Design and construction of miRNA sponges for treatment of Glioblastoma

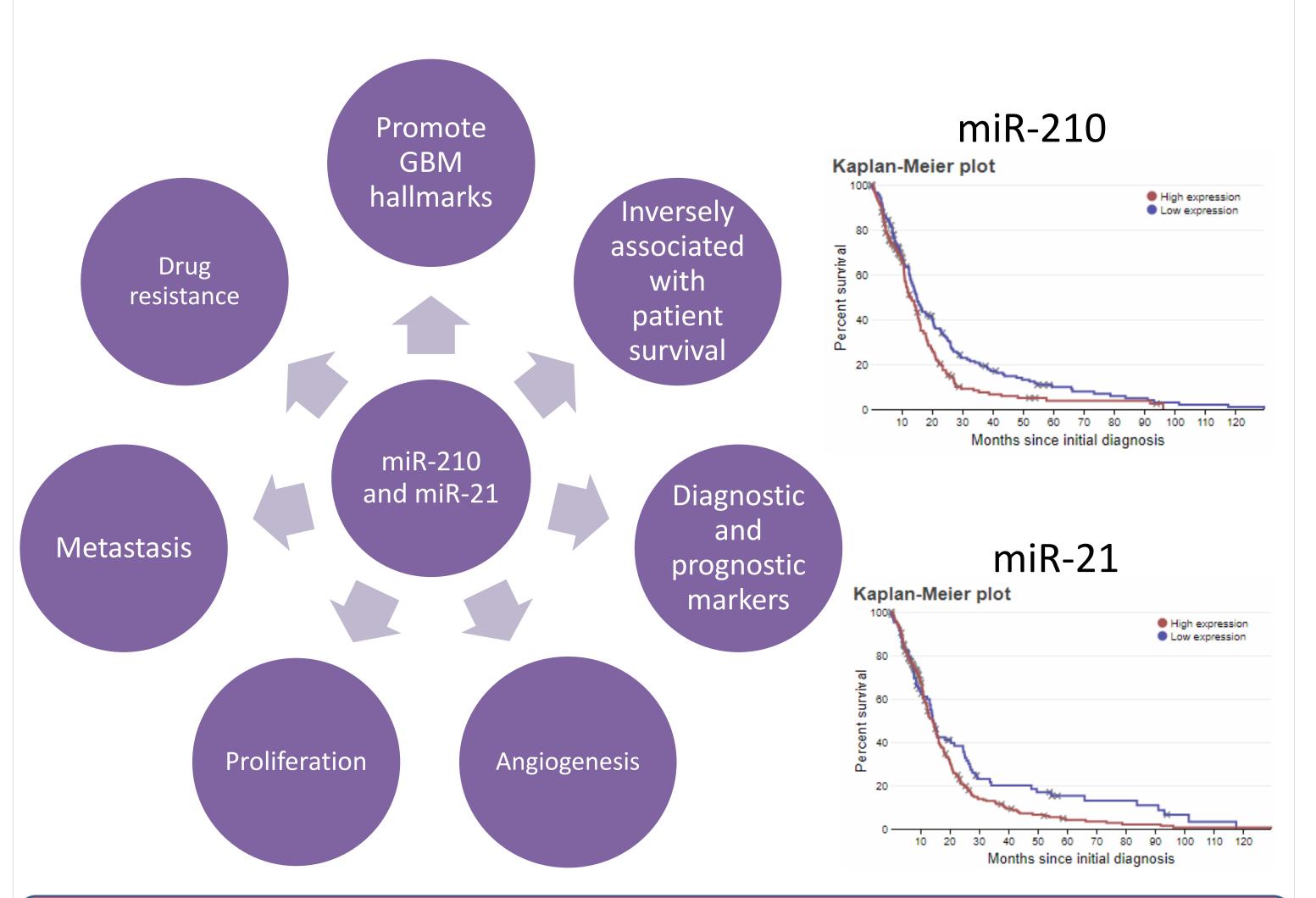
Avlokit Kumar, Kshitij Sahu, Aniket Patel, Yukti Makhija, Indranil Mondal, Omkar Suhas Vinchure, Dr. Ritu Kulshreshtha*



Abstract

MicroRNAs are small, highly conserved non-coding regulatory RNA molecules. They have been shown to control many fundamental biological processes such as development, differentiation, proliferation, apoptosis and post-transcriptional gene regulation in cells. Compelling evidences have demonstrated that miRNA expression is deregulated in Glioblastoma (GBM) through various mechanisms. miRNAs may function as either oncogenic or tumour suppressor. The oncogenic role of miR-21-5p promoting various hallmarks of GBM has been well studied. Apart from this, our lab has identified miR-210-3p as a hypoxia-regulated miRNA in GBM that also has pro-tumor properties. Thus, various methods for miRNA modulation have been explored. miRNA sponge based approach to inhibit miRNAs had initially shown promise, and has been extensively studied in our lab. The results of the sponge based therapy technique showed an inhibition rate of 25-30% in oncogenic miRNA levels. Here, we aim to develop novel and more effective therapy techniques to effectively target oncomiRs miR-21-5p and miR-210-3p in GBM.

