RESEARCH PAPER

Gal-1 silenced trophoblast tumor cells (BeWo) show decreased syncytium formation and different miRNA production compared to non-target silenced BeWo cells

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ABSTRACT
Galectin-1 (gal-1), a member of the mammalian β-galactoside-binding proteins, exerts biological effects by recognition of glycan ligands, including those involved in cell adhesion and growth regulation. In previous studies, we demonstrated that gal-1 induces cell differentiation processes on the membrane of choriocarcinoma cells BeWo, including the receptor tyrosine kinases (RTKs) REarranged during Transfection (RET), Janus Kinase 2 (JAK2) and Vascular endothelial growth factor receptor 3 (VEGFR3). Furthermore, Mitogen-Activated Protein Kinases (MAPK) and serine/threonine kinases were phosphorylated by gal-1. In addition, gal-1 in trophoblast cells in vitro induced syncytium formation especially after concentration dependent stimulation of the cells with this galectin. This is in contrast to MAPK-inhibitor U0126 that reduced syncytium formation of BeWo cells. The aim of this study was to analyze the syncytium formation abilities of BeWo cells that were gal-1 silenced. We found a significantly reduced syncytium formation rate in gal-1 silenced BeWo cells. In addition, these cells show a different miRNA expression profile. In summary, we found that gal-1 is a major trigger for fusion processes in BeWo cells. This function is accompanied by different regulation of miRNA synthesis in the BeWo cell culture model.

KEYWORDS
BeWo; galectin-1; miRNA; syncytium formation

Introduction
Galectin-1 (Gal-1), a member of β-Galactoside-binding proteins, forms a non-covalently bound homodimer with 2 carbohydrate recognition domains (CRD) in order to bind with Galβ1-4GlcNAc1 and Galβ1-3GlcNAc.2 Based on its molecular structure gal-1 belongs to the group of prototype galectins. It has been shown to display an immuno-modulatory function for trophoblast cells of placenta and in the process of tumorigenesis.3 Gal-1 contributes to the immunologic privilege of the trophoblast by eliminating maternal cytotoxic T cells via apoptosis.4 On the other hand gal-1 is suggested to be a marker for trophoblast invasion based on its co-expression in decidua and trophoblast5 and its increased expression in malignant changes of trophoblast.6

Gal-1 binds to Thomsen-Friedenreich (TF) antigen, which is expressed by syncytiotrophoblast (SCT), extravillous trophoblast (EVT) and trophoblast tumor cell line BeWo.7 This binding leads to decreased hormone production in TF positive cells. However trophoblast tumor cells JEG-3, which are negative for TF show no effect after gal-1 stimulation concerning hormone production,8 thus pointing at an alternative way of stimulation by gal-1. Further research revealed gal-1 influencing proliferation of TF positive BeWo cells.9 Contrarily to activated T cells, BeWo cells showed no higher rates of apoptosis after gal-1 incubation and therefore inhibition of BeWo cells seems also in this case to be independent from apoptosis.10 Research at term placentas, in cases of intrauterine growth restriction (IUGR), preeclampsia (PE) and HELLP syndrome showed significantly higher gal-1 expression in decidua and EVT.11 Especially in placentas of cases of PE increased gal-1 expression was accompanied by an elevated expression of TF at the membranes of the EVT. As gal-1 binds preferentially to TF in trophoblast cells12 elevated expression of gal-1 in EVT at the membrane border could be explained by increased binding of gal-1 at EVT.11

Syncytium formation, which takes place only in myo- blasts, osteoclasts and SCT, displays a very interesting
process. In myoblasts, absence of gal-1 leads to decreased and less effective fusion of cells. However, underlying pathways have not been elucidated completely so far. Influences of gal-1 stimulation on various components and markers of syncytium formation in BeWo cells have been shown; by now information on syncytium formation in gal-1 silenced cell lines is still lacking.

