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Regulation of cell proliferation by large-conductance calcium-activated potassium and volume-sensitive chloride channels in human cardiac fibroblasts

ML He, CP Lau, HF Tse, GR Li
Department of Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong

Background: Cardiac fibroblasts play a central role in the maintenance of extracellular matrix in the normal heart and as mediators of inflammatory and fibrotic myocardial remodelling in the injured and failing heart. Excessive fibroblast proliferation and increase in the extracellular matrix increase myocardial stiffness and cause ventricular dysfunction and subsequent heart failure. Our previous study demonstrated that multiple ion channels were heterogeneously expressed in human cardiac fibroblasts, including IK_{Ca} (large-conductance calcium-activated potassium current), $I_{Cl.vol}$ (volume-sensitive chloride current), and sodium current (I_{Na}). Little is known about the functional involvement of these ion channels in cardiac fibroblasts, and the present study was therefore designed to examine the possible involvement of these ion channels in proliferation of human cardiac fibroblasts.

Methods and results: Using MTT assay and ^{3}H-thymidine incorporation assay, we found that the I_{Na} current blocker tetrodotoxin had no effect on cell proliferation; however, the specific big conductance I_{KCa} blocker paxilline (1 and 3 μM) and the volume-regulated chloride channel blocker 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid disodium (DIDS, 100-200 μM) remarkably suppressed proliferation of human cardiac fibroblasts with 48 h incubation. Knockdown of KCa1.1 or CLC3 with specific siRNAs significantly reduced I_{KCa} or $I_{Cl.vol}$ channel protein levels, the cell proliferation was decreased by the corresponding siRNA. Flow cytometry analysis showed that human cardiac fibroblasts retained at G_0/G_1 phase (control, 55.8%) by paxilline (3 μM, 79.2%, $P<0.01$) or DIDS (200 μM, 72.8%, $P<0.05$) or the corresponding siRNAs; meanwhile distribution of cells in S phase was decreased. Western blot analysis revealed a reduced expression of the cell cycle regulatory proteins cyclin D1 and cyclin E.

Conclusion: Our results demonstrate that I_{KCa} and $I_{Cl.vol}$ channels, but not I_{Na} channels, participate in the regulation of proliferation of human cardiac fibroblasts by promoting cell cycle progression via modulating cyclin D1 and cyclin E expression.

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Treatment of endothelial progenitor cells dysfunction in patients with type 2 diabetes with human embryonic stem cells–derived endothelial progenitor cells

JCY Ho, WH Lai, MF Li, NLY Wong, MC Ip, KW Au, CW Siu, HF Tse
Division of Cardiology, Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Clinical studies suggest that endothelial progenitor cells (EPCs) can enhance angiogenesis and improve cardiac function in patients with myocardial ischaemia. However, the use of autologous EPCs therapy in those patients with diabetes is limited by the functional impairment in their EPCs. Recent advance in the availability of EPC derived from human embryonic stem cell (HES) could be a potential cell source to overcome the hurdles of the intrinsic scarcity and phenotypic deficiencies of EPCs.

Methods and Results: We investigated the cytokine profile and angiogenic potential of EPCs isolated from peripheral blood of type 2 diabetic patients (DM-EPC) and healthy controls (C-EPC). Cultured human umbilical vascular endothelial cells (HUVEC) and endothelial cells derived from HES (HES-EPC) were also assessed to evaluate their therapeutic potential. Furthermore, tube formation assay was performed to assess the in-vitro angiogenic potential of different source of EPCs. Using cytokine profiling, two angiogenic factors, vascular-endothelial growth factor (VEGF, 197 vs 664 pg/mL; $P<0.001$) and angiogenin (418 vs 866 pg/mL; $P<0.001$) were shown to be down-regulated in DM-EPC compared with C-EPC. Tube formation assay revealed functional impairment of DM-EPCs which could be rescued by the replenishment of VEGF and angiogenin. More importantly, condition medium from cultured HES-EC, but not from HUVEC could also rescue the functional impairment of DM-EPC ($P<0.05$). When injected into the hind muscles in immunodeficient SCID mice subjected to unilateral ischaemia, $CD34^+CD31^+$ HES-EPC cells but not angiogenic factors cocktail improved limb salvage and haemodynamic ($n=6$, $P<0.05$).

Conclusion: Our results demonstrate that angiogenic factors secreted from HES-EPC can restore the function of EPCs in patients with type 2 diabetes, which can potentially improve the clinical efficacy of EPC therapy in those patients.

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