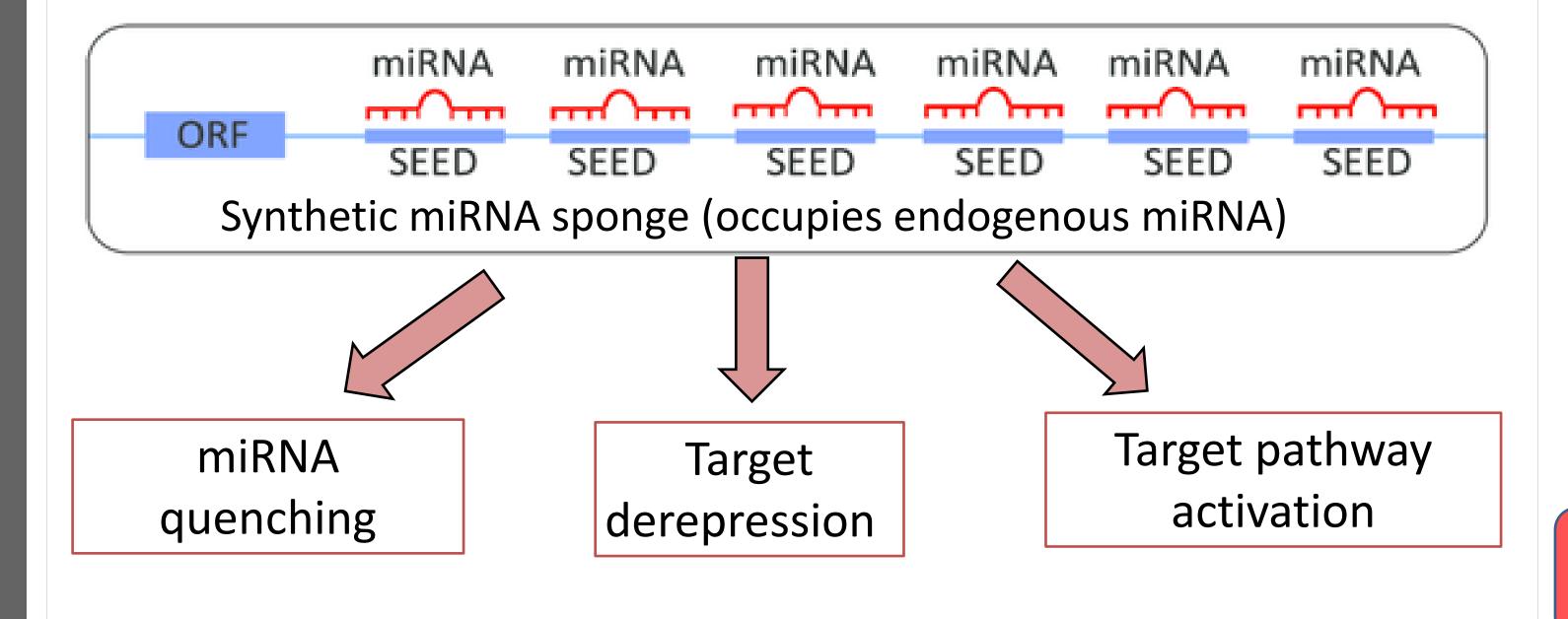
miR-21-5p and miR-210-3p: OncomiRs in GBM