Materials and methods

Cell culture of gal-1 silenced BeWo cells

The gal-1 silenced choriocarcinoma cell lines BeWo was obtained from Q-tech (Sirion GmbH, Munich, Germany). For gal-1 knock out cultures BeWo cell lines with knock down vector Ad-shGal1-379 at KA efficiency of 99% were used. BeWo cell suspensions at 1 x 10⁵ cells/ml DMEM medium (Dulbecco’s Modified Eagle Medium, Biochrom, Germany, 3.7 g/l NaHCO₃, 4.5 g/l D-Glucose, 1.028 g/l stable glutamine, Na-Pyruvate, supplemented with 10% heat-inactivated FCS (fetal calf serum) without antibiotics and antimycotics) were incubated for up to 96 h in chamber slides. As controls cells of a non-target transfected BeWo cell line (Sirion) was used; furthermore called control cells. Cell fusion was not induced by any reagents and therefore spontaneous fusion was implicated.

Isolation of RNA for qPCR

Total RNA was prepared using the acid NucleoSpin RNAII Kit (Macherey-Nagel, Nr. 740955.50) according to manufacturer’s protocol: Cell-lysis was induced with RA1 and β–Mercaptoethanol (3.5μl). With NucleoSpin-filter, the lysat was filtrated. For RNA binding NucleoSpin RNAII (Machery Nagel) was used. Membrane Desalting Buffer was added and once more centrifuged. With adding of rDNAse and Reaction Buffer for rDNA (1:10) DNA is digested. The mixture is washed 3 times with RA2 or RA3 Buffer and eluted with RNAse free water.

cDNA-Synthesis/Reverse transcription

Reverse transcription for cDNA-Synthesis was performed with TaqMan_ EZ RT-PCR Kit (PE, Applied Biosystems). The quantitative real time PCR (qPCR) reactions were performed with a final volume of 20 μl consisting of 10 μl 2xRT-Mastermix and 10 μl mRNA. The reaction conditions were 10 min at 25°C, 120 min at 37°C, 5 s at 85°C and 4°C on hold and were performed with Eppendorf Mastercycler gradient (Eppendorf, Hamburg, Germany).

qPCR

For one Probe, a volume of 20 μl containing 1 μl TaqMan® Gene Expression Assay 20× (Table 1; Applied Biosystems, Weiterstadt, Germany), 10 μl TaqMan® Fast Universal PCR Master Mix 2x (Applied Biosystems), 1 μl cDNA template and 8 μl H₂O (DEPC treated DI water, Sigma, Taufkirchen, Germany) was used. The mix was applied on an Optical Fast 96-Well-plate (Applied Biosystems) and covered by optical caps (Applied Biosystems). PCR-assays were performed with Taqman 7500 Fast (Applied Biosystems). Enzyme activation was performed at 95°C for 20 sec at hold. At 95°C 40 cycles of qPCR was started with 3 sec of denaturing and followed by 30 sec of annealing at 60°C. For the Results the RQ = 2⁻ΔCt method was used. Ct is defined as the first fluorescent signal reaching statistical significance. ΔCt values were calculated by normalizing to an endogene control, for which β-actin (ACTB) and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as housekeeping genes were used.

Immunocytochemistry

After fixation with Ethanol/Methanol (1/1) for 15 min, slides were washed with PBS (phosphate buffered saline, pH 7.4, 5 min) and blocked with horse serum (30 min, room temperature, 1:100). The slides were afterwards stained with the primary antibodies (see Table 2) for 18 h at 4°C. After washing, sections were detected with the biotinylated secondary antibodies (30 min, room temperature). The Vectastain Elite ABC-Kit (Vector Laboratories, UK) was used for visualization according to manufacturer instructions. In between all steps, PBS was used for washing the sections 3 times. Then, the slides were stained with AEC plus (3-Amino-9-Ethylcarbazole, Dako, Denmark) (12 min, room temperature) and washed in tap water. Finally, slides were counterstained with hemalaun (2 min, room temperature), washed in tap water and then covered. Sections were examined using a Leica photomicroscope (Solms, Germany). Images were obtained with a digital camera system (JVC, Victor Company of Japan, Japan). A total of 9 fields were analyzed with the size of 1000 mm x 1000 mm.

