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KRUPPEL-LIKE FACTOR 4 PROMOTES DIFFERENTIATION OF ENDOTHELIAL PROGENITOR CELLS INTO ENDOTHELIAL CELLS BY UP-REGULATING ENDOTHELIAL NITRIC OXIDE SYNTHASE

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Objective The differentiated function of Endothelial progenitor cells (EPCs) may play a pivotal roles in endothelial repair and reendothelialisation replacement of postinjury endothelium. However, the mechanism of differentiation of EPCs remains unclear. One of zinc finger transcription factors family members, KLF4, was involved in the regulation of numerous biological processes including proliferation, differentiation, development, and apoptosis. The present study was designed to investigate whether transcription factor, Krüppel-like factor 4 (KLF4), is involved in the differentiation of EPCs derived from bone marrow (BM-EPCs) into endothelial cells (ECs).

Methods Mononuclear cells were obtained from Sprague–Dawley rats bone marrow by Ficoll density gradient centrifugation and cultured with medium DMEM/F12 in culture dishes, the secondary attached cells were characterised as adherent cells which were double positive for Dil-ac-LDL uptake and lectin binding by direct fluorescent staining demonstrated under a laser scanning confocal microscope. Flow cytometry (FACS) analysis was performed using antibodies against rat CD133 and CD34. The KLF4 and eNOS mRNA expression with reverse transcription-polymerase chain reaction (RT-PCR) methods in differentiated endothelial progenitor cells.

Results We analysed the expression of KLF4 in differentiating EPCs and found that KLF4 expression was low during the early stages of differentiation but had increased significantly by day 14 of culture. The staining revealed that KLF4 was localised predominantly to the cytoplasm and nucleus. Overexpression of KLF4 up-regulated the expression of endothelial nitric oxide synthase (eNOS) and of markers of mature ECs in EPCs. In addition, overexpression of KLF4 dramatically increased the number of cells that took up both DiL-labelled acetylated low density lipoprotein and FITC-labelled Ulex europaeus agglutinin-1. Expression of the markers of mature ECs, was inhibited dramatically in EPCs transfected with KLF4-specific siRNA. The effects of KLF4 could be blocked by treatment with L-NAME, an inhibitor of eNOS.

Conclusions KLF4 promotes the differentiation of EPCs into ECs by increasing the expression of eNOS and the availability of nitric oxide, which might represent, at least in part, a novel mechanism of EPC differentiation.