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Quo vadis, trophoblast? Exploring the new ways of an old cell lineage

Sascha Drewlo, Ph.D [a, *], D. Randall Armant [a, b]

[a] Department of Obstetrics and Gynecology, Wayne State University, School of Medicine, Detroit, MI 48201, USA
[b] Department of Anatomy and Cell Biology, Wayne State University, School of Medicine, Detroit, MI 48201, USA

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ABSTRACT

Trophoblast cells are the first embryonic lineage to differentiate during human development, and are needed to sustain fetal life through their role in constructing a placenta. As the fetus grows, the trophoblast rapidly expands and further differentiates to produce an extravillous subtype that invades the maternal tissues. Some of the extravillous trophoblast cells find their way into the reproductive tract, and can be safely captured by noninvasive collection from the endocervical canal, similarly to a Pap smear. We are developing a new technology for investigating trophoblast cells residing in the cervix to better understand their development, and to glean information from them about pregnancy status. Trophoblast retrieval and isolation from the cervix (TRIC) efficiently isolates hundreds of trophoblast cells without limitations due to early gestational age, maternal obesity, or uteroplacental insufficiency disorders. Cells that appear to be extravillous trophoblast, based on their molecular phenotype, can be purified from Pap smears obtained between 5 and 20 weeks of gestation, using magnetic nanoparticles coupled to an antibody recognizing HLA-G that they specifically produce. Information about fetal genotype and adverse pregnancy outcomes has been obtained using TRIC, and could one day provide assessment of maternal and fetal risk of disease. As perinatal interventions for placental disorders and inherited congenital disorders emerge, TRIC could provide a key diagnostic tool for personalize precision pregnancy management.

1. Introduction

Placental cells are found primarily near the conceptus, residing either in the placenta itself or the maternal uterine wall. A small number of trophoblast cells are displaced from the conceptus during pregnancy by mechanisms not yet fully understood. Some extravillous trophoblast (EVT) cells enter the uterine cavity, and eventually accumulate in the cervix where they can be safely collected by a simple pap smear. We introduced "TRIC," (trophoblast retrieval and isolation from the cervix), a method to isolate trophoblast like cells expressing human leukocyte antigen G (HLA-G) that reside in endocervical specimens [1]. The presence of trophoblast cells in the reproductive tract has been known for many years [2], but a lack of means to provide these cells in numbers and purity sufficient for unambiguous analysis hindered their use in clinical testing and biological investigation. TRIC provides trophoblast like cells with an EVT-like phenotype in numbers suitable for most molecular analyses [1]. Here, we discuss our investigation of applications that are possible using EVT-like cells residing in the cervix. They have the potential for diagnosing fetal genotype and risk for pregnancy complications, as early as 3 weeks post-conception. Furthermore, we suggest how assessment of placental health by TRIC during ongoing pregnancies will facilitate testing and development of proposed interventions to limit or prevent pregnancy complications. With further exploration, TRIC could have a place in the coming age of personalized and precision pregnancy management.

2. Trophoblast subtypes and origins

Trophoblast cells are a key component of the placenta that include two major lineages, villous and extravillous, with distinct functional roles to ensure a successful pregnancy [3]. The villous trophoblast (VT) lineage generates an elaborate cellular network

Abbreviations: (TRIC), Trophoblast retrieval and isolation from the cervix; (PE), Preeclampsia; (HLA-G), human leukocyte antigen G; (EVT), Some extravillous trophoblast; (VT), villous trophoblast; (STR), small tandem repeat; (SNV), single nucleotide variant; (PPARG), peroxisome proliferator-activated receptor gamma.

* Corresponding author. C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, 275 E. Hancock Avenue Detroit, MI 48201-1415, USA.
E-mail address: sascha.drewlo@wayne.edu (S. Drewlo).

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that creates the outer layer of the villous tree, a structure exposed to maternal blood for exchange of gases, nutrients and waste with the growing fetus [4]. As gestation progresses, the syncytiotrophoblast layer sheds apoptotic nuclei [5] into the maternal circulation and releases exosomes that communicate their cargo [6], which includes microribonucleic acids targeting several maternal organ systems. EVT cells disperse from the placenta at the anchoring villi to invade the maternal decidua; thus, producing an interface between the placenta and the uterine wall, as well as ensuring maternal blood flow into the intervillous space. As EVT cells migrate from the implantation site, they further differentiate into what appear to be functionally distinct subtypes that can be identified by both their location and marker expression [4]. We do not fully understand how many EVT subtypes exist within the basal plate and placental bed in addition to the columnar (proximal and distal), interstitial, and endovascular. The endovascular EVT cells are thought to facilitate spiral artery remodeling and immune modulation at the fetal-maternal interface. Histological features of placentas delivered by women with disease related to insufficient perfusion of the placenta suggest a prior disruption of EVT function in the first trimester [7], characterized by reduced trophoblast invasion, insufficient remodeling of the spiral arteries, and EVT cell death [8–11].

