Role of somatic *ETNK1* mutation in the mitochondrial activity

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Background Atypical chronic myeloid leukemia (aCML) is a clonal disorder belonging to the myelodysplastic/myeloproliferative syndromes. About 13% of aCML cases carry somatic mutations in *ETNK1* gene, encoding for H243Y and N244S substitutions[1]. In *ETNK1*-positive aCML primary samples the intracellular level of phosphoethanolamine (p-ET), the product of the ETNK1 kinase, was 5.2-fold lower than in the non-mutated ones[1].

Objectives Since p-ET is essential for the synthesis of phosphatidylethanolamine (PE)[2] and given that PE is one of the most abundant phospholipids in the inner membrane of mitochondria[3], we focused our attention on mitochondrial activity.

Methods The CRISPR/Cas9 system was used on 293 Flp-In™ cell line to generate clones carrying heterozygous N244S mutation and homozygous *ETNK1* functional deletion (KO cells). MitoTracker™ Red CMXRos was used to visualize mitochondrial activity. CellROX™ Green Reagent was used to assess ROS production. ATP levels were quantified by using ATPlite Luminescence Assay System. DNA damage was detected by quantifying γ-H2AX foci formation, and mutation frequency was determined by 6-thioguanine assay.

Results In both N244S and KO cells, mitochondria change their morphology from an elongated, tubular shape to a round, swollen one. Moreover, N244S and KO cells show a significant increase in mitochondrial activity (1.78 and 2.13 fold increase, respectively; $p= 0.0096$ and $p=0.0050$) compared to WT, and also in ROS (1.66 and 1.74 fold increase, respectively; $p<0.0001$) and ATP production (1.67 and 1.68 fold, respectively; $p<0.0001$ and $p=0.0082$). γ-H2AX analysis reveals a higher number of foci ($p<0.0001$) in N244S and KO cells (2.60 ± 0.22 and 2.89 ± 0.27) compared to WT (0.56 ± 0.08). In line with these data, 6-thioguanine assay shows a higher mutation rate in N244S and KO cells ($8.09*10^{-7}\pm9.6*10^{-8}$ and $8.20*10^{-7}\pm1.28*10^{-7}$; $p=0.0060$ and $p=0.0264$) compared to WT ($2.98*10^{-7}\pm8.2*10^{-8}$).

Conclusion Taken together, our results show that impairment of ETNK1 function causes an increase in mitochondrial activity, which in turn leads to increased production of ROS driving the accumulation of DNA mutations.

References

1. Gambacorti-Passerini, C.B. et al. Recurrent ETNK1 mutations in atypical chronic myeloid leukemia. Blood 125, 499-503 (2015).
2. Kennedy, E.P. & Weiss, S.B. The function of cytidine coenzymes in the biosynthesis of phospholipides. J Biol Chem 222, 193-214 (1956).
3. Vance, J.E. Phospholipid synthesis and transport in mammalian cells. Traffic 16, 1-18 (2015).