ARTICLE IN PRESS

Placenta xxx (2015) 1–5

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Cervical trophoblasts for non-invasive single-cell genotyping and prenatal diagnosis

I. Pfeifer [a, 1], A. Benachi [b, 1], A. Saker [a], J.P. Bonnefont [c], H. Mouawia [a], L. Broncy [a], R. Frydman [d], M.L. Brival [e], B. Lacour [f], R. Dachez [g], P. Paterlini-Bréchot [a, f, *]

[a] INSERM Unit 1151 – Team 13, Paris Descartes University, Paris, France
[b] Department of Gyncology, Antoine Béclère Hospital, Clamart, France
[c] Laboratory of Medical Genetics, Necker-Enfants Malades Hospital, Paris, France
[d] Department of Reproduction, Foch Hospital, Suresnes, France
[e] Department of Maternity, Les Lilas, France
[f] Laboratoiry of Biochemistry A, Necker-Enfants Malades Hospital, Paris, France
[g] Alfred Fournier Institute, Paris, France

ARTICLE INFO

Article history:
Received 15 May 2015
Received in revised form
3 November 2015
Accepted 5 November 2015

Keywords:
ISET®
Trophoblasts
Non-invasive prenatal diagnosis
Cystic fibrosis
Spinal muscular atrophy

ABSTRACT

Objective: We aimed at developing a method to recover trophoblastic cells from the cervix through a completely non-invasive approach and obtaining a genetic proof of their fetal nature implying that they can be used for non-invasive prenatal diagnosis (NIPD).
Methods: We studied obstetrical samples from 21 pregnant women between 8 and 12 weeks of gestation scheduled for chorionic villus sampling or undergoing elective termination of pregnancy. A cytobrush was used to extract cells from the external parts of the cervix and transferred to 10 ml of preservative solution. Cells were layered on filters with 8 microns pores using the ISET system (Isolation by SizE of Tumor/Trophoblastic cells) and stained. Putative fetal cells were collected by single cell laser-assisted microdissection and identified as fetal or maternal cells by Short Tandem Repeat genotyping. NIPD was blindly performed on 6 mothers at risk of having a fetus with Cystic Fibrosis or Spinal Muscular Atrophy.
Results: Trophoblastic cells were recovered from all tested cervical samples with a frequency of 2–12 trophoblasts per 2 ml. NIPD was blindly obtained and verified in 6 mothers at risk of having a fetus with Cystic Fibrosis or Spinal Muscular Atrophy.
Discussion: Although larger confirmation studies are required, this is the first report providing a solid proof of principle that trophoblasts can be consistently and safely recovered from cervical samples. Since they are a source of pure fetal DNA, i.e. fetal DNA not mixed with maternal DNA, they constitute an ideal target to develop NIPD of recessive diseases, which is a technical challenge for methods based on cell free DNA.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

In order to avoid the risk of miscarriage linked to amniocentesis and chorionic villus sampling (CVS) [1], fetal DNA can be, in principle, retrieved non-invasively from three sources: circulating fetal cells in maternal blood [2], transcervical trophoblastic cells [3] and cell-free fetal DNA in maternal blood [4]. The analysis of cell-free fetal DNA has allowed developing reliable non-invasive tests for prenatal detection of aneuploidies [4]. However, the use of cell-free fetal DNA, which is mixed with maternal cell-free DNA in variable proportions, for non-invasive prenatal diagnosis (NIPD) of single-gene disorders and recessive diseases is particularly challenging [5]. In this setting, targeting genetic tests to the pure fetal DNA contained in fetal cells remains an attractive aim.

The presence of fetal cells in the endocervix was first demonstrated by Shettles in 1971 [6]. However, until now the rarity of

* Corresponding author. Unité Inserm 1151 – équipe 13, Université Paris Descartes, Bâtiment Leriche, Porte 14, rue Maria Helena Vieira Da Silva, 75014 Paris, France.patriziapaterlini@gmail.com
E-mail address: patriziapaterlini@gmail.com (P. Paterlini-Bréchot).
[1] Authors I. Pfeifer and A. Benachi contributed equally to this work.

http://dx.doi.org/10.1016/j.placenta.2015.11.002
0143-4004/© 2015 Elsevier Ltd. All rights reserved.

