Synthesis and Delivery of Morpholino Oligonucleotides: Translational Research

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The present work relates to the synthesis of phosphorodiamidate morpholino oligomers called PMOs or morpholinos. PMOs are antisense oligonucleotides, used to study gene functions in vitro and in vivo models. The PMOs are only available with Gene Tools LLC, USA (www.gene-tools.com) and the cost is 400 $ for 300 nmol. There is no synthetic method for PMOs except their patent [1] and also the poor cellular transfection property precludes PMO’s therapeutic applications and perhaps the reason FDA has given conditional approval of PMO-based drug Eteplirsen (AVI-4658) to Sarepta Therapeutics, USA on 26th September 2016 [2] for the treatment of Duchenne Muscular Dystrophy (DMD). Though P-PMO and Vivo PMO have been developed by Gene Tools through conjugation of PMO with guanidinium-based transporters, however, these transporters develop toxicities, nonspecific interactions due to the presence of too many flexibly attached guanidinium groups and hydrolyzed by peptidases in the case of CPPs. Here, I describe the synthesis of exocyclic-amine-protected 7′-hydroxy and 7′-chlorophosphoramidate activated morpholino monomers of A, T, G, and C and a PMO oligomer of 25-mer by solid-phase synthesis [3]. To overcome the delivery problem of PMOs, we report here a novel internally tetraguanidinium linked nonpeptidic cellular transporter (IGT) having conformationally rigid backbone composed of pharmacologically compatible heterocyclic six-membered rings which internalizes efficiently into cells in full growth medium and ubiquitously distributed into zebrafish injected embryos. It efficiently transports antisense PMOs in cells and exhibits antisense effect against Gli1 in hedgehog signaling pathway and shows temporal regulation of no tail gene in zebrafish embryos. Comparing with Gene Tools Vivo PMO, our IGT-conjugated PMOs show better antisense efficacy and has evoked interest from both academia and industries regarding further research and commercial venture.

References