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Characterization of Fetal Cells from the Maternal Circulation by Microarray Gene Expression Analysis – Could the Extravillous Trophoblasts Be a Target for Future Cell-Based Non-Invasive Prenatal Diagnosis?

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Fetal cells in maternal blood · Extravillous trophoblast · Microarray · Gene expression

Abstract
Introduction: Circulating fetal cells in maternal blood provide a tool for risk-free, non-invasive prenatal diagnosis. However, fetal cells in the maternal circulation are scarce, and to effectively isolate enough of them for reliable diagnostics, it is crucial to know which fetal cell type(s) should be targeted. ***Materials and Methods:*** Fetal cells were enriched from maternal blood by magnetic-activated cell sorting using the endothelial cell marker CD105 and identified by XY fluorescence in situ hybridization. Expression pattern was compared between fetal cells and maternal blood cells using stem cell microarray analysis. ***Results:*** 39 genes were identified as candidates for unique fetal cell markers. More than half of these are genes known to be expressed in the placenta, especially in extravillous trophoblasts (EVTs). Immunohistochemical staining of placental tissue confirmed CD105 staining in EVTs and 76% of fetal cells enriched by CD105 were found to be cytokeratin-positive. ***Discussion:*** The unique combination of mesodermal (CD105) and ectodermal (cytokeratin) markers in EVTs could be a potential marker set for cell enrichment of this cell type in maternal blood and could be the basis for future cell-based non-invasive prenatal diagnosis.

Introduction

Prenatal screening for chromosomal aneuploidies in the fetus has so far mainly been achieved by sampling of fetal cells from the conceptus itself, either by chorionic villus sampling or amniocentesis. A major disadvantage

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of both of these procedures is, however, that the sampling procedure is associated with a risk of unintended pregnancy loss in the range of 0.5–1% [1]. Therefore, researchers have for a long time tried to use maternal blood samples for non-invasive prenatal diagnosis (NIPD) since it is envisioned that fetal cells isolated from the maternal circulation can replace cells from amnionic fluid or chorionic villi in diagnosis for common chromosomal aneuploidies using fluorescence in situ hybridization (FISH)-based techniques [2]. However, fetal cells in the maternal circulation are extremely rare with estimates of 1–2 cells/ml blood [3, 4]. For this reason several research groups have tried to establish technologies for the enrichment and identification of a range of fetal cell types, including nucleated red blood cells, certain types of fetal white blood cells and placenta-derived cells but with limited success [5–9]. One possible reason for this lack of success is limited knowledge on the specific types of the fetal cells present in maternal blood, leading to suboptimal markers for enrichment and identification.

Due to these problems, some years ago we initiated a more targeted strategy for determining the type of the fetal cells in maternal blood. This strategy involved generation of expression profiles based on microarray analysis of cDNA from circulating fetal cells originating from male fetuses. These fetal cells were initially enriched by partial negative enrichment by removing subgroups of maternal blood cells and identified by Y chromosome-specific FISH on samples from pregnancies with a male fetus. We identified, in this previous investigation, a set of 28 genes with higher expression in fetal cells compared to maternal blood cells [10]. Several of these 28 genes are expressed in the placenta which led us to suggest that at least a fraction of the fetal cells in maternal blood are derived from the placenta.

The aim of the present communication has been to extend these microarray gene expression studies with the primary aim to define one – or at most a few – relatively frequent fetal cell types in maternal blood with the ultimate goal of developing a cell-based NIPD based on these cells. This has in the present communication been achieved by moving from mainly negative selection of maternal cell types in our previous communication to a magnetic-activated cell sorting (MACS)-based positive enrichment based on endothelial cell surface markers. The reason for choosing endothelial cell markers in this communication was based on our previous finding that placental and endothelial genes were abundant among the genes highly expressed in circulating fetal cells.

More specifically we have in the present communication developed a fetal cell enrichment procedure based on the endothelial cell marker CD105. Considering that CD105 is highly expressed in placenta we – as would be expected – find that the fraction of placental genes is now even higher among the genes highly expressed in the enriched fraction. But we furthermore find that a substantial fraction of these placental genes are expressed in extravillous trophoblasts (EVTs). We therefore hypothesize that the majority of the fetal cells caught by CD105 are EVTs, an assumption that is supported by the finding of both cytokeratin and CD105 in fetal cells from maternal blood and in EVTs in placental sections.

