OTR.19 - TET2 mutation in cellular reprogramming and hematopoietic differentiation

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Introduction:

Primary myelofibrosis (PMF) is a myeloproliferative neoplasm characterized by an clonal myeloproliferation. In PMF, driver somatic mutations occur in *JAK2* , *MPL* or *CALR* genes. Mutations in epigenetic regulators as *TET2* and *ASXL1* that could lead to loss-of-function were frequently identified. In this context, induced pluripotent stem cells (iPSC) could be used to study clonal heterogeneity and to recapitulate *in vitro* some hematological features of PMF. *Tet2* deletion in mouse embryonic fibroblast was shown to reduce cellular reprogramming efficiency.

Objective:

The main goal of this work was to assess the impact of somatic mutations in *CALR/TET2* in both cellular reprogramming and hematopoietic differentiation using iPSC

Methodology:

We used next generation sequencing to screen a cohort of Brazilian PMF patients for myeloid somatic mutations. One of the patients granulocytes (P1) were shown by Sanger sequencing to harbor $CALR^{ins5}$ and $TET2^{G898X}$ mutations. CD34+ primary cells were isolated from P1 or a healthy donor control (C1) and erythroblasts were differentiated in vitro. We generated iPSC from P1 (P1-iPSC) or from the control (C1-iPSC) erytroblasts using the Sendai virus system. The pluripotency was confirmed in iPSC colonies by the expression of embryonic stem cells markers. The capacity to form germ layers was evaluated by embryonic body formation and layer specific markers detection by immunohystochemistry. Hematopoietic differentiation was performed on feeder-free culture supplemented with cytokines and CD43+CD34+ progenitors sorted at day 10-14. Myeloid Colony-Forming Units (CFU) were quantified in methylcellulose assays, under microscopic evaluation on day 12. CFU-Megakaryocytes were scored in plasma clots assays, after 9 days of culture and labeling with anti-CD41a antibody and alkaline phosphatase staining. Granulocytes were differentiated from CD34+CD43+ progenitors in liquid culture during 20 days.

Results:

After cellular reprogramming, 32 P1-iPSC clones were obtained, 20 $CALR^{ins5}$/$TET2^{wt}$ and 12 $CALR^{ins5}$/$TET2^{G898X}$ homozygous. The different genotypes observed for iPSC reflect the clonal diversity present in the PMF primary sample. We confirmed that *TET2*wt as well as *TET2*G898X iPSCs displayed pluripotency and all three germ layers markers. We next sought to study the role of these mutations in the hematopoietic differentiation. We observed that all type of myeloid colonies were generated in methylcellulose assays for P1-iPSC and C1-iPSC. Our preliminary results show that an increased number of granulocyte/monocyte colonies derived from CD34+CD43+ progenitors of P1-$TET2^{G898X}$ iPSCs, when compared both with iPSC P1-$TET2^{WT}$ iPSCs and C1-iPSC. Using plasma clot assay, we observed higher numbers of large CFU-MK colonies derived from P1-iPSC versus C1-iPSC.

Conclusion:

Our results suggest that the $TET2^{G898X}$ did not impair cellular reprogramming, since iPSC harboring this mutation displayed all the features of bona fide iPSC and that mutations in *CALR* and *TET2* have an impact on hematopoietic differentiation of iPSC.

Keywords: iPSCs; Primary myelofibrose; epigenetic regulators