Evaluation of staining was completed by using the semi-quantitative assay as described in previous studies. The IRS calculation consists of multiplying the optical staining intensity (graded as 0 = no, 1 = weak, 2 = moderate and 3 = strong staining) and the fraction of...
Table 2. Antibodies used for cytochemistry and immunofluorescence staining.

| Antigen                        | Antibody                                                                 |
|--------------------------------|---------------------------------------------------------------------------|
| Immunochemistry                | Anti-β-Catenin, rabbit IgG (dilution: 1:600)                              |
|                                | Anti-E-Cadherin, mouse IgG 1/2 (dilution: 1:500)                          |
| Immunofluorescence             | Anti-Galectin-1, goat IgG                                                |
|                                | Anti-Syncytin, Rabbit IgG                                                |
|                                | Donkey-Anti-Goat-Cy-3 IgG                                               |
|                                | Goat-Anti-Rabbit-Cy-2 IgG                                               |
|                                | Diagnostic BioSystems; Pleasanton, USA                                   |
|                                | Calbiochem; Beeston Nottingham, UK                                       |
|                                | R&D Systems, Abingdon, UK                                               |
|                                | Abnova, Heidelberg, Deutschland                                         |
|                                | Dianova; Hamburg, Deutschland                                           |
|                                | Dianova; Hamburg, Deutschland                                           |

positive stained cells (0 = no staining, 1 ≤10% of the cells, 2 = 11-50% of the cells, 3 = 51-80% of the cells and 4 = ≥81% of the cells). Two observers working independently examined the slides.

Extent of antigen expression for β-catenin staining, was evaluated by examining the percental changing of the membrane-staining compared to the cell number (100% = all membranes are positive, 0% = no membrane is positive).

For data collection, processing, and statistical analysis SPSS/PC software package, version 15.01 (SPSS, Germany) was used. The non-parametric Wilcoxon’s signed rank tests for comparison of the means was used for statistical analysis. P < 0.05 values were considered statistically significant.

Immunofluorescence staining of gal-1 silenced BeWo cells and non-target BeWo cells

The experiments were done in quadruplicates. Slides were fixed with Aceton for 10 min, washed with PBS and stained with Ultra V Block (Labvision, USA, 15 min, room temperature). Then, the slides were stained with the primary antibody for either gal-1 (goat IgG [1:75], Table 2) or syncytin (Rabbit IgG [1:20], Table 2) overnight at 4°C. After washing, chamberslides for gal-1 or syncytin were stained with either Cy3 labeled antibody Donkey-Anti-Goat IgG (1:100; room temperature, 30 min) which will appear red or Cy2 labeled antibody Donkey-Anti-Rabbit IgG (1:100; room temperature, 30 min) which will appear green. The slides were finally covered with mounting medium containing 4′,6′-diamino-2-phenylindole (Vectorshild mounting medium DAPI, Vector Laboratories, Denmark) and examined with a photomicroscope (Zeiss Axioshot, Germany). Images were obtained with a digital camera system (Axiocam, Zeiss, Germany). A total of 9 fields were analyzed with the size of 1000 μm × 1000 μm.

ELISA

For the quantification of sE-cadherin and β-Catenin in cell lysates immunoassays (all R&D Systems, Abingdon, UK) were used as a 2-site sandwich ELISA (Table 3). The β-Catenin ELISA detects total β-Catenin in cell lysates whereas the sE-cadherin ELISA detects only the soluble form of this protein, liberated from the cell surface by proteases in processes like cell fusion during syncytium formation. In brief, an antibody specific for either sE-cadherin or β-Catenin has been pre-coated onto a microplate, respectively. Standards and samples are added and any sE-cadherin or β-Catenin present is bound by the immobilized antibody. After washing away unbound material, a biotinylated detection antibody recognizing sE-cadherin or β-Catenin is used for detection utilizing a standard streptavidin-HRP format. Substrate solution is added to the wells and color develops in proportion to the amount of sE-cadherin or β-Catenin bound in the initial step. The color development is stopped and the intensity of the color is measured.

