

In vivo evaluation of optimized anti-hypoxia miRNA-6883 lipid nanoparticles in colorectal and breast cancer for CDK4/6 and HIF silencing

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Background*:
MicroRNA 6883 (miR-6883) inhibits colorectal and breast cancer growth through the silencing of targets including checkpoint kinases CDK4/6 and antiapoptotic protein BCL-XL. miR-6883 also decreases expression of Hypoxia Inducible Factor 1α (HIF1α), a crucial regulator of angiogenesis and cancer cell survival in low-oxygen environments. This effect may be mediated by the ubiquitin ligase SMURF2. We previously optimized a lipid nanoparticle formulation for the delivery of miR-6883 to improve tumor uptake and in vivo half-life. Here, we investigate the anti-hypoxia effect of miR-6883 in a murine colorectal carcinoma xenograft model.

Methods*:
To assess on-target silencing, a luciferase-CDK4 3'UTR fusion was transduced into HCT116 colorectal carcinoma and SKBR3 triple negative breast cancer cells. The CDK4 3'UTR contains a miR-6883 binding site, allowing the model to report miR-mediated silencing. A hypoxia response element (HRE) (Addgene #118706) was transduced into HCT116 and SKBR3 to monitor HIF activity under miR-6883 treatment. For in vivo experiments, 2 million HCT116-HRE cells were injected into nude mice subcutaneously. Groups consisted of untreated (n=3), 1µg (n=4), or 10µg (n=8) of LNP-encapsulated miRNA, administered via intratumoral injection. Luminescence was recorded at 0, 3, and 7 days.

Results*:
Our miR-6883 5-component LNP formulation attenuated luciferase signal in CDK4 3'UTR reporter cell lines after 48h in vitro. In the HCT116-HRE xenograft model, a 94.3% reduction in luciferase activity was observed 3 days post-injection with 1µg/mouse miR-6883 LNP compared to baseline, whereas luminescence in untreated controls increased 38.8%. After 7 days, luminescence in the 10µg-treated group remained 61.9% reduced versus baseline.

Conclusion*:

The present experiments confirm on-target in vivo activity of our optimized miR-6883 LNPs, evidenced by a marked decrease in HIF signaling. Future in vivo experiments will determine efficacy in breast cancer xenograft models, the ability of miR-6883 LNPs to inhibit tumor growth, and potential combination therapies.