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研究課題名（和文）Scaffold-assisted tissue engineering of early human placenta derived from embryonic stem cells

研究課題名（英文）Scaffold-assisted tissue engineering of early human placenta derived from embryonic stem cells

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研究成果の概要（和文）：本研究の目的は、ヒトES細胞由来の栄養芽幹細胞を立体マトリックスで培養し、胎盤状の構造(絨毛状構造=villus等)を生み出す条件を発見するということだ。突起状のPDMS(ポリジメチルシロキサン)マトリックスにプラズマ処理を行うとヒトES細胞がマトリックスに結合、増殖、栄養芽幹細胞に分化できた。更に、マトリックスを培養地に浮かせて、その裏面で培養すると、非増殖性の絨毛状構造及び胎盤が発する合胞体性結節=syncytial knotに似た多核細胞塊の発生が観察できた。現在、その多核細胞塊がどれほど実際の合胞体性結節であるかを細胞学的に調べている。

研究成果の概要（英文）：The goal of this study was to find optimal conditions for formation of placenta-like structures in differentiated trophoblast cells derived from human ES cells grown on a solid matrix. We tested whether providing a shaped scaffold (micro-bumps of polydimethylsiloxane, PDMS) encouraged the outgrowth of trophoblast cells from human ES cells. We found that plasma treatment of the PDMS mold greatly enhanced the ability of human cells to adhere and grow. Using plasma-treated molds we succeeded in attaching human ES cells and differentiating them into trophoblast-like precursors. We found that growing cells underneath a PDMS mold floating on the media surface encouraged outgrowth of nonproliferative villus-like structures and shedding of syncitial knot-like clusters of multinucleated cells. We are currently cytologically characterizing these clusters to determine whether they are actual differentiated syncitial knots.

研究分野：細胞生物学

キーワード：trophoblast　human ES cells　tissue engineering

１．研究開始当初の背景

(1) It has been difficult to study early human placenta owing to both practical and ethical difficulties in obtaining material. One potential solution to this problem is to grow early placenta tissue derived from progenitor stem cells *in vitro*. In the case of trophoblast stem (TS) cells, since they belong to the extraembryonic lineage which normall does not descend from embryonic stem (ES) cells, direct differentiation from ES cells has been challenging. Recent studies have pointed at ways to achieve direct ES>TS differentiation using inhibitors of Nodal and Activin to inhibit development of embryonic cell layers, forcing the cells to go down the extraembryonic trophoblast pathway. .

(2) Even with successful differentiation into TS cells, however, actual placental structures have been difficult to achieve in a culture dish. We and other groups have now turned to 3D scaffold creation to mimic the early developmental environment of the placenta to determine whether correct cell differentiation could occur.

２．研究の目的

(1) The goal of this study was to find optimal conditions for formation of placenta-like structures in differentiated trophoblast cells derived from human ES cells grown on a solid matrix. By recapitulating early differentiation steps of human placenta, we aimed to create an *in vitro* model for the study of placental disorders.

(2) In particular, we were interested in whether our *in vitro* system would show the formation of two structures: villi, which are protrusions of the placenta that act as a barrier between the placenta and maternal blood, and syncytial knots, clusters of cells that are shed from the placenta into the maternal bloodstream. In particular, syncytial knots show an increased incidence in conditions such as preeclampsia, a dangerous condition during childbirth. By changing culture conditions and observing the rates of knot formation, we hoped to discover conditions that could suppress the creation and shedding of syncytial knots.

３．研究の方法

(1) We grew human ES cells on a variety of PDMS (polydimethylsiloxane) scaffolds (Figure 1) and tested their ability to differentiate correctly in to TS cells and other cells descended from TS cells that make up the placenta: cytotrophoblast (CyT) and syncytiotrophoblast (SynT) cells. Testing was performed by single-molecule RNA FISH using known markers of trophoblast development such as CDX2 for cytotrophoblasts (**Figure 2**) as well as immunostaining for proteins such as the proliferation marker KI67 (**Figure 3**). Additionally, microarray analysis was performed on cells before and after differentiation to characterize gene expression profiles and confirm that trophoblast-like fate was adopted.

