LABORATORY OF CELL SOCIOLOGY †

Assistant Professor
HAMADA, Yoshio

Technical Assistant: *GONDA, Naoko*

Mammals have evolved placentae that facilitate the transport of nutrients and oxygen into the fetus. Primates and rodents have an ancestral type of placenta in which maternal blood is not contained within endothelial cell lined vessels but rather is in direct contact with epithelial cells of the fetal placenta (so called hemochorial placentation) that are derived from the trophoblast cell lineage (Figure 1). How maternal blood vascular circuits develop in the hemochorial placenta without immunological rejection has been a longstanding mystery in biology.

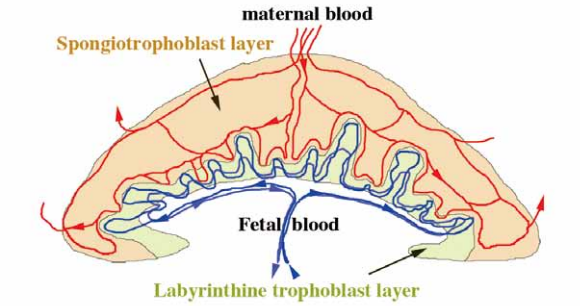

Figure 1. Fetal and maternal blood flow in the mouse placenta. Feto-maternal exchanges take place in the labyrinthine layer (green). Polyploid trophoblasts are observed in the spongiotrophoblast layer (orange) where *Notch2* is expressed.

The *Notch2* null mutant dies at mid-gestation due to impaired maternal circuit formation. Two mechanisms for the formation of spaces for the maternal circuit have been speculated. One is by polyploid Trophoblast Giant Cells (TGCs) that retain the ability to form cavities, analogous to endothelial cells. Notch signaling mutations reduce the population or cause dysfunction of TGCs. The other is by deletion of trophoblasts, which we have proposed. Notch mutation would lead the trophoblasts to resist the deletion.

We carried out a survey of authentic cell death, apoptosis and necrosis, related to the formation of the maternal blood circuit, but failed to find meaningful signals. Therefore, we suspected that an unknown type of cell death or deletion might be involved. Because histological studies of developing mouse placentae show that formation of open spaces begins at E10.5 and become bigger at later stages in the spongiotrophoblast layer (Figure 2), it was rational to speculate that cell death or deletion occurs in the spongiotrophoblast layer. Study of the nuclear size of spongiotrophoblast cells shows a constant and substantial population of polyploid trophoblast cells throughout development.

How is the population of polyploid trophoblast cells kept constant? Considering that diploid spongiotrophoblast cells differentiate into polyploidy during normal development, the supply from diploid trophoblast cells and deletion could maintain a constant population. Now, our working hypothesis

†: This laboratory was closed on 31 March, 2016.

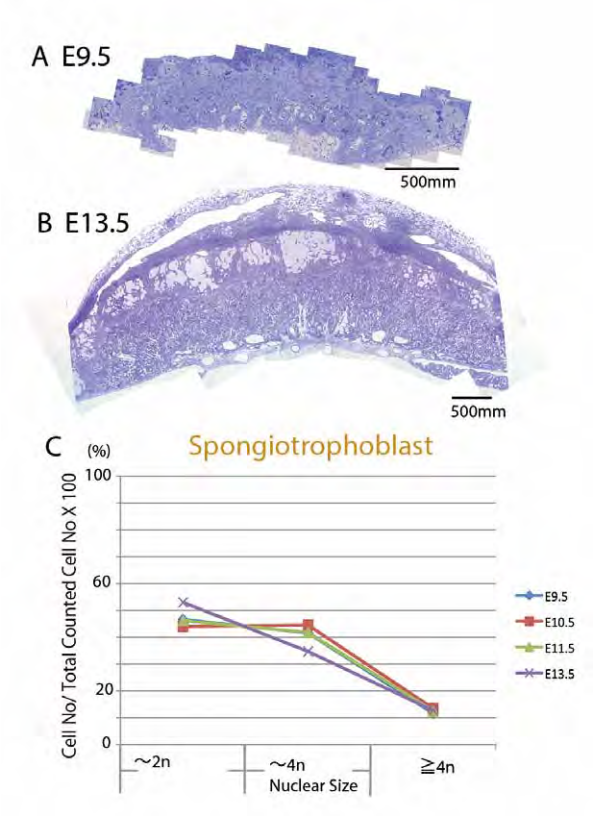

Figure 2. Drastic morphological change and unchanged population of polyploid trophoblast cells. Open spaces are barely seen at E9.5 (A) and become large enough to contribute to half of the spongitotrophoblast layer at E13.5 (B). In contrast to the morphological changes, the proportion of diploid, replicating dipoloid and polyploidy is unchanged throughout E9.5-E13.5 (C).

became testable. Polyploid trophoblasts should be deleted and *Notch2* mutation should cause them to resist the deletion.

Although tetraploid embryo derived trophoblasts are able to support ES cell derived embryos to grow to term, they are deleted from the placenta by E11.5 if they co-exist with diploid trophoblasts (Figure 3). Contrastingly, *Notch2* null mutant tetraploid embryo derived trophoblasts resist the deletion and even dominate diploid trophoblasts. From these results we conclude that maternal blood spaces throughout the trophoblast are created by polyploid trophoblast cell deletion.

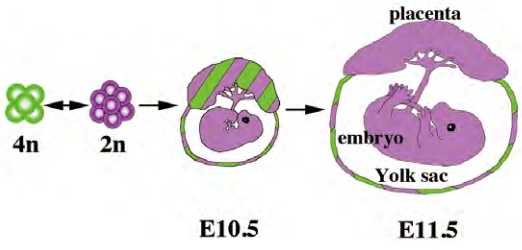

Figure 3. Fate of tetraploid trophoblast cells in chimera embryos. A tetraploid embryo was aggregated with a diploid embryo *in vitro* and transplanted onto a pseudo-pregnant female's uterus. While the 4n embryo contributes to the placenta and yolk sac, it does not contribute to the embryo proper by E10.5, 4n embryo derived trophoblasts disappear by E11.5.