Placenta xxx (2017) 1–8

Contents lists available at ScienceDirect

Placenta

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

Implantation and extravillous trophoblast invasion: From rare archival specimens to modern biobanking

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ARTICLE INFO

Article history:
Received 30 November 2016
Received in revised form
1 February 2017
Accepted 7 February 2017

Keywords:
Placenta
Human implantation
Extravillous trophoblast
Uterine gland
Uterine vein
Endoglandular trophoblast
Biobanking

ABSTRACT

Extravillous trophoblast invasion serves to attach the placenta to the uterus and to enable access to nutrients for the embryo throughout pregnancy – secretions of the uterine glands in the first trimester, maternal blood in the second and third trimester. For assessing extravillous trophoblast invasion, histology (in combination with immunohistochemistry) still plays a major role in placental research. This is especially true for the re-assessment of rare archival specimens from early human implantation sites or placenta *in utero* with the background of recent knowledge which may help to strengthen current hypotheses. This review summarizes the recently expanded picture of extravillous trophoblast invasion, gives an overview about fundamental archival specimens in placental research, presents new images of archival specimens, gives insights into the latest developments in the field of biobanking and provides insight into the current situation on sample usage in the absence of biobanks. Modern techniques allow expanding our hitherto believed concept of extravillous trophoblast invasion, which is not restricted to spiral arteries: Extravillous trophoblasts also invade into uterine glands and uterine veins and thereby connect all these luminal structures with the intervillous space. All biomedical research dramatically depends on the quality of the assessed biological samples. Hence, researchers should be aware that the time between collection of a sample from a body and the beginning of analysis (pre-analytical phase) may have more impact on the outcome of a study than previously assumed.

1. Introduction

Although there is a huge variety of highly sophisticated omics technologies available today, one can still learn a lot from histology and morphological assessment of tissues and organs. In placental research, histology in combination with immunohistochemistry and electron microscopy still plays a major role in identifying new players in the interaction between mother and fetus. Especially, histology of rare archival specimens from early human implantation sites or placenta *in utero* can be of great value.

At the same time, science has evolved and has led to the development of structured and systematic collections of samples to be used in research, biobanks. Today, biobanks have taken over a major role in biomedical research, offering the hope to enable higher sample quality, better linkage to clinical data and less garbage in – garbage out when analyzing human-derived samples.

Looking at placental development, extravillous trophoblasts originate from the distal side of trophoblast cell columns derived from cytotrophoblasts of anchoring villi. Within these cell columns proliferative but non-invasive cytotrophoblasts switch to an invasive but non-proliferative extravillous phenotype and start to enter maternal tissues (interstitial trophoblast) [1]. From a histological point of view one of the most important characteristics of extravillous trophoblasts is their expression of major histocompatibility complex, class I, G (HLA-G) [2–4]. Only HLA-G is a specific marker for extravillous trophoblasts, rather than the still commonly used cytokeratin 7. The latter also reacts with maternal glandular epithelial cells [5]; this may lead to the misidentification of maternal uterine glands and maternal vessels where in the latter the endothelium is replaced by extravillous trophoblasts. Researchers should take care when choosing antibodies against HLA-G, since not all antibodies recognize HLA-G1, which is the only isoform expressed at the cell surface [6]. The anti-HLA-G antibody we recommend to use is the clone 4H84; it recognizes all isoforms of HLA-G, including HLA-G1 (which is the full length, membrane

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http://dx.doi.org/10.1016/j.placenta.2017.02.007
0143-4004/

bound isoform, expressed on the cell surface) [5].

This review is divided in three parts, the first part summarizes the latest developments in the field of extravillous trophoblast invasion towards uterine glands and vessels. The second part gives an overview about fundamental archival specimens in placental research for "placental rookies". For well-established placental researchers some new images of archival specimens are discussed in the context of the latest literature. In the third part, this review aims at extending this view to the latest development in the field of biobanking and provides insight into the current situation on sample usage in the absence of biobanks.

2. Extended view on extravillous trophoblast invasion into uterine vessels and glands

It is commonly accepted, that extravillous trophoblasts start their invasive pathway by invading the decidual stroma (interstitial trophoblast) [7] and thereby anchor the placenta to the uterus. It is also long accepted that extravillous trophoblasts invade and transform uterine spiral arteries (endovascular trophoblast) [8,9]. This leads to plugging of such vessels during the first trimester of pregnancy and after release of the plugs enables flow of maternal blood towards the placenta starting with the beginning of the second trimester. Only recently, the morphological appearance of trophoblast plugs have been described in detail [10].

