LABORATORY OF CELL SOCIOLOGY

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Animal organs are made up of several types of cells, and organized in an orderly fashion wherein the proportion of each cell type is constantly maintained. This orderly cell arrangement and proportion are built up during organogenesis by cell-cell interactions. Since it has been postulated that *Notch* plays a role in cell fate decisions by mediating cell-cell interactions, we are endeavoring to discover the cellular and molecular mechanisms at work during organogenesis by studying the function of *Notch*.

Organogenesis of the mouse placenta occurs during early pregnancy, embryonic days 7-9, before the establishment of molecular transport mechanisms in the definitive placenta takes place. Trophoblasts not adjacent to the inner cell mass differentiate into trophoblast giant cells and lie at the outside, forming an interface with the maternal deciduas. The polar trophectoderm gives rise to the cells of the chorion as well as the ectoplacental cone; these produce the labyrinthine and spongiotrophoblast layers, respectively. While maternal red blood cells begin to perfuse into trophoblast cell layers and reach the labyrinthine layer by E9.5, the invasion of embryonic allantoic mesenchyme into the labyrinthine layer and the differentiation of fetal red blood and endothelial cells which line the fetal capillary take place around E9.5.

The *Notch2* null mutation results in embryonic lethality by embryonic day 11.5 due to the formation of poor maternal vascular beds. The mutant placenta shows a normal invasion of angiogenic allantoic mesenchyme followed by premature formation of fetal blood vessels in the mutant placentas as early as E9.0. However, the specification of trophoblast subtypes appears not to be drastically disturbed. Thus, in the developing mouse placenta, *Notch2* is likely not involved in cell fate decisions, but rather participates in the formation of circulatory systems in the labyrinth layer where the expression of *Notch2* is detected. Although inadequate formation of maternal vascular beds was partially restored by aggregating mutant diploid embryos with wild type tetraploid embryos, networks of maternal vascular beds appeared still compromised in the 4N chimeric placenta. These results indicate that *Notch2* promotes vasculogenesis.

How maternal vascular beds are formed in the developing mouse placenta has yet to be explored. The simplest way to form the beds among tightly adhered labyrinthine trophoblasts is through their cell death. We studied a spatiotemporal appearance of dead cells in the developing placenta. While vasculogenesis does not occur in the presumptive labyrinth layer at E8.5, some dying cells were detected. At E9.5, extensive trophoblast cell death took place around newly forming maternal blood beds. In contrast to the wild type placenta, extensive cell death did not occur in the E9.5 mutant placenta (Fig. 1). It is likely that *Notch2* plays a role in vasculogenesis through being involved in the process of trophoblast cell death. We are now carrying out studies on how Notch2 participates in the cell death process and how the gene is activated in the trophoblast in cell culture.

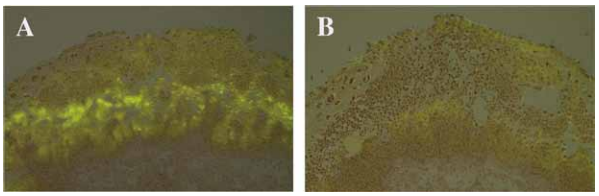

Figure 1. Programmed cell death in the developing mouse placenta. Dying trophoblast was visualized by staining with a fluorescent dye at E9.5. Wild type placenta showed extensive cell death around newly forming maternal blood beds which were surrounded by Notch2 expressing trophoblast (A), but cell death was scarce in the mutant placenta.