STUDY OF NOVEL MODULATORS OF STORE-OPERATED CALCIUM ENTRY AS A THERAPY FOR TUBULAR AGGREGATE MYOPATHY

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Introduction: Calcium is a "life-and-death" signal as its fine cytosolic regulation can trigger numerous signalling pathways, at times with opposite cellular outcomes. Cells have therefore evolved to use specific toolkits, taking into consideration that changes in the spatial, temporal and amplitude characteristics of the cytosolic Ca^{2+}-rises encode for specific signals. Among these proteins, Orai and STIM mediate store-operated Ca^{2+}-entry (SOCE), i.e. the ability of cells to sense a decrease in endoplasmic reticulum luminal Ca^{2+} and induce Ca^{2+} -entry across the plasma membrane. Genetic defects of STIM1and Orai1proteins that give rise to loss-of-function (associated to immunodeficiencies) and gain of function (GoF) disorders have been described. Gof mutations affect primarily skeletal muscle and platelets, although other organs may also be affected. Both STIM1and Orai1mutations are linked to three separate, but overlapping, disorders: tubular aggregate myopathy (TAM), Stormorken syndrome (STRMK) and York platelet syndrome (YPS). While the mutations have different clinical presentations, quality of life of patients is severely hampered and no curative trial is at present on-going suggesting that these represent highly neglected diseases. Nevertheless, it is well known that the core cellular defect in TAM is an increased SOCE. SOCE inhibitors are tolerated in mouse models of acute pancreatitis and this has led to five clinical trials that are at present undergoing, thereby providing evidence that this pharmacological strategy could be tolerated by TAM patients.

Materials and methods: We have generated cell lines bearing the most common mutations leading to these disorders (F108I, I1151F, H109R for STIM1and P245L, S97C for Orai1) by site-specific mutagenesis approach, which represent in vitro tools to investigate TAM. Furthermore, we have generated a mouse colony bearing the p.I115F (c.A343T) constitutive knock-in point mutation (KI-STIM1^{I115F}) on a C57Bl/6background. This is among the most frequent mutations reported (present also in Italy), although it is not the most severe mutation in patients.

Results: First, we demonstrated that Hek cells over-expressing wild-type STIM1display a moderate increase in SOCE compared to controls while the increase is significantly larger in extent and rate in cells expressing any of the mutated STIM1or Orai1proteins. In order to translate the information to patients, we have also shown that myotubes from a patient (p.S97C on Orai1) display a significantly higher SOCE, alongside spontaneous oscillations compared to control myotubes. Second, we have demonstrated that KI-STIM1^{I115F} mice reflects the human physiopathology with (i) impaired body and muscle growth, (ii) enhanced SOCE in myotubes, (iii) muscle weakness, (iv) platelets count alteration, (v) increased monocytes population.Last, we have characterized a new series of SOCE inhibitors termed pyrtriazole, that are efficacious against GoF mutations in heterologous systems, and in patient and KI- STIM1^{I115F} myotubes. The best-characterized compound, BV37, displays specificity, has been characterized electrophysiologically, and, most importantly, is efficacious in reducing cerulein-induced acute pancreatitis, another disorder characterized by excess of SOCE activity. This provides reassurance that it can be tested in the KI- STIM1^{I115F} mouse model.

Discussion and conclusions: In conclusion, we have completely characterized the KI-STIM1^{I115F} mouse model and we have discovered series of inhibitors of SOCE that mitigate in vitro and *ex vivo* the effect of the defect and one of them is efficacious in cerulein-induced acute pancreatitis. The aims of this proposal are to: (i) establish whether these GoF disorders are amenable to pharmacological treatment with SOCE inhibitors, (ii) provide both indications of mechanisms underlying the phenotype downstream of Ca^{2+}-dys-homeostasis and possible surrogate biomarkers to follow the disorders.