing proteinase inhibitors mixture (1 mM PMSF, 5 mM $NaVO_4$) with cell scraper. The lysate was then heated at 100°C for 10 min, sonicated on ice for 5 min, and centrifuged (12,000 ×*g*, 10 min, 4°C) for the supernatant. For nuclear fractions, after getting rid of cytoplasmic fraction as described [8], the pellet was suspended with high-salt buffer C (20 mM Hepes pH 7.9, 400 mM NaCl, 1 mM EDTA, 1 mM EGTA, 25% glycerol, 1 mM DTT, and fresh proteinase inhibitors mixture), vortexed 10 s at the highest setting, and incubated for 2 h on ice. Finally, the sample was subjected to centrifugation (12,500 ×*g*, 10 min, 4°C) and the supernatants were used as nuclear extracts.

Immunofluorescence The cells were cultured on coverslips and transfected with plasmids. After 48 h, they were then rinsed three times in cold PBS, fixed with 3.8% paraformaldehyde at room temperature for 30 min, washed, and permeabilized with 0.5% Triton X-100/PBS for 10 min. The cells were blocked with 1% BSA/TBST for 30 min and stained with anti-β-catenin antibody for 2 h. Then, the cells were washed four times with TBST for 5 min each time, followed with incubation with Texas Red-conjugated anti-Rabbit antibody in darkness for 1 h, and the nuclei were stained with DAPI for 6 min. In the end, the coverslips containing the cells were mounted on glass slides with VectaShield. Cells were then viewed with a Nikon Eclipse 400 fluorescence microscope.

Immunoprecipitation Cells were washed twice with ice-cold PBS and lysed in cold RIPA lysis buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, and 2 mM EDTA) with protease inhibitors (1 mM PMSF, 5 mM $NaVO_4$, 10 μg/ml leupeptin, and 10 μg/ml aprotinin) and then incubated on ice for 20 min. The lysates were centrifuged (12,000 rpm, 20 min, 4°C) and the supernatant was precleared with normal rabbit/mouse IgG and sepharose-protein G, (Pharmacia) or LCA-agarose, and then incubated with corresponding antibody at 4°C for 2 h on a rotator. Protein–antibody complexes were then collected with pre-washed sepharose-protein G beads or the lysates were incubated with pre-washed LCA-agarose (Vector Laboratories, Inc.) at 4°C overnight. The pellet collected by centrifugation (12,000 ×*g*, 30 s) was then washed three times with ice-cold RIPA, and finally resuspended in the SDS sample buffer (62 mM Tris–HCl, pH 6.8, 2% SDS, 100 mM DTT, and 15% glycerol). After being boiled at 100°C for 5 min, the immunoprecipitated complexes were released for western blot analysis.

Western Blot Proteins were mixed equally with 2× SDS loading buffer and resolved on SDS-PAGE gel. Proteins on the gel were then transferred onto PVDF membranes (Gelman). After blocking with 5% milk protein in TBST for 2 h, the membranes were incubated with specific

primary antibodies for 4 h at 4°C. Then the blots were washed for three times with TBST, and incubated for 2 h with HRP-conjugated secondary antibodies. Immunolabeling was detected by ECL (Pierce) after washing with TBST. Antibodies employed in the analysis were as follows: anti-β-catenin, α-catenin (Neomarkers), anti-c-myc (Invitrogen), anti-phospho-tyrosine PY20 (ICN) and anti-phospho Y654 of β-catenin (Abcam), anti-histone H4 (Upstate), normal rabbit IgG, anti-cyclin D1, anti-E-cadherin, anti-GAPDH, anti-Histone H4, anti-mouse, anti-rabbit secondary antibodies (Santa Cruz). Polyclonal antibody against Fut8 was raised in our laboratory by immunizing rabbits using the peptide LVRDNDHPDHSSREL mapping from 29 to 43 near N terminus of human Fut8.

Flow cytometric analysis Cell preparation and staining with fluorescein isothiocyanate (FITC)-conjugated LCA were as previous report [6] in both transfected 95C and 95D cells. Analysis was conducted on 20,000 cells per measurement using a FACScalibur machine (Dickinson).