Materials and Methods

Study Population and Clinical Material (fig. 1)

The blood samples used in this study were derived from 120 pregnant women coming for routine nuchal translucency scans at gestation weeks 11–13 at Aarhus University Hospital in Skejby, Denmark. Our inclusion criteria were singleton pregnancies with a low-risk assessment of aneuploidies (combined screening >1: 300). Written informed consent was given by all participants, and the project was approved by the local Danish science ethics committee. For immunohistochemical studies of placental sections, first-trimester placentas were obtained from elective terminations of pregnancies (gestational age 10–12 weeks). Written informed consent was obtained from each patient with approval of the local ethics committee.

Fetal Gender Determination

The gender of the fetus was determined by the procedure previously described (polymerase chain reaction procedure [10]). Only samples from women expecting a male fetus were used for fetal cell enrichment.

Blood Preparation and Fetal Cell Enrichment

All tubes and pipette tips used in this procedure were pre-coated with a coating buffer (2% BSA in PBS w/o Ca^{2+} and Mg^{2+}). All centrifugations were at 500 *g* for 10 min at 4°C unless otherwise stated.

Blood samples (24 ml, heparinized) were fixed within 15 min of drawing in 4% formaldehyde in PBS (Gibco, pH 7.4, w/o Ca^{2+} and Mg^{2+}) for 10 min at room temperature (RT). Red blood cells were lysed in 0.1% Triton X-100 in PBS slowly rotating for 45 min at RT. Nucleated cells were subsequently washed in 2% BSA in PBS (4°C) and then centrifuged (15 min). Pellets were resuspended in 4°C cold PBS and stored overnight at 4°C. Cells were permeabilized in 50% methanol (–20°C) at 4°C for 10 min, centrifuged and transferred to two tubes containing 2 × 10 ml PBS buffer with 0.5% BSA and 2 mM EDTA, centrifuged again and resuspended in 370 or 600 µl MACS buffer (PBS pH 7.2, 0.5% BSA, 2 mM EDTA).

For testing the capability of different endothelial markers to enrich fetal cells, MACS was performed using four different kinds

