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Efficient microRNA inhibition in a highly invasive orthotopic glioblastoma model

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Introduction

Up-regulated microRNAs (miRs) are promising new targets in glioblastoma (GBM) therapy. In vitro and in vivo inhibition has been shown to diminish both proliferation and invasion. However, most often the tumor models used have been non-orthotopic and poorly resemble the invasive GBM in patients. Moreover, miR inhibition has been performed in ways that are difficult to translate into clinical use. The purpose of this study was to determine if convection enhanced delivery (CED) of an anti-miR (a chemically modified oligonucleotide complementary to a miR) could efficiently inhibit a miR in a highly translational tumor model.

Conclusion

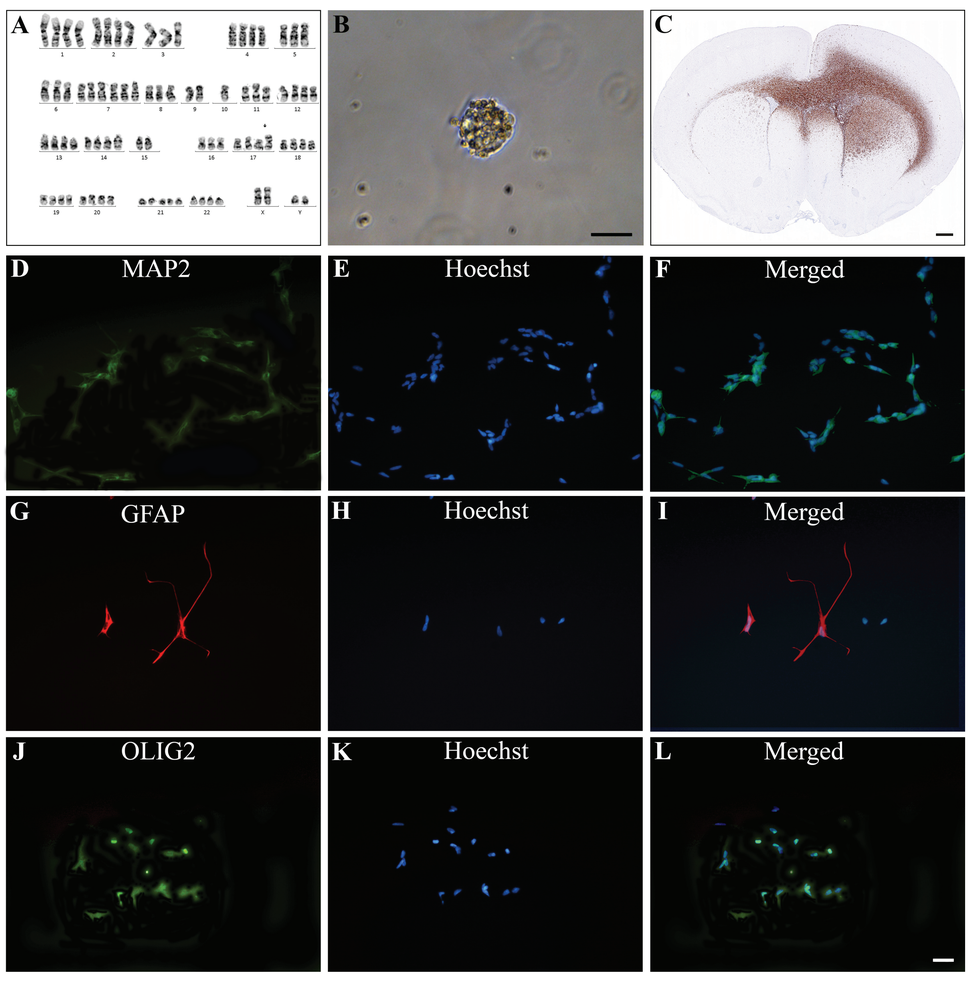
In conlusion, we found that intratumoral convection-enhanced delivery of anti-miR-let-7a:

- Was well tolerated
- Preserved the integrity of the anti-miR
- Caused efficient target de-repression.

As a proof of concept the results hold promise for future miR oligonucleotide anti-sense strategies in clinical neuro-oncology.

Material and Methods

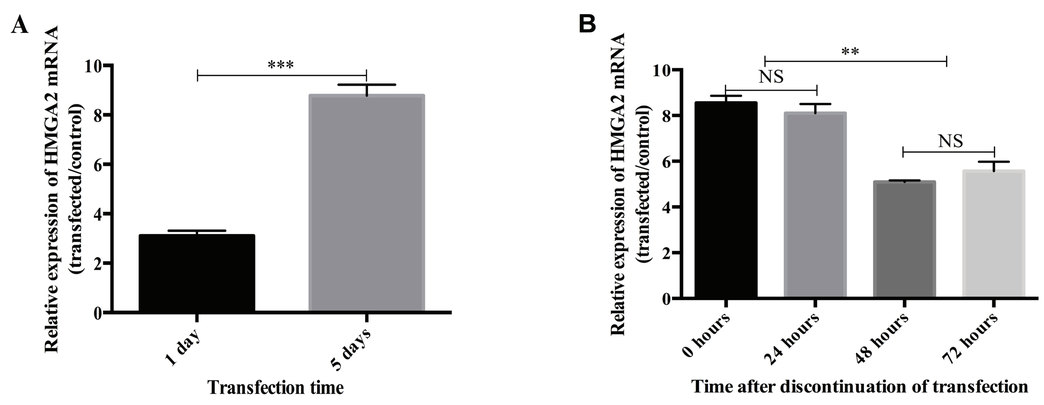
In vitro, miR-let-7a was inhibited in a patient-derived glioblastoma stem cell line by free uptake with 10µM anti-miR (a chemically modified oligonucleotide complementary to let-7a, Regulus Therapeutics) for 1 or 5 days to test transfection efficacy and stability. In vivo, non-transfected cells were inoculated into the brains of nude rats and post-tumor formation, a micro infusion pump (iPRECIO) was implanted and anti-miR-let-7a or saline was infused for 31 days. Then tumors were removed and tumor mRNA level of the miR-let-7a target High-mobility group AT-hook 2 (HMGA2) was determined by quantitative real-time PCR (qRT-PCR). Extracted residual anti-miR from the pump reservoirs was analysed with mass spectrometry and spectrophotometry to assess integrity and concentration of the anti-miR.