Luciferase Assay Cells were seeded into 24-well plates and next day transiently transfected with 0.4 μg luciferase reporter plasmids: TOPflash or FOPflash (Upstate), 0.6 μg specific expression vectors, 0.1 μg pRL-TK (Promega). The cells were incubated for 24 h at 37°C, washed once with PBS, and then lysed to measure luciferase reporter gene expression by dual-luciferase reporter assay system (Promega). The intensity of luminescence was measured by a TD-20/20 luminometer (Turnerbiosystems). pRL-TK was used as internal control, and the luciferase activity was normalized to *Renilla* luciferase activity and then averaged over three independent experiments for each point. Tcf-mediated gene transcription was determined by the ratio of TOPflash over FOPflash luciferase activity.

Results

Core fucosylation of E-cadherin and regulation of nuclear β-catenin

E-cadherin expressed highly in 95C lung cancer cells (low metastasis potential) and core-fucosylated E-cadherin expressed lowly (Fig. 1A). However, core-fucosylated E-cadherin expressed highly in 95D lung cancer cells, which was consistent with our previous results [6]. 95C and 95D lung cancer cells were from the same patient with lung cancer, but possess different metastasis potential. Thus, we transfected 95C cells with Fut8 cDNA expression vector and transfected 95D cells with Fut8-RNAi construct to construct two pairs of cell models: 95C/Fut8 (Fut8 over-expression in 95C cells) Vs 95C/M (mock transfectants in 95C cells), 95D/si-Fut8 (95D with Fut8-RNAi in 95D cells) Vs 95D/Control (scrambled sequence transfectants in 95D cells). After transfection, Fut8 mRNA and protein in these two pairs of cell models were detected by RT-PCR and Western blotting and shown in Fig. 1B, which suggested successful transfection and well working of the vectors. Unrelated RNAi constructs did not yield the same result as Fut8-RNAi. The products of Fut8 in Fut8-RNAi transfectants were also confirmed by flow cytometric analysis lectin blot with LCA staining (Fig. 1D,E). Stably transfected cells were used for cell function study and transiently transfected cells were used for signaling study. Fut8 over-expressed 95C cells grew slowly.

Then we investigated whether E-cadherin was core-fucosylated in these two cell models. In the results, E-cadherin in Fut8-RNAi-transfected 95D cells was less core-fucosylated (Fig. 1C), but more core-fucosylated in Fut8-transfected 95C cells. E-cadherin expression was not affected by both Fut8 transfection and Fut8 RNAi. Interestingly, the level of β-catenin in the nucleus was greatly elevated in 95D cells with Fut8 RNAi, but reduced in Fut8-transfected 95C cells compared with their corresponding control cells in the immunofluorescence observation (Fig. 2a). In order to confirm this phenomenon, we further isolated nuclei and applied the total nucleus proteins for Western blot. The result shown in Fig. 2b indicated that more nuclear β-catenin was expressed in 95D cells with Fut8 RNAi, while less β-catenin was seen in the nucleus fraction of 95C cells with Fut8 overexpression, although the total β-catenin did not change greatly. In order to know the dynamics of nuclear β-catenin accumulation, nuclear β-catenin protein level in transiently Fut8-RNAi-transfected 95D cells was investigated at three pre-selected time points, *i.e.*, 24 h, 48 h, 72 h. As in Fig. 2c, nuclear accumulation occurred as early as 24 h after Fut8-RNAi transfection and remained stable during the following period. After the core fucosylation, E-cadherin association with β-catenin was quite enhanced in Fut8-transfected 95C cells. In Fut8 RNAi-transfected 95D cells, less β-catenin (Fig. 2d) was noted in the complex. Such recruiting of β-catenin to the membrane binding complex might contribute to the reduction of nuclear β-catenin level.