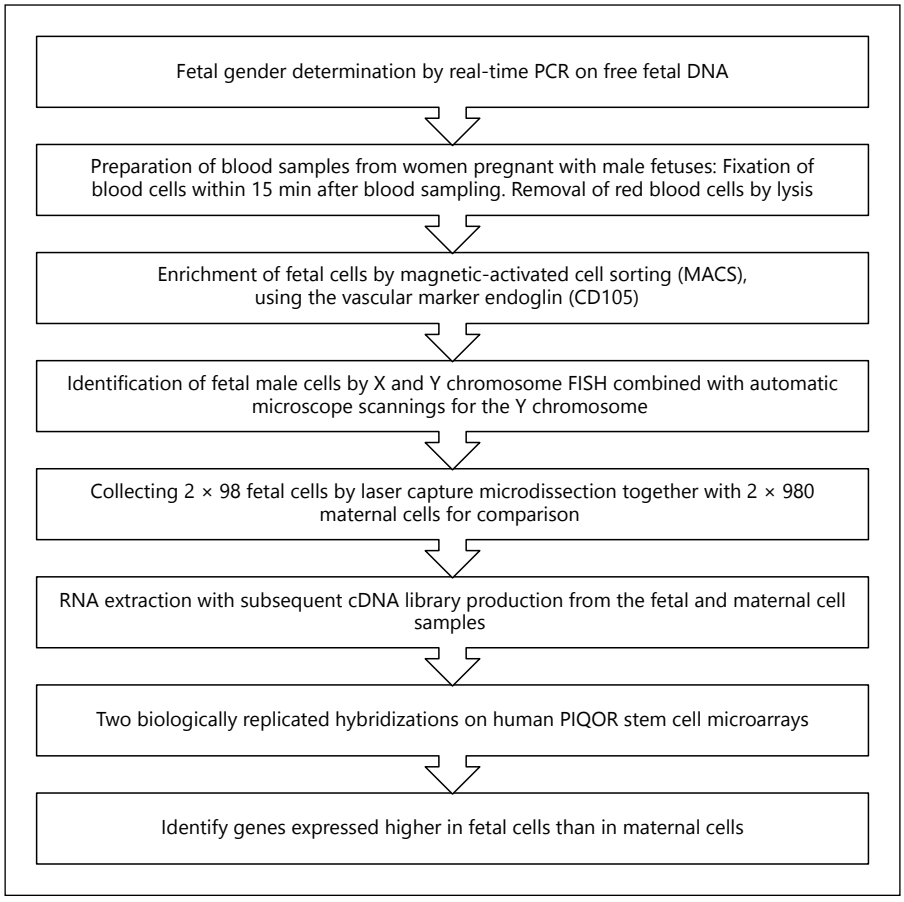

Fig. 1. Overview of the methodology. Workflow of the different steps performed in enrichment and analysis of fetal cells from maternal blood.

of microbeads: CD34, CD105, CD141 and CD146 (which are all known endothelial cell surface markers) according to the basic protocols (with LD columns) provided by the manufacturer (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) with slight modifications. For the experiment aiming at the identification of fetal cell markers, only microbeads labeled with antibodies against CD105 (endoglin) were subsequently used (see Results section). After the MACS procedure, the enriched cell samples were smeared on poly-L-lysine-coated microscope slides in 40-µl aliquots (approx. 50,000 cells/slide).

Identification of Male Fetal Cells by X and Y Chromosome FISH and Automated Scanning

Cells on slides were fixed, permeabilized and X and Y chromosome-specific FISH was performed as described [11] with omission of the rehybridization step. Automated microscope scanning for finding cells with a Y chromosome was also performed as described previously [11].

Generation of Complementary DNA Libraries

Laser Capture of Fetal Cells from Microscope Slides

Laser capture of fetal cells was performed as described previously [10] on a PALM MicroBeam system at Carl Zeiss MicroImaging GmbH, Munich, Germany. For each of the two replicates, 98 fetal cells were collected in the same cap. For each fetal cell collected, 10 surrounding maternal nucleated blood cells were collected in another tube giving a total of 980 maternal control cells. This resulted in four tubes: two with 98 fetal cells each and two with 980 matched maternal cells each.

SuperAmp™ RNA Amplification and Complementary DNA Microarrays

RNA extraction and SuperAmp™ RNA amplification, production and amplification of cDNA from RNA, as well as microarray experiments were performed as previously described [10]. In short, cDNAs from fetal and matched maternal control cells were labeled with Cy5-dCTP (fetal cDNA) and Cy3-dCTP (maternal cDNA) and hybridized in 1:1 mixtures to PIQOR™ stem cell microarrays as described previously [10].

In situ Immunohistochemistry on Placental Tissue Sections

Placental samples were collected, fixed in HOPE (Hepes-glutamic acid buffer mediated Organic solvent Protection Effect; DCS, Hamburg, Germany) and embedded in paraffin. Sections (5 µm thickness) were deparaffinized according to standard procedures and incubated in PBS including 0.05% Tween (Merck) for 5 min. Non-specific background was blocked by incubation with Ultra V Block (Lab Vision/Thermo Fisher Scientific, USA) containing 10% human AB-Serum for 7 min. Primary antibodies (pan-cytokeratin (Sigma), Cytokeratin 7 (Dako), CD105 biotinyl-

ated (R&D Systems), and HLA-G(4H84)) were diluted 1:100 (alone or in combination) in Antibody Diluent (Dako, USA) and incubated for 45 min at RT. Secondary antibodies (rabbit anti-mouse IgG Alexa Fluor 488, Invitrogen; Streptavidin Alexa Fluor 555, Invitrogen) were diluted 1:200 (alone or in combination) in PBS and sections were incubated for 30 min at RT in the dark. Nuclei were stained with DAPI (diluted 1/2,000 in PBS; Invitrogen, Molecular Probes, Eugene, Oreg., USA) for 5 min. Finally, slides were washed with PBS, dried and mounted with ProLong Gold antifade reagent (Invitrogen). Fluorescence microscopy was performed using a Leica DM 6000B Microscope and an Olympus DP 72 Camera.

