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ENDOTHELIAL LINEAGE DIFFERENTIATION FROM IPS CELLS IS REGULATED BY MIRNA-21/AKT AND TGF-Β2 PATHWAYS

E Di Bernardini, P Campagnolo, A Margariti, A Zampetaki, M Mayer, Y Hu, Q Xu

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Background Endothelial death/dysfunction is a critical process in the development of cardiovascular diseases. Finding a source of

endothelial cells (ECs) for regenerative medicine is a challenging yet fundamental issue. Induced pluripotent stem cells (iPSCs) constitute an attractive source of cells for transplantation because of their high proliferation and differentiation potential.

Methods and Results In this study, we investigated the mechanisms regulating iPSC differentiation into ECs, focusing on miRNAs and their targets. Firstly, we established a differentiation protocol using collagen and VEGF to drive the functional differentiation of iPSCs into ECs. After 7 days, the cells strongly expressed EC markers such as VE-cadherin, Flk-1, vWF and eNOS and formed tubes on Matrigel culture. A parallel study was conducted on embryonic stem cells (ESCs) and iPSCs to analyze the synergistic effect of VEGF and shear stress in improving EC differentiation. Next, we compared the miRNA signature of undifferentiated and differentiated cells with VEGF for 3 days. Amongst the 5 validated miRNAs we focused on miR-21, previously shown to be involved in angiogenesis. Overexpression of miR-21 (pre-21) in pre-differentiated iPSCs induced EC marker upregulation, while inhibition miR-21 (LNA-21) produced the opposite effect. Accordingly, in vitro and in vivo capillary formation was increased by pre-21 and reduced by LNA-21. Interestingly, miR-21 overexpression increased TGFβ2 mRNA and secreted protein level, which was consistent with the strong upregulation of TGFβ-2 during VEGF-induced iPSC differentiation. In addition treatment of iPSCs with TGFβ-2 induced EC marker expression and in vitro tube formation through induction of VEGF secretion. Inhibition of SMAD3, a downstream effector of TGFβ-2, strongly decreased VE-cadherin expression, indicating the importance of the TGFβ-2 pathway in the differentiation process. Furthermore, TGFβ-2 neutralizing antibody inhibited miR-21 induced EC marker upregulation, indicating that TGFβ-2 is a required downstream effecter for miR-21-mediated EC differentiation. In silico target screening revealed PTEN as one of the predicted targets of miR-21. Our results confirmed that pre-21 significantly inhibited PTEN expression whereas LNA-21 induced it. Luciferase assay confirmed that miR-21directly binds to the 3' UTR of PTEN, which is inhibited by a site-specific mutation. Since PTEN is the upstream negative regulator of AKT signaling pathway, we then confirmed that pre-21 induced Akt phosphorylation, while LNA-21 decreased it. Functionally, it was found that inhibition of miR-21 in vivo Matrigel assay revealed the significant influence on angiogenesis in animal models.

Conclusion These data demonstrate that miR-21 directly targets the PTEN/Akt pathway, in which TGFβ-2 pathway regulated by miR-21 is also involved. Thus, the molecular mechanisms elucidated in this work might provide the basic information for stem cell therapy for vascular disease, e.g. tissue engineering and endothelial repair in damaged vessels.