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Differences in Iron Distribution in Patients with Myelodysplastic Syndromes Carrying *SF3B1* Mutations Using Energy-dispersive X-ray Spectroscopy (XEDS) and Electron-Energy Loss Spectroscopy (EELS)

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Ring sideroblasts (RS) are erythroblasts with iron-loaded mitochondria visualized by Prussian blue staining (Perls' reaction) as a perinuclear ring of blue granules [1]. RS is a cardinal feature of refractory anemia with ring sideroblasts (RARS) and RARS associated with thrombocytosis (RARS-T), two subtypes of myelodysplastic syndrome (MDS) and MDS/ myeloproliferative neoplasm. RARS and RARS-T are frequently associated with mutation of *SF3B1*, a component of the RNA splicing machinery [2]. Aside from its phenotypic (RS) association, *SF3B1* has been associated with good outcomes in MDS. In order to explain the clinical phenotypes we investigated the consequences of *SF3B1* dysfunction in MDS. We hypothesized the followings: 1) *SF3B1* mutation is a founder mutation in RS formation and results in changes in RNA splicing of crucial genes in erythropoiesis and mitochondrial pathways. 2) *SF3B1* mutations cause changes in composition/chemical valence of iron deposition leading to free radical elevation and DNA damage which would limit the viability of neoplastic cells favoring good outcome. If the mitochondrial function is compromised by *SF3B1* mutation, the reduction of ferric iron to ferrous (Fe^{2+}) iron is attenuated and ferric iron may accumulate, resulting in mitochondrial iron overload [3,4]. In order to analyze the iron deposition in *SF3B1* mutants and wild type (WT) patients, we thus applied two complementary analytic techniques usually used in the characterization of material elements in inorganic specimens: energy-dispersive X-ray spectroscopy (XEDS) and electron-energy loss spectroscopy (EELS). The results revealed that the amounts of the iron depositions are significantly different between WT and *SF3B1* mutants (**Figure 1**). However, Fe_2O_3 is the most prevalent iron form in both WT and *SF3B1* mutants [2] (**Figure 2**).

In conclusion, we applied, for the first time, XEDS and EELS in order to investigate the iron composition in *SF3B1* mutant and WT RARS patients. *SF3B1* mutant patients have different iron composition compared to WT patients. It is likely that Fe_2O_3 is the chemical form of the iron involved in the pathogenesis of RS.

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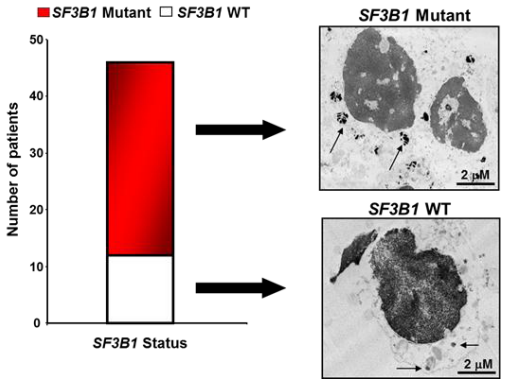

Figure 1. Iron composition in *SF3B1* WT and mutant patients.

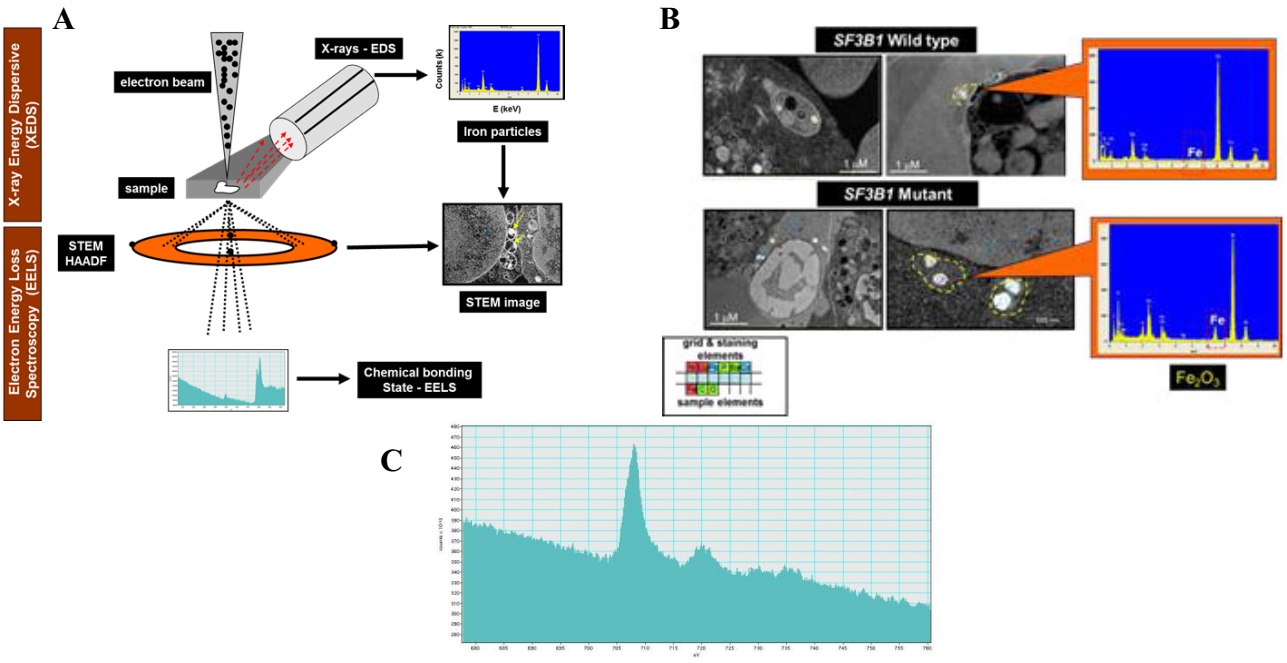

Figure 2. Elemental analysis of in *SF3B1* WT and mutant patients using XEDS and EELS. A) Schematic of sample analysis, B) Iron particles found in *SF3B1* WT and mutant patients, C) EELS spectrum of iron oxide particles.

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