Antibody Staining of Fetal Cell-Enriched Fractions of Maternal Blood

All incubations on slides were performed under coverslips in a humidity chamber at RT. Microscope slides containing male fetal cells (X and Y chromosome-positive cells identified by FISH) were rinsed once with 4 × SSC/0.1% Tween 20 and incubated for 30 min with blocking buffer (4 × SSC, 0.5% blocking reagent (Boehringer), 1% BSA, 10% goat serum) followed by 30 min in 100 µl of a mixture of anti-cytokeratin 7 (Dako) and anti-pan-cytokeratin (Sigma) diluted 1:100 in blocking buffer. Slides were rinsed 3 × 5 min in 4 × SSC/0.1% Tween 20 and incubated for 30 min with 100 µl Alexa Fluor 488 rabbit anti-mouse IgG (Invitrogen) diluted 1:200 in blocking buffer. Slides were subsequently rinsed 3 × 5 min in 4 × SSC/0.1% Tween 20 and incubated for 30 min in 100 µl of Alexa Fluor 488 goat anti-rabbit IgG (Invitrogen) diluted 1:200 in blocking buffer. Finally, slides were rinsed 2 × 5 min in 4 × SSC/0.1% Tween 20, 5 min in 2 × SSC and mounted in Vectashield with 0.6 µg/ml DAPI (Vector Laboratories).

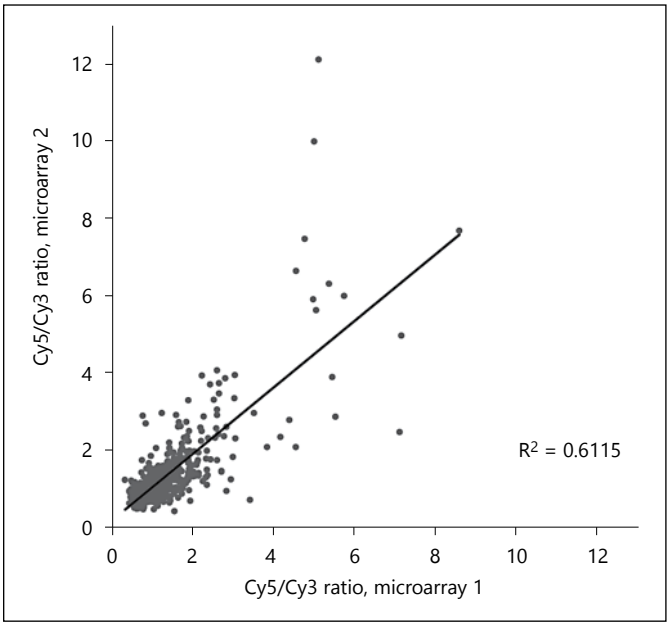

Fig. 2. Correlation of the two replicated PIQOR microarrays. Fetal/maternal (Cy5/Cy3) ratios of the two biological replicates plotted against each other. Genes that were blank in either array as well as two outliers were omitted. Gene ratios with no CV or a CV >60% in either array were omitted (666 genes are plotted). R^2 is the coefficient of correlation for the two datasets.

Results

Specific Enrichment of Fetal Cells Using Endothelial/Vascular Markers

Magnetic selections using the markers CD146, CD34, CD105 and CD141 were performed on 4–7 blood samples from women pregnant with male fetuses. CD105 antibody resulted in the highest number of fetal cells (Y chromosome-positive cells), with an average of 3.2 fetal cells per 10 ml of maternal blood, whereas the other markers retrieved only 0.36 (CD34), 0.56 (CD141) and 0 (CD146) fetal cells per 10 ml of maternal blood (details are given in online suppl. table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000356073). Consequently, CD105 antibody was chosen for subsequent enrichments.

Analysis of Expression Libraries from CD105-Positive Fetal Cells in Maternal Blood

From 34 maternal blood samples enriched by MACS with antibody against CD105, 196 cells of fetal origin were identified by X and Y chromosome-specific FISH. These fetal cells were collected in two replicates of 98 fetal cells together with a 10-fold excess of surrounding maternal nucleated blood cells for comparison. The RNA profiles of these cells were analyzed by means of microarray analysis on PIQOR stem cell arrays containing 930 oligos. Each oligonucleotide target was present in quadruples and the mean of the four values was used in subsequent analyses after exclusion of oligonucleotide targets where the coefficient of variation (CV) of the quadruples was >60%. From mean values fetal/maternal ratios were calculated for all sites. An XY plot of fetal/maternal ratios of all sites with values on both replicates showed a high degree of correlation ($R^2 = 0.61$) (fig. 2), indicating a reasonable quality of the array data.