Detection of cell fusion by cell-labeling

Both, the gal-1 silenced BeWo cell line and the control cell line were treated according to following procedure:

A total of 2.5×10⁵ cells were labeled with 8 μg/ml DiO (1,1′-dioctadecyl-indocarbocyanine perchlorate) fluorescent cell-labeling solution (Vybrant Cell-Labeling Solutions, Molecular Probes, Scotland) in serum-free DMEM medium (Dulbecco’s Modified Eagle Medium, Biochrom, Germany, 3.7 g/l NaHCO₃, 4.5 g/l D-Glucose, 1.028 g/l stable glutamine, NaPyruvate) for 20 min at 37 °C without CO₂. Further 2.5×10⁵ cells were labeled with 4 μg/ml DiI (1,1′-dioctadecyl-3,3,3′-tetramethylindocarbocyanine perchlorate) fluorescent cell-labeling solution (Vybrant Cell-Labeling Solutions, Molecular Probes, Scotland). Cells

Table 3. ELISAs used for detection of proteins in cell culture supernatants.

| ELISA                | Order number          |
|----------------------|-----------------------|
| sE1-Cadherin         | DCADE0                |
| Total human β-Catenin| SUV1329               |

¹ sE-Cadherin: soluble E-Cadherin.
were washed with serum-free DMEM medium 3 times. After washing, cells were re-suspended in DMEM medium supplemented with 10% heat-inactivated FCS, 250 μg/ml Amphotericin B and 10000 U/10000 μg/ml Penicillin/Streptomycin. DiO and Dilabeled cell suspensions were mixed in one well of a 24-well plate. Finally, cells of both cell lines (gal-1 silenced and non-silenced) were incubated for up to 96 h at 37°C. The amount of cell fusion was evaluated in 10 randomly chosen fields of each well using Zeiss Axiolab 40 CFL fluorescent microscope (Zeiss, Germany) as described recently. Images were obtained with a digital camera system (Power Shot A620, Canon, Japan). The degree of cell fusion was detected by determining the amount of yellow cells as fusion of green colored cells and red colored cells results in yellow staining in fluorescence.

**miRNA extraction**

Cells were seeded in 6-well plates, allowed to attach overnight and serum deprived for 2h before total RNA extraction using TRIZol® reagent following manufacturer's instructions (Invitrogen, Darmstadt, Germany). RNA concentration and purity were determined on NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, USA). Samples with A260/A280 values greater than 1.8 were stored at −80°C until being processed.

**qPCR for mature miRNA**

qPCR was performed using Taqman microRNA assays (hsa-miR-517c, Assay ID: 001153; hsa-miR-519d, Assay ID: 002403; miR-512-3p, Assay ID: 001823; miR-525-3p, Assay ID: 002385; miR-191, Assay ID: 000490; RNU-48, Assay ID: 001006) and Taqman Universal PCR master mix reagents (Applied Biosystems, Darmstadt, Germany). qPCR was run in duplicates on Mx3005P QPCR System (Applied Biosystems, Darmstadt, Germany). miRNA expression was normalized to RNU48 transcript levels by using the $2^{-\Delta\Delta Ct}$ method. Results are expressed as relative expression to the mean of sh-control group (mean ± SEM) of 6 independent experiments.

**Statistics**

The SPSS/PC software package, version 15.01 (SPSS, Germany), was used for collection, processing, and statistical data analysis. Statistical analysis was performed using the non-parametrical Wilcoxon signed rank tests for comparison of the means. $P < 0.05$ values were considered statistically significant.