Please cite this article in press as: I. Pfeifer, et al., Cervical trophoblasts for non-invasive single-cell genotyping and prenatal diagnosis, Placenta (2015), http://dx.doi.org/10.1016/j.placenta.2015.11.002

ARTICLE IN PRESS

these cells and the difficulty to collect them through a completely non-invasive approach has prevented their implementation for NIPD. Different methods, all collecting fetal cells from the inner part of the cervix and/or the lower pole of the uterine cavity, called transcervical cells (TCC) sampling, were developed, including: intrauterine lavage, endocervical lavage, endocervical mucus aspiration as well as endocervical sampling by a cytobrush [7–18]. Studies have established that uterine and endocervical lavage are the most effective methods to yield fetal cells as early as 5 weeks of gestation [3,7–11]. All these methods however present one major risk: fetal loss [3,19,20]. On the one hand, samples were collected immediately prior to termination of pregnancy in the majority of studies, hence their safety has not been sufficiently examined. On the other hand, establishing the sampling method's safety on a large casistic is difficult for evident reasons.

These observations prompted us to test another approach. We reasoned that the Papanicolaou (PAP) test is currently performed on pregnant women during the first trimester of pregnancy [21], and that its safety has been extensively demonstrated throughout the world. We have thus tested if this sampling approach could consistently detect trophoblastic cells. Interestingly, no study using the PAP-test sampling method to collect cervical trophoblasts had been previously reported.

We also combined this method with our genetic approach to reliably identify trophoblastic cells in blood [22] and avoided the use of antibodies which could lower the sensitivity of trophoblasts detection.

In this study, we aimed at developing a completely non-invasive method to recover trophoblastic cells from the cervix and obtaining a genetic proof of their fetal nature implying that they can be used for non-invasive prenatal diagnosis (NIPD).

2. Materials and methods

We have tested 21 pregnant women (between 8 and 12 weeks of gestation, including 6 (Necker-Enfants Malades Hospital Paris, France), tested immediately before CVS, being at risk for having a baby affected by Cystic Fibrosis or Spinal Muscular Atrophy, and 15 women tested before elective termination of pregnancy (Antoine Béclère, Clamart Hospital, France; Maternity “des Lilas”, Les Lilas, France).

Cells were obtained with the use of a cytobrush, but unlike the reported transcervical cells sampling methods [3], the brush was not inserted into the endocervical canal but rather rotated at the external os, as done during a routine PAP test. Cytobrushes were transferred to 10 ml of a specific preservative solution (Cytofix, Rarecells® Diagnostics, Paris, France). We also obtained 1 ml of blood (collected on EDTA) from each woman and from the father for genomic DNA extraction and testing.

ISET was carried out as previously described [2,22–25] with only minor modifications. In order to layer the cells and eliminate the liquid, 1 ml of each Cytofix sample was diluted 50 fold in bi-distilled, sterile water and subsequently filtered through the Rarecells® Device using a Rarecells® consumable containing an 8 microns pores filter (Rarecells® Diagnostics, Paris, France; www.rarecells.com). The filter was then stained with a 0.1% nuclear fast red stain/5% aluminum sulphate solution (Sigma–Aldrich, St. Louis, MO, USA), incubated for 2 min and then thoroughly rinsed with water. Filters were dried on air.

We became aware of the morphology of cytotrophoblasts by microdissecting putative cytotrophoblasts and analyzing them by short tandem repeat (STR) genotyping. Single cells displaying a cytotrophoblast-like or syncytiotrophoblast-like morphology were retrieved directly from the ISET filters by laser-capture microdissection using the Nikon TE 2000-U (Nikon Paris, France and MMI Zurich, Switzerland) laser-equipped microscope. Each single cell was lifted from the filter and transferred onto the lid of a microfuge tube suited for PCR.