In the following analyses we defined genes preferentially expressed in fetal cells as the 10% with the highest fetal/maternal ratio within the array dataset. This corresponded to genes which were expressed 2.22-fold or higher in fetal cells compared to maternal cells for the first biological replicate (microarray 1) and 1.96-fold or higher in the second replicate (microarray 2). By using this 10% cut-off value, microarray 1 gave 75 genes overexpressed in fetal cells and microarray 2 gave 78 genes overexpressed in fetal cells. Out of these, 39 genes were found

Table 1. List of genes overexpressed in fetal cells from maternal blood

Gene	Cy5 to Cy3 ratio average, array 1	Cy5 to Cy3 ratio average, array 2	Gene	Cy5 to Cy3 ratio average, array 1	Cy5 to Cy3 ratio average, array 2
CD33	8.57	7.69	FIGD	3.03	3.36
CTNNB1	7.14	4.98	PUM2	2.83	2.62
SOX13	7.10	2.49	TNC	2.80	3.88
OLIG2	5.73	6.01	CRIP1	2.77	2.38
COL1A2	5.44	3.91	HMGA1	2.65	3.75
DLX5	5.36	6.32	GATA5	2.65	3.48
PBLD	5.10	12.12	MMP9	2.63	2.43
HAMP	5.04	5.64	MCAM	2.60	3.07
SMC3	4.99	10.00	SMAD3	2.60	4.08
ACTB	4.97	5.92	EN1	2.60	2.93
CRABP1	4.76	7.48	JUNB	2.58	2.58
CDC42_1	4.55	6.65	TCF3	2.55	2.34
IL7R	4.54	2.10	F11R	2.52	3.32
CD9	4.38	2.80	ATF4	2.43	3.72
PF4	4.16	2.36	SELPLG	2.38	2.33
CRYL1	4.04	2.88	CD68	2.35	2.00
NCAM1	3.83	2.10	KCNQ4	2.27	2.89
SELE	3.51	2.98	FOXA2	2.23	3.95
PDGFC	3.05	2.32	CLDN6	2.22	2.51
JUN	3.04	3.96			

The 39 common genes with the 10% highest fetal/maternal ratio (Cy5/Cy3 ratio, average value) in both microarray 1 and 2, listed in descending order with respect to ratio average of microarray 1.

overexpressed in both arrays. Table 1 lists the names of the 39 genes with information on the gene expression ratios found in the two microarrays.

At this point a literature search was performed on the 39 fetally overexpressed genes to determine their general protein functions as well as their potential role in placenta or endothelium (online suppl. table 2). Information about the function of the proteins encoded by the 39 genes overexpressed in fetal cells was found in Entrez and/or UniProtKB/Swiss-Prot via the homepage http://www.genecards.org/. The search revealed that 22 of the 39 genes were expressed in placenta and of these 22 genes, 13 were expressed in EVTs.

Immunohistochemical Investigations

Two immunohistochemical studies were performed. In the first of these studies, staining of first-trimester placental tissue sections revealed that EVTs (identified by positive staining for cytokeratin and HLA-G) do express the mesodermal marker CD105 on their surface (fig. 3a–d). In the second immunohistochemical study, cytokeratin staining using a pan-cytokeratin antibody was performed on 21 male fetal cells enriched from maternal blood samples by MACS using CD105 antibody and verified as male cells by FISH. 16 out of 21 (76%) fetal cells stained positive with the pan-cytokeratin antibody whereas no maternal cells stained positive for cytokeratin. A typical example of cytokeratin staining of a fetal cell is shown in figure 3e.

Discussion

In the present study, we firstly demonstrated that the vascular marker CD105 can be used for enriching fetal cells from the maternal circulation, and that the majority of fetal cells enriched in this way are cytokeratin-positive. CD105 is a transmembrane glucoprotein of mesodermal origin located on the surface of vascular endothelial cells and is a very well established vascular marker. For this reason, CD105 are expressed in most organs but the highest expression is found in placenta, which is not surprising considering the extremely high degree of vascularization in this organ.

