The Role of the Fibronectin Receptor Embigin in Cell Adhesion and Metabolism in Human Embryonic Kidney Cells

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CELL BIOLOGY

1. Abstract

Embigin (EMB, GP70) is a fibronectin receptor and a member of the immunoglobulin superfamily. Embigin is a cell membrane protein and involved in cell adhesion. In addition, embigin plays a role in cell metabolism as it is a chaperon-like protein for monocarboxylate transporters (MCTs) and forms a complex with them in the plasma membrane. MCTs transport monocarboxylates such as L-lactate and pyruvate across the plasma membrane. Embigin is known to assist MCT2 in localisation, but it also assists MCT1, 3, 4 and 7.

The aim of this research was to demonstrate whether the silencing of the embigin expression would have an impact on the cell adhesion or the expression of the MCTs.

2. Methods

- The used cell line was the 293T clone of the human embryonic kidney cells (HEK293T)
- The expression of embigin and MCTs were characterised in HEK293T cells with western blot and PCR, respectively
- The silencing of embigin was done with RNA interference (RNAi) and the result was confirmed with western blot
- The cell adhesion was studied by microscopy and xCELLigence
- The expression of embigin and MCT1 was studied with confocal microscopy

3. Results

HEK293T Cells Express Embigin and Monocarboxylate Transporters

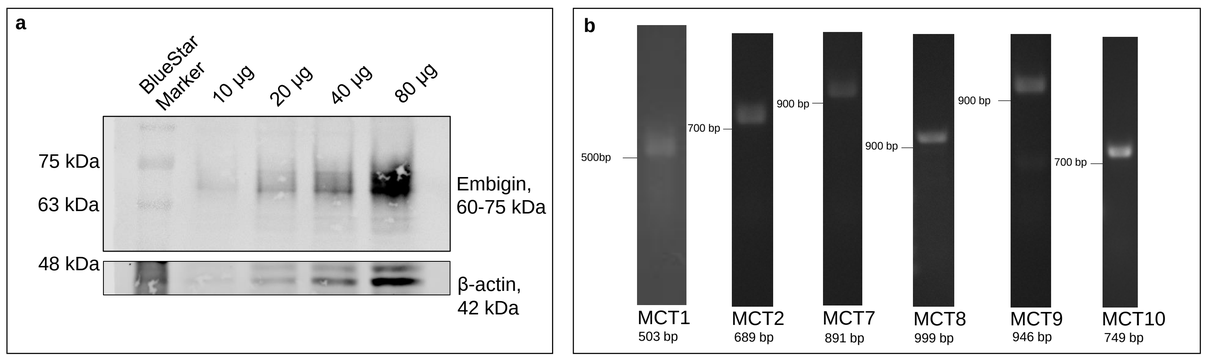

Figure 1. a The expression of embigin in HEK293T cells was confirmed with western blot (8% SDS-page). Different protein concentrations (10, 20, 40 and 80 µg) were loaded on the gel. The gradient nature of the observed embigin band (60-75 kDa) is due to the glycosylation of the protein.
b The expression of the MCTs 1, 2, 7-10 was determined with PCR. The RNA of HEK293T cells were isolated and converted into cDNA. The results were analysed with agarose gel electrophoresis (1.25 % (w/v)).

