Unverricht-Lundborg disease: development of splicing therapeutic approaches for a patient with an homozygous mutation in the cystatin B gene

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Unverricht-Lundborg disease (ULD or EPM) is the most common form of progressive myoclonic epilepsy (PME) worldwide. It is an autosomal recessive neurodegenerative disorder characterized by action myoclonic jerks, generalized tonic-clonic seizures, marked photosensitivity and juvenile ataxia with gradual worsening. Actually, there is no etiologic treatment for ULD, symptomatic pharmacologic and rehabilitative management are the mainstay of patient care (1).

ULD is caused by mutations in the cystatin B gene (CSTB) localized on chromosome 21q22.3 and encodes an inhibitor of several lysosomal cathepsins (2-4). Thirteen CSTB mutations have been described as causal of EPM (5). The most common one, responsible for about 90% of the abnormal alleles is an unstable expansion of a CTCGAC repeat in the 5′ UTR promoter region (6). The remaining twelve mutations are missense, nonsense, frameshift and splice-site mutations that may lead to abnormal RNA processing (5). Most frequently, splice-site mutations interfere with exon recognition in pre-mRNA transcripts and lead to exon skipping or intron retention. In general, more than 90% of disease-causing point mutations affect classical splice-sites (7,8). A mutation in a splice donor site (5′SS) often interferes with proper complex formation of the splice factor complex 5′SS–min-5′SS–mRNA (9). The part of the U1 snRNP that binds the SDS by Watson-Crick base pairing is called U1 snRNA (U1). After U1 has recognized a SD5, the complex recruits other components of the spliceosome to further promote splicing of the pre-mRNA (10). The increasing knowledge of RNA biology is stimulating new approaches of RNA-based strategies to achieve therapy in alternative to conventional gene replacement therapies. The vast majority of RNA-based approaches have exploited, in vitro and in vivo, antisense sequences to either mask natural splice sites, to induce skipping of deleterious exons, or newly generated cryptic sites, to favour the use of the canonical ones (11). On the other hand, the use of U1snRNA complementary to the mutated site has been described as a potentially therapeutic strategy to correct 3′ splice site defects dependent on U1 binding (12,13).

Recently, our group described a Portuguese ULD patient who is homozygous for a new synonymous mutation c.666G>A (p.G222G) located at the last nucleotide of exon 1 which leads to missplicing of CSTB transcripts (14). Here, to overcome the pathogenic effect of the SD5 mutation, we have exploited both antisense oligonucleotide and U1 snRNA mediated therapeutic strategies to correct the splice defect in the patient cell line.

\textbf{Study of the impact of the CSTB gene mutation at cDNA level}

\textbf{Development of Splicing Therapeutic Approaches for an ULD patient with the synonymous mutation c.666G>A}

\textbf{U1 snRNA-mediated Gene Therapeutic Approach}

\textbf{U2 snRNA-mediated Gene Therapeutic Approach}

\textbf{Antisense Oligonucleotide (LNA) Therapeutic Approach}

\textbf{References}

\textbf{Acknowledgments}

\textbf{Figures}

\textbf{Table}

\textbf{Supplementary Material}