# Therapeutic Potential of miR-3189-3p Against Glioblastoma

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## Abstract

Glioblastoma is an aggressive type of brain tumor that responds poorly to standard radio- and chemo-treatments. MicroRNAs are short single-stranded non-coding RNAs that regulate gene expression in both normal and pathological conditions. Using *in vitro* and *in vivo* models we have previously found that miR-3189-3p has anti-tumoral activity against glioblastoma through inhibition of cell growth and migration. We also found that miR-3189-3p-induced inhibition of cell growth was mediated by the downregulation of the splicing factor SF3B2, while impairment of migration was mediated by the downregulation of p63RhoGEF. Altogether, those findings indicate a potential use for miR-3189-3p as a novel therapeutic approach, whether alone or in combination with existing therapies.

Given the strong biological effect of miR-3189-3p on glioblastoma cells, and considering the fact that microRNAs can have several targets, we sought to investigate the implication of other gene targets in the miR-3189-3p-mediated effects. The cancer susceptibility candidate 3 (CASC3) is among the top predicted targets of this microRNA. CASC3 is a core component of the splicing-dependent multiprotein exon junction complex (EJC), a complex deposited at the exon-exon junction and functions in nonsense-mediated mRNA decay (NMD). Here, we found that overexpression of miR-3189-3p results in downregulation of CASC3 and parallel upregulation of the TIA1 cytotoxic granule-associated RNA binding protein-like 1 (TIAL1, also called TIAR), a MYC translational repressor. Using RNA immunoprecipitation (RIP) technique, we further found that increased levels of TIAR resulted in increased MYC mRNA bound to TIAR. Interestingly, under these experimental conditions, Myc mRNA levels were unchanged. Overall, these findings suggest a mechanism by which miR-3189-3p-mediated downregulation of CASC3 results in stabilization and translational repression of MYC mRNA through increased expression of TIAR.

## Nonsense Mediated Decay

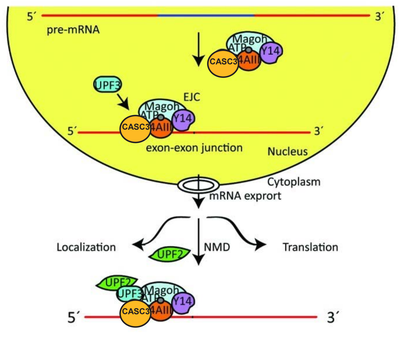


**Figure 1. Nonsense mediated decay exhibits post translational termination**
Nonsense mediated decay is a mechanism to degrade targeted mRNAs that are undergoing premature translation termination. The exon-junction complex plays an important role in NMD by attaching to the mRNA during splicing. The EJC is then transported to the cytoplasm with the mRNA where it is determined if it will undergo NMD or complete translation.
*Adapted from Alexandra Z. Andreou et al. .*

## Western Blot Analysis

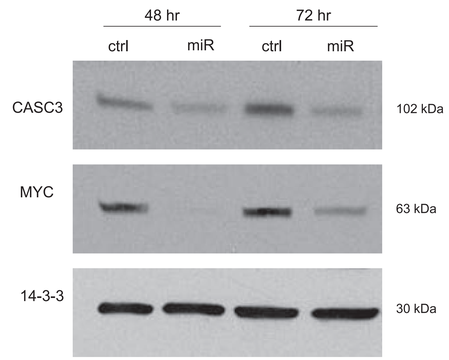


**Figure 2. CASC3 and Myc proteins are downregulated in the presence of miR-3189-3p**
Western blots showing expression levels of CASC3 and MYC in U87-MG cells that were transfected with miR-3189-3p or scramble control miRNA for 48h and 72h. 14-3-3 was used as loading control.

## Immunofluorescence

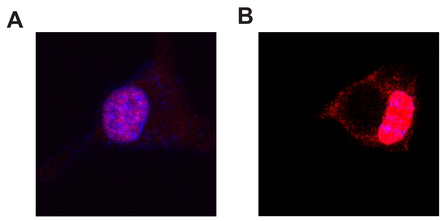


**Figure 3. miR-3189-3p upregulates TIAR levels in U87-MG cells.**
Representative images of U87-MG cells transfected with scrambled control miRNA or miR-3189-3p. Immunofluorescence microscopy was performed 48 hours post-transfection to determine the localization and distribution of TIAR. Note the increased TIAR levels (red) in both cytoplasm and nucleus of miR-3189-3p transfected cell. **A.** Scramble control miRNA, 50 nM. **B.** miR-3189-3p, 50 nM. Nuclei were stained with DAPI (blue).