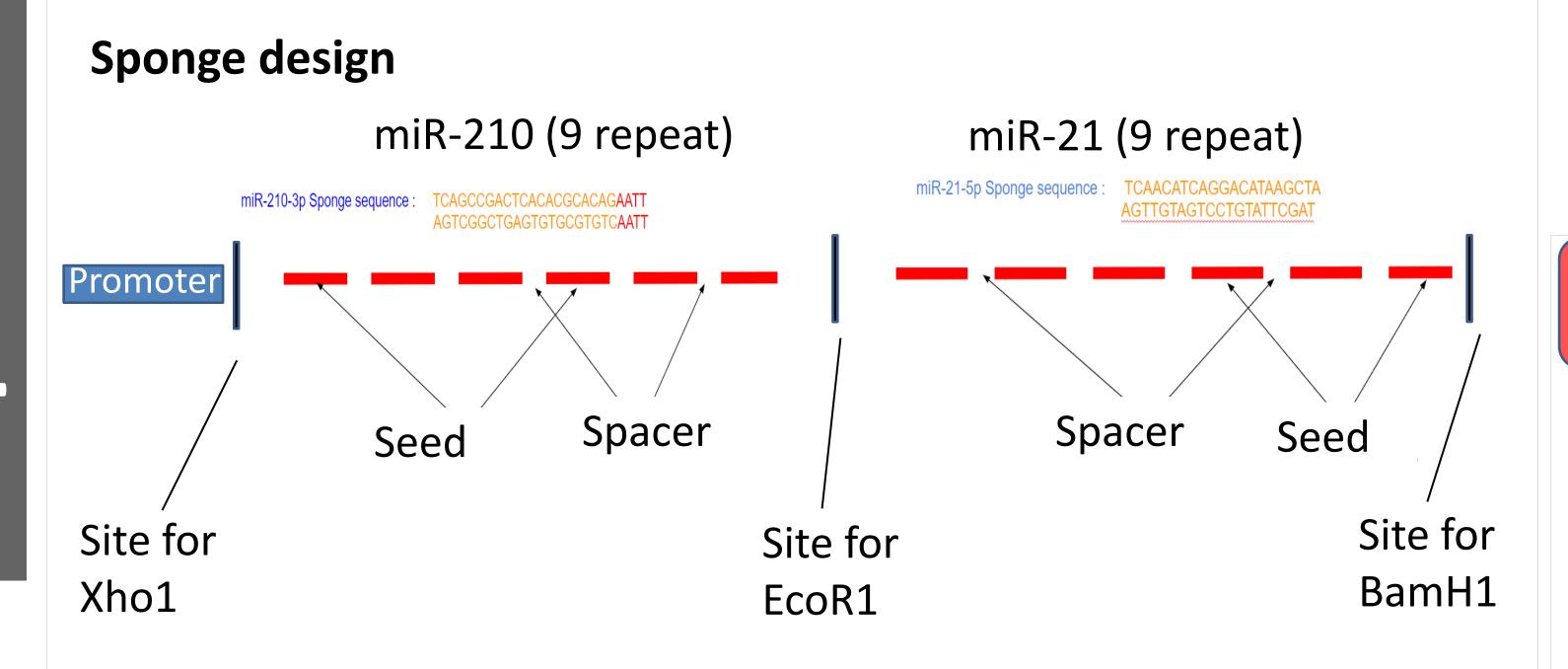
What are miRNA sponges?

- miRNA based sponges are specialized expression constructs that express miRNA-specific near-complimentary repeat sequences (seed sequences) as a part of a single transcript.
- These repeats serve as miRNA binding sites that 'quench' the available miRNAs and limit their bioavailability in the cell thereby leading to derepression of their cognate targets.