Accumulation of nuclear β-catenin was related to E-cadherin core fucosylation

Based on the results above, we wonder if the regulation of nuclear β-catenin is related to E-cadherin core fucosylation or not. Thus we did experiments to observe nuclear β-catenin accumulation in either E-cadherin-negative MDA-MB-231 cells or positive cells MDA-MB-468, MCF-7. Both MDA-MB-231 and MCF-7 breast cancer cells are

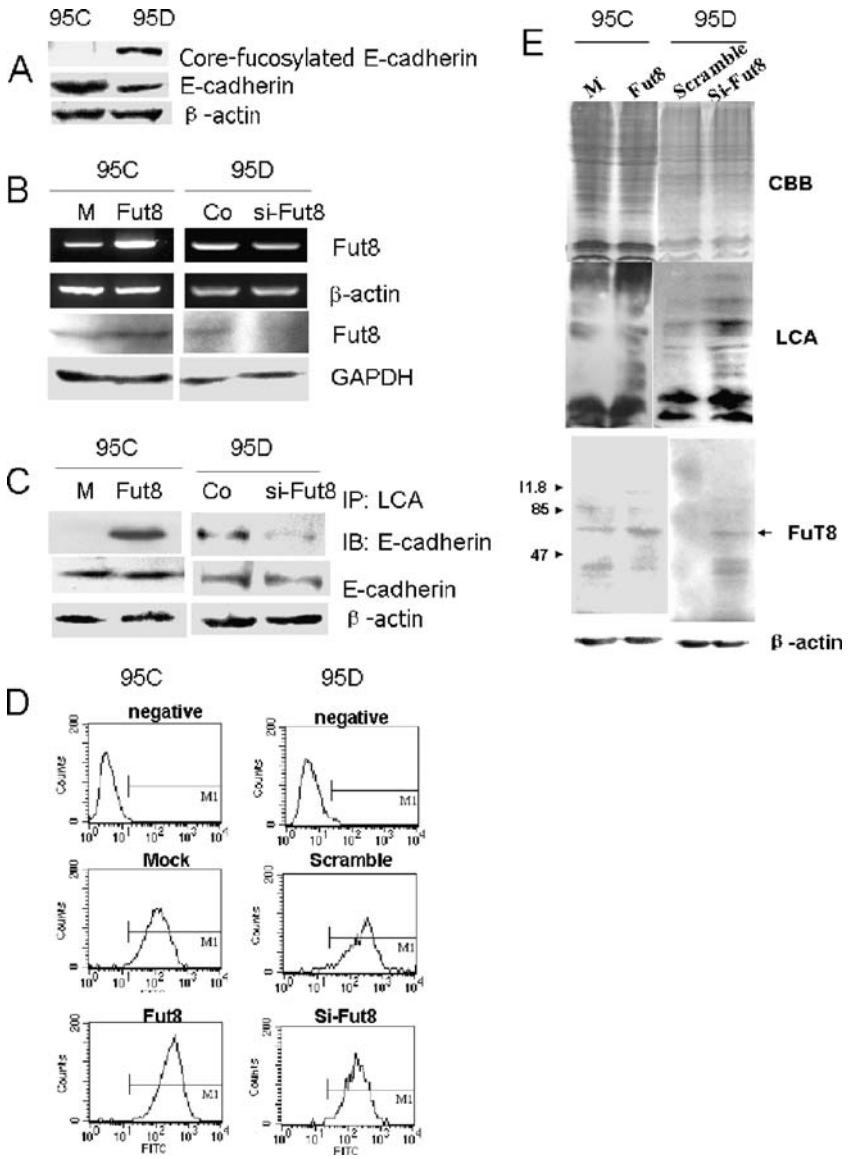


Fig. 1 E-cadherin core fucosylation in lung cancer cells. **A** E-cadherin was highly core-fucosylated in 95D cells in LCA precipitation with E-cadherin antibody blot. **B** In both Fut8 and Fut8-RNAi transfectants, the expression of Fut8 mRNA was confirmed by RT-PCR and Western blotting. M (Mock) was the control for Fut8 by using the vector plasmid. Scramble was the control for si-Fut8 by using scrambled sequence. **C** Core-fucosylated E-cadherin was over expressed in Fut8-transfected 95C cells and down regulated in Fut8 RNAi (si-Fut8) 95D cells. The assays were repeated more than three independent experiments. **D** The transfected cells were stained with FITC-conjugated LCA and analyzed with flow cytometry. The *top panel* was the negative control, *middle* vector control (Mock for Fut8 transfection, scramble for si-Fut8), and the *low panel* the transfectants with Fut8 or si-Fut8. **E** The transfected cells were assayed by SDS-PAGE and stained with Coomassie brilliant blue (SBB; *top panel*), LCA lectin blot (*middle panel*), and Western blot with Fut8 antibody staining. The *arrow at the left side* indicated molecular weight of kilodalton and the *arrow at the right side* indicated Fut8 position. The experiments were repeated at least three times, independently

