

## Circulating endothelial progenitor cells from patients with renal cell carcinoma display aberrant VEGF regulation, reduced apoptosis and altered ultrastructure

Germano Guerra<sup>1</sup>, Valentina Poletto<sup>2</sup>, Marco Biggiogera<sup>3</sup>, Silvia Dragoni<sup>3</sup>, Dmitry Lim<sup>4</sup>, Camillo Porta<sup>5</sup>, Stefania Montagnani<sup>6</sup>, Domenico Tafuri<sup>7</sup>, Vittorio Rosti<sup>2</sup>, Francesco Moccia<sup>3</sup>

<sup>1</sup> Department of Medicine and Health Sciences "Vincenzo Tiberio", University of Molise, Campobasso, Italy -

<sup>2</sup> Center for the Study of Myelofibrosis, Biotechnology Research Laboratory, Fondazione IRCCS Policlinico San Matteo, 27100, Pavia, Italy - <sup>3</sup> Department of Biology and Biotechnology, University of Pavia, 27100, Pavia, Italy

- <sup>4</sup> Department of Pharmaceutical Sciences, University of Piemonte Orientale "Amedeo Avogadro", 28100, Novara, Italy - <sup>5</sup> Medical Oncology, Fondazione IRCCS Policlinico San Matteo, 27100, Pavia, Italy - <sup>6</sup> Department of Public Health, University of Naples "Federico II", 80131, Napoli, Italy - <sup>7</sup> Department of Sport Sciences and Wellness,

University of Naples "Parthenope", 80133, Napoli, Italy

Endothelial colony forming cells (ECFCs) are the only endothelial progenitor cells (EPCs) subtype belonging to the endothelial phenotype and capable of forming neovessels *in vivo*. We have recently shown that the intracellular Ca<sup>2+</sup> machinery plays a key role in ECFC activation and is remodeled in ECFCs isolated from patients suffering from renal cellular carcinoma (RCC-ECFCs). More specifically, ECFCs upregulate the store-operated Ca<sup>2+</sup> entry (SOCE) machinery, while they seemingly show a reduction in the Ca<sup>2+</sup> concentration within the endoplasmic reticulum ([Ca<sup>2+</sup>]<sub>ER</sub>). Metastatic RCC patients are commonly treated with an anti-vascular endothelial growth factor (VEGF) therapy, but they show either intrinsic or adaptive refractoriness, which ultimately leads to their death. Herein, we assessed whether and how the rearrangement of the Ca<sup>2+</sup> machinery impacts on the pro-angiogenic Ca<sup>2+</sup> response to VEGF, which stimulates normal ECFCs (N-ECFCs) through an oscillatory Ca<sup>2+</sup> response. We found that VEGF stimulates the nuclear translocation of p65/RelA, a major component of the Ca<sup>2+</sup>-dependent transcription factor NF-κB, in N-ECFCs. This process is blocked by the pharmacological abrogation of VEGF-induced Ca<sup>2+</sup> oscillations. We further showed that NF-κB controls VEGF-induced protein expression of E-selectin, VCAM-1 and MMP9. Likewise, VEGF-induced expression was also inhibited by the pharmacological suppression of the accompanying Ca<sup>2+</sup> spikes. Thus, VEGF induces a Ca<sup>2+</sup>-dependent, NF-κB-mediated protein expression in N-ECFCs. VEGF did not trigger protein expression in RCC-ECFCs despite the fact that VEGFR-2 was normally expressed and auto-phosphorylated. Our subsequent studies employed the targeted recombinant Ca<sup>2+</sup>-sensitive photoprotein aequorin to confirm that [Ca<sup>2+</sup>]<sub>ER</sub> is lower in RCC-ECFCs; surprisingly, electron microscopy analysis revealed that the endoplasmic reticulum cisternae are enlarged rather than shrunk in these cells. These results show for the first time that VEGF fails to stimulate tumor-derived ECFCs: these findings could therefore help to understand the relative failure of anti-VEGF treatment in RCC patients.

### References

- [1] Lodola F et al. (2012) Store-operated Ca<sup>2+</sup> entry is remodelled and controls *in vitro* angiogenesis in endothelial progenitor cells isolated from tumoral patients. *PLoS One* 7(9):e42541 doi: 10.1371/journal.pone.0042541.

### Keywords

Renal carcinoma; VEGF; Endothelial Progenitor Cells (EPCs); Ca<sup>2+</sup> machinery.