It was somewhat to our surprise that CD146 (MCAM), which we found to be overexpressed in fetal cells in ma-

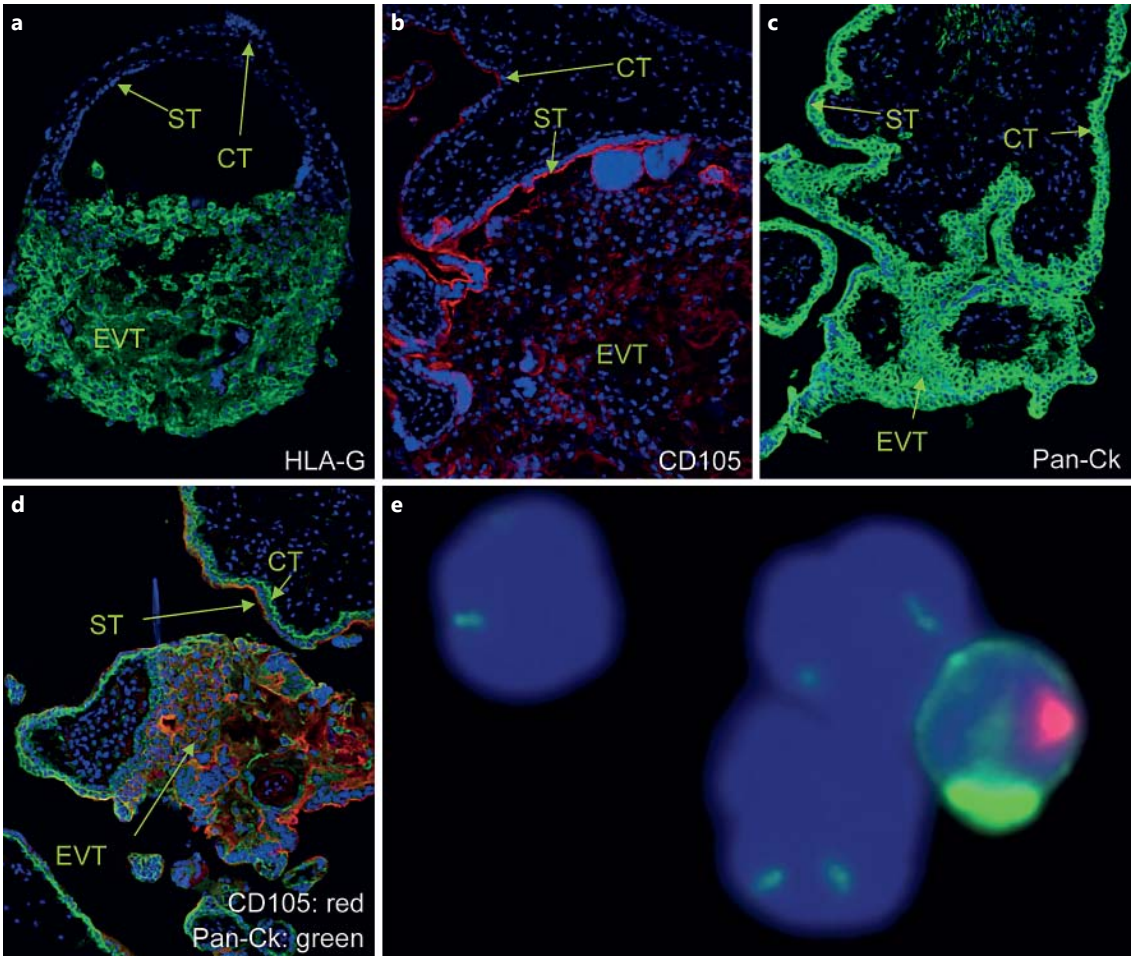

Fig. 3. Immunohistochemistry on villous sections from placental biopsies and fetal cell from maternal blood at 10–12 weeks' gestational age. HLA-G is used as a marker of EVTs. Nuclei are stained with DAPI. ST = Syncytiotrophoblast; CT = villous cytotrophoblast; EVT = extravillous trophoblast. **a** HLA-G staining of EVT. **b** CD105 staining of EVT and ST. **c** Pan-cytokeratin (Pan-Ck) staining of EVT, CT and ST. **d** CD105 and pan-cytokeratin staining colocalization on EVT and ST. **e** Image of a cytokeratin-stained fetal cell together with three maternal cells. The cells had undergone FISH with green probe for the X chromosomes and red probe for the Y chromosome followed by staining with a pan-cytokeratin antibody in green. The fetal cell with a red FISH signal for the Y chromosome exhibits green cytoplasmic cytokeratin stain. Nuclei are stained blue with DAPI.

ternal blood in our previous study, did not significantly enrich fetal cells in our enrichment series. This could suggest that we should be careful in directly extrapolating from mRNA/cDNA data to protein levels.