well documented to be negative [9, 10] and positive [11] respectively in E-cadherin expression. Here, we further tested E-cadherin in MDA-MB-468, MCF-7 cells, and in MDA-MB-231 cells, but no E-cadherin protein expression was seen in MDA-MB-231 cells at all (Fig. 3a). The nuclear accumulation of β-catenin after Fut8 transfection or Fut8-RNAi was then investigated in these cell lines with either negative or positive E-cadherin expression. The result was consistent with our hypothesis and we observed that in MDA-MB-231, transfection with either Fut8 or Fut8 RNAi constructs didn't make any change in nuclear β-catenin expression (Fig. 3b), but in MCF-7 cells, a marked nuclear accumulation occurred after transient transfection with Fut8 RNAi. A similar accumulation was also observed in MDA-MB-468 (data not shown).

To further demonstrate our hypothesis, we constructed the following experiment that was to rescue E-cadherin expression in MDA-MB-231 cells, which are negative in E-cadherin expression by transfection with the E-cadherin full length cDNA expressional vector, and to cotransfect the cells with either Fut8 or Fut8 RNAi construct. Then the expression level of nuclear β-catenin was analyzed and the result was summarized in Fig. 3c. Cotransfection with Fut 8 and E-cadherin made the cells express both Fut8 and E-cadherin (Fig. 3d) simultaneously and inhibited nuclear β-catenin accumulation interestingly. However, transfection with E-cadherin alone didn't cause any difference from those with mock plasmid transfection. The transfectant with E-cadherin and Fut8 RNAi constructs also did not cause a significant accumulation of nuclear β-catenin, which suggested that the transfected E-cadherin had not well been core-fucosylated at the time and the core fucosylation was involved in the regulation of nuclear β-catenin accumulation.

