Introduction: An important feature of type 1 diabetes (T1D) is the existence of the so-called "angiogenic paradox", a phenomenon in which the same organism presents exacerbated vascularization in certain organs (e.g. kidney) and impaired angiogenesis in others (e.g. heart). AMPK is an energy sensor that targets carbohydrate and lipid metabolism by modulating gene expression and is an insulin sensitizing molecule, rendering this kinase an ideal therapeutic target.
Objectives: To study the effects of administration of AICAR, Compound C and Metformin on the angiogenic behavior of human microvascular endothelial cells (HMEC-1), as well as, to analyze the possible changes in expression of genes related with metabolism.
Methodology: HMEC-1 were cultured in with two different glucose concentration: 5.5mM D-Glucose (low glucose (LG)) or 20mM D-Glucose (high glucose (HG)) and treated with AICAR, Compound C or Metformin to evaluate angiogenic behaviour by proliferation, migration and tube capillary formation assays. Alterations of genes related with metabolism and angiogenesis were verified by q RT-PCR.
Results: All treatments induced a decrease in proliferation, migration and tube formation in HMEC-1 when subjected to both LG and HG. The analysis of metabolic and angiogenic gene expression showed some alterations: Kdr, Pfkfb2, Tgfb2, Timp2 and Jag1 gene expression were increased when treated with all compounds *vs* control group; Smad5 reduced with AICAR treatment, but increased with Compound C and Metformin in LG condition. Timp2 expression was increased with AICAR and Compound C treatment and decreased with Metformin administration when compared to LG control.
Conclusion: Administration of AICAR, Compound C and Metformin alters the angiogenic behavior of HMEC-1 and can modulate the expression of important genes related to angiogenesis and cellular metabolism. These preliminary results bring new insights in the crosstalk between endothelial metabolism and angiogenic behavior and may be useful to develop new therapeutic approaches to counteract diabetic complications.

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16 – Endothelial metabolism impacts on vascular behaviour in type 1 diabetic mice

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Introduction: "Angiogenic paradox" is a vascular phenomenon common in type 1 Diabetes (T1D), a metabolic disorder characterized by chronic hyperglycaemia associated with micro and macrovascular complications in several organs. Accordingly, in the same patient, angiogenesis is exacerbated in some organs (e.g. in the retina and kidney) as well as impaired in others (e.g. heart).
Objectives: Analyze the expression profile of genes associated to metabolism and angiogenesis in renal and cardiac endothelial cells of T1 diabetic mice.
Methodology: T1DM was induced in C57Bl/6 mice by administration of streptozotocin. Kidneys and hearts were isolated ten weeks after T1D development and endothelial cells (ECs) were isolated by FACS. Total RNA samples were submitted to Angiogenic and AMPK Signaling PCR Arrays. Microvessel density (MVD) in kidney and heart tissue were quantified by immunohistochemistry with CD31 staining.
Results: Upregulation of 5 genes were found upon AMPK Signaling PCR Array analysis: Adra1a, Cpt1a, Pfkfb2, Strada and Rb1cc1 in the kidney ECs. Inversely, Cab39, Akt2, Rps6kb2, Adra2c, Pnpla2, Prkacb transcripts were down-regulated in heart ECs. The analysis of the Angiogenesis PCR Array showed Tgfb2, Kdr and Timp2 down-regulated in the kidney, while in the heart Notch ligand, Jag1, was downregulated whereas Smad5 expression was upregulated. MVD analysis showed a significantly increased of the number of CD31-positive ECs in kidneys of diabetic mice when compared to healthy animals, whereas in heart there was the slight reduction.
Conclusion: Imbalances in mTOR, Akt and PI3K signaling, as well as growth factors involved in angiogenesis were found in ECs from the two organs, implying metabolic changes. Elucidating the crosstalk between endothelial metabolism-vascular complications will enable novel therapeutic approaches.

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17 – Role of sex hormones in the innate immunity against prostate cancer cells

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Background: Innate immunity and inflammation may increase the risk of prostate cancer. Sex hormones influence the state of inflammatory immune responses, previous studies show that hormonally active androgens are anti-inflammatory, whereas estrogens are pro-inflammatory thus sex hormones can modulate macrophage response. Chronic inflammation can be a major contributor to prostatic cancer and the link between them of major importance to the development of novel therapeutical approaches via molecular targeting of inflammatory mediators and immunotherapy-based approaches.
Objectives: In the present study we investigated the effects of testosterone and estradiol on macrophage activation of the innate immune response using macrophages-like cells (RAW 264.7).
Methods: Induced cytotoxic and antitumor response of RAW 264.7 to M1 phenotype by exposure to E. coli lipopolysaccharide. After M1 phenotype induction macrophages were exposed to sex hormones. This will trigger various activation rates and different outcomes in prostate cancer cell line (PC3).
Results: It was observed an increase in the activation status of RAW 264.7 with a concentration of $1x10_{-6}$M both in estradiol and in testosterone. Although in co-culture there aren't significant differences when compare with the control. It seems that hormonal supplementation overlaps to the immunotoxic response.
Conclusions: This cellular model represents a good study model to future *in vitro* studies in immunotherapies, especially in combine therapies.