LABORATORY OF CELL SOCIOLOGY

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Animal organs are made up of several types of cells, 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 decision by mediating cell-cell interactions, we are endeavouring 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 showed 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 appeared not to be drastically disturbed. Thus, in the developing mouse placenta, *Notch2* is likely not involved in cell fate decision, but rather participates in the formation of circulatory systems in the

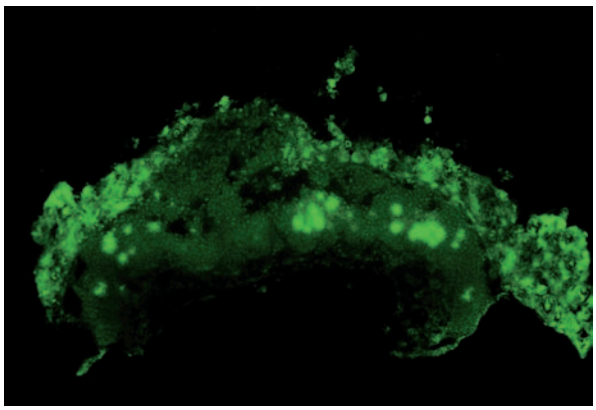

Figure 1. Cell death in *Notch2*$^{+/LacZ}$ mouse placenta at E8.5.
Acridine Orange (AO) positive signals were mostly observed in presumptive labyrinthine trophobast layer.

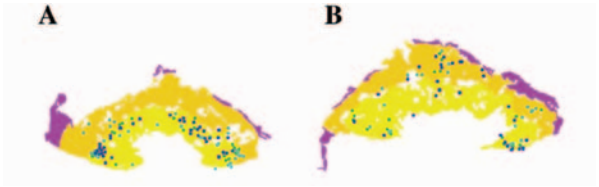

Figure 2. Distribution of AO positive signals in E9.5 *Notch2*$^{LacZ/LacZ}$ placenta (A) and *Notch2*$^{+/LacZ}$ placenta (B). Red, orange and yellow color indicates giant cell layer, spongiotrophoblast layer and labyrinthine trophoblast layer, respectively. Dots indicated in light blue, dark blue, and green show dying cells in three serial sections.

labyrinth layer where the expression of *Notch2* was 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 been unexplored. 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 by staining with a fluorescent dye, acridine orange, which has been employed to detect apoptotic cells in *Drosophila* (Figure 1). It was found that the appearance of dying cells correlated well with maternal vasculogenesis and was delayed in the mutant placenta (Figure 2). Identification of a factor which is responsible for the induction of *Notch2* expression in trophoblasts around newly forming maternal blood beds is another project in our laboratory. When both projects are accomplished, we should obtain some insights into *Notch2* gene function in maternal vasculogenesis in the developing mouse placenta.

Publication List

〔Original paper〕

● Tang, H., Brennan, J., Karl, J., Hamada, Y., Raetzman, L., and Capel, B. (2008). Notch signaling maintains Leydig progenitor cells in the mouse testis. Development *135*, 3745-3753.