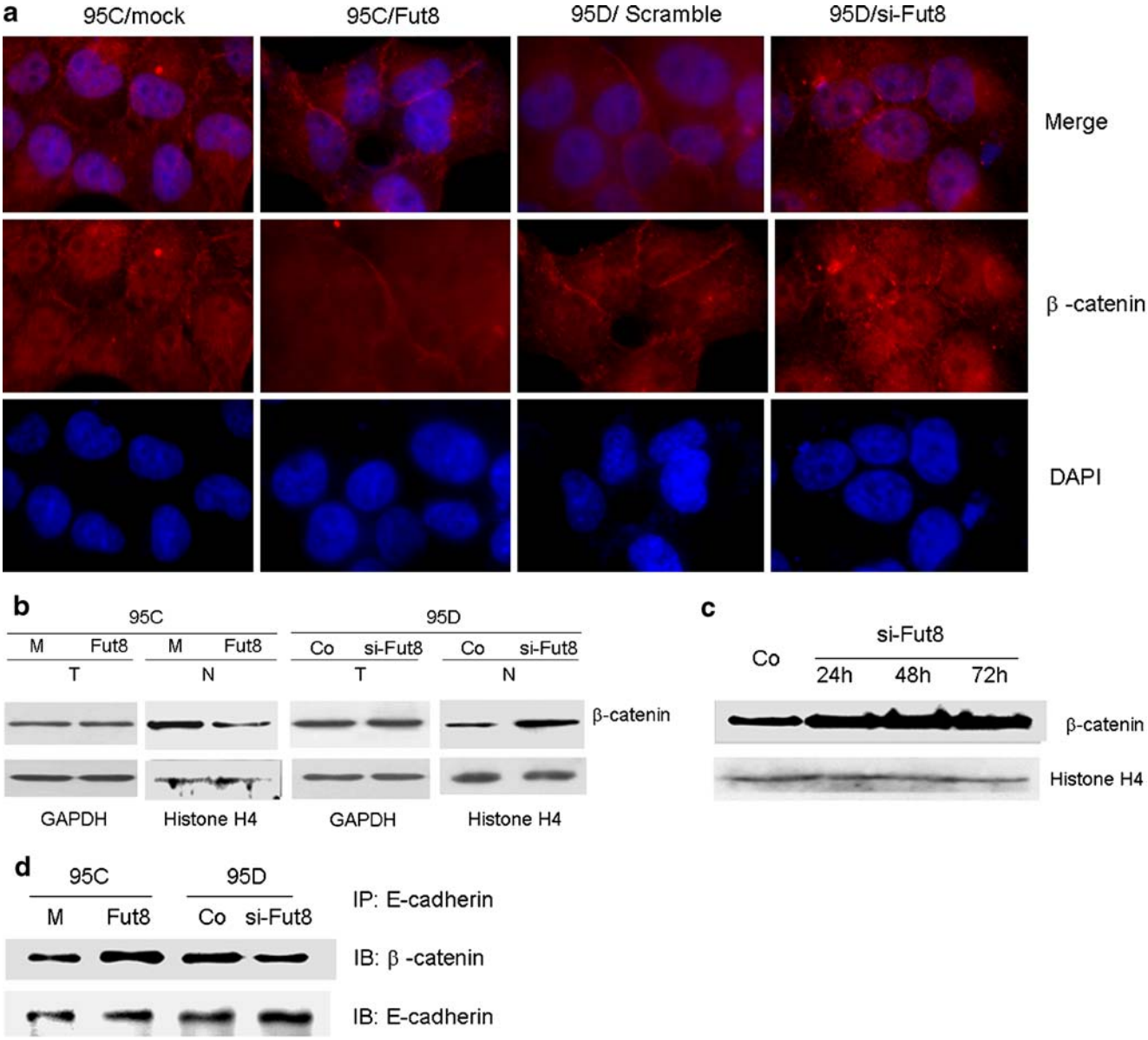
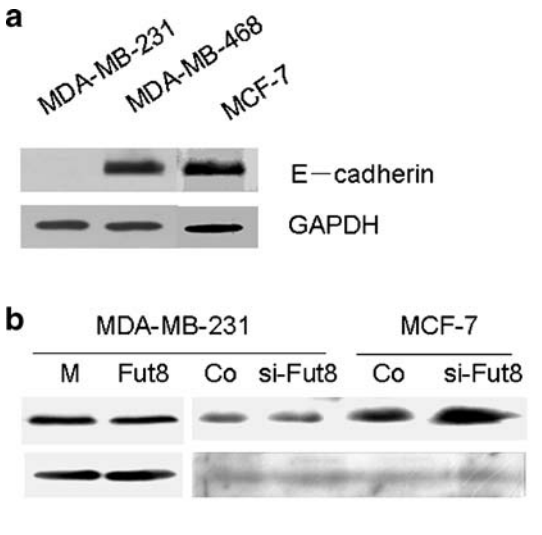


Fig. 2 Fut8 transfection regulates the nuclear β-catenin expression. **a** Subcellular localization of β-catenin in both Fut8-transfected 95C and Fut8 RNAi 95D cells was analyzed. β-catenin was stained as *Red* in both subcellular and nuclei compartment. The nuclei were stained by DAPI as *Blue* in the figure. The image was captured by IX2-BSW software, then merged with Image-pro Plus. **b** The total cellular and nuclear extracts were analyzed by Western blotting. One representative blot out of three independent experiments leading to similar results was shown in all experiments. **c** Nuclear accumulation in 95D cells with Fut8 RNAi could be observed as early as 24 h after transfection, and lasted for more than 48 h. **d** The transfected cell lysates were immunoprecipitated (IP) with mouse monoclonal antibody against E-cadherin, then the immunoprecipitates were immunoblotted (IB) with β-catenin or E-cadherin antibody respectively

Core-fucosylated E-cadherin has effects on β-catenin/Tcf activity Nuclear β-catenin activity usually depends on the complex forming with transcriptional factors. Thus, measurements of β-catenin/Tcf transcriptional activity represented nuclear β-catenin in another way. Compared with the data in control cells, the β-catenin/Tcf transcriptional activity determined by the ratio of TOPflash over FOPflash luciferase activity (Fig. 4a), was not only decreased significantly in Fut8 over-expressed 95C cells, but also increased in Fut8 RNAi-transfected 95D cells significantly, which was consistent with the data above. These were further corroborated by the expression changes of its target genes, such as c-myc, cyclin D1 as reported from documents [12, 13]. The results of c-myc and cyclin D1expression in 95C and 95D transfectants were summarized in Fig. 4b. Compared with the control cells, the levels of c-myc and cyclin D1 were significantly down regulated in Fut8 over-expressional 95C cell, but dramatically up-



