ETNK1 mutations increase mitochondrial activity and promote DNA damage through ROS production

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Diletta Fontana[1], M. Mauri[1], A. Niro[1], L. Massimino[1], M. Bertagna[1], G. Zambrotta[1], M. Bossi[1], S. Citterio[2], B. Crescenzi[3], G. Signore[4], V. Piazza[4], F. Fanelli[5], C. Mecucci[3], G. Cavaletti[1], D. Rea[6], C. Gambacorti-Passerini[1,7], R. Piazza[1,7]

[1] Department of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy.
[2] Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milano, Italy.
[3] Centro Ricerche Emato-Oncologiche, University of Perugia, Perugia, Italy.
[4] Center for Nanotechnology Innovation @NEST, Istituto Italiano di Tecnologia, Pisa, Italy.
[5] Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy.
[6] Service d'Hématologie adulte, Hôpital Saint-Louis, Paris, France.
[7] Hematology and Clinical Research Unit, San Gerardo Hospital, Monza, Italy.

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, N244S and G245V substitutions. In *ETNK1*-positive aCML primary samples the intracellular level of phosphoethanolamine (p-ET), the product of ETNK1 kinase, was 5.2-fold lower than in controls. Since p-ET is essential for phosphatidylethanolamine (PE) synthesis, one of the most abundant phospholipids in mitochondrial inner membrane, we focused our attention on mitochondrial activity. We generated CRISPR/Cas9 clones carrying heterozygous N244S mutation and homozygous ETNK1 deletion (KO cells). In both N244S and KO cells, mitochondrial morphology changed 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). A similar increase in γ-H2AX ($p=0.0037$) is present in primary aCML patients samples carrying ETNK1 mutation compared to ETNK1-WT ones. 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 ($2.98*10^{-7}\pm8.2*10^{-8}$). The hierarchical reconstruction of somatic mutations in ETNK1-mutated aCML patients reveals that ETNK1 variants invariably occur very early in the evolution history of aCML patients. In conclusion we 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 further oncogenic mutations.