Two 50 nM siRNAs Silence Embigin at 48 and 72 h time-points

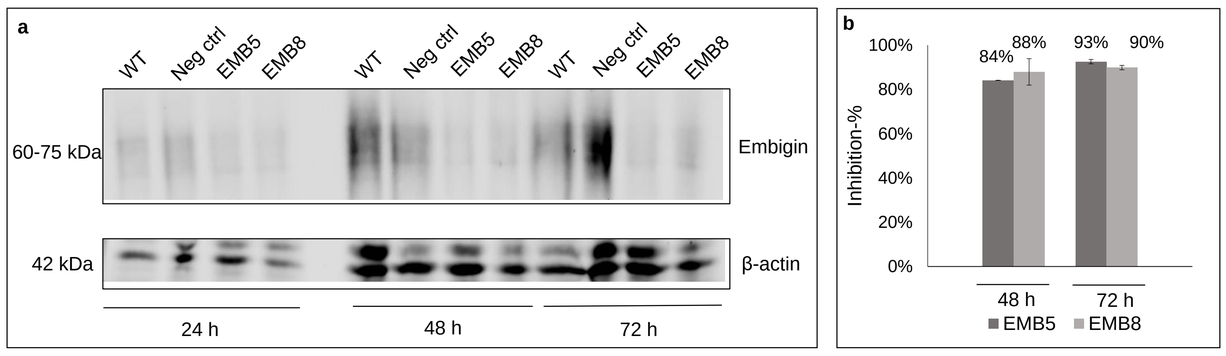

Figure 2. a Embigin is successfully silenced in HEK293T cells by using two different small interfering RNAs (siRNAs) (EMB5 and EMB8; 50 nM) in 3 time-points (24, 48 and 72 h). The result was confirmed with western blot (8% SDS-page). The cell number was low at the 24 h time-point. The house-holding protein β-actin was used as a loading control. The wild type (WT) HEK293T was used as a positive control and 50 nM negative control siRNA (AllStar, QIAGEN) as a negative control (neg ctrl). **b** The inhibition percent of EMB5 and EMB8 were 84 ± 0 %, 88 ± 6 % (48 h); 93 ± 1 %, 90 ± 1 % (72 h), respectively. β-actin was used in the quantitation and the inhibition percent was calculated based on the neg ctrl.

The Silencing of Embigin might have an Impact on the Cell Adhesion

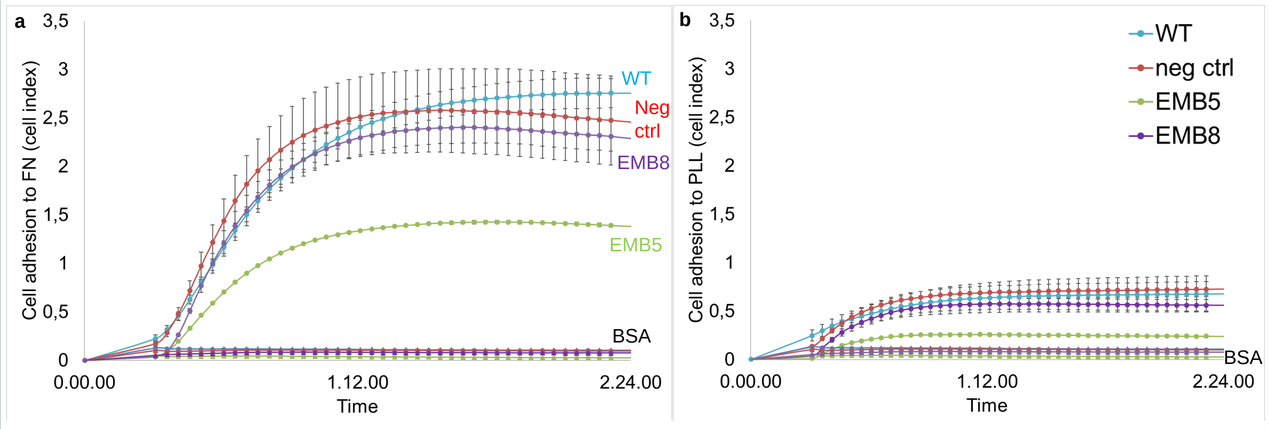

Figure 3. The spreading of the WT and the siRNA treated HEK293T (neg ctrl siRNA, EMB5 and EMB8; 50 nM, 48 h incubation time) cell lines was studied with xCELLigence on two different matrixes (5 µg/cm^2): (**a**) fibronectin (FN) and (**b**) poly-L-lysine (PLL). The WT HEK293T and the neg ctrl siRNA treated cells spread on the both matrixes better than the silenced cell lines. The spreading of EMB8 treated cells were slightly lower compared to controls while the spreading of EMB5 treated cells was lower compared to other cell lines. Bovine serum albumin (BSA) (0.1%) was used as a negative control.
xCELLigence generates a cell index based on the number, the shape and the size of the cells. The figures shows the steepest state of the curve during which the cells spread on the matrix (until 2 h 24 min time-point). After the spreading phase, the cells are in a plateau state and then they start to proliferate.