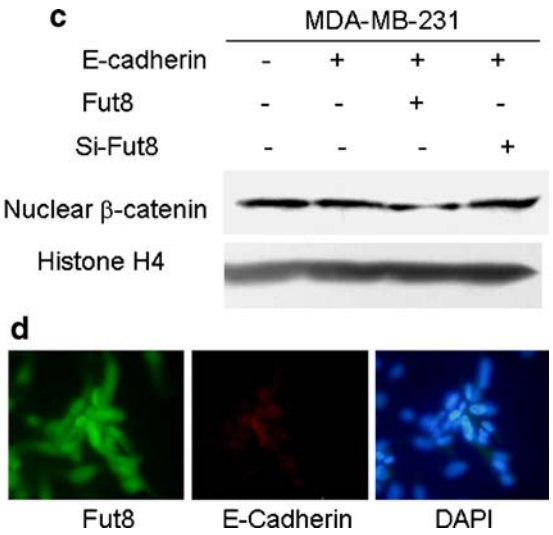


Fig. 3 Regulation of nuclear β-catenin is related to E-cadherin in Fut8 transfectants. **a** E-cadherin expression was not seen in MDA-MB-231 cells, but well expressed in MDA-MB-468 and MCF-7 cells. **b** No change of nuclear β-catenin level was observed in MDA-MB-231 cells transfected with either Fut8 or Fut8 RNAi, while in MCF-7, a great elevation of nuclear β-catenin expression was seen after Fut8 RNAi. **c** MDA-MB-231 cells were transfected with control plasmids (*lane 1*), E-cadherin only (*lane 2*), E-cadherin plus Fut8 (*lane 3*), and E-cadherin plus Fut8-RNAi (*lane 4*), respectively. **d** Fut8 (*green*) and E-cadherin (*red*) were expressed in the same cells after the cotransfection of MDA-MB-231 cells with Fut8 and E-cadherin constructs out of three independent experiments

regulated in Fut8 RNAi 95D cells (Fig. 4b). These results indicated that core-fucosylated E-cadherin mediated accumulation reduction of nuclear β-catenin which was accompanied by downregulation of c-myc and cyclin D1 protein expression.

Core fucosylation effects on β-catenin tyrosine phosphorylation

E-cadherin, as a single-pass transmembrane protein, its extracellular domain mediates the homophilic interaction, while its intercellular region mediates the association with catenin proteins of which binds tightly to E-cadheirn and links it through α-catenin to actin cytoskeleton. As core fucosylation of E-cadherin could affect the interaction with β-catenin, whether it could also affect the phosphorylation of β-catenin was our next question. Interestingly, the content of α-catenin in the complex was remarkably regulated in both Fut8 and Fut8 RNAi transfectants (Fig. 5A). The binding of α-catenin would regulate the phosphorylation of β-catenin. Therefore, we further investigated the phosphorylation on β-catenin. Tyrosine phosphorylation of β-catenin has an effect on E-cadherin-mediated adhesion and is associated with the transcriptional activity. In results, it was noted that the level of Y654 phosphorylated β-catenin was remarkably decreased in Fut8-transfected 95C cells, but increased slightly in Fut8 RNAi 95D cells compared with their control cells (Fig. 5B). The total tyrosine-phosphorylated β-catenin level was in the same change trend with Y654 phosphorylated β-catenin. Meanwhile, the transfected cells were also directly analyzed by Western blot with anti-Y654/β-catenin monoclonal antibody. A similar result was achieved (data not shown).

