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Development of placenta in a rodent – model for human placentation

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1. ABSTRACT

Rodents provide an excellent experimental model to study human placental development. In this review, our aim was to explain major events that underlie the placental development in mammals in general, and specifically in rodent. Those events include trophoblast cell proliferation, decidual reaction and contact between the mesenchyme of the allantois with ectoplacental cone, all orchestrated by activation of a series of genes. We also aimed to compare molecular and genetic events of rodent and human placentation. Employing the rodent placenta development model will yield better understanding of these processes in other mammals, especially in humans.

2. INTRODUCTION

The purpose of this paper is to give an overview of rodent placenta development as model for normal human embryonic development. After fertilization, the zygote starts the process of cleavage, consisting of a number of mitotic divisions, giving rise to the blastocyst. In the blastocyst, the separation into embryoblast and trophoblast tissues represents the first differentiation and appears very early in development (1). The prospective trophoblast becomes polarized in the outer layer of the embryo as early as at 16-cell stage, when genes responsible for its differentiation start to be expressed (2). The marker of the future trophoblast cells is *Cdx-2* gene (caudal type

homeobox 2) (3); and its expression in the embryonal stem cells is sufficient for their differentiation into trophoblast (4,5), whereas Cdx-2 knockouts, although capable of initiating cell cleavage, cannot support the development of the trophoblast to completion (6,7). Its protein product is homeodomainic transcriptional factor which acts as repressor for *Oct4* (Octamer 4; POU class 5 homeobox 1) and *Nanog2* (Nanog homeobox) genes, markers of embryoblast and embryonal stem cells that are indispensable for their differentiation (8). In embryoblast cells, Oct4 is hypomethylated and acetylated, whereas in the trophoblast cells is hypermethylated and deacetylated and inhibits its differentiation (9).

The blastocyst has to implant to form placenta and thus enable successful progress of pregnancy. The relative importance of throphoblast cells for implantation is apparent from the fact that, e.g. in the mouse preimplantation embryo, composed of 64 blastomeres, only 13 cells represent the future cells of the embryoblast, whereas all the other cells future trophoblast (10). Unlike humans, where implantation and placentation occured on the same side, in rodents, the implantation takes place at the antimesomethral wall of the uterus during the fifth day of embryonic development, and the final placenta is formed on the mesomethral wall of uterus (11). Placenta is of chorioallantoic type because the blood vessels which supply chorion originate from the mesenchyme of the allantois (12). In rats, before formation of the chorioallantoic placenta takes place, the placental functions are performed by the yolk sac, which represents the only place for metabolic exchanges and the main nutritive organ until the 11th day of gestation. After that period the yolk sac functions jointly with the chorioallantoic placenta (13). In humans, these features are similar but not so pronounced while still retaining its haematopoetic task (14).

3. GENES INVOLVED IN THE PLACENTA DEVELOPMENT

The ectoplacenta emerges on the eighth day of gestation from the trophoblast cells which form the ectoplacental cone, and during the ninth day maternal lacunas without the endothelium arise within this ectoplacenta (Figure 1) (15,16). At the beginning of the 11th day of gestation, the mesenchyme of the allantois establishes the contact with the ectoplacenta, and due to its ability of angiogenesis, it differentiates into fetal mesenchyme and bloodvessel channels supplied with endothelium (17). This mesenchyme penetrates deeply into ectoplacental trophoblast, among the already formed maternal lacunas, thus creating labyrinth, i.e. the region where maternal and fetal blood circulation coexist and where the exchange of metabolites takes place (18). This is a very sensitive period during development of placenta, when the coordination between trophoblast and maternal lacunas is crucial for development of the labyrinth. There is no successful continuation of gestation without a well developed labyrinth. Therefore, it is not surprising to find a large number of the already known genes to be involved in this process. Myriam Hemberger and James C. Cross have classified these genes into four groups, responsible for particular phase of placental development: 1) genes responsible for differentiation of the trophoblast giant cells, (TGC), 2) genes important for chorioallantoic fusion, 3) genes responsible for the initiation of villous branching and vascularisation of trophoblast, and 4) genes for the transport of matter through the labyrinth (19). We will briefly summarize each of these groups as to have a more detailed view into events taking place in each one of the aforementioned phases.