Although the presence of trophoblast in the reproductive tract and cervix was first recognized 45 years ago [12], their exact origin and phenotypic diversity is understudied. One suggested source of trophoblast cells in the reproductive tract is that EVT cells migrating radially from the embryo early in pregnancy could break through the capsularis [13]. An alternative mechanism is now emerging, based on the proposal that secretions from the endometrial glands support placental growth and development in the first ten weeks of pregnancy before influx of the maternal circulation [14]. Evidence demonstrates that EVT cells actively invade into uterine glands so they communicate directly with the intervillous space [15,16]. We can speculate that EVT cells entering glands at the margins of the placenta are carried by glandular secretions into the uterine cavity and flow to the cervical canal. Endoglandular trophoblast might provide a constant pool of placental cells moving through the cervical canal during the first half of gestation, the period when they can be successfully obtained by TRIC [17]. Further investigation is needed to test this hypothesis and determine whether the endoglandular trophoblast can be distinguished by their molecular profile from the other EVT subtypes.

Until recently, the biology of cervical trophoblast cells was largely unknown due to the inability to purify them from the cervix in numbers and purity sufficient for detailed evaluation [2]. TRIC provides 100–1000 EVT-like trophoblast cells with an average purity of >90% (maternal cells are present in excess), and the amount of cells obtained is unaffected by body mass index or gestational age between 5 and 20 weeks of gestation [17]. Single cell molecular analysis of cells isolated by TRIC from pregnancies bearing a male fetus confirms the purity of isolated trophoblast like cells [1], with only an occasional female (i.e., maternal) cell detected (278 of 280 cells from 6 patients were XY by FISH; 89–100% of cells from 9 patients were positive for the SRY gene by single cell PCR). The same study revealed that trophoblast like cell obtained by TRIC from the cervix have properties expected of differentiated EVT cells residing in the decidua that produce HLA-G, α1 integrin subunits, PECAM1, VE-cadherin, and MMP9. Moreover, their protein expression pattern differs from VT that express α6 integrin subunits, E-cadherin, and PSG1. Although hundreds of these trophoblast like cells can now be obtained, it remains unclear 1) whether they are a truly homogenous population, 2) if there are differences based on gestational age, and 3) if the endocervical environment alters their molecular expression profile. Interestingly, the isolated trophoblast like cells are alive based on a lack of reactivity with TUNEL [1], and their ability when isolated in culture medium to invade Matrigel (unpublished observation). While there is much to learn about these EVT-like cells, they are promising candidates for prenatal clinical applications.

3. Prenatal genetic testing at five weeks of gestation

We have investigated whether trophoblast like cells isolated by TRIC from Pap specimens could serve as a barometer for fetal health, placental function, and pregnancy outcome, beginning with an examination of their utility for interrogating the fetal genome. To determine whether the genotype of trophoblast cells from the endocervical canal matches that of the fetus, we examined 20 consecutive specimens collected between 5 and 19 weeks of gestation to compare maternal and trophoblast like (HLA-G positive) cells separated by TRIC to the placental genome [18]. We applied a targeted next-generation sequencing approach (ForenSeq, Illumina) that uses small tandem repeat (STR) and single nucleotide variant (SNV) sequences with high heterozygosity to distinguish individuals, similar to approaches used routinely in forensics. In total, 94 SNVs and 59 STRs located across the whole genome were investigated. The sample population had an average gestational age of 8.3 weeks, containing 9 male and 11 female fetuses. Trophoblast like cell purity, determined by immunofluorescence labeling of β-hCG, was on average 89.2%, and correlated well with the fetal DNA fraction of 92.2% calculated from the sequencing data [18]. The genotypes of cervical trophoblast like cells and placenta cells matched 100%, and were distinct from, but related to, the maternal cell genotypes. The success of fetal genotyping with DNA obtained by TRIC introduces a new approach for prenatal genetic testing that can be performed earlier than any of the invasive method, such as amniocentesis and chorionic villous sampling (Fig. 1). TRIC is noninvasive, and provides nucleotide-specific resolution not available from cell-free fetal DNA screening with maternal plasma [19]. TRIC technology now needs to be tested in the clinical setting to demonstrate its reliability for identifying genomic abnormalities, while considering its limitations, which include the challenges of multiple gestations and confined placental mosaicism.

4. A window into the health of the pregnancy

Because EVT-like cells obtained by TRIC reside outside the placenta and in a different tissue environment, there is the prevailing question whether the cells could provide a window into the ongoing pregnancy. The development of technologies to assess risk of pregnancy complications at the beginning of gestation could help clinicians to more effectively anticipate and manage adverse pregnancy outcomes. It can be argued that, due to their location, EVT cells residing in the cervix cannot be expected to reflect placental pathophysiology or pregnancy status. This implies that the maternal environment proximal to the placenta is required to maintain the physiological state of the EVT cells with respect to pregnancy status. The reason for abnormal EVT invasion is not well understood, but an intrinsic defect of trophoblast function could contribute due to abnormal gene expression, which likely is expressly true for severe early onset disease.

We examined whether HLA-G positive trophoblast cells isolated by TRIC are a proxy for placental function, based on the contribution of altered EVT differentiation to placental insufficiency syndromes. In two pilot studies [20,21], we showed that protein signatures of EVT cells in endocervical specimens from women who later developed an early pregnancy loss or clinical symptoms of preeclampsia or fetal growth restriction differed from healthy,

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