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# Gene Expression Profiles by Microarray Analysis during Matrigel-induced Tube Formation in a Human Extravillous Trophoblast Cell line: Comparison with Endothelial Cells

K. Fukushima [a,*], M. Murata [a], M. Hachisuga [a], K. Tsukimori [a], H. Seki [b], S. Takeda [c], K. Kato [d], N. Wake [a]

[a] Department of Obstetrics and Gynecology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
[b] Department of Obstetrics and Gynecology, Saitama Medical Center, Saitama Medical University, Saitama, Japan
[c] Department of Obstetrics and Gynecology, Juntendo University, Tokyo, Japan
[d] Division of Molecular and Cell Therapeutics, Department of Molecular Genetics, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan

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ABSTRACT

Endovascular differentiation of extravillous cytotrophoblasts (EVT) during placentogenesis induces remodeling of spiral arteries that increases blood flow toward the intravillous space and is required for maintaining pregnancy. To address the molecular mechanisms involved in this differentiation, we investigated the gene expression profile during matrigel-induced tube formation in TCL1 cells, a human immortalized EVT cell line, and HUV-EC-C, human umbilical vessel endothelial cells, and compared their profiles.

The numbers of genes that showed significant up-regulation (>3-fold expression at both 3 and 6 h, and/or >5-fold expression at either 3 or 6 h) during tube formation and significant down-regulation (0.33-fold expression at both 3 and 6 h, and/or less than 0.2-fold expression at either 3 or 6 h), were 969 and 659 in TCL1, respectively. In HUV-EC-C, the numbers of genes that showed significant up-regulation and down-regulation were 86 and 65, respectively. Only 73 of 1628 genes that showed significant expression changes in TCL1 were common with HUV-EC-C. The genes showing significant expression change specifically in TCL1 were associated with cellular, metabolisms, proliferation, anti-apoptosis, proteolysis adhesion, and some known to be involved in EVT differentiation or related to angiogenesis.

The gene expression profile in EVT during tube formation is very different from that of endothelial cells. Further investigations based on the current data may help to elucidate mechanisms of normal and abnormal placentogenesis.



## 1. Introduction

Differentiation of extravillous cytotrophoblasts (EVT) has two modes: one is endovascular differentiation, in which EVT replaces and remodels spiral arteries resulting in increased blood flow toward the intervillous space. The other is interstitial invasion, which promotes placental anchorage and is accompanied by endovascular invasion [1,2]. The significance of these differentiation pathways is shown by the fact that in preeclampsia, in which both interstitial and endovascular invasions are abnormally shallow, EVT cells show significant defects in differentiation [3,4]. Concurrent with these differentiations, several molecules act as differentiation markers and change their expression patterns. For example, integrin subunit conversion during interstitial and endovascular differentiation is well characterized [5,6] and this conversion is defective in patients with placental insufficiency. These gene expression alterations corresponding to different differentiation pathways are essential for organization of the feto-maternal interface during early development.

We have previously investigated the important role of several molecules as regulators in this differentiation using matrigel-induced endovascular differentiation of TCL1 cells, immortalized human trophoblasts. Inhibiting the up-regulation of hypoxia-inducible factor 1 alpha (HIF1A) suppressed hypoxia-responsive element transcriptional activity, VEGF induction, integrin αVβ3 (ITGAV/ITGB3) aggregation and was accompanied by the inhibition of tube formation in TCL1 cells [7]. Tumor necrosis factor alpha (TNFA) can switch integrin expression and induce apoptosis in a human EVT cell line, with apoptosis being suppressed by signals via integrin β1 (ITGB1) [8]. Vascular endothelial growth factor

* Corresponding author. Department of Obstetrics and Gynecology, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. Tel.: +81 92 642 5395; fax: +81 92 642 5414.
*E-mail address:* kfuku@gynob.med.kyushu-u.ac.jp (K. Fukushima).

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(VEGF) strengthens ITGAV/ITGB3 adhesion [9]. These results also suggested that several soluble factors, together with extracellular matrix (ECM), collaboratively regulate biological behavior of EVT in early human pregnancy. However, it is still unclear how these molecules interact and regulate EVT differentiation.

In the present study, to identify molecules involved in endovascular differentiation of EVT, we performed microarray analysis during tube formation on matrigel using the human EVT cell line, TCL1, and compared its gene expression profile with that of human endothelial cells.

