In vitro approach for placental drug transport studies using induced pluripotent stem cells

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Scope of the lecture:

There are a number of precautions for medication administration during pregnancy. Although pregnant women requiring medication should be treated adequately, the potential effects of a drug treatment on the fetus cannot be ignored. Evaluation of placental drug transport is the first step in chemotherapeutic safety evaluation during pregnancy. However, information on the placental permeability of drugs is difficult to accumulate, and well-established *in vitro* models are not available. In this lecture, I will outline the use of induced pluripotent stem cells (iPSCs) in developing an *in vitro* approach for placental drug transport studies, highlighting our latest research results.

Learning objectives:

1. Japanese drug administration guidance during pregnancy
2. Past knowledge relating to *in vitro* placental drug transport models
3. Differentiating conditions of iPSCs and trophoblast cell lines

Extended abstract:

The provision of information is crucial to ensure that an "informed decision" on a medication choice is made by a consulting patient during pregnancy. It has been difficult that healthcare providers search conclusive precautions in this field. One of the explanations was little information can be obtained from Japanese product information documents of drugs. In the survey of Yokohama Teratology Monitoring Center, a member of the International Clearinghouse for Birth Defects Surveillance and Research (ICBDSR), congenital abnormalities were reported in approximately 3% of regular birth in humans. An important point on the fetal safety of drugs is whether they increase the incidence rate of these congenital abnormalities. Recently, the descriptions in product information documents of drugs have been revised to include fetal safety information in Japan as well. In this revision, the precautionary points of "administration to pregnant women, parturient women, and nursing mothers" will be explicitly divided into the following precautionary points: "administration to pregnant women," "administration to men and women of reproductive age," and "administration to nursing mothers." In addition, further detailed fetal safety information will be included such as placental permeability, teratogenic potency, results admitted from the amount of fetal exposure and timing of pregnancy exposure, and alternate drugs. This revision will be effective from 2019.

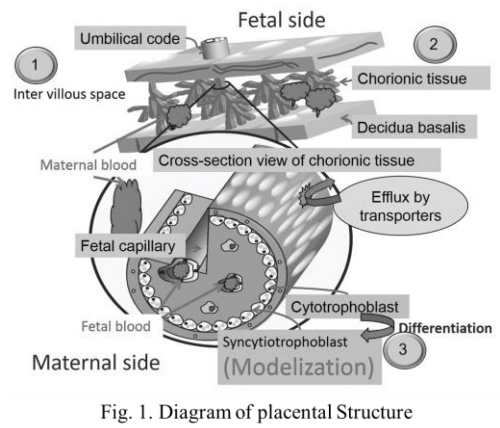

Fig. 1. Diagram of placental Structure

In this precautionary information considering the administration of medication during pregnancy, placental drug permeability has not been systematically organized. Therefore, we have focused on constructing an *in vitro* placental drug transport model, aimed at accumulating placental drug permeability information.

Drugs are transported to the fetal circulation across the syncytiotrophoblast layer (3), which constitutes the outermost cell layer of the chorionic tissue (2) in the intervillous space (1, Fig. 1). Several studies on *in vitro* placental drug transport models have used choriocarcinoma, JEG-3, and BeWo cells. These studies have demonstrated that JEG-3 cells differentiated (DJEGs) using CSC® medium, a marketable medium for human umbilical vein endothelial cells, exhibited the desired features of secretion of human chorionic gonadotropin (hCG), high expression levels of breast cancer resistance protein (BCRP), and acquisition of cell-to-cell fusion function, as observed in syncytiotrophoblasts *in vivo*[1].

However, in some instances, the drug concentrations observed in the fetal side of the DJEGs model were not reflective of the predicted *in vivo* fetal drug concentrations. Therefore, it would be necessary to reduce the variation between syncytiotrophoblasts and the *in vitro* evaluation model for this model to be a useful marker of placental drug transport. Therefore, we focused on the *in vivo* similarities of differentiating iPSCs. It is well known that after treatment with a high concentration of bone morphogenetic protein 4 (BMP4), iPSCs achieve a syncytiotrophoblast-like form and secrete hCG.

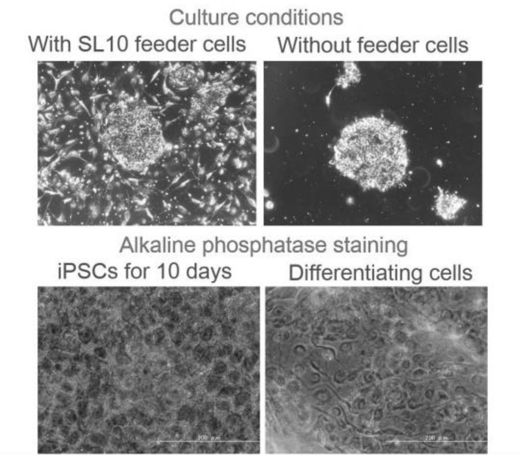

Fig. 2. Microscopy of iPSCs cultured without feeder cells and alkaline phosphatase staining.

Firstly, the conditions required for the differentiation of iPSCs[2] into syncytiotrophoblasts were investigated (Fig. 2). The iPSCs differentiated by BMP4 could also mature into tissue cells, growing from the endoderm and mesoderm. Therefore, it was difficult to obtain a single layer of syncytiotrophoblasts. In contrast, iPSCs differentiated by retinoic acid (RA) efficiently mature into hemocytoblast. Additionally, iPSCs treated with RA have been shown to secrete hCG, notably. These findings suggest that differentiating iPSCs treated with RA could be expected to be a useful *in vitro* placental drug transport model.

BMP4 is an efficient, but costly differentiation induction reagent. The discovery of alternative factors for inducing iPSCs into syncytiotrophoblasts would be useful for studying this differentiation machinery. We demonstrated that iPSCs treated with RA for 7 days were induced to differentiate into syncytiotrophoblasts, as confirmed by their marked hCG secretion, *BCRP* gene expression, and immunofluorescence staining of cytokeratin 7 (Fig. 3), an accurate intracellular marker for assessing the purity of human placental villous trophoblast cells. In the future, we will optimize the differentiation conditions for iPSC-derived syncytiotrophoblast cell layers and establish efficient maintenance culture conditions for *in vitro* placental drug transport evaluation studies. In this lecture, I will present a series of background information and research results relating to an *in vitro* approach for placental drug transport studies using iPSCs.

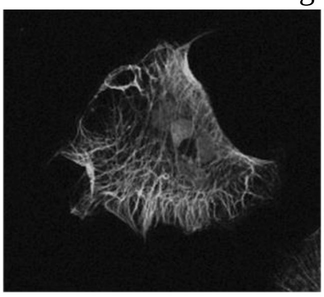
Fig. 3. Cytokeratin 7 immunofluorescence staining of iPSCs differentiated to syncytiotrophoblasts.

1) Ikeda K et al., (2011) *In vitro* approaches to evaluate placental drug transport by using differentiating JEG-3 human choriocarcinoma cells. Basic Clin. Pharmacol. Toxicol. 108: 138–145.
2) Okita et al., (2011) A more efficient method to generate integration-free human iPS cells. Nat. Methods.