

Stem Cell Approaches in Pregnancy Associated Disease

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Editorial

Eutherian mammals give birth to developmentally advanced progeny by utilizing an intrauterine organ, placenta. The placenta is essential to implantation of embryo, transportation/exchange of nutrients, gases and wastes to support fetal development. It is also an endocrine organ that regulates maternal physiology to establish and maintain a pregnancy and promote fetal growth by producing of both steroid and polypeptide hormones. Trophoblast cell lineage, derived from trophoblast of the blastocyst as the first lineage differentiation, gives rise to specialized cell types of placenta. Placenta is the most diverse organ in mammalian organs and has been classified based on the structural and functional features. The human and rodent bear some degree of resemblance each other and share hemochorial placental type that forms direct contact with maternal circulation and tissues [1].

The human placenta contacts maternal cells through primarily two types of trophoblasts, 1) Extra Villous Trophoblast (EVT) and 2) Syncytio Trophoblast (STB). Both of the trophoblast subsets are derivatives from a stem cell-like subpopulation of cytotrophoblast.

Preeclampsia is a most common hypertensive syndrome of pregnancy. This disease affects ~3-7 % of pregnancies and is responsible for 15% of pre-term births and for ~18 % of maternal deaths in the United States [2,3]. Criteria for diagnosis of preeclampsia include maternal hypertension, proteinuria and pulmonary edema. They represent the late manifestation the disease when the symptoms are often prominent in the last few weeks of gestation [4]. However, the etiology of disease is vascular remodeling defects and poor placental perfusion between mother and fetus that begins very early in pregnancy [5]. The poor vascular remodeling stems from failure of EVT to invade deeply and to modify maternal spiral arteries. STB also contributes to the pathophysiology by releasing cytokines, anti-angiogenic proteins [6-8] and placental debris [9] that are elevated in mothers diagnosed with preeclampsia. Although such new findings have been accumulated, precise mechanisms of failures in the remodeling and perfusion continue to be poorly defined [10].

As mentioned earlier, the rodent placenta has some resemblance to that of the human. Numbers of rodent models are able to recapitulate some of pathophysiological conditions of preeclampsia. However, comparative morphological and physiological inferences may have limitations to address about human placental development and to understand etiology of the disease. Because preeclampsia cannot be diagnosed at the time when the placenta is being established, term placenta is of little value in study. There remains a need for *in vitro* systems that can mimic early events in the development of the human placenta [2]. We [11] and others [12,13] have pointed out that models useful for studying human trophoblast development need to be better than immortalized or cancerous trophoblast cell lines and primary cultures of placenta. These models are unable to study transition between undifferentiated cell and early trophoblast stages when differentiation process to the EVT and STB can be examined.

Treating human Embryonic Stem Cells (ESC) with the growth factor, Bone Morphogenetic Protein (BMP) 4 (termed hESC/BMP4), is first described Xu et al. [14]. This model allows generating trophoblast and provides a series of temporal and spatial snapshots into the changes that accompany initial differentiation from a precursor pluripotent

cells and the subsequent formation of sublineages as they arise from cytotrophoblast precursors.

Recent attempts to improve differentiation to trophoblast can be achieved by blocking the signaling systems essential for maintaining pluripotency [15,16]. We have observed an enhanced conversion to trophoblast following BMP4 addition by blocking both activin A and FGF signaling simultaneously by including A83-01 (an inhibitor of activin A signaling) [17] and PD173074, a fibroblast growth factor receptor inhibitor [16]. When hESC were treated with either BMP4 alone or BMP4 plus the two inhibitors (BMP4/inhibitors), a morphological switch to an epithelial phenotype started by day 2 under both treatment regimens, although the process occurred more rapidly under BMP4/inhibitors than with BMP4 alone. Consistent with the morphological differences, production of markers of the advanced trophoblast sub-lineage, STB: chorionic gonadotropin, progesterone and placental growth factor and EVT: HLA-G, were also higher in cells treated with BMP4/inhibitors. Some of transcription factors implicated in trophoblast lineage emergence were up-regulated immediately in both treatments and increased over a period of days especially cells cultured under 20% O₂ than cells exposed to physiological oxygen condition (4% O₂). In uterus, EVT invading into the uterine wall will also be exposed to increasingly higher O₂ tensions as it approaches maternal arterioles. When a hypoxic response is introduced in the placenta in experimental models, such models appear to recapitulate multiple aspects of preeclampsia. Such variable O₂ conditions employed in the hESC/BMP4 model seem reasonable to include.

Recently the hESC/BMP4 model has been challenged by Bernardo et al. [18]. The study asserts the hESC/BMP4 model does not generate trophoblasts at all, but it derives only to mesoderm derivatives. However, the assertion has been retorted by us [19] and others [20] and the cell culture condition they employed has deviations from the initial study [14]. In our hands, the hESC/BMP4 model generates greater than 95% of cells expressed KRT7, a general marker of trophoblast, while markers for endoderm and mesoderm were generally absent [19].

If the differentiation system can be applied to induced Pluripotent Stem Cells (iPSC), it opens ways to study connections between genetic or epigenetic background and pregnancy disorders associated with trophoblast phenotype that have been difficult to address until now. The results obtained so far show that iPSC can be efficiently differentiated into trophoblasts by the same BMP4/inhibitors exposure used for hESC. Therefore, iPSC derived from babies whose pregnancies were affected by placental pathologies can now be used as a source of experimental material. We are currently attempting to re-create trophoblasts from infants born to mothers with preeclampsia by generating iPSC from

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discarded umbilical cord and converting these pluripotent cells to cytotrophoblast, EVT and STB by the BMP4/inhibitors approach. The cells will be examined for features of sublineage differentiation under varied oxygen tensions and supplementation factors for mimicking pathophysiological conditions and compared the phenotypes of cells derived from control cases. This approach may also provide insight into the molecular mechanisms that control these developmental transitions.

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