Differentiation of the trophoblast giant cells (considered here are the secondary giant cells only, not the primary ones, which surround the embryo at the very beginning of gestation, immediately upon the implantation) that are responsible for the endovascular invasion of the trophoblast into the endothelium of maternal spiral arteries, is determined by the interaction of two genes. The first is *Mash 2*, which stimulates throphoblast proliferation and prevents its differentiation and is, thus, responsible for the maintenance of its cells (20). In contrast, *Hand 1* stimulates the diferentiation of trophoblast cells into giant cells; thus by blocking its expression in the mouse. Riely has obtained mutant animals that can reach, at most, the seventh and a half day of embryonic development (21,22). The Hand1 null mice do not form trophoblast giant cells (TGC) and display an exceedingly small ectoplacental cone (23,24). Products of both of these genes are basic „*helix-loop-helix*„ proteins (acting as transcription factors), which are localized in the ectoplacental cone and in the basal layer (spongiotrophobast). These areas represent the layers of future placenta which contain the precursors of trophoblast giant cells indicating that the antagonistic activities of Mash2 and Hand1 have to be coordinated (25,26).

To make the further discussion on genes required for successful placentation more comprehensible it is necessary to remind that placenta is composed of three completely different tissue types: epithelial cells derived from the trophoblast, and stromal and blood vessel cells derived from the extraembryonic mesoderm (in rodents they are derived from the mesoderm of allantois). Thus, one could say that placenta is the product of fusion of two extraembryonic structures, chorion and allantois (27).

For this fusion to take place, two proteins seem important: the vascular cell adhesion molecule 1 (VCAM-1) (the transmembrane glycoprotein, a member of the group of adhesive molecules of the immunoglobulin gene superfamily) present on the surface of allantois, and its ligand, alpha4 integrin, present on the basal surface of chorion (28). Cells that lack functional expression of both of these genes, develop seemingly normal allantois and chorion, but are unable to establish stable contact between these two types of tissues (29).

Once the chorio-allantoic fusion is succesfully established, the next phase in development of placenta takes place, which is characterized by initiation of villous branching and vascularization of trophoblast. The main actor during initiation of villous branching is the mammalian homologue of the *Gcm1* (*glial cell missing 1*) gene, originally discovered in *Drosophila* (30). Studies of

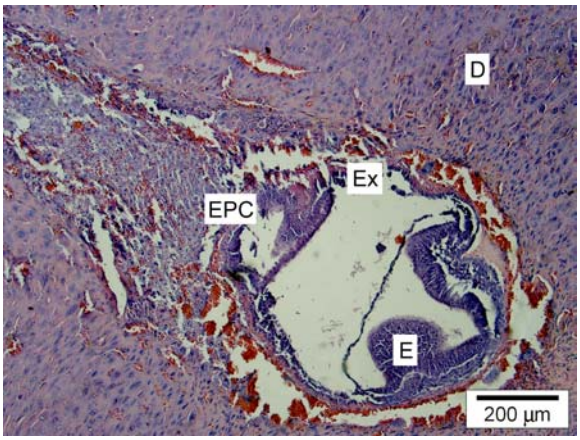

Figure 1. Deciduoma containing 9th day old rat embryo (vaginal plug designated day 0 of gestation). D - decidua, E - embryo, Ex - extraembrional part, EPC- ectoplacental cone (Fisher rat strain).