Each microdissected cell was lysed in 15 μL of lysis buffer (100 mmol/L Tris–HCl, pH 8; 400 μg/mL proteinase K) for 2 h at 60 °C, followed by proteinase K inactivation at 94 °C for 15 min. For primer extension preamplification (PEP) [26], to the lysed cell we added 5 μL of a 400 μM solution of random primers (Kit genPEP 75 OD, Genetix, Boston, USA), 6 μL of PCR buffer (25 mM MgCl2/gelatin (1 mg/mL), 100 mM Tris–HCl, pH 8.3, 500 mM KCl), 3 μL of a mixture of four dNTPs (each at 2 mM) and 1 μL (5 U) of Taq polymerase (Applied Biosystem, Foster City, CA, USA) in a final volume of 60 μL. Single-cell genotyping was performed to identify cells having a fetal genome by using STR primers found to be informative through the analysis of paternal and maternal genomic DNA. For genotyping we used 10 different sets of STR genotyping primers from 10 selected STR regions shown in Table 1. Amplification was performed in 60 μL containing 6 μL of the PEP product, 10 mM Tris–HCl, 50 mM KCl, 2.5 mM MgCl2, 200 μM of each deoxynucleotide, 0.5 μM of each STR ‘outer’ primer and 2 U of Taq Gold (Applied Biosystems, Foster City, CA, USA). 2 μl of a 1:10 diluted PCR outer product were re-amplified in a nested PCR in 20 μL final volume using ‘inner’ fluoresceinated STR primers and the same PCR protocol. One μL of the 1:20 diluted inner PCR product (amplicon) was then mixed with 13.5 μL of deionised Hi-Di formamide and 0.5 μL of Genescan 400 HD (ROX) marker (Applied Biosystems) and loaded into an ABI Prism 3100 automated sequencer (Applied Biosystems). Profiles were analyzed using the Genescan and Genotyper software programs (Applied Biosystems).

The NIPD of CF and SMA was performed blindly and carried out as described in other studies [2,24,25].

Invasive diagnoses were carried out at Hôpital Necker-Enfants Malades, Laboratoire de Génétique Médicale, Paris, France.

3. Results

We screened a total of 21 cervical samples from pregnant women between 8 and 12 weeks of gestation, including 6 tested before CVS, at risk for having a baby affected by Cystic Fibrosis or Spinal Muscular Atrophy, and 15 undergoing elective TOP. In all cases cervical samples were obtained by cytobrush and retrieving cells exclusively at the level of the external os, as in the completely safe PAP test. As shown in Fig. 1B, an exocervical squamous epithelial cell (marked with an arrow) is easily morphologically recognized in microscopic images. We were looking for cells displaying a cytotrophoblast-like morphology: round cells with large, irregular hyperchromatic nuclei (Fig. 1B). However, some rare maternal endocervial cells and fetal cytotrophoblasts may have a similar morphology and are therefore much harder to differentiate morphologically (see Fig. 1A and B) without genetic tests. Fetal genotypes were all verified by fluorescent PCR analysis of informative STR markers (Fig. 1C, Table 1). Syncytiotrophoblasts were very rarely found (Table 1). They have dense nuclei and are multinucleated (Fig. 2).

We identified fetal cells (either cytotrophoblasts or cytotrophoblasts and syncytiotrophoblasts) in all 21 samples, with a frequency of 2–12 fetal cells per 2 ml of sample (Table 1). We found approximately 1 cytotrophoblast every two microdissected cells (Table 1).

In order to show that our previously published protocols for NIPD of Cystic Fibrosis and Spinal Muscular Atrophy can be successfully applied to fetal cells isolated from the cervix, NIPD was blindly performed in six cases of pregnant women at risk for having a baby affected by Cystic Fibrosis or Spinal Muscular Atrophy (Table 1). NIPD of Cystic Fibrosis was based on the presence or

Please cite this article in press as: I. Pfeifer, et al., Cervical trophoblasts for non-invasive single-cell genotyping and prenatal diagnosis, Placenta (2015), http://dx.doi.org/10.1016/j.placenta.2015.11.002

Download English Version:

https://daneshyari.com/en/article/5894353

Download Persian Version:

https://daneshyari.com/article/5894353

Daneshyari.com