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Dysregulation of store-operated calcium entry in myelofibrosis-derived monocytes

Olga Kulemina[1], Ekaterina Klimenko[1], Ksenia Levchuk[1], Roman Vlasik[1], Leonid Khordzhasov[1], Elza Lomaia[1].

[1]Almazov National Medical Research Centre, Saint-Petersburg, Russian Federation

INTRODUCTION

The store-operated calcium entry (SOCE) is a key mechanism for maintaining STIM-ORAI signaling and Ca^{2+} balance, which is necessary for cell proliferation, including tumor stem cells. The role of SOCE in myelofibrosis (MF) pathogenesis and response to treatment is not well understood[1,2]

AIM

To study the role of SOCE in the pathogenesis and treatment response in MF

METHOD

Whole blood samples were obtained from healthy donors (HD; n=19) and patients with MF associated with JAK2^{V617f} or CALRI (n=15) after local ethical approvement and subjects' informed consent. Monocytes were isolated from peripheral blood mononuclear cells by 24h-plastic adherence. Fluorescent measurements of calcium were performed on Zeiss Observer.Z1 using 2 μM Fluo-4AM and were normalized to basal intensity. For endoplasmic reticulum (ER) depletion, 1 μM thapsigargin was used. The amplitudes of ER depletion and SOCE were analyzed in MF- compared to HD-monocytes and according to treatment: hydrea (HU) or ruxolitinib (RUX). Dynamics of SOCE activity was measured in CALR-mutated monocytes before and during 16 months of RUX treatment. Expression profile of SOCE components was analyzed by flow cytometry with anti-TRPC1-AF594, anti-Orai1-AF647 and anti-STIM1-AF680 antibodies. Statistical analysis was performed using GraphPad Prism 10 software.

RESULTS

The SOCE was 1.7-2.2-fold decreased in both treated JAK2-MF and CALR-MF ($p<0.001$) and, conversely, was 1.7-fold increased in untreated CALR-MF monocytes compared to HD ($p=0.04$; Figure 1). Simultaneously, ER depletion amplitude had a 2.5- to 3.1-fold increase in HU-treated JAK2-MF and both RUX-treated and untreated CALR-MF monocytes ($p<0.0001$).

The improvement of both SOCE and ER-depletion amplitude was observed at 4 mo of RUX, reaching HD values and corresponded to clinical improvement of MF. The ER depletion amplitude had a 2.5- to 3.1-fold increase from 4 to 10 mo of RUX ($p<0.0001$), whereas SOCE amplitude tended to fall (1.4- to 0.4-fold decrease; $p<0.05$). At the last time point, both SOCE and ER-depletion amplitude returned to the untreated CALR-MF levels, consistent with disease progression (Figure 3&4).

The overexpression of Orai1 and STIM1 was observed on both treated JAK2- and CALR-MF monocytes ($p<0.05$; Figure 5&6).

Fig. 1 SOCE amplitude

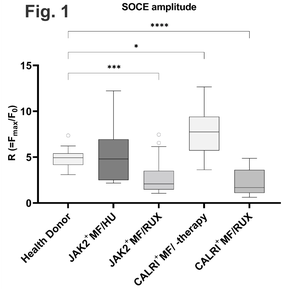


Fig. 2 ER-depletion amplitude

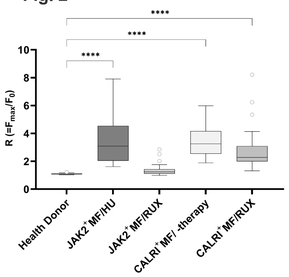


Fig. 3 SOCE amplitude

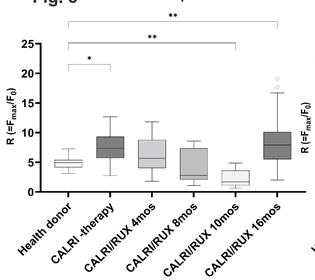


Fig. 4 ER-depletion amplitude

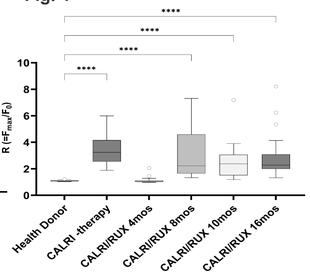


Fig. 5 Orai1 Monocytes

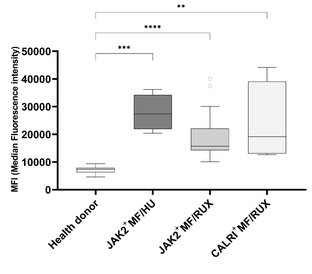
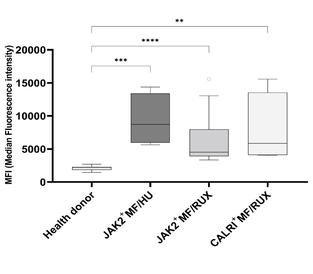


Fig. 6 STIM1 Monocytes



SOCE – store-operated calcium entry; MF – myelofibrosis ; ER – endoplasmic reticulum; RUX- ruxolitinib treatment; HU – hydroxyurea treatment; * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<0.0001$

CONCLUSIONS

We demonstrated significant dysregulation of SOCE in myelofibrosis. The SOCE was inhibited during RUX treatment and changed drastically at the end time point. We supposed that overexpression of Orai1 and STIM1 can be compensatory to inhibition of SOCE by treatment. The concordance of ER-depletion to HD levels at the first time point with its decreasing to untreated level together with decreasing of SOCE amplitude could represent the ER-stress during treatment of MF.

REFERENCES

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2. Pinto, Mauro Cunha Xavier et al *Cellular signalling* vol. 27,11 (2015): 2139-49.

CONTACT INFORMATION

Olga Kulemina, MD: *olkulem@gmail.com*