

## Targeting of NAADP-mediated calcium signaling affects VEGF-induced angiogenesis

Annarita Favia<sup>1</sup>, Guido Gambarà<sup>1</sup>, Alessio D'Alessio<sup>3</sup>, Marianna Desideri<sup>2</sup>, Donatella Del Bufalo<sup>2</sup>, Elio Ziparo<sup>1</sup>, Fioretta Palombi<sup>1</sup> and Antonio Filippini<sup>1</sup>

<sup>1</sup> Department of Anatomy, Histology, Forensic Medicine and Orthopaedics, Section of Histology and Medical Embryology, SAPIENZA University of Rome, Via A. Scarpa 16, 00161 Rome, Italy

<sup>2</sup> Experimental Chemotherapy Laboratory, Regina Elena National Cancer Institute, Rome, Italy

<sup>3</sup> Istituto di Istologia ed Embriologia - Facoltà di Medicina e Chirurgia "A. Gemelli", L.go Francesco Vito 1, Roma

Vascular endothelial growth factor (VEGF) and its transmembrane receptors VEGFR1 and VEGFR2 play a key role in controlling both physiological and pathological angiogenesis, including vascularization of solid tumours. We have identified a novel and crucial signalling mechanism through which activation of VEGFR2 in human endothelial cells (HUVEC) selectively triggers the intracellular release of calcium from acidic compartments, operated by the second messenger NAADP (nicotinic acid adenine dinucleotide phosphate). Live imaging of calcium fluxes in cells treated with VEGF in the presence of specific inhibitors have shown that 1) VEGF-activated calcium stores are different from IP<sub>3</sub> and ryanodine sensitive compartments and are of acidic nature, which strongly indicates the involvement of NAADP signalling 2) NAADP inhibition by its specific antagonist Ned-19 abolishes VEGF-induced calcium response. This inhibition is accompanied by impaired phosphorylation of downstream targets ERK1/2, Akt, eNOS, JNK (but not p38) and results in significant reduction of cell proliferation, migration and capillary-like tube formation *in vitro*. Interestingly, when the angiogenic response to VEGF was assayed *in vivo* utilizing Matrigel plugs subcutaneously implanted in mice, Ned-19 was found to dramatically inhibit VEGF-induced angiogenesis. Altogether our data showing that NAADP plays a key role in the control of VEGF-induced angiogenesis could potentially contribute to identify new targets for antiangiogenic therapeutic strategies, a goal to which much scientific effort has long been devoted but still awaiting ultimate success.

---

### Key words

Endothelial cells, angiogenesis, calcium signalling, tumour angiogenesis.