

**TTRV30M oligomeric aggregates inhibit proliferation of renal progenitor cells but maintain their capacity to differentiate into podocytes *in vitro***

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In Familial Amyloidotic Polyneuropathy, the amyloid deposition of mutant transthyretin TTR V30M can lead to renal complications. An unexplored mechanism is the toxicity of oligomeric TTR aggregates. A subset of renal progenitor cells (RPC) in the adult human kidney can induce regeneration of podocytes and tubular structures of the nephron, which can be critical for preventing irreversible renal failure. We assessed whether RPC are vulnerable, *in vitro*, to TTRV30M oligomers. RPC proliferation was reduced by 16.3±9.7% and 32.6±6.3% after 48 and 72 hours, respectively, in the presence of the oligomers. However, oligomers did not induce apoptosis or alterations in cell cycle to any significant extent, and did not influence RPC differentiation into podocytes. From this first attempt, we can say that TTRV30M oligomers inhibit RPC proliferation but do not influence their capacity to differentiate into mature podocytes, and thus should not compromise tissue regeneration.

**INTRODUCTION**

Familial Amyloidotic Polyneuropathy Type I (FAP-I) is an autosomal dominant disease characterized by systemic extracellular amyloid deposition of a mutant transthyretin, TTR V30M [1], affecting particularly the peripheral nervous system. Renal complications can also occur, including nephrotic syndrome and end-stage kidney failure [2]. An unexplored mechanism is the toxicity of early oligomeric TTR aggregates [3].

A subset of renal progenitor cells (RPC) with self-renewal and multidifferentiation potential exist at the urinary pole of Bowman's capsule in the adult human kidney [4]. These resident progenitor cells can induce regeneration of podocytes and tubular structures of different portions of the nephron, which can be critical for preventing irreversible renal failure, and could be useful in cell therapies.

We intend to evaluate the influence of oligomeric TTRV30M aggregates on proliferation and differentiation of RPC in order to understand if their regenerative potential could be compromised.

## METHODS

Recombinant human TTRV30M was produced using an *E. coli* expression system and purified by affinity, ion exchange and gel filtration chromatographies. Oligomerization was induced as described by Lindgren *et al*, and assessed by chemical cross-linking and thioflavin T assay.

Viability and proliferation of RPC were evaluated by MTT assay. RPC were treated with soluble and oligomeric TTRV30M for 24, 48 and 72 hours. The results were represented as the ratio: (ODsample – ODblank)/(ODcontrol – ODblank). Average values and SD were calculated from triplicate determinations.

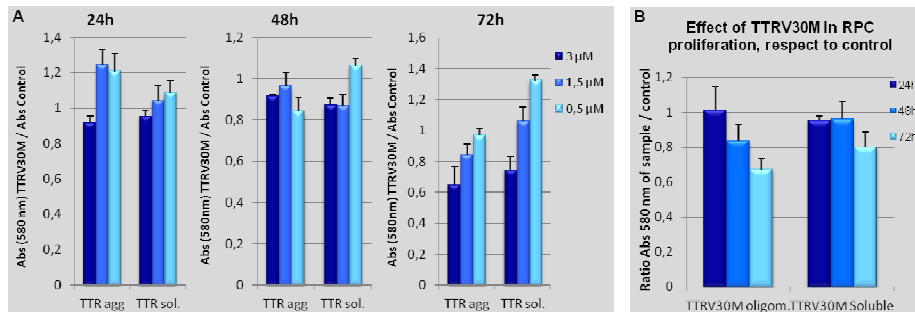
Fluorescence-activated cell sorting (FACS) analysis of annexin V and propidium iodide was used to assess induction of apoptosis. Staining with propidium iodide was used for cell cycle analysis. RPC were treated with 3  $\mu$ M of oligomeric TTRV30M for 48 and 72 hours.

RPC differentiation into podocytes *in vitro* was induced with VRAD medium: DMEM-F12, 0,5% FBS, vitamin D3 and all-trans retinoic acid. For the non differentiation controls, cells were treated with EBM 0,5% FBS. The oligomeric TTRV30M was diluted in the correspondent media at a final concentration of 3  $\mu$ M. Cells were stimulated for 24 and 48 h. Relative expression levels of the nephrin gene were measured by real time pcr.

## RESULTS

The proliferation of RPC treated with oligomeric and soluble TTRV30M for 24 hours was not significantly affected, except for the highest protein concentration (Figure 1A). The treatment with 3  $\mu$ M oligomeric TTRV30M for 48 and 72 hours reduced RPC proliferation, respectively, 10 and 33%, when compared to the controls. Soluble TTRV30M reduced 25% the proliferation of RPC after 72 hours, what may reflect the natural instability of the tetramer caused by the V30M mutation.

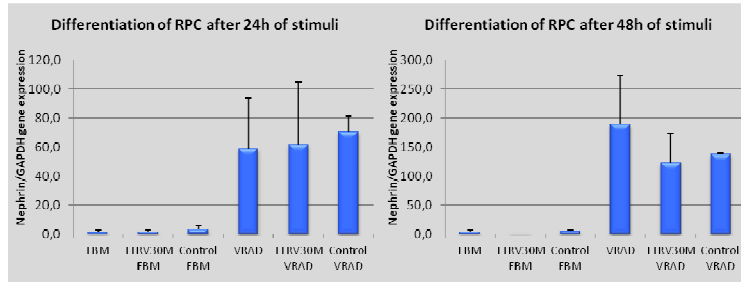
RPC from different patients were treated with 3  $\mu$ M TTRV30M. On average, the oligomeric TTR reduced RPC proliferation, respectively, 16.3 $\pm$ 9.7% and 32.6 $\pm$ 6.3% after 48 and 72 h (Figure 1B).



**Figure 1.** Viability of RPC exposed to TTRV30M, measured by MTT assay. A - RPC treated with 0,5  $\mu$ M, 1,5  $\mu$ M and 3  $\mu$ M TTRV30M for 24, 48 and 72h; B - RPC treated with 3  $\mu$ M TTRV30M for 24, 48 and 72h; results are shown as mean with indicated standard deviation of triplicate samples from three independent assays.

FACS analysis showed no significant differences between the control and the cells exposed to the TTRV30M oligomers, either for apoptosis or cell cycle progression, either at 48 and 72h (data not shown).

Relative expression levels of the nephrin and the housekeeping gene GAPDH were measured by real time pcr. The results varied between experiments, but on average we obtained similar results between the controls and the cells exposed to TTRV30M aggregates (Figure 2).



**Figure 2.** Differentiation of renal progenitor cells into podocytes. Assessment by quantitative RT-PCR of fold increase mRNA levels for the podocyte marker nephrin and the housekeeping gene GAPDH.

## DISCUSSION

The mutant TTRV30M reduced proliferation and metabolic viability of renal progenitor cells. However, the FACS analysis of annexin V and propidium iodide did not show induction of apoptosis or necrosis to any significant extent. Also, cell cycle progression was not significantly influenced by the oligomers. The expression level of the nephrin gene, a marker for podocytes, showed no alterations relatively to controls, so the inherent capacity of these progenitor cells to differentiate into podocytes was apparently not affected by the oligomers.

Further studies are needed to elucidate the mechanisms of toxicity in the kidney, particularly the role of oxidative stress. From this first attempt, we can say that TTRV30M oligomers inhibit the proliferation of renal progenitor cells but do not influence their capacity to differentiate into functionally mature podocytes, and thus should not compromise tissue regeneration.

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