## RNA-Immunoprecipitation (RIP)

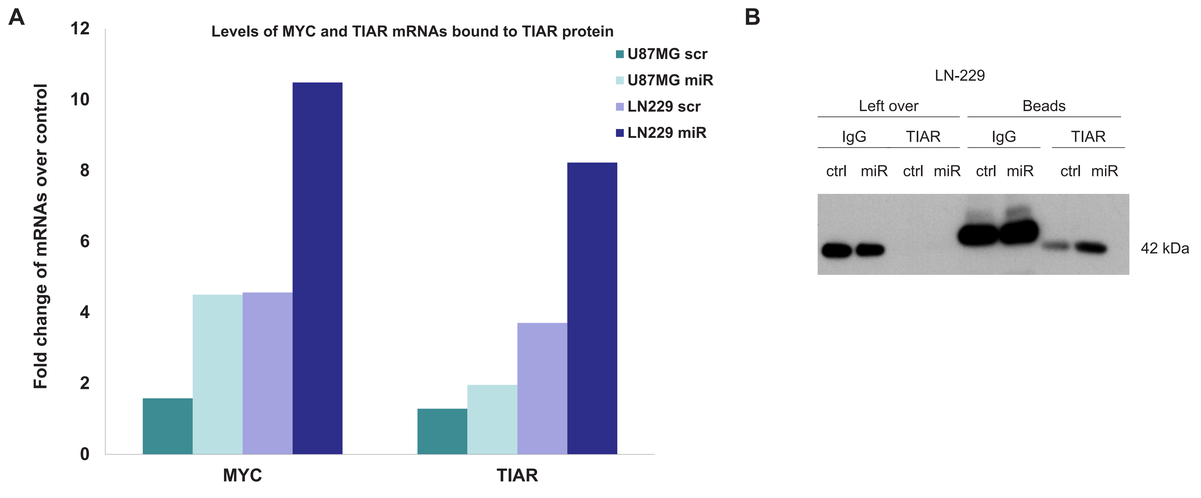


**Figure 4. Increased levels of MYC and TIAR mRNAs are bound by TIAR protein following transfection of glioblastoma cells with miR-3189-3p**
**A.** U87-MG and LN-229 cells were transfected with miR-3189-3p for 48h. Cellular lysates were subjected to immunoprecipitation using a TIAR specific antibody and control IgG. After the immunoprecipitation, half of the beads-IP-complexes were used for protein extraction and Western blot analysis and the other half was used for RNA extraction. After cDNA synthesis and real-time quantitative PCR, we observed increased association of MYC and TIAR mRNAs in immunoprecipitated TIAR-complexes. **B.** Western blot analysis of leftover lysates and IP magnetic beads demonstrates that TIAR is efficiently immunoprecipitated by anti-TIAR antibody.

## Conclusions

- CASC3 and MYC proteins are significantly down-regulated in the presence of miR-3189-3p
- TIAR, a known target of CASC3, is increased in glioblastoma cells following miR-3189-3p transfection
- The miR-3189-3p-mediated downregulation of MYC protein is the result of inhibition of translation, as determined by increased association of MYC mRNA with TIAR.

## Working model

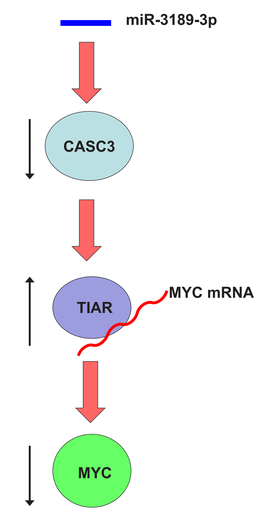


**Figure 5.** Diagram showing the working model of the miR-3189-3p-mediated downregulation of the MYC oncogene.

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