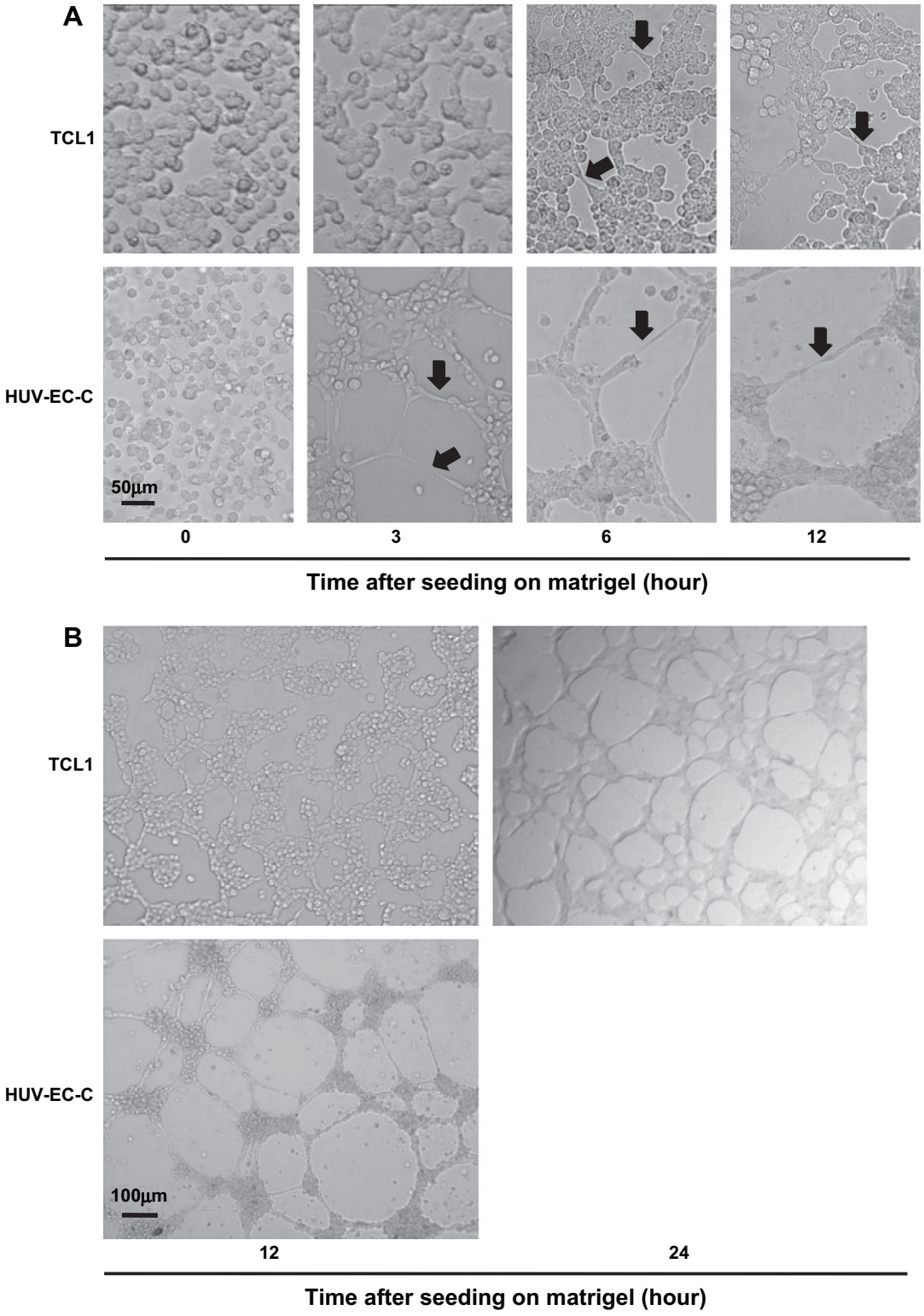


**Fig. 1.** Morphological comparison of tube formation between TCL1 and HUV-EC-C. (A) Chronological morphology during tube formation by TCL1 cells (upper panel) and HUV-EC-C cells (lower panel) after 0, 3, 6, and 12 h seeded on polymerized matrigel, Bars: 50 μm. Capillary networks (arrow). (B) Long observation (more than 12 h) with low magnification, Bars: 100 μm. Twenty-four hour observation of HUV-EC-C is not available because almost all of the cells had already detached.