Abstract

Friedreich Ataxia (FRDA) is an autosomal recessive systemic disease with prominent neurologic and cardiac manifestations. It is principally caused by a GAA triplet expansion in intron1 of the frataxin gene. The long expansion causes chromatin rearrangement and epigenetic silencing of the locus, resulting in decreased mRNA transcription and consequently in low protein level. Frataxin is a mitochondrial protein involved in Fe handling, FeS cluster assembly and respiratory chain functioning and it is highly conserved among eukaryotes as well as prokaryotes. There is no cure for Friedreich ataxia, but in the last decades different approaches, aimed to restore normal frataxin protein levels and genome editing approaches, have demonstrated encouraging results in cellular and animal models, providing a proof-of-principle of the efficacy of frataxin restoration. These approaches are at different stages of pre-clinical or early clinical development. In this work a deep investigation on frataxin gene locus (FXN) has been performed. From the frataxin gene multiple transcript isoforms derive, controlled by two different Kozak sequences. Such Kozak sequences show opposite efficiency in regulating translation and the multiple transcript isoforms are differentially expressed among different tissues. Moreover, the isoforms transcripts, controlled by the strong Kozak, are not translated into protein. Furthermore, this study proposes a new potential therapeutic approach, consisting in the selective elimination of the alternative splicing site present in exon4-exon5 junction, using base editors. This modification leads to the elimination of *FXN2* alternative transcript and to the increase of the canonical one, with a consequent increase of frataxin protein level. Moreover, human induced pluripotent stem cells (hiPSCs) have been reprogrammed and validated as patients based cellular models in which base editing approach can be tested.