3. Results Continues

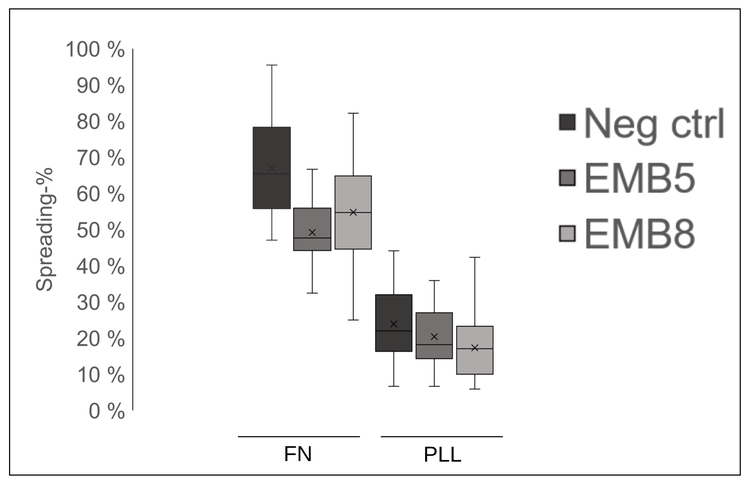

Figure 4. The spreading of HEK293T cells was studied with phase-contrast microscopy. The experiment was performed with cell lines treated with the neg ctrl siRNA and embigin-targeting siRNAs (EMB5 and EMB8) (50 nM siRNA, 48 h incubation time) on two different matrixes (5 µg/cm^2): FN and PLL.
The spreading percent of the neg ctrl was higher than embigin-silenced cell lines, being 67% (average value) on FN whereas the spreading of silenced cell lines were 49% (EMB5) and 55% (EMB8). There was also a slight decrease in the adhesion to PLL, being 24% in neg ctrl, 20% in EMB5 and 17% in EMB8. Cells did not spread on the control coating BSA as expected.

The percentage of the spread cells was calculated based on the total number of the cells on the counting area. The calculations included results from three different experiments. Each experiment had three parallel sample and cells were counted from four different counting areas from each sample.

The Silencing of Embigin might have an Impact on the Expression Level of MCT1

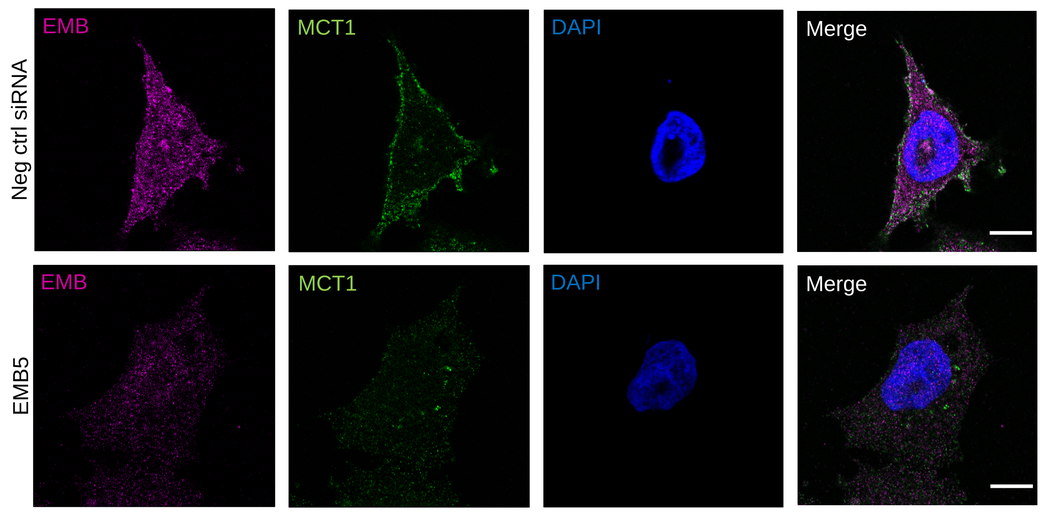

Figure 5. The expression of embigin and MCT1 in the siRNA treated cell lines (neg ctrl siRNA and EMB5; 50nM, 48 h incubation time) were studied with confocal microscopy Zeiss LSM880, 40x objective (zoomed (x2) pictures). The cells were let to spread on FN (10 µg/mL) for 2h before fixing them with 4% PFA for 20 min at room temperature. Scale bars, 10 µm.
The silencing of embigin might decrease the expression of MCT1. However, this requires further investigation.
The cells were permiabilized with TritonX-100 (0.2%) and stained with EMB (1:100; abcam, ab179801) and MCT1 (1:1000; Merk, AB1296-I) primary antibodies and Alexa Fluor secondary antibodies (1:200). The nucleus was stained with DAPI (5 min). The negative controls used in image settings were only stained with secondary antibodies. The images were edited with ImageJ.

4. Conclusions

- HEK293T cells express embigin and MCTs 1, 2 and 7-10
- Embigin can be successfully silenced with two different siRNAs
- The silencing of embigin might have an impact on the adhesion of HEK293T cells on fibronectin and poly-L-lysine
- The silencing of embigin might have an impact on the expression of MCT1, but this requires further investigation

Main references: **Talvi et al.**, (2024) Embigin deficiency leads to delayed embryonic lung development and high neonatal mortality in mice, iScience, Vol.27 (2), 108914-108914; **Sipilä et al.**, (2022) Embigin is a fibronectin receptor that affects sebaceous gland differentiation and metabolism, Developmental Cell, Vol.57, 1453-1465; **Xu et al.**, (2022) Embigin facilitates monocarboxylate transporter 1 localization to the plasma membrane and transition to a decoupling state, Cell reports (Cambridge), Vol.40 (11), p.111343-111343.

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