A novel role of GW182 in regulating miRNA stability

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Abstract

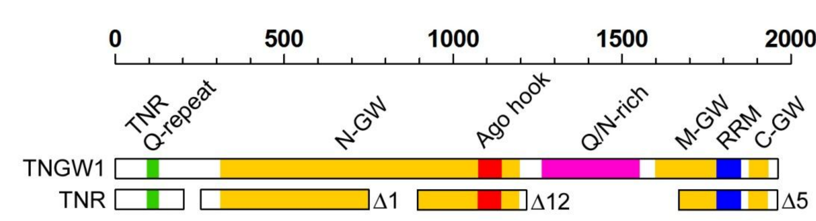
Although miRNA-mediated gene silencing is known to require functional Ago2 and GW182, the requirements for miRNA stability and exportation remain largely unknown. This study examined the role of GW182 in regulating miRNA stability and exosome mediated secretion using lentiviral shRNA-induced GW182-knockdown (KD) HEK293 cells. Stable GW182-KD cells, compared to their parental cells, demonstrated more rapid elimination of synthetic miR-146a (51.1% 6h; 29.4% 24h), miR-132 (57.4% 6h; 40.4% 24h) and endogenous miR-16 (69.1% 24h). This was not observed in the negative control cells transduced with lentiviral shRNA containing sequence mutations that did not affect GW182 levels. GW182 paralog TNRC6B, but not TNRC6C knockdown, also reduced miRNA half-life in early time points. Replenishment of GW182, its longer isoform TNGW1, its paralogs TNRC6B and TNRC6C, as well as one of its silencing domain Δ12, can significantly rescue/extend the synthetic miRNA half-life. The elimination of miRNA in GW182-KD cells was not due to enhanced secretion into extracellular space via exosome but rather degraded endogenously by yet-to-be identified exonuclease. The present study revealed a novel role of GW182 in maintaining miRNA homeostasis.

Introduction

GW182 protein was first identified and characterized by our laboratory in 2002 as a novel protein using an autoimmune disease patient serum ***(1)***. It is a 182 kDa protein characterized by multiple glycine (G) and tryptophan (W) repeats. GW182 likely serves as an essential component of GW bodies (GWBs, also known as mammalian Processing bodies, or P bodies) because knockdown of this protein leads to the disassembly of these foci ***(2)***. GW182 interacts with Ago2 in RISC complex and plays a critical role in miRNA mediated mRNA silencing function ***(3-6)***. In our previous study, two non-overlapping domains, Δ12 and Δ5, were identified to trigger repression comparable to full length GW182 in a tethering functional assay ***(7)***.

Besides miRNA silencing mechanism, a tremendous amount of effort has also been put into understanding how miRNA is regulated but current knowledge is primarily limited to the control of miRNA biogenesis without much information on the turnover of mature miRNA. A family of small RNA degrading nucleases (SDNs) was first reported in *Arabidopsis* for their ability to degrade mature miRNA ***(8)***. Another exonuclease, XRN-2, was subsequently identified to have similar function in *C. elegans* ***(9)***. A recent report demonstrated that miRNA possesses differential stability in human cells. The exosome 3' to 5' exoribonuclease complex was identified as the primary nuclease involved in particular miRNA decay with a more modest contribution by the Xrn1 and no detectable contribution by Xrn2 ***(10)***. However, the detailed mechanism and proteins involved in regulating miRNA stability in human cells remain elusive.

Although the silencing activity of GW182 was well characterized, little is known of the role of GW182 in regulating miRNA stability and secretion. By using lentiviral-based shRNA strategy, GW182 stable knockdown HEK293 cells were generated to observe its long term effects on miRNA stability. The effect was monitored primarily by transfected synthetic miRNA and thus avoiding the interference by transcription. Our data suggested that synthetic miRNA was more rapidly eliminated when GW182 was depleted and this effect can be rescued by re-expressing GW182 and its longer isoform, paralogs, and one silencing domain Δ12. The endogenous and overexpressed miRNA can be primarily secreted through secretory vesicle termed exosomes. However, depletion of GW182 reduced miRNA secretion into extracellular space suggests its potential regulatory role in this process.