**Results**

**Expression of gal-1 on mRNA level**

Efficiency of gal-1 silencing was tested in both, the gal-1 silenced BeWo cell line and the control cell line. We found a 0.01-fold gal-1 mRNA expression in gal-1

![Gal-1 mRNA Expression](image-url)

*Figure 1.* Gal-1 silencing proved to be efficient as gal-1 silenced BeWo cells showed a 0.01-fold mRNA expression compared to control BeWo cells. The comparative results are depicted graphically as block bars and show relative quantification for both cell lines.
silenced BeWo cell line compared to the control BeWo cell line (Fig. 1).

**Immunocytochemistry**

As shown in Figure 2 gal-1 silenced BeWo cells showed high expression of E-cadherin (A) after 96h of cultivation compared to control cells (B). We identified significant differences between the 2 cell types (p < 0.001). A summary of staining is shown in Figure 2C. Results for E-cadherin staining are displayed graphically in Figure 2C as box plot.

Staining with β-catenin showed also stronger expression for this peptide in gal-1 silenced BeWo cultures (D) in comparison to control cells (E). Differences between both cell types were significant (p < 0.001). A summary of staining is shown in Figure 2F. Results for β-catenin staining are displayed graphically in Figure 2F as box plot.

**Immunofluorescence staining**

Control staining for gal-1 showed significant knock down for gal-1 in specifically silenced BeWo cell culture (< 1% staining of gal-1, Fig. 3A) compared to control BeWo cells (> 90% staining for gal-1, p < 0.001, Fig. 3B).

Syncytin protein expression was significantly increased from 1-5% (in gal-1 silenced BeWo cells SEM = 1.0) to 78% in control cells (p = 0.011, SEM = 0.71) as demonstrated in Figs. 3C–D.

**Quantification of β-Catenin and soluble E-cadherin with ELISA**

The total β-Catenin ELISA showed significant higher expression of this protein in cell lysates of gal-1 silenced trophoblast cells BeWo (mean 4.33 ± 0.80pg/ml) compared to control cells (mean 2.77 ± 0.23pg/ml) after 96 h of cultivation (Fig. 4A, p < 0.001).

In contrast, the ELISA detecting the soluble form of E-cadherin showed high values of sE-cadherin in control BeWo cells (mean 3.38 ± 0.17 pg/ml) compared to gal-1 silenced trophoblast cells BeWo (mean 1.95 ± 0.46 pg/ml) after 96 h of cultivation (Fig. 4B, p < 0.001).

**Detection of cell fusion by cell-labeling**

The number of cells showing fusion of both colors was significantly increased in the control cell culture in vitro compared to gal-1 silenced cells (p = 0.006) after 96 hours of cultivation. BeWo cells (gal-1 silenced and

![Figure 2](image-url). High expression of E-cadherin could be found in gal-1 silenced BeWo cells (A). In comparison to this finding control cells showed decreased expression of E-cadherin (B). This difference was statistically significant (p < 0.001) and is depicted as box plot in (C). Similar results could be reached for β-catenin staining, which showed stronger expression in gal-1 silenced cultures (D) than in control BeWo cells (E). Staining results, which are all statistically significant (p < 0.001), are displayed as box plots (F). The boxes represent the range between the 25th and 75th percentiles with a horizontal line at the median. The bars on top and below depict the 5th and 95th percentiles. Values more than 1.5 box lengths from the 75th percentile are indicated by circles and values more than 3.0 box lengths from the 75th percentile are indicated by the asterisk.
non-target silenced) were both stained with DiO (green) or Dil (red), respectively and mixed afterwards. Cell populations with both colors are seen in gal-1 silenced BeWo cells, 0 h cell culture in vitro, (Fig. 5A), and in control cells, 0 h (Fig. 5B). Fusion of red and green colored cells results in in yellow staining because of close proximity. After fusion of cells, gal-1 silenced BeWo cells appear in yellow, 96 h (Fig. 5C), significantly higher numbers of fused cells are seen in control BeWo cells, 96 h (Fig. 5D).

