Preventing Preeclampsia: The Effect of Vascular Endothelial Growth Factor on Trophoblast Differentiation

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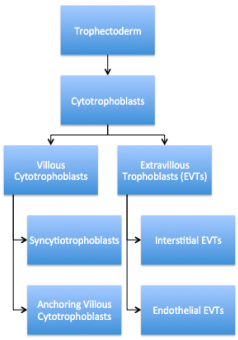
Introduction/Background

Embryonic trophoblast cells play an integral role in the adplantation, implantation and placentation of the embryo.[1] To perform these roles trophoblast cells must undergo a rigorously controlled differentiation process to form trophoblast subtypes[1] (see figure 1). One subtype is the extravillous trophoblast (EVT), which is involved in the invasion of the maternal decidua and altering the maternal vascular, to ensure an adequate supply of blood to the growing embryo. EVTs are characterised by their motile invasive phenotype. Poor EVT formation and invasion of the maternal decidua and spiral arteries is associated with an increased risk of preeclampsia.[1] Vascular endothelial growth factor (VEGF) has been thought to affect the process of trophoblast differentiation, EVT formation and behaviour.

Figure 1: Trophoblast differentiation forming trophoblast subtypes



Objectives

1.To develop an in vitro system for trophoblast growth using the SGHPL-4 and TEV-1 trophoblast cell line
2.To identify the role of VEGF in trophoblast differentiation and behaviour

SGHPL-4 and TEV-1 Trophoblast Cell Line

SGHPL-4 is a well characterised trophoblast cell line transfected with oncogenes from SV40 and were provided by Prof G.S. Whitley.[2] TEV-1 is another trophoblast cell line transfected with HPV16.[3] Both cell lines are well proven to differentiate into EVTs.

References

[1]Ji, L., Brkić, J., Liu, M., Fu, G., Peng, C., & Wang, Y. L. (2013). Placental trophoblast cell differentiation: physiological regulation and pathological relevance to preeclampsia. Molecular Aspects of Medicine, 34(5), 981-1023.
[2]Steinberg, M. L., & Robins, J. C. (2016). Cellular models of trophoblast differentiation. Seminars in Reproductive Medicine, 34(1), 50-56.
[3]Feng, H. C., Choy, M. Y., Deng, W., Wong, H. L., Lau, W. M., Cheung, A. N., et al. (2005). Establishment and characterization of a human first-trimester extravillous trophoblast cell line (TEV-1). Journal of the Society of Gynecologic Investigation, 12(4), 21-32.
[4]Bunnell, T.M., Burbach, B.j., Shimizu, Y., & Ervasti, J.M. (2011). b-Actin specifically controls cell growth, migration, and the G-actin pool. Molecular Biology of the Cell, 22(21), 4047-4058.

METHOD

SGHPL-4 and TEV-1 trophoblast cell lines were grown and maintained using established in vitro tissue culturing techniques. A known quantity of cells were treated with 0ng/ml, 10ng/ml or 50ng/ml of VEGF. Cells were then observed at 24, 48 and 72 hours after exposure for changes in behaviour. Cells were immunochemical stained for β actin, a marker of motility[4], which indicates increased EVT formation. Images were taken and examined at a set brightness threshold to quantify the presence of β actin.

Results

Figure 2: The number of trophoblast cells (out of 100) that express β actin above a set brightness threshold

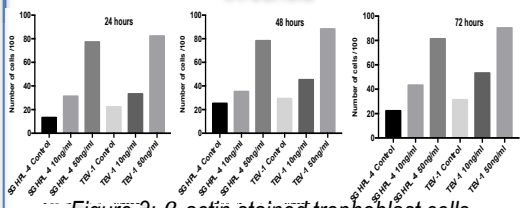
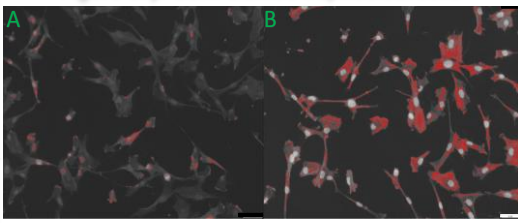


Figure 3: β actin stained trophoblast cells



The results of these experiments show a clear increase in the number of trophoblast cells that reach the threshold with higher concentrations of VEGF (See figure 2). Figure 3 shows images cells after the brightness threshold was applied. Image A and B are examples of the TEV-1 cells exposed to 24 hours of 0ng/ml and 50ng/ml of VEGF, respectively. Image B shows a much higher affinity for the staining. This indicates that β actin is more prominent when trophoblast cells have been exposed to VEGF. These results suggest that VEGF make trophoblast cells more motile. The increased motility is a sign of increase EVT formation.

Conclusion

Exposure to VEGF increase motility of trophoblasts, indicating a strong correlation between VEGF and trophoblast differentiation to form EVTs.