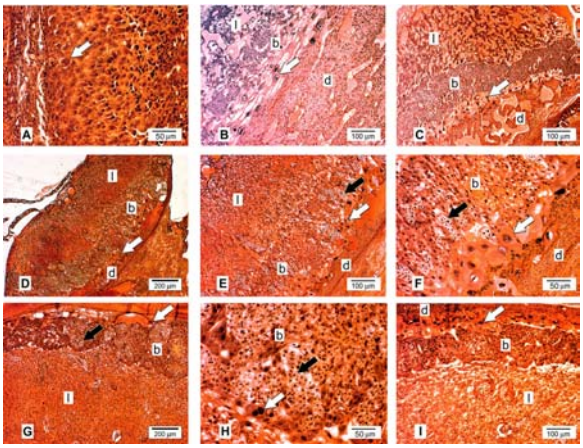

Figure 2. Development of rat placenta; A 12th day, B 14th day, C 15th day, D - F 17 th day, G and H 18 th day, I 20 th day of geatation, l – labyrint, b – basal plate, d – decidua, white arrow – TGC (trophoblast giant cell), black arrow – glycogen cells (Fisher rat strain)

its distribution in mammals have shown that *Gcm1* is highly expressed specifically in placenta (31). In the phase immediately prior to chorio-allantoic fusion, *Gcm1* mRNA was detected in rare trophoblast cells of chorion (32). Its protein product, however, starts to be synthetized only following the chorio-allantoic fusion by a mechanism of adhesion-dependent signaling from cell membrane to translation control site in the cytoplasm. This type of post-transcriptional control is found in embryonal development of other organs, as well as in tumorigenesis (33).

Whereas the *Gcm1* gene serves at the initiation stage of villous branching, for the continuation of branching and vascularization of the trophoblast a large number of genes seems to be responsible. Of those, two seem to be important for the growth of labyrinth (the main part of rodent placenta), based on its defective development when gene is mutated: *Gab 1* (signal adaptor molecule for a range of proteins like the Fibroblast growth factor receptor – *Fgfr2*, Epidermal growth factor receptor – *Egfr*, receptor for leukemia inhibitory factor (*LIF*)) and *Sos 1* (guanine exchanger or GDP/GTP exchanger) (19).

Finally, it remains for us to consider those genes responsible for transplacental transport of various substances and molecules. Among molecules that appear critical for such a function are connexins Cx26 and Cx31 (34). Connexins Cx26 function as bridges linking neighboring cells and they have been identified as channels for transport of glucose across hemochorial barrier (35). Connexin Cx31 plays the role in the differentiation of the trophoblast cells (36).

Our experiments using DNA demethylation agent 5-azacytidine (5azaC), which disrupts normal gene expression, point to the importance of orderly gene expression during placental development in rats. Treatment of rats with 5azaC at different time points during gestation produces changes in morphology of the placenta. We observed reduced or entirely absent labyrinth, placental mass and proliferation capacity of throphoblast cells (37). In addition, we have found significant changes in glycosylation of cytosolic and membranous proteins (37,38). DNA methylation is unquestionably important for the process of placentation and in humans as indicated by many research (39). Novakovic and collaborators discover data which strongly implicates epigenetic regulation of the DNA methyltransferase gene family in the establishment of the unique epigenetic profile of extraembryonic tissue in humans (40). Examination of invasive choriocarcionoma cell lines revealed altered methylation patterns consistent with a role of methylation change in gestational trophoblastic disease. The distinct pattern of tumour-associated methylation implicates a coordinated series of epigenetic silencing events, similar to those associated with some tumours, in the distinct features of normal human placental invasion and function (41).

4. FEATURES OF THE RODENT PLACENTA

Similar to humans, rodent placenta is of hemochorial type, i.e., the trophoblast cells originating from chorion are in direct contact with the erythrocytes, of the maternal blood (42,43). In hemochorial placenta, trophoblast invasion takes place in the uterine endometrium (44). Responsible for this invasion are two types of cells: the trophoblast giant cells (TGC) (Figure 2) and the glycogen-rich trophoblast cells (45) (Figure 2 E, F, G, H; analyzed in our laboratory on Fisher rat). The glycogen-rich trophoblast cells form structure analogous to the extravillous invasive interstitial trophoblast in humans, and are thus responsible for the interstitial invasion of uterine endometrium (46). Trophoblast giant cells of the rat are analagous to the endovascular trophoblast in humans and as such enable invasion into the maternal blood circulation system (47). These processes are associated with redistribution of cell-cell and cell-substrate adhesion molecules, cross talk between external extracellular matrix through adhesion molecules and the expression of several proteolytic enzymes, including matrix metalloproteases and

serine proteases (48,49). Defects in these processes may lead to diseases such as gestational preeclampsiae. Preeclampsia is a hypertensive disorder unique to human pregnancy that can result in significant morbidity and mortality for mother and fetus (50). One possible mediator may be the matrix metalloproteases, a family of proteinases typically recognized for long term tissue remodelling (51).

