Targeting Nonsense Mutations: NV848, NV914, and NV930 for REP1 protein restoration in Choroideremia

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Choroideremia is an X-linked, rare genetic disease caused by mutations in the CHM gene on the X chromosome, with an estimated incidence of approximately 1:50,000 to 1:100,000 worldwide. This disease is characterized by progressive night blindness and degeneration of the visual fields, initially affecting the peripheral areas and later the central ones. The evolving degeneration of the retinal pigment epithelium (RPE), photoreceptors, and atrophic choroid leads to serious peripheral field loss in adolescence and adulthood, central visual loss during adulthood, and total blindness during the last decade of life[2]. The CHM gene encodes for Rab Escort Protein 1, referred to as REP1, which is the A subunit of the Rab Geranyl-geranyl transferase II (RabGGTII), involved in the geranylation of Rab proteins[3]. Among the several mutations, 34% are represented by nonsense mutations in the CHM gene; these ones lead to the formation of a premature termination codon (PTC) in the CHM mRNA coding sequence, with the absence or a truncated and non-functional REP1 protein[4]. REP1 protein is not only involved in the vesicular trafficking and the correct prenylation on Rab protein[5], but also in several pathways involved in cancer[6], oxidative stress[7], autophagy[8,] and apoptosis[9]. Nowadays there is no cure or treatment against nonsense mutations, but promising result come from suppression therapy, based on the use of translational readthrough inducing drugs (TRIDs), that can overall the PTC when the ribosome go on it, introducing a near-cognate tRNA in correspondence of the PTC, allowing the continuation of the translation instead of termination, this approach are showing promising results against this kind of mutation[10]. Recently, the Pibiri-Lentini group has synthesized three TRIDs molecules, 1,2,4-oxadiazoles, namely NV848, NV914, and NV930, derivatives of Ataluren (Known as PTC124), a well-known readthrough agent, that are showing *in vitro* and *in vivo* very interesting results[11,12,13]. Our work is focused on studying the possible readthrough activity and the effect of these molecules on different Choroideremia nonsense models. Especially, to evaluate the above-mentioned molecules' activity on CHM nonsense mutations, two transient cell lines, CHM R293X and CHM R253X, have been produced. To evaluate the readthrough activity of these compounds and the rescue of the REP1 protein, immunofluorescence analyses have been performed after 48h of treatment. Furthermore, to investigate their activity on primary cell culture, 72h chronic treatments on Fibroblasts CHM^{R253} have been performed, and REP1 protein rescue by Immunofluorescence has been evaluated. The results show the rescue of the REP1 protein in all the models analysed, confirming the promising results in fighting nonsense mutations.

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