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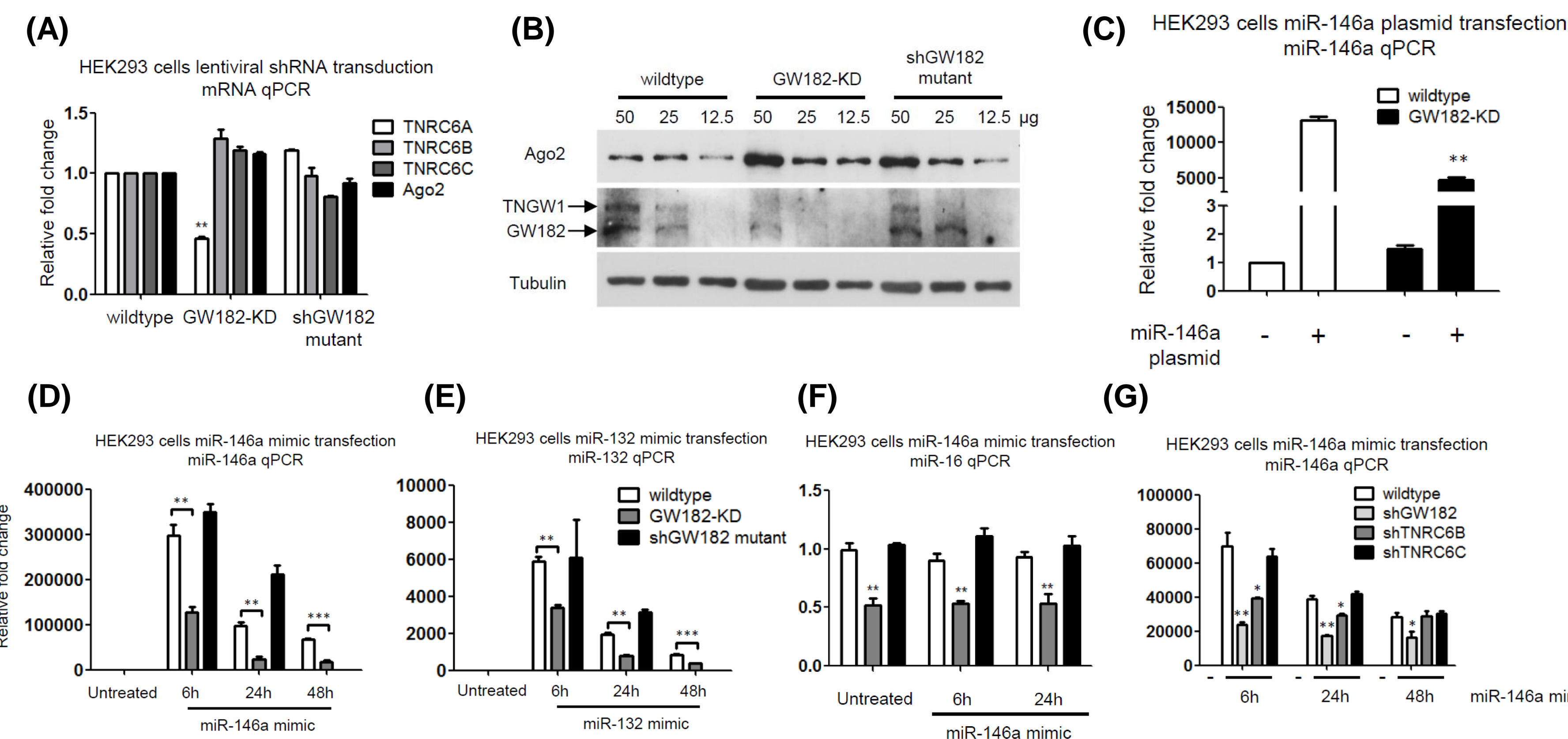