Our finding that the fetal cells in maternal blood also express cytokeratin is striking since expression of markers of different germ layers (here ectoderm and mesoderm) in the same cell is unusual and in generally only seen in connection with epithelial-mesodermal transition which is a rare phenomenon under normal conditions. However, one normal condition where epithelial-mesodermal transition takes place is during placentation where highly invasive EVTs of placental (ectodermal) origin are invading the maternal spiral arteries displacing maternal endothelial cells, whereby the placenta can take control of the maternal blood supply to ensure proper growth of the fetus [reviewed in 12, 13]. Zhou et al. [14] have shown that during this process EVTs change their surface proteins to adopt a vascular (mesodermal) phenotype. But the epithelial-mesenchymal transition is not complete, since EVTs still maintain expression of cytokeratin [14]. This is in agreement with our finding (fig. 3a–d) that EVTs in placenta sections stain positive for both CD105 and cytokeratins. These EVTs are in very close contact with the

maternal circulation [13, 14] and therefore, from an anatomical point of view, it seems likely that a part of the fetal cells present in maternal blood around the beginning of the second trimester are EVTs, exhibiting expression of markers with invasive capability, for endothelial cell-to-cell adhesion and at the same time retaining epithelial markers.

Positive staining for both CD105 and pan-cytokeratin, during pregnancy is however also seen in the multinucleated syncytiotrophoblast layer (fig. 3), which raises the question if some of the circulating cells are fragments of syncytiotrophoblasts (syncytical knots) that are caught and identified by the CD105/pan-cytokeratin combination. During normal pregnancy, multinucleated syncytial knots are shed from the syncytiotrophoblast but they normally do not reach the peripheral blood system because they are removed in the lungs [15, 16]. However, on rare occasions, we have observed clumps containing many condensed nuclei which we presume are syncytial knots, but these structures are clearly different from the mononucleated fetal cells that were used for the microarray analysis in the present study.

In the microarray analyses, we – like in our previous study [10] – used the PIQOR stem cell arrays, on the expectations that the fetal cells would have stem cell properties. This assumption is partially based upon our previously identified fetal cell markers but also on several reports of fetal cell microchimerism, where fetal cells have been found in different maternal tissues, adapting different tissue-specific markers, including endothelial cell markers [17–19].

We chose to perform two biological replicates of the expression profiles which enabled us to document the reproducibility of the method. Based on the replicates, we conclude that the expression data obtained are reliable in spite of the fact that the fetal cells have gone through a long analytical procedure. This conclusion is based on the data shown in figure 1, illustrating both qualitative and quantitative similarities among the two biological replicates especially with regard to the fraction of mRNAs that gave strong signals and were found to be differentially expressed (for further data quality assessment, see online suppl. fig. 1, 2). These mRNAs are most likely among the more stable mRNAs and other fetally overexpressed genes could very well remain undetected due to more pronounced mRNA degradation.

Among the genes overexpressed in fetal cells, we found several genes which code for proteins known to be involved in the maintenance of the endothelium. Thus, *F11R, CLDN6, MCAM* and *CTNNB1* play roles in tight junctions and endothelial cell to cell adhesion [20–24] while *MMP9, VEFG-D*, *ATF4, CD9, ILR7* and *TNC* are active proteins in angiogenesis [25–30]. Likewise, *JUN, JUNB, CDC42, MCAM, NCAM, TCF3, CD9, MMP9, HMGA1, CTNNB1* and *SELPG* are all proteins known to be important for trophoblast invasion and migration [31–45].

Altogether, our literature studies revealed that 22 of the 39 fetally overexpressed genes (56%) are expressed in placenta and 13 of these 22 genes are expressed in EVTs (online suppl. table 2).