A summary of the fusion experiments is shown in Fig. 5E.

qPCR for miRNA

qPCR for miRNA was performed for comparison of BeWo cell cultures silenced for gal-1 to those with non-target silencing. The following miRNAs were detectable in both cell cultures: miR-519d, miR-517c, miR-503, and miR-509-3p. A summary of the qPCR results is shown in Fig. 5E.

Figure 3. In gal-1 silenced BeWo cell culture a knock down for gal-1 was shown in control immunofluorescence staining. (A). Strong staining (red) could be shown for gal-1 in control BeWo cells (>90% staining for gal-1; p < 0.001) (B). Also expression of syncytin protein (stained green) appeared increased from gal-1 silenced BeWo cells (C) to control cell culture (D). Results were statistically significant with p < 0.011 and SEM = 0.71.

Figure 4. Absolute quantification of β-Catenin via ELISA in cell lysates of gal-1 silenced cell cultures (A) showed higher expression (mean 4.33 ± 0.80pg/ml) when compared with control BeWo cells (mean 2.77 ± 0.23pg/ml). Results were statistically significant (p < 0.001) and are depicted as bar graphs. Contrary to this finding the ELISA for sE-cadherin (soluble E-cadherin) displayed high concentration of this protein in control BeWo cells (mean 3.38 ± 0.17 pg/ml) and lower results for gal-1 silenced cells (mean 1.95 ± 0.46 pg/ml). Again all results were statistically significant with p < 0.001 (B) and are depicted as bar graphs with absolute quantification.
miR-512-3p, miR-525-3p and miR-191 (see Fig. 6). Only miR-519d expression showed a statistically significant 2 fold increase in gal-1 silenced BeWo cells compared to control cells (p = 0.031). The expression of miR-517c appeared also to be increased in gal-1 silenced BeWo cells but did not reach statistical significance p = 0.084. MiR-512-3p, miR-525-3p and miR-191 displayed no differences in expression between both cell groups.

**Discussion**

The immunologic effects of gal-1 and its role in gestational diseases like PE and HELLP syndrome have
already been shown.\textsuperscript{4,11} Additionally, galectins have been demonstrated to be essential for cancer progression and developing metastases.\textsuperscript{16}

In recent in vitro studies on BeWo cells and human cytotrophoblast (CTB) cells gal-1 stimulation promotes down regulation of β-catenin and E-cadherin, the latter being an indicator of syncytium formation in terms of shedding during the process of cell fusion;\textsuperscript{15} this was also confirmed by our findings. Gal-1 also induces Elk1 activation, being a transcription factor activated by mitogen-activated protein kinases (MAPK) pathways. Additional results showed gal-1 induced signaling in BeWo cells involves phosphorylation of MAP kinases, which are necessary for syncytium formation in trophoblast cells.\textsuperscript{17}

Inhibition of β-catenin and E-cadherin expressions has been shown in the 48 h BeWo cell culture before using double immunofluorescence after stimulation with gal-1. As an indicator for syncytium formation in BeWo cells E-cadherin disappeared from the cell-borders parallel to down regulation of the mRNA.\textsuperscript{18} Our results demonstrate the contrary effect in gal-1 silenced BeWo cells and show again the involvement of gal-1 in syncytium formation.