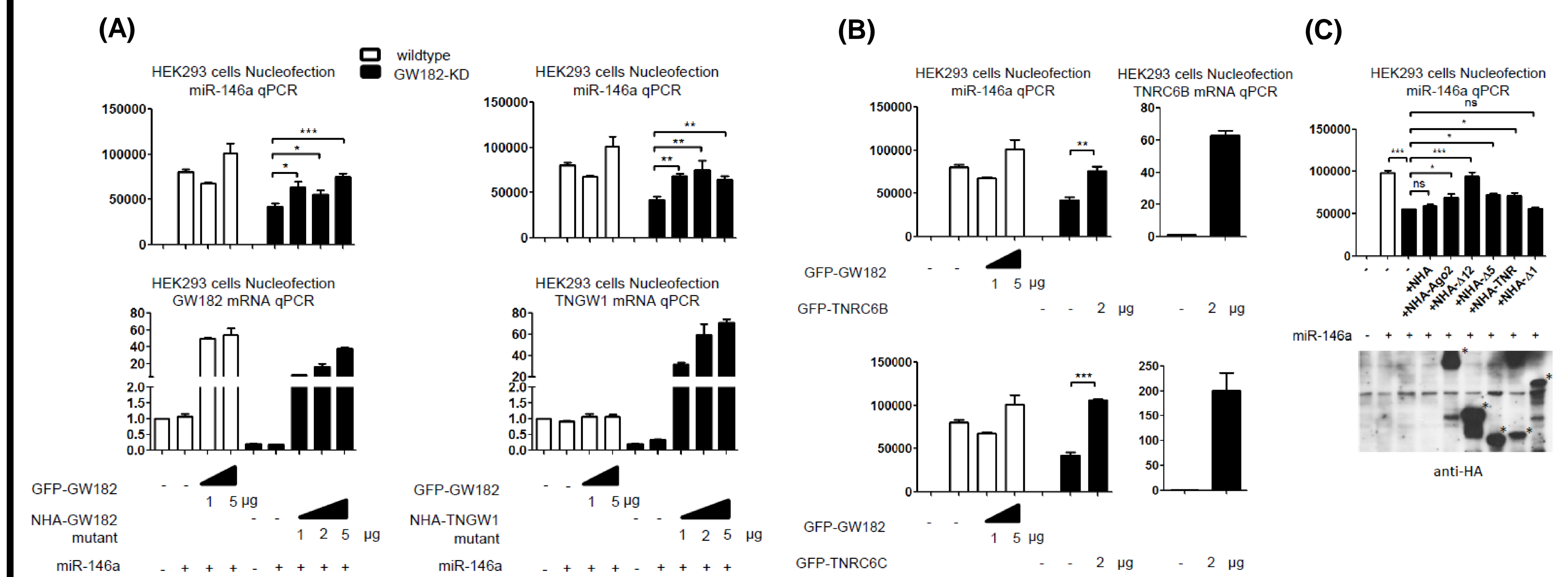
1. Knockdown of GW182 and TNRC6B impaired miRNA half-life



