Candidate: Emanuela Pupo
Advisor: Prof. Luca Munaron

Suggested referees:

- Prof. Mohamed Trebak
- Prof. Thierry Capiod

Pro-angiogenic calcium signals in tumor-derived endothelial cells

Tumor vascularization is a very peculiar and complex event, very different from the physiological one and fundamental for cancer progression. Several groups have recently focused their attention on tumor-derived ECs (TEC) as a more adequate model to study tumor angiogenesis. Interestingly, TECs display a distinct and unique phenotype different from normal vascular ECs at molecular and functional levels. In particular TECs obtained from breast carcinomas (B-TECs) have been established and well characterized showing an immature proangiogenic phenotype with enhanced proliferation, migration, and capillary-like tube formation compared with 'normal' ECs.
We recently showed that arachidonic acid (AA) triggers intracellular calcium increase in B-TECs and that the observed Ca^{2+} entry involved in early steps of tumor angiogenesis *in vitro*. In the first part of my thesis we investigated the multiple roles of the nitric oxide (NO) and cyclic AMP/protein kinase A (PKA) in the regulation of AA-mediated Ca^{2+} signaling in B-TECs. B-TEC stimulation with 5 μmol/L AA resulted in endothelial NO synthase (NOS) phosphorylation at Ser1177, and NO release was measured with the fluorescent NO-sensitive probe DAR4M-AM. PKA inhibition by the use of the membrane-permeable PKA inhibitory peptide myristoylated (PKI_{14-22}) completely prevented both AA- and NO-induced calcium entry and abolished B-TEC migration promoted by AA. Furthermore AA-dependent calcium entry and cell migration were significantly affected by both the NOS inhibitors suggesting that NO release is functionally involved in the signaling dependent on AA. Interestingly, AA-induced Ca_c increase is significantly lower in 'normal' ECs and is not able to promotes HMVECs migration conversely to what was observed in B-TECs.
In the second part we focused on hydrogen sulfide (H_2S) which is now considered, together with CO and NO, the third gasotransmitter in biology and medicine. At the cardiovascular level H_2S is a known vasorelaxant thank to its effect on the smooth muscular tissue and it decreases myocardial contractility both in vitro and in vivo in fact knockout mice for the enzyme that endogenously produce H_2S (CSE) develop hypertension. Otherwise in several papers its role in cardioprotection from strokes has been demonstrated. Since H_2S has been recently proposed to exert a role in angiogenesis progression, we investigated the effects of H_2S on B-TEC. Ca^{2+} imaging shows that acute perfusion with NaHS, a widely employed H_2S donor, triggers cytosolic calcium increase (Ca_c) in B-TECs: such response is drastically lower in 'normal' human endothelial cells (HMVECs). Moreover NaHS failed to promote either migration or proliferation on HMVECs, while B-TEC migration was enhanced at low micromolar NaHS concentrations (1–10 μM). Remarkably H_2S mediates tumor pro-angiogenic signaling, as suggested by the dramatic inhibitory effect on VEGF-induced B-TEC migration and calcium signaling exerted by DL-propargylglycine, an inhibitor of H_2S producing enzyme cystathionine-γ-lyase.
Interestingly the functional relevance of both AA- and H_2S-induced calcium entry could be restricted to tumor-derived endothelial cells (EC). In fact both molecules evoked a smaller calcium entry in normal human microvascular ECs compared with B-TECs, and even more importantly, were unable to promote cell motility in wound healing assay. This evidence opens an intriguing opportunity for differential pharmacologic treatment between normal and tumor-derived human ECs.