The indication that the EVTs are circulating in maternal blood is somewhat surprising considering that several research groups have investigated if the fetal cells in maternal blood could be trophoblasts but without success [7, 46] and it has also previously been suggested that the circulating trophoblasts may be extravillous trophoblast cells [47]. However, in all these studies HLA-G was used as marker for enrichment of EVTs, which may be the reason for lack of success perhaps because the HLA-G epitope is not stable on circulating cells [46, 47].

CD105 has also previously been attempted as a marker for isolation of circulating fetal cells in maternal blood but also with limited success [48, 49]. However, these studies were conducted on unfixed Ficoll-Pague™ isolated mononuclear blood cells, a method we also have tried without success, most likely due to fetal cell fragility (data not shown). In the present communication we chose to prefix whole blood and only after this step started lysis of red blood cells and then proceeded to MACS on the remaining cell pellet. This quick fixation is most likely diminishing destruction of the fetal cells.

We therefore believe that a procedure based on enrichment of fetal cells by CD105 antibody followed by fetal cell identification using cytokeratin staining and finally FISH screening of the fetal cells for common chromosome aneuploidies can be developed into an alternative method for defining at risk pregnancies. There are, however, also outstanding problems, both biological and technical, before a clinical test can be introduced. The biological problems relate primarily to the number of fetal cells which is very low. We have partly overcome this problem by increasing the volume of blood drawn, but we believe that 25 ml of blood is the limit. Another possibility is to improve the selection efficiency either be improving the MACS system or by incorporating more antibodies. Here especially those of the markers described in the present communication that are of surface located and of mesodermal origin could be of interest. Also the fact that we target a placental cell type can be a problem since it has

been repeatedly shown that there can be a confined mosaicism in the placenta, giving false positive results [50]. To what extent this will be a problem has to be investigated in larger series. With regard to technical problems then, these also relate to the very low number of fetal cells which means that the enrichment procedure will be critical but our experience is that early fixation as described above can solve many of these problems. But as with other procedures involving very few cells, strict procedures to prevent contamination are mandatory.

Given the clear benefits of achieving prenatal chromosome screening without an invasive technique it is not surprising that also other ways of collecting fetal material without an invasive approach have been tried. In this respect the most promising alternative is the analysis of the free fetal DNA circulating in maternal blood. Opposite to fetal cells the free fetal DNA is, however, in most respects identical to the excess of free maternal DNA in blood. The only consistent difference between fetal and maternal DNA in blood is the pattern of CpG methylations in certain regions. Therefore, several strategies based on initial enrichment of fetal DNA based on methylation differences have been tried [51, 52] but with varying success. A much more efficient approach has been to measure relative amounts of e.g. chromosome 21 DNA sequences compared to DNA sequences from reference chromosomes without prior enrichment and then look for a small increase in chromosome 21 sequences in trisomic cases. Many groups and companies have focused on this approach using different ways of doing the DNA quantifications. At present, molecular counting based on next-generation sequencing (NGS) has shown by far the best results [53–56] with sensitivities and specificities sufficiently high to introduce this technique for risk screening for chromosomal aneuploidies. However, the drawback of an NGS approach is firstly that there is a fraction of women where the analysis fails and secondly that the NGS approach involves centralization of the analytical procedures which can pose logistic problems.

In conclusion, we have characterized a fraction of mononuclear fetal cells circulating in maternal blood in gestation weeks 11–14 and we believe that these are EVTs. They can be identified in maternal blood based on combinations of mesodermal and ectodermal markers, a combination that is not generally expected to be seen in maternal blood cells. This combination may therefore constitute a unique marker set that can achieve sufficient fetal cell enrichment and identification to form the basis for a non-invasive prenatal diagnostic procedure for genetic analysis. Future studies have to demonstrate in a larger, blinded clinical setting if these markers can give rise to enough fetal cells and thereby provide the basis for determination of the chromosomal status of the fetus.

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Disclosure Statement

The experiments described in this article were performed in and funded by the private company FCMB ApS, in which the authors M.B., R.S., L.H., K.M. and B.C. were employed. S.K. and B.C. are founders of this company. The ultimate goal of FCMB ApS was to develop a new method for non-invasive prenatal diagnostics based on isolating fetal cells from the maternal circulation. The authors M.B., R.S., L.H., B.C. and S.K. have filed patent applications on the isolation and identification of fetal cells in maternal blood for non-invasive prenatal diagnostics.

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