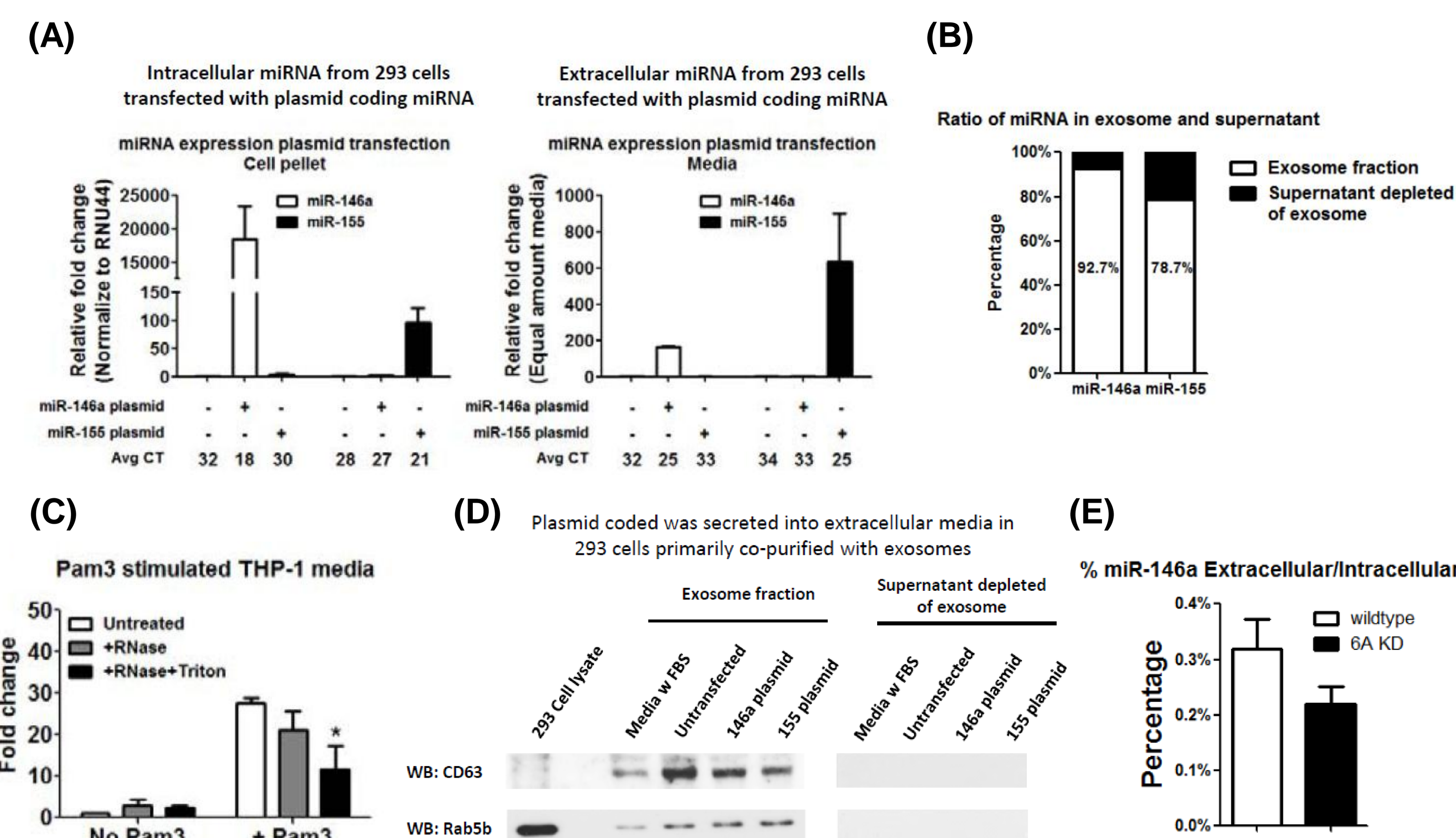
(A). shRNA against GW182/TNGW1 (shGW182) was fused with miR-30 stem loop and cloned into pTYF-PAC lentiviral vector with GFP marker for generating stable knockdown HEK293 cells. shRNA sequence mutation (shGW182 mutant) was cloned based on the same strategy serving as a negative control. Lenti-shGW182 effectively and specifically knocks down GW182 without significantly affecting its paralog TNRC6B, TNRC6C and its interaction partner Ago2. shGW182 mutant did not significantly affect any GW182 family member nor Ago2. **(B).** Western blot showed shGW182 specifically depleted GW182 and TNGW1 protein levels. **(C).** Plasmid coding pre-miR-146a, **(D). (E).** Synthetic miRNA mimic, **(F).** Endogenous miR-16 was significantly reduced in GW182-KD cells compared to wildtype but not shGW182 mutant control. **(G).** TNRC6B but not TNRC6C stable knockdown cells also showed reduced synthetic miRNA level. *t*-test was used for statistics. *, $p<0.05$; **, $0.01<p<0.05$; ***, $p<0.01$. n=3.

