Identification of new therapeutic candidates targeting representative RNA probes of Steinert's disease by affinity capillary electrophoresis

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Myotonic dystrophy type 1 (DM1), also named Steinert's disease, is a genetic dominantly inherited degenerative disease. The pathology is caused by the expansion (>50) of a trinucleotide $[CTG]_n$ located on the 3'UTR non-coding region of the DMPK gene (Dystrophia Myotonica Protein Kinase, chromosome 19q13.3).

The pathogenesis of DM1 is not fully understood but most of the evidence indicate a RNA gain-of-function mechanism. The transcription of the expanded $[CTG]_n$ trinucleotide in a toxic $[CUG]_n$ RNA, forming hairpins, leads to RNA *foci* in the nucleus which can interact and sequestrate various molecules and proteins. That phenomenon leads to misfunction of some proteins and finally to the symptoms that are observed in the pathology. Currently, only symptomatic treatments are administrated even though several therapeutic strategies are considered. One of them hypothesis the use of small molecules that can selectively target the RNA sequence and release the sequestrating proteins [1].

The aim of this project is to demonstrate the suitability of an Affinity Capillary Electrophoresis (ACE) method to highlight ligands that bind specifically to DM1-representative RNA probes.

The first part of this study focuses on RNA probes generation. These are obtained with bacterial amplifications of suitable plasmids, containing 95 and 14 [CTG] repetitions, followed by linearization, purification, and finally *in vitro* transcription. The RNA $[CUG]_{95}$ and $[CUG]_{14}$ synthetized fragments are used as pathological and non-pathological probes, respectively.

The second part consists to the development of the ACE method. In ACE the affinity constants can be extracted from migration times which are modified due to the formation of complexes between ligands and analytes. The most important drawback of this mode of operation concerns the repeatability of data between analyses. The optimization of the method allows a good repeatability in terms of migration times. To achieve this goal, a dynamic coating process using polyethylene oxide as coating agent is performed.

Finally, the last part aims to test libraries of small molecules to identify their eventual affinity towards the pathological RNA probes, with the developed ACE method. Those interactions have been quantified by fitting the data by a non-linear regression (plotting $\Delta\mu$ as function of [ligand]) and compared to the ones obtained using the non-pathological RNA probes.

The optimized ACE method constitutes a simple and quick tool to highlight new therapeutic candidates for the clinical treatment of the Steinert's disease. The results will be corroborated by fluorescence microscopy assays to confirm the validity of the ACE method.

References

[1] M.López-Morató, J. Brook, M. Wojciechowska, *Frontiers in neurology*, **2018**, volume 9.