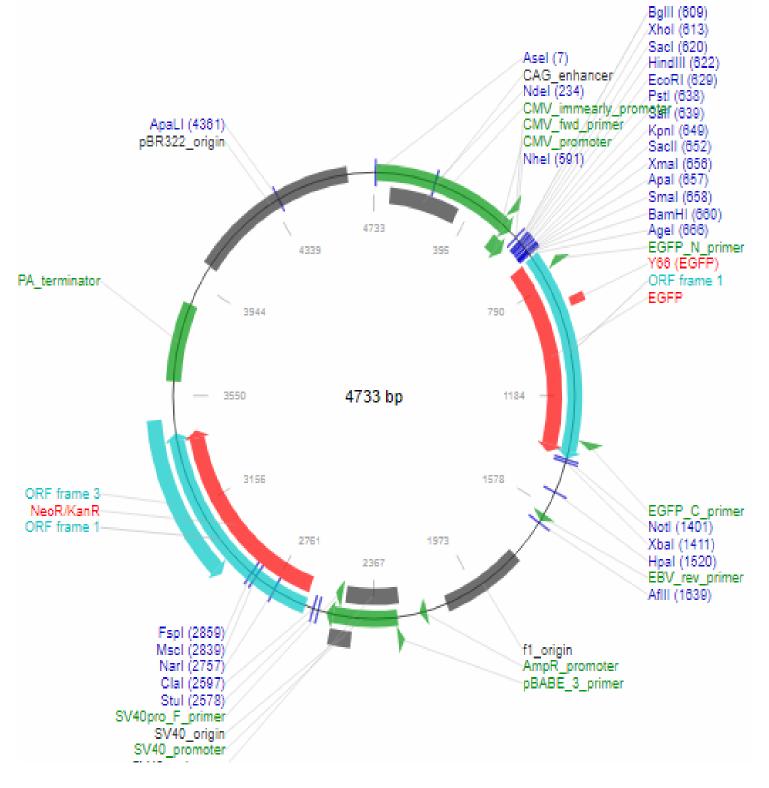
miRNA sponge: Mode of action



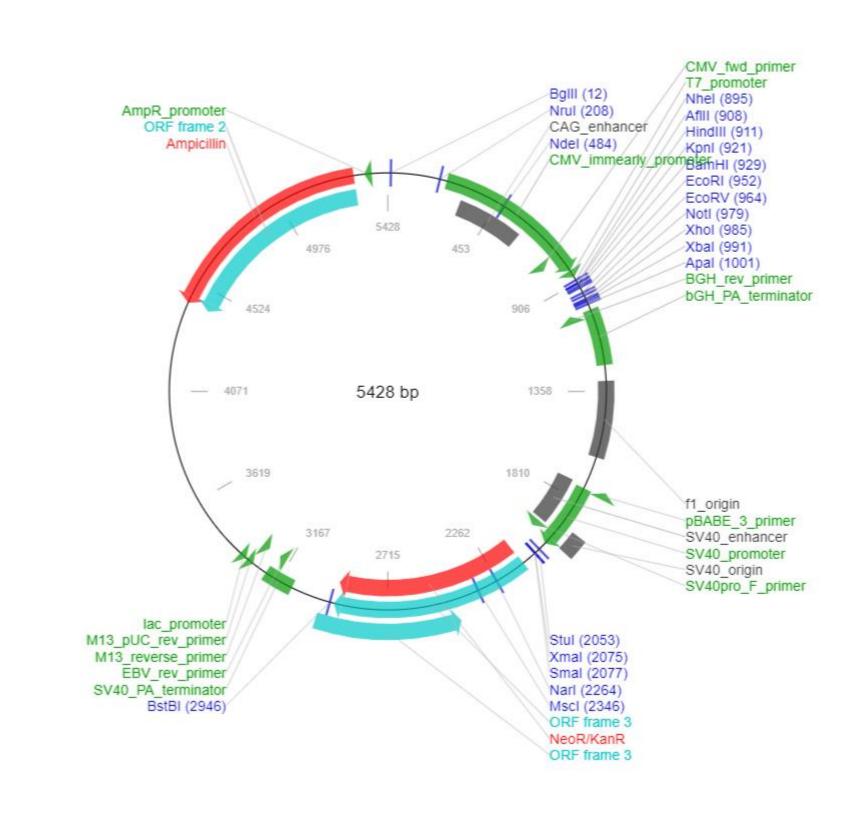
Results



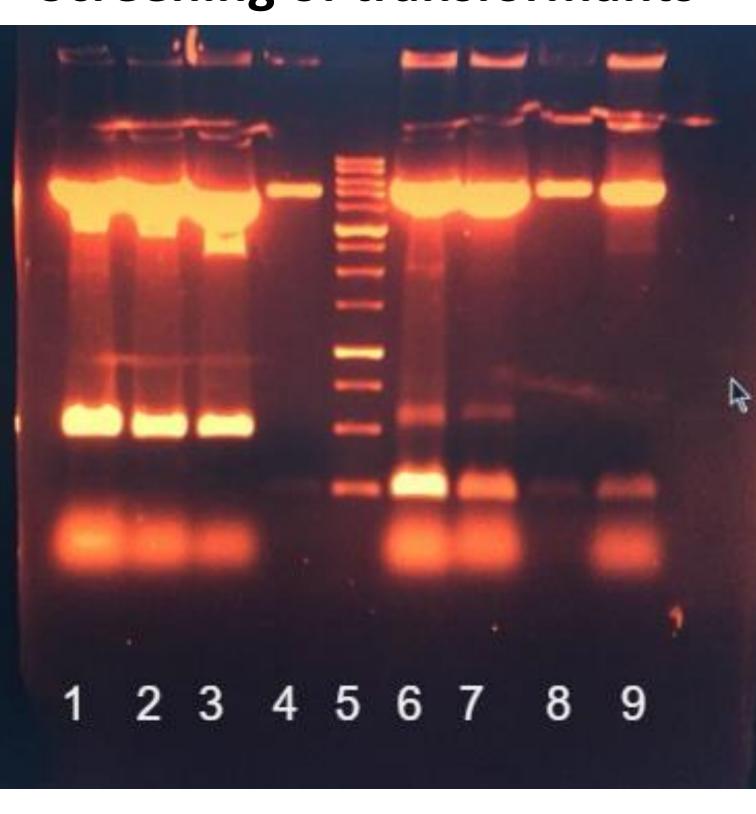
PEGFP N1 Vector



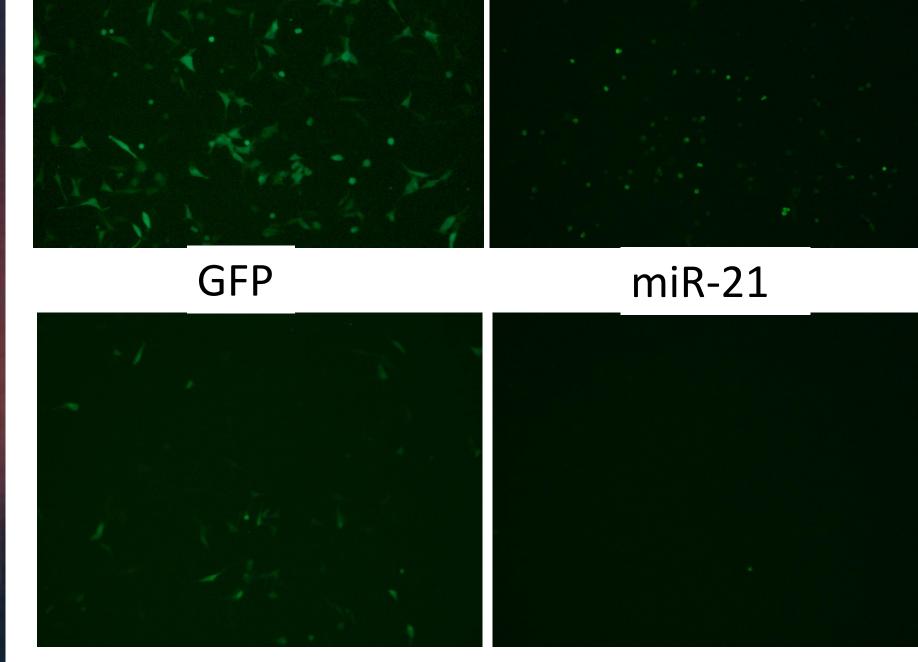
pcDNA3.1 (-)



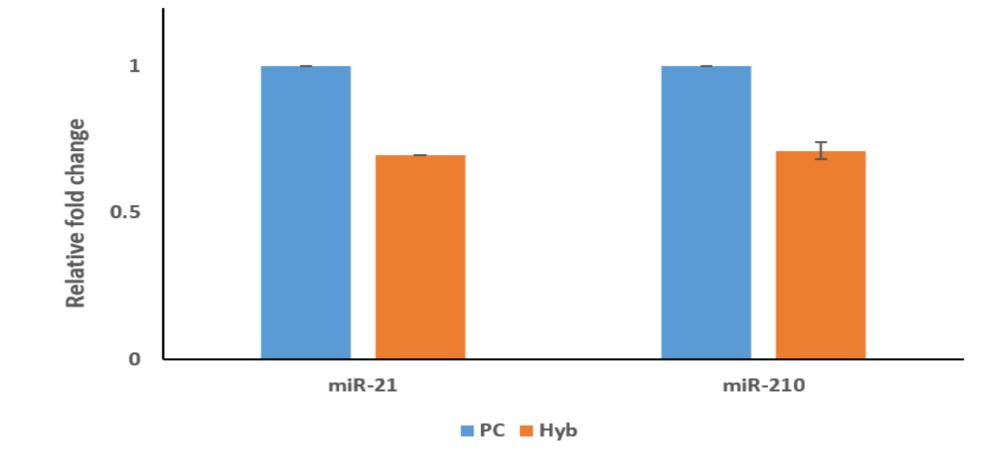
Screening of transformants



Transfection



miR-210 Hybrid



Conclusion and Future plan

- Partial repression of miRNA levels was observed.
- GFP expression was not evident when hybrid construct was used.
- Repression of individual miRNAs was observed only using hybrid sponge.
- Expression of miRNA target genes will also be analyzed.
- miRNA masking approach will also be considered.
- A vector with a stronger promoter can also be used.

References

- Bartel, D. P. MicroRNAs: Genomics, Biogenesis, Mechanism, and Function. *Cell* 116, 281–297 (2004).
- Kulshreshtha R, Ferracin M, Wojcik SE, Garzon R, Alder H, Agosto-Perez FJ, Davuluri R, Liu C-G, Croce CM, Negrini M, Calin GA, Ivan M: A microRNA signature of hypoxia. *Mol Cell Biol* 27:1859–1867 (2007).