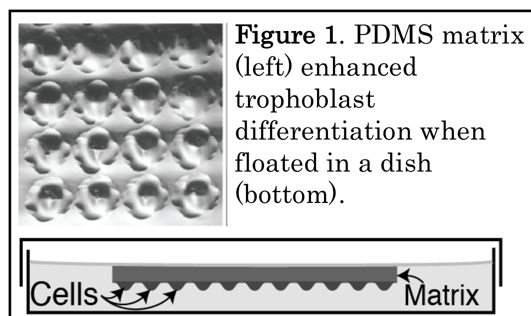

Figure 1. PDMS matrix (left) enhanced trophoblast differentiation when floated in a dish (bottom).

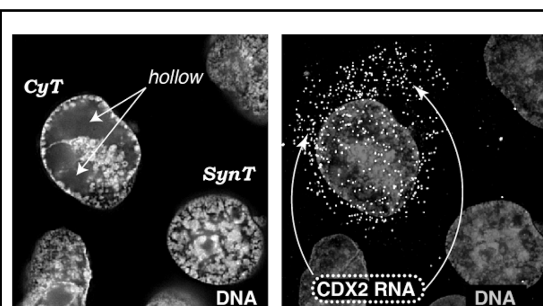

Figure 2. Single-molecule RNA FISH shows that putative cytotrophoblast cell (CyT) has correct expression of CDX2

４．研究成果

(1) We first identified growth conditions which would lead to the correct differentiation of human ES cells into TS cells. In accordance with previous work, we found that stimulating cells with BMP4 while simultaneously inhibiting the FGF and Activin pathways led to reproducible loss of pluripotency and differentiation into a trophoblast-like fate. This was confirmed cytologically by immunofluorescence and single-molecule RNA FISH, as well as with microarray expression data. In particular, we found by single-molecule RNA FISH that that the protein Nanog (a marker of pluripotency) was completely lost by 24h after treatment with differentiation media. This demonstrates our model is an excellent system for examining the earliest

steps of pluripotency loss.

(2) Although nearly 100% of cells went on to adopt the trophoblast fate when grown on a glass surface, they did not elaborate structures such as villi found in early placenta, as had been previously shown. We therefore next tested whether providing a shaped PDMS scaffold encouraged the outgrowth of trophoblast cells into structures such as placental villi. Although a mouse TS cell line readily attached and grew on the matrix, we found human cells would not attach to the matrix.

(3) In the final year of this study, we found that plasma treatment of the PDMS mold greatly enhanced the ability of human cells

