

## **“VEGF induces human endothelial progenitor cells proliferations by eliciting oscillations in intracellular Ca<sup>2+</sup> concentration”**

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Endothelial progenitor cells (EPCs) traffic from the bone marrow to the site of tissue regeneration and sustain neo-vascularization after acute vascular injury and upon the angiogenic switch in solid tumors. Therefore, they represent a suitable tool for cell-based therapy in regenerative medicine and provide a novel promising target in the fight against cancer. The main stimulus responsible for EPC egression from the bone marrow and engraftment within neovessels is vascular endothelial growth factor (VEGF). Intracellular Ca<sup>2+</sup> signals regulate numerous endothelial functions, such as proliferation, migration, and differentiation, and underpin VEGF effect on mature endothelium. We have recently shown that EPC growth is governed by a store-dependent Ca<sup>2+</sup> entry (SOCE) pathway on the plasma membrane, which is activated by depletion of the inositol-1,4,5-trisphosphate (InsP<sub>3</sub>)-sensitive Ca<sup>2+</sup> pools<sup>1</sup>. The present study aimed at investigating the nature and the role of VEGF-elicited Ca<sup>2+</sup> signals in EPCs. All the putative SOCE mediators (i.e. TRPC1, TRPC4, Orai1 and Stim1) were present in EPCs. VEGF induced long lasting Ca<sup>2+</sup> oscillations, however, removal of external Ca<sup>2+</sup> (0Ca<sup>2+</sup>) and SOCE inhibition with BTP-2 reduced the number of Ca<sup>2+</sup> spikes. Blockade of phospholipase C- $\beta$  (PLC- $\beta$ ) with U73122 and emptying the InsP<sub>3</sub>-sensitive Ca<sup>2+</sup> pools with cyclopiazonic acid (CPA) prevented the Ca<sup>2+</sup> response to VEGF. Accordingly, the Ca<sup>2+</sup> response to VEGF was inhibited by superfusing CPA during the ongoing oscillations. Notably, VEGF induced EPC was abrogated by SOCE inhibition with BTP-2. Similarly, VEGF promoted NF- $\kappa$ B translocation into the nucleus in a BTP-2-sensitive manner. Thus, VEGF causes an initial InsP<sub>3</sub>-dependent Ca<sup>2+</sup> discharge followed by SOCE-mediated Ca<sup>2+</sup> entry in cEPCs. SOCE, in turn, controls store refilling and induces cell proliferation by recruiting NF- $\kappa$ B.

Keywords: Calcium flux, Cellular proliferation, Endothelial progenitor cell, Tube formation, VEGF