2. Replenishment of GW182 and its paralogs restored the shortened synthetic miRNA half-life



(A). Overexpression of GW182 and its isoform TNGW1 significantly restored synthetic miRNA half-life **(B).** Transfection of GW182 paralogs TNRC6B and TNRC6C significantly restored synthetic miRNA half-life **(C).** Overexpression of GW182 silencing domain Δ12 significantly restored synthetic miRNA half-life. Other GW182 fragments as well as Ago2 only modestly affected synthetic miRNA half-life.

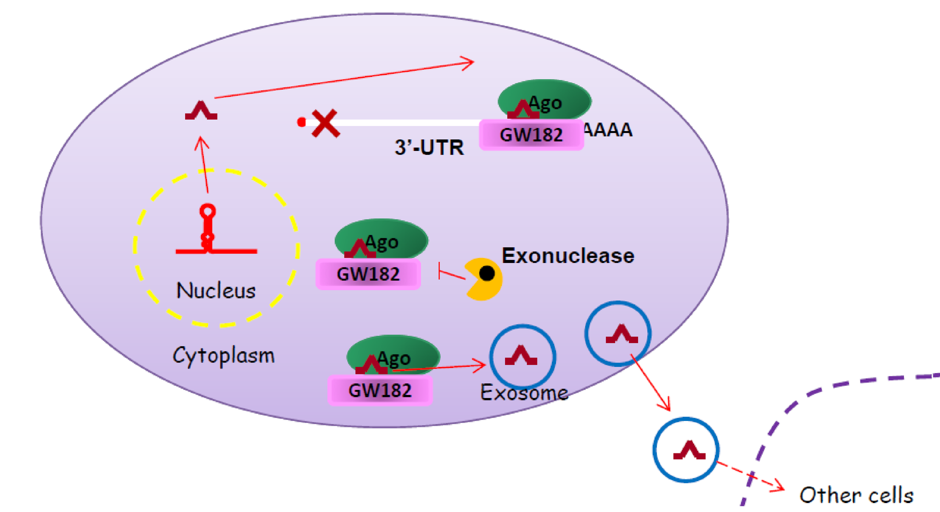
3. Depletion of GW182 reduced miRNA secretion via exosomes



(A). Overexpressed miRNA secreted into extracellular space. **(B).** Secreted miRNA was primarily via secretory exosomes. **(C).** miRNA in exosomes are protected from ribonuclease, **(D).** Overexpressed miRNA did not alter the quantity of exosomes **(E).** GW182 depletion reduced miRNA secretion.

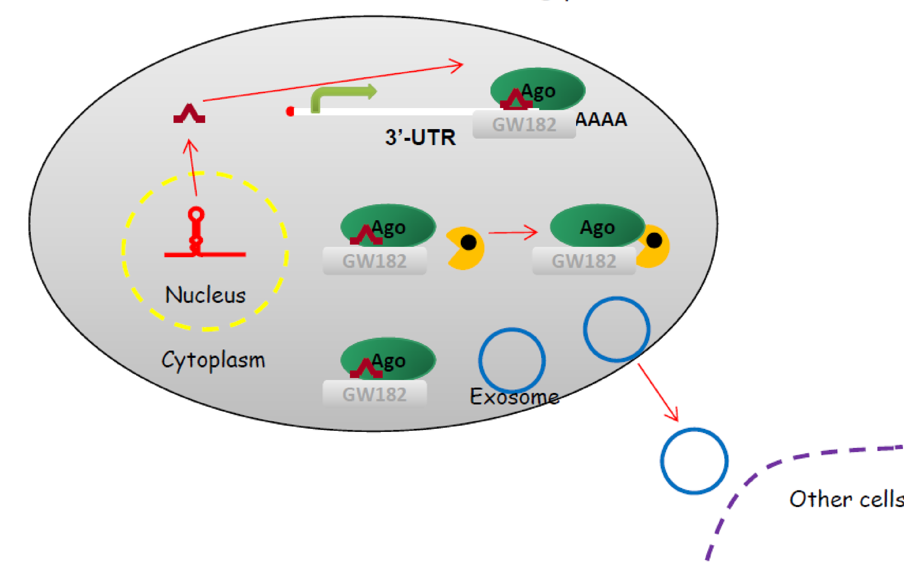
4. Current working model

GW182 in normal level – maintain miRNA mediated homeostasis



- ❑ GW182 silences target mRNA
- ❑ GW182 protects miRNA from degradation in favor of gene repression
- ❑ GW182 helps miRNA packed into exosome in favor of silencing adjacent cells?

Reduced GW182 level – lose silencing potential



- ❑ GW182 depletion de-represses target mRNA
- ❑ GW182 depletion causes miRNA shortening half-life in favor of gene de-repression or activation
- ❑ GW182 depletion reduces miRNA secretion

Summary

1. GW182 and TNRC6B depletion reduced miRNA half-life.
2. Overexpression of GW182 and its paralogs, isoform and middle silencing domain extended miRNA half-life.
3. Depletion of GW182 reduced miRNA secretion through exosomes.

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Acknowledgments

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