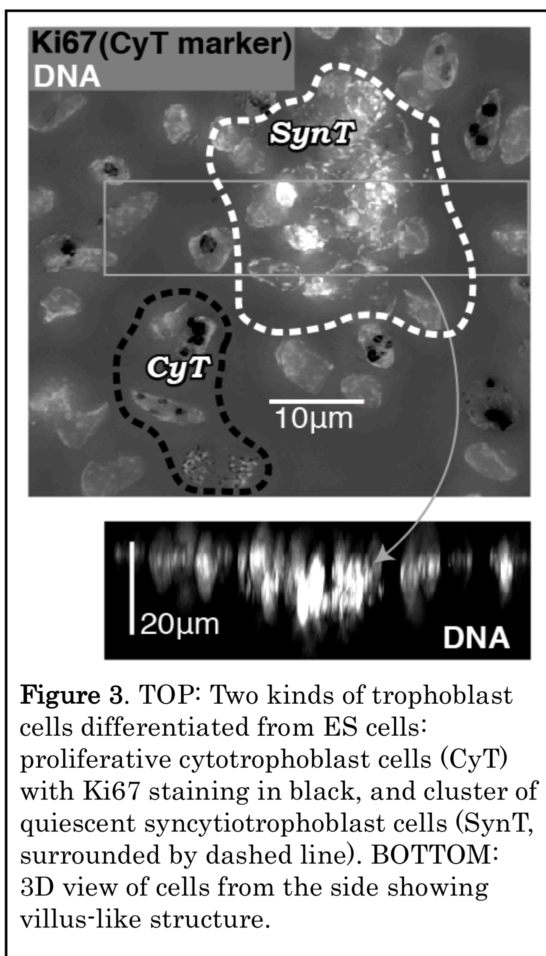

Figure 3. TOP: Two kinds of trophoblast cells differentiated from ES cells: proliferative cytotrophoblast cells (CyT) with Ki67 staining in black, and cluster of quiescent syncytiotrophoblast cells (SynT, surrounded by dashed line). BOTTOM: 3D view of cells from the side showing villus-like structure.

to adhere and grow. Using plasma-treated molds we succeeded in attaching human ES cells and differentiating them into trophoblast-like precursors.

(4) We next developed conditions for culturing trophoblast cells on these molds that could recapitulate placental structures. We found that growing cells underneath a PDMS mold floating on the media surface **(Figure 1)** encouraged the

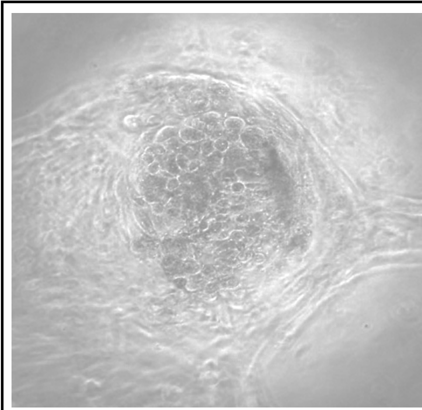

Figure 4. A placental villus-like structure observed attached to a "bump" on the floating PDMS mold

outgrowth of villus-like structures and shedding of syncytial knot-like clusters of multinucleated cells. The villus structures (**Figure 4**) were positive for trophoblast markers PL1 and cytokeratin 7. Further evidence for cell differentiation to a syncitiotrophoblast state was provided by the cell proliferation marker KI67, which was present in the

bottom layer of cells but not found in the upper layer. This strongly resembles the organization of syncitiotrophoblast in *in vivo* placental villus structures.

(5) To assess whether these villus structures were also able to shed syncytial knots, we examined the culture dishes for

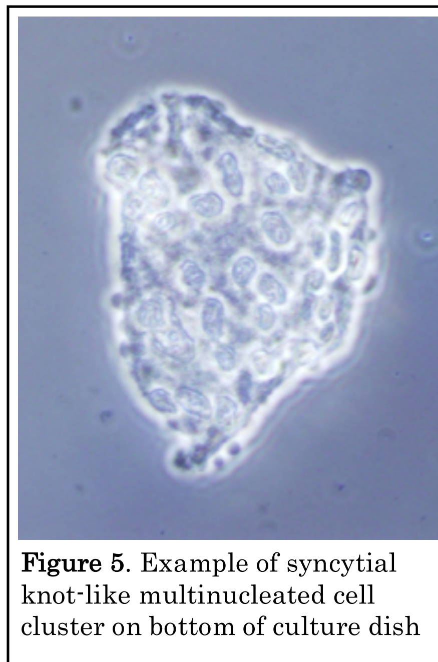

Figure 5. Example of syncytial knot-like multinucleated cell cluster on bottom of culture dish

floating or submerged cell clusters. We indeed discovered clusters of cells at the

bottom of the culture dishes. The clusters of cells were multinucleated, and of approximately the same size as actual syncitial knots **(Figure 5)**. We are currently characterizing these clusters using immunostaining after cell sorting to determine whether they are actual differentiated syncitial knots.

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

Our manuscript from this work is still in preparation.

〔雑誌論文〕（計　　件）

〔学会発表〕（計　　件）

〔図書〕（計　　件）

〔産業財産権〕

○出願状況（計　　件）

名称：
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権利者：
種類：
番号：
出願年月日：
国内外の別：

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〔その他〕
ホームページ等
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