The result of trophoblast invasion is the creation of functional placenta with three essential parts: the basal zone, the labyrinth and decidua (52). Decidua grows at smaller rate as gestation progresses since the labyrinth reaches its maximum size in the third part of gestation (53) (Figure 2; shown on Fisher rat analyzed in our laboratory). The basal zone of the placenta consists of trophoblast stem cells and three types of differentiated cells: trophoblast giant cells, spongyotrophoblast cells and glycogen-rich cells (54). This second generation of giant cells (secondary giant cells) emerges in the border between the basal zone and the decidual part of placenta (55). Here there are no fetal blood vessels nor the chorioallantoic mesenchyme, only the blood circulation system which exclusively belongs to the mother (56).

The labyrinth zone is located on the fetal side of placenta and represents the space where one can find both maternal lacunes and fetal blood vessels (57) (Figure 2B, 2C; Fisher rat). The labyrinth is constructed of cytotrophoblast stem cells which differentiate at all times into trophoblast giant cells (TGC) or agglomerate into syncytium (58) (Figure 2E, 2F, 2H; Fisher rat). Many genes involved in TGC development and function are conserved between rodents and humans, such as transcription factors, proteases and cell adhesion molecules (59).

System of fetal capillaries and maternal lacunas form two separate blood circulations separated by the placental membrane (60). In rats, this membrane is composed of three layers: the outer layer of trophoblast bathed directly by maternal blood and inner two layers which are multinucleated and syncytial in nature (61). For this reason the rat placenta belongs to the group of hemotrichorionic placentas, as opposed to human placenta which is monochoryal (62). In monochoryal placentas, the villi have a nearly complete cytotrophoblast layer underlying the surface layer of syncytial trophoblast during most of the first trimester. As gestation proceeds the cytotrophoblast layer becomes discontinuous and the cytotrophoblast cells become more stellate, at which time the placenta is considered to be villous hemomonochorial (63). Syncytiotrophoblast's function is to produce proteins and steroid hormones (64).

In rat hemotrichorionic placenta, the outer layer, throphoblast, later develops into trophoblast giant cells. These cells (TGC) show reduced levels of p53 and Rb protein synthesis which are indispensable for their exit from the cell cycle and entry into the endocycle of genomic amplification, and synchronous rise in synthesis of cyclin E, which is indispensable for the transition into the S-phase (65,66). Towards the end of pregnancy the TGC-layer loses its regular shape and thins down on some places to sheer bilayer of cell membranes (67). Similar thinning of the hemochorial barrier happens generally in all placentas in order to increase transport efficiency for better growth of the fetus (68).

This fact is especially interesting in light of the toxicological studies on the placenta. Reproductive and developmental toxicology studies have yielded evidence that metals from tobacco smoke can act as endocrine-disrupting chemicals in reproductive tissues (69). The results were initially obtained in rat placenta, and then confirmed at the human (70,71).

Because of all these characteristic, i.e. haemochorial and chorioallantois placentation as well as the depth of trophoblast invasion (especially rats) and a number so far discovered genes in human and rodent (especially mouse) performing similar functions, we can generally consider the rodent placenta as a suitable model for studying not only human placentation, but also diseases that are associated with it.

5. CONCLUDING REMARKS AND PERSPECTIVES

Thanks to the progress in medicine and to the fact that placenta is increasingly becoming an interesting organ to study, primarily in its obstetric sense and function, but also in its evolutionary, immunological, and genetic sense, there appears a necessity for the animal experimental model whose placental development resembles closely to that of humans. We suggest that the development of placenta in a rodent is such a model, uniquely helpful to investigate trophoblast cell invasion as well as the impacts of various teratogenic agents on development of placenta, as the exclusive reproductive organ in mammals.

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