Furthermore, gal-1 has been proven to be of importance in trophoblast invasion in vitro. A direct correlation of gal-1 concentration with cell invasiveness could be shown by supplementing recombinant gal-1 and blocking endogenous gal-1. Therefore, gal-1 appears essential for trophoblast invasion.\textsuperscript{19} Using the invasive trophoblast cell line HTR-8/SVneo an in vitro model for trophoblast invasion has been created to show effects of blocking gal-1 by an anti-gal-1 antibody, demonstrated in other settings.\textsuperscript{20} Contrary effects in terms of improved trophoblast invasion could be seen after applying 2 recombinant forms of gal-1.\textsuperscript{21} These findings of gal-1 stimulated human trophoblast show opposite effects compared to mouse knock-down results. However, there is increasing evidence for species specific glycans thus differing in functional relevance.\textsuperscript{22} Gal-1 is meanwhile considered to play a role in cancer cell invasiveness; in hepatocellular cancer existence of metastasis is significantly correlated with increased gal-1 expression.\textsuperscript{23} Overexpression of gal-1 has also been shown in trophoblast of invasive mole, trophoblastic tumor and chorion carcinoma.\textsuperscript{24} Reduced invasion in highly metastatic cancer cells was reached by knocking down gal-1 with small interfering RNA hereby showing interference of gal-1 with matrix metallo proteinase expression (MMP).\textsuperscript{25,26} Abnormal activity and deregulated expression of matrix metallo proteinase 2 (MMP-2) are involved in impaired trophoblast invasion in hypertensive pregnancies.\textsuperscript{27} This proteinase is an immediate target of miR-519d-3p, a microRNA (miRNA), that belongs to a class of endogenous, non-coding RNA of about 20-25 nucleotides acting as negative post-transcriptional factor for gene expression. The miR-519d-3p belongs to the placenta-related chromosome 19 miRNA

\textbf{Figure 6.} Results of qRT PCR for miRNA show expression of miR-519d, miR-517c, miR-512-3p, miR-525-3p and miR-191 in both gal-1 silenced and control BeWo cells. Statistically significant increase was shown only in miR-519d expression in gal-1 silenced BeWo cells compared to control cells ($p = 0.031$). Increased expression of miR-517c in gal-1 silenced BeWo cells did not reach statistical significance $p = 0.084$.
cluster (C19MC) which also includes the here tested miRNAs miR-525-3p, miR-512-3p and miR-517c. Compared to placentas of normal pregnancies, those miRNAs have been found to be differentially expressed in pregnancy pathologies including preeclampsia. We and others have demonstrated that overexpression of miR-519d-3p leads to down regulation of MMP-2 mRNA and decreased protein expression and subsequently impaired trophoblast invasion. Our results of qPCR for miRNA display similar results as in gal-1 silenced BeWo cell cultures miR-519d-3p is increased compared to non-target silenced BeWo cells, thus pointing at a pathway of gal-1 influencing trophoblast invasion and cell migration.

As mentioned above mechanisms of trophoblast membrane fusion are not fully understood. So far, involvement of cadherin 11 and connexin 43 as proteins of cell to cell communication has been demonstrated. Furthermore the membrane glycoprotein syncytin has been proven to induce syncytium formation on interaction with D-mammalian retrovirus receptor RDR. Syncytin expression has been demonstrated to be increased after gal-1 stimulation in BeWo cells confirming the suggested role of gal-1 in syncytium formation. Our results furthermore support these findings as staining of syncytin was significantly decreased in gal-1 silenced BeWo cells. Syncytin, necessary involved in syncytium formation, is in its expression directly affected by the presence and expression of gal-1. As to underline the complexity of syncytium formation in trophoblast recent findings have shown that 2 members of the vasohibin family vasohibin-1 (VASH1) and vasohibin-2 (VASH2) are contrarily involved in syncytium formation via arteriogenesis without changing expression of syncytin in BeWo cells. TF also being expressed by endometrial glandular cells is a ligand for gal-1 depending on menstrual cycle and therefore gal-1 is suspected to play a role in blastocyste adhesion. Therefore, binding of gal-1 to the TF epitope appears to be implicated in 2 main processes during human pregnancy: the attachment of the trophectoderm to the endometrium within the window of implantation and the fusion of villous CTB into syncytium at the placenta. Conclusion

Gal-1 displays immediate proportional influence on soluble E-cadherin concentrations in BeWo culture conditions. Non-target cell cultures showed better fusion capability than BeWo cell cultures silenced for gal-1. This effect became visible through β-catenin, E-cadherin and syncytin staining and additionally by higher concentrations of shed E-cadherin in cell clearance of non-target silenced BeWo cells and lower concentrations of total β-catenin in the same cell population. Obviously gal-1 exerts direct influence on the fusion process of trophoblast cells.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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