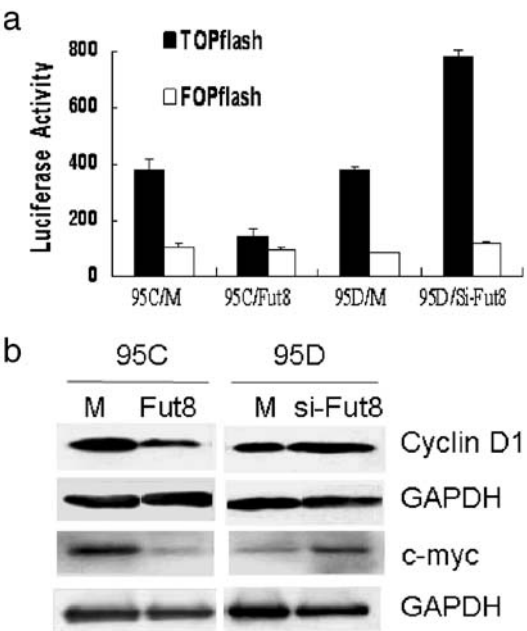


Fig. 4 The transcriptional activity of β-catenin/Tcf (T-cell factor) and the target gene expression. **a** 95C and 95D cells were cotransfected with reporter plasmid (TOPflash) harboring Tcf binding sites or its control (FOPflash) with a mutant Tcf-binding site respectively, and *Renilla* luciferase pRL-TK as well as Fut8-related or control plasmids. Luciferase activity was determined 48 h post transfection, normalized against values for the corresponding *Renilla* luciferase activity. *Columns*: means of three representative experiments; *bars*: Standard error (*SE*). **b** Cyclin D1 and c-myc expressions after the transfection were assessed by Western blotting analysis of total lysates by corresponding antibodies. Three independent assays were performed

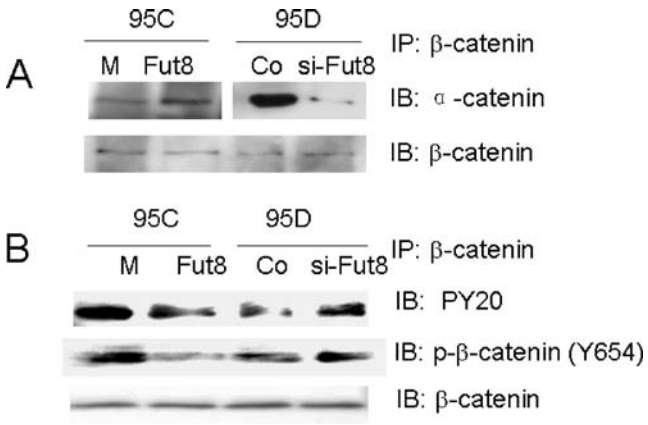


Fig. 5 Tyrosine phosphorylation of β-catenin and its association with α-catenin. **A** The transfected cell lysates were immunoprecipitated with rabbit anti-β-catenin antibody and the immunoprecipitates were immunoblotted with anti-α-catenin antibody. **B** The transfected cell lysates were immunoprecipitated with rabbit anti-β-catenin antibody, then immunoblotted with antibodies against tyrosine phosphorylation. One representative blot out of three independent experiments leading to the results is shown

Discussion

N-glycans in various glycoproteins, such as adhesion molecules, contribute greatly to the function of folding, stability, and other biological functions. In hepatoma cells, an elevated level of Fut8 was observed and core-fucosylated α-fetoprotein has been reported as a marker for early diagnosis and poor prognosis of the disease [14, 15]. The glycan structure of E-cadheirn has its significant effect on the regulation of the molecule. Cytoplasmic *O*-glycosylation blocks the transport of E-cadherin during apoptosis, resulting in reduced intercellular adhesion and ER stress [16]. *N*-glycans, especially the complex oligosaccharides on the ectodomain 4, change the molecular organization of E-cadheirn, thus destabilize adherens junctions (AJs) [17]. Recently, core fucosylation has also been reported to regulate the activation of EGFR [4], TGFR [3], low-density lipoprotein receptor-related protein [18] as well as integrin α3β1 [19]. Core fucosylation does modulate the function of such molecules and plays an important role in regulation of these proteins. However, it is not clear how the core fucose of the glycan regulates the function of E-cadherin and the downstream signaling. Our results provided evidence showing that core fucosylation of E-cadherin affected the molecule binding affinity and regulated nuclear β-catenin accumulation.