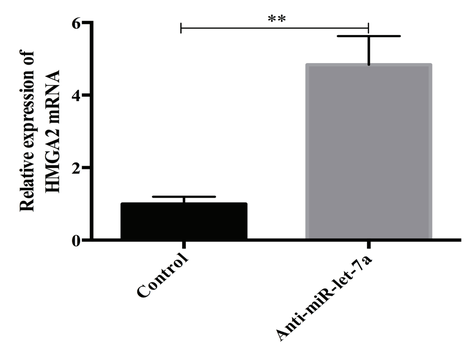
Characterization of the patient-derived T87 GBM stem cell line used in all experiments. (A) The T87 karyotype showed gain of chromosome 7 and loss of chromosome 10, typical for GBM. (B) T87 cells were grown in serum-free medium and when trypsinated to single cells they formed spheroids at clonal density. (C) In vivo xenografting of 300,000 T87 single cells into the right striatum of immunodeficient rats produced highly infiltrative tumors. The tumors were identified by immunohistochemical anti-human vimentin staining of paraffin embedded coronal sections of the brains 6 weeks post tumor cell inoculation (D-L). Differentiation assay with the neuronal marker MAP2, the astrocytic marker GFAP and the oligodendrocyte marker OLIG2. Upon culturering in serum-containing medium T87 cells had the ability to differentiate into neurons (D-F), astrocytes (G-I) and oligodendrocytes (J-L). Scale bar 100µm (B), 1mm (C), 50µm (D-I).

Results

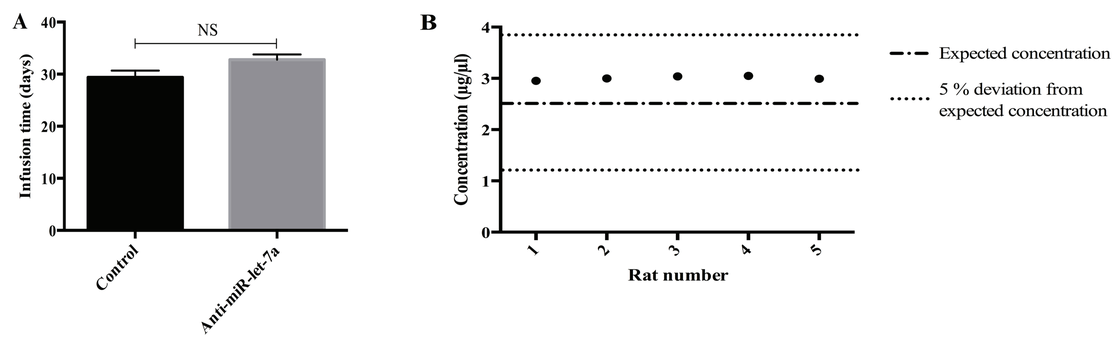
In vitro, five day transfection was more efficient then 1 day at de-repressing HMGA2 (Fig. 1A) and the transfection was stable for 24 hours after removal of the anti-miR (Fig. 1B). In vivo the tumor HMGA2 level was significantly de-repressed 4.8 fold (Fig. 2). Residual anti-miR from the pump reservoirs was non-degraded (Fig. 3A-C) and no animals showed signs of adverse effects.



qRT-PCR mRNA relative expression levels of HMGA2 in anti-miR-let-7a transfected T87 cells relative to control transfected T87 cells. β-actin was used as housekeeping gene. (A) Five days free uptake transfection period with anti-miR-let-7a caused more efficient target de-repression than 1 day. (B) After discontinuation of 5 days transfection the target de-repression was stable for 24 hours where after it dropped. Error bars represent SEM from 3 independent transfections. NS $p>0.05$, ** $p<0.01$, *** $p<0.001$.



qRT-PCR mRNA relative expression levels of HMGA2 in the tumor samples from the control (n=5) and anti-miR-let-7a (n=5) infused rats. A significant target de-repression of 4.8 (+/-1.77) fold was seen. Human GAPDH was used as housekeeping gene. Values represent means (+/-SEM) in each group. NS $p>0.05$, ** $p<0.01$.



Anti-miR concentration and integrity after storage in pump reservoirs. (A) The mean infusion time in the control (n=5) and anti-miR-let-7a (n=5) infused rats were similar. (B) Upon euthanization residual anti-miR-let-7a was extracted from the pump reservoirs and the concentrations were determined using the extinction coefficient and the optical density read at 260 nm. In no animals (n=5) did the concentrations differ more than 5% from the intended concentration. (C) Mass spectrometry showed no signs of anti-miR degradation confirmed by the large peaks at 13–14min. and no other peaks apart from the internal standard for calibration at 4.5 min. NS $p>0.05$.

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