2.1. Endoglandular trophoblast

In the last few years, the picture of trophoblast invasion has been massively expanded. Over the last six years it has been demonstrated that extravillous trophoblasts also invade into uterine glands [11,12]. Already in 2002 Burton and colleagues have demonstrated that secretion products of uterine glands fill the intervillous space of the placenta during the first trimester of pregnancy facilitating histiotrophic nutrition of the embryo prior to hemotrophic nutrition with the onset of maternal blood flow within the placenta [13–15]. In the same year Craven at al described extravillous trophoblasts surrounding uterine glands [16]. Thirty years earlier, Knoth and Larsen have already described close contact between invading trophoblast and uterine glands [17]. However, at that time it was totally unclear how glands are opened towards the intervillous space. It was only in 2010 when Moser et al. detected that trophoblast invasion into uterine glands (endoglandular trophoblast) leads to the connection of the glandular lumen to the intervillous space allowing secretion products to enter this space [11].

Additionally, these authors took a closer look into publicly available archival human implantation sites and also looked for archival material at their own site. Using this approach these authors identified a very close connection between an implanting embryo and subjacent uterine glands already at day 10 of pregnancy [12]. Fig. 1 shows images of an implantation site retrieved from the archive of the Institute of Cell Biology, Histology and Embryology at Medical University of Graz, Austria. These serial sections reveal how a uterine gland below the implantation site is widening and seems to open towards the very early placenta of that specimen (Fig. 1, images 13 and 14). So far, it was hypothesized that it is the extravillous trophoblast, developing in week 3 post conception, that invades into luminal structures. Looking at the archival material presented in Fig. 1, this view needs to be revisited: The evidence for a direct connection between the implanting blastocyst and uterine glands already at the time of implantation (Fig. 1, images 13 and 14) opens new ways for looking at early erosion of uterine luminal structures by the trophoblast.

2.2. Endovascular trophoblast stratified into endoarterial and endovenous trophoblast

Very recently another route of trophoblast invasion has been discovered. Although denied and disputed for decades, Moser et al. (2016) have identified, that using the correct markers (like EphB4 for venous endothelium and desmin as well as smooth muscle actin for the vascular smooth muscle layer) also invasion into uterine veins can be detected [18]. This of course is more than logical, as also veins need to be connected and opened towards the intervillous space. Only by opening and connecting these vascular structures, maternal blood flowing into the placenta via eroded spiral arteries can flow back into the maternal circulation. Two other groups have been able to prove this invasion into veins (personal communication, J. Pollheimer, Medical University of Vienna, Austria and He et al 2017 [19]). Besides that, even in the classic paper by Harris et al. 1966 the authors stated "*large multinucleated giant cells are frequently encountered within the veins*" and "*In one specimen … the trophoblast had opened up a vein in the myometrium*" [20]. Also Craven et al. [21] have proposed invasion of maternal decidual veins by trophoblast since they found trophoblasts in the lumen of such vessels. The concept of venous invasion does not question but rather broadens the concept of arterial trophoblast invasion, which is associated with extensive remodeling, transformation and plugging of the arteries.

This new route of invasion into uterine veins asks for a new definition of the terminology of extravillous trophoblast. So far, trophoblast cells within the wall or lumen of spiral arteries have been termed endovascular trophoblasts. With the invasion into uterine veins, this term is no longer specific enough. Hence, we propose a new definition of extravillous trophoblasts (Fig. 2): Those trophoblasts invading glandular structures are termed "endoglandular trophoblast", those trophoblasts invading vascular structures (arteries and veins) are termed endovascular trophoblasts. The latter are further stratified into endoarterial trophoblasts (invading into and transforming spiral arteries) and endovenous trophoblasts (invading into uterine veins). An example for endovenous invasion is presented in Fig. 3. Beside that, we have recently collected strong evidence for extravillous trophoblast invasion into lymphatic vessels (unpublished data and also personal communication, J. Pollheimer, Medical University of Vienna, Austria and He et al. 2017 [19]).

3. Fundamental archival human implantation sites

3.1. Publicly available archival human implantation sites

As can be seen from the achievements above, revisiting archival material with current knowledge may well enable detection of new, so far undetected features. Unfortunately, today only a few images from a handful of human early implantation sites are available for the scientific (and public) community. As an example we describe the image collections of the well-known collections that are publicly available at the Centre for Trophoblast Research (http://www.trophoblast.cam.ac.uk/Resources). These image collections include:

- The Boyd Collection, comprising histological sections (no blocks) of placenta-in-situ specimens and isolated placental specimens. As is outlined on the website of the Boyd Collection: "*The placenta-in-situ specimens often demonstrate low-lying placentas, and may have resulted from hysterectomy for antepartum hemorrhage. The isolated placental samples are likely to have been obtained from spontaneous miscarriages*." The

Please cite this article in press as: G. Moser, B. Huppertz, Implantation and extravillous trophoblast invasion: From rare archival specimens to modern biobanking, Placenta (2017), http://dx.doi.org/10.1016/j.placenta.2017.02.007