In this study, we observed that core fucosylation of E-cadherin regulated E-cadherin intracellular downstream signaling, since overexpression of Fut8 in 95C cells led to increase of core fucosylated E-cadherin and silence of Fut8 by target siRNA resulted in decrease of core fucosylation in E-cadherin in 95D cells in which E-cadherin was highly core-fucosylated. At the same time, a reduction of nuclear β-catenin was observed in Fut8-transfected 95C cells through not only immunofluorescent assay but also Western blot, although total cellular β-catenin expression was not significantly affected. Nuclear β-catenin accumulation was noted in si-Fut8-transfected 95D cells, which was coincident with the change of core-fucosylated E-cadherin. These indicated that there was some association between core fucosylation modification on E-cadherin and β-catenin accumulation in the nucleus, although there was no evidence showing β-catenin was also core-fucosylated. However E-cadherin was known to be core-fucosylated in both our previous study and current research. Then we ask if core-fucosylated E-cadherin was responsible for the regulation of β-catenin accumulation. Another pair of cell lines with E-cadherin negative (MDA-MB-231) or positive (MDA-MB-468, MCF-7) expression were selected to study this issue. Our data indicated that such a regulation by core fucosylation was related with E-cadherin. Nuclear β-catenin did not change in MDA-MB-231 breast cancer cells with Fut8 transfection or target siRNA. However, transfection with E-cadherin and Fut8 together reduced nuclear β-catenin level in such cells, which suggested that E-cadherin played a key role in the regulation of nuclear β-catenin accumulation and the regulation of nuclear β-catenin accumulation was related to E-cadherin core fucosylation. Transfection with E-cadherin alone or with E-cadherin and Fut8 RNAi did not change nuclear β-catenin significantly, which suggested the rescued E-cadherin had not been well core-fucosylated in MDA-MB-231 cells.

We next ask whether the regulation of nuclear β-catenin could lead to transcriptional activity change or not, because nuclear β-catenin could form a transcriptional complex with TCF/LEF to regulate target gene expression such as cyclin D1 and c-myc [12, 20]. Our proceeding data served as a strong support to this. We noted that at the time of E-cadherin core fucosylation in Fut8-transfected 95C cells the activity of the TCF promoter-reporter construct, TOPflash, reduced significantly. Similarly, when E-cadherin was less core-fucosylated by Fut8 target silence, the activity of TOPflash got significantly higher than the control. This was further supported by the changes of c-myc and cyclin D1 expression. In our previous study one clone of the stable transfection with Fut8 RNAi aggregated greatly into a sphere like stem cells and the cell growth retarded. The other clones did not. 95D cells with transient transfection of si-Fut8 grew faster.

To understand the inhibition of nuclear β-catenin accumulation by core fucosylation of E-cadherin, E-cadherin affinity to catenin was investigated after the fucosylation. Our results showed that core-fucosylated E-cadherin bound much more β-catenin with α-catenin. This

indicated that core fucosylation of E-cadherin enhanced the formation of E-cadheirn-β-catenin-α-catenin complex. Concomitantly, E-cadherin bound less catenins to form the complex in Fut8-RNAi transfectants. This suggested core fucosylation not only could affect the dimerization of E-cadheirn [6], but also promoted the intracellular combination with catenins. We also noted the reduction of tyrosine 654 phosphorylation of β-catenin in Fut8-transfected 95C cells, which was related with the binding to E-cadherin since Tyr654 phosphorylation of β-catenin decreases the molecule binding to E-cadherin [21]. Modification of β-catenin tyrosine 142 decreases binding to α-catenin [21]. In our result a remark decrease in binding to α-catenin was observed after Fut8 target silence in 95D cells, which might be associated with the tyrosine phosphorylation modification in β-catenin. Loss of α-catenin would further promote β-catenin binding of galectin-3 and enhance transcriptional activity by formation of a β-catenin-T-cell factor-DNA complex in the nucleus [22, 23]. The expression regulation of c-myc and cyclin D1 after Fut8 overexpression or silence further supported the promotion of β-catenin transcriptional activity.

In summary, our data presented here demonstrated that E-cadherin in 95C,D lung cancer cells could be core-fucosylated by Fut8 and such core fucosylation on E-cadherin regulated the molecule function and its down stream signaling.

Acknowledgement This work was supported by Natural Science Foundation of China (30070183, 30570414), Shanghai Leading Academic Discipline Project (B110), and Wenzhou Scientific and Technological Bureau (H2006023).

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