

NFAT molecular profiles of VEGF-induced-dental-stem-cells cultured on human amniotic membrane

Siti Nurnasihah Md Hashim, Muhammad Fuad Hilmi Yusof, Wafa' Zahari, Khairul Bariah Ahmad Amin Noordin and Ahmad Azlina*

School of Dental Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.

azlinakb@usm.my

Abstract

Nuclear factor of activated T-cells (NFAT) signalling pathway involved in endothelial differentiation have been described in the literature. However, the information about the involvement of NFAT pathway on the stem-cell-endothelial differentiation is limited. Thus, we investigated the NFAT pathway using an *in-vitro* 3D-construct. The construct that mimicked the endothelial-induced environment were made of extracellular matrix (ECM)-rich scaffold, human amniotic membrane (HAM) and pro-angiogenic growth factor, vascular endothelial growth factor (VEGF). In this study, stem cells from human exfoliated deciduous teeth (SHED) were cultured on HAM with an addition of VEGF. Cyclosporine A (CsA) inhibitor was used to inhibit the NFAT pathway. The expression level of genes associated with the NFAT signalling pathway was quantified using One-Step Real-Time PCR. SHED induced with VEGF (SHEDi) acted as a control group, while the treatment groups were SHEDi cultured on HAM (SA), SHEDi cultured on HAM treated with VEGF (SAV), and SHEDi cultured on HAM treated with VEGF and CsA (SAVC). The result showed that CsA boosted the gene expression of all gene markers (*Cox-2*, *IL-8* and *RCAN1.4*) at day 1, but not *ICAM-1*. While at days 7 and 14, *IL-8*, *ICAM-1* and *RCAN1.4* were down-regulated by CsA inhibitor. The cell morphology was undetermined, but the presence of filament-like structures could be seen through an inverted microscope. This study provides an insight on the effect of angiogenic microenvironment contributed by HAM scaffold on SHED-endothelial differentiation. Besides, this study accomplishes in showing the involvement of the NFAT pathway of the current *in-vitro* 3D-construct.

Keywords: Endothelial gene markers; human amniotic membrane; nuclear factor of activated T-cells pathway; stem cells from human exfoliated deciduous teeth; vascular endothelial growth factor

*Author for Correspondence