Anti-Diabetic Activity of miR-103/107 Anti-miRs

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ABSTRACT

miR-103/107 are a family of microRNAs encoded in the introns of the pantothenate kinase genes. These microRNAs affect insulin sensitivity in mouse models of diabetes, and their expression inversely correlates with insulin sensitivity in humans, which demonstrate that these microRNAs represent potential targets for the treatment of type 2 diabetes. We have observed improved glucose homeostasis in both diet-induced obese (DIO) mice and leptin receptor-deficient db/db mice. It has been proposed that miR-103/107 inhibit adipocyte differentiation and that anti-miR treatment improves insulin sensitivity by increasing adipocyte differentiation. A number of laboratories have now observed a correlation between miR-103/107 expression and adipocyte size. We have performed time course studies in DIO mice treated with miR-103/107 anti-miRs to determine if changes in adipocyte size correlate with the onset of improvements in glucose metabolism. While we observed an increase in the number of small adipocytes and a decrease in large adipocytes following anti-miR treatment, glucose homeostasis improved prior to these morphological changes in the adipose tissue. These results suggest an additional mechanism may be responsible for the improved insulin sensitivity with anti-miR treatment. To this end, we are examining the metabolic effects of anti-miR-103 compounds *in vivo* using hyperinsulinemic-euglycemic clamp studies to determine which tissues contribute to the improvement in insulin sensitivity.

BACKGROUND

miR-103: 5'-AGCAGCAUUGUACAGGGCUAUGA-3'
miR-107: 5'-AGCAGCAUUGUACAGGGCUAUCA-3'
Seed

- miR-103 and miR-107 (miR-103/107) are highly expressed in brain, lung and white and brown adipose tissue with relatively low expression in liver
- Hepatic miR-103/107 levels are increased in mouse models of type 2 diabetes (ob/ob & DIO) and a positive correlation exists between hepatic miR-103/107 levels and insulin resistance in humans with fatty liver diseases [1]
- Viral and transgenic over-expression of miR-103/107 impair glucose tolerance and antagomiRs against miR-103/107 improve glucose tolerance [1,2]
- miR-103 transgenic mice have increased adipocyte size, while adipocyte size decreases after treatment with miR-103/107 antagomiRs [1]
- Inhibition of miR-103/107 may improve insulin sensitivity by increasing adipocyte differentiation

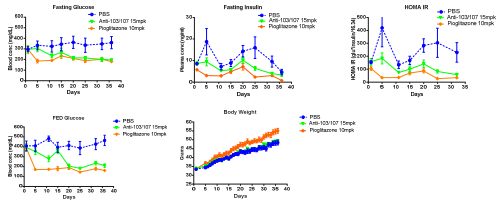
METHODS

- Anti-miR-103/107 is a 2'fluoro/2'-methoxyethyl modified oligonucleotide with a phosphorothioate backbone
- The anti-miR was administered subcutaneously to diabetic mice at 5 or 15 mg/kg, twice weekly. Pioglitazone was administered by oral gavage at 10 mg/kg, daily.
- Oral glucose tolerance – 4-6 hr fast; oral 2 g/kg 50% dextrose. Blood glucose measured by hand held glucose meter. Insulin measured by ELISA (Alpco).
- Liver triglycerides extracted with acetone and measured by colorimetric assay (Infinity Triglyceride Liquid Reagent).
- Euglycemic-hyperinsulinemic clamp - Indwelling catheter in jugular vein established 4-5 days before clamp. 2-hr hyperinsulinemic-euglycemic clamp performed on conscious, overnight fasted mice with a primed and continuous infusion of human insulin (2.5 mU/kg/min)[3]. 20% glucose infused at a variable rate. Continuous infusion of [3-^{3}H]glucose and bolus (10mCi) 2-deoxy-D-[1-^{14}C]glucose at 75 minutes to assess whole body glucose turnover and insulin-stimulated glucose uptake in individual tissues, respectively.

1 Trajkovski et al Nature 2011 474 649
2 Ohno et al Science Report 2013 3 2553
3 Kim et al Diabetes 2004 53 1060

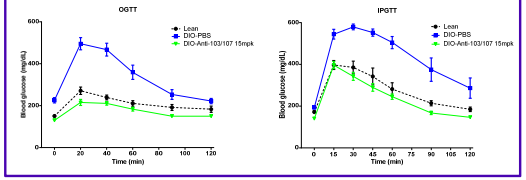
RESULTS: db/db Mice

Anti-miR-103/107 treatment improves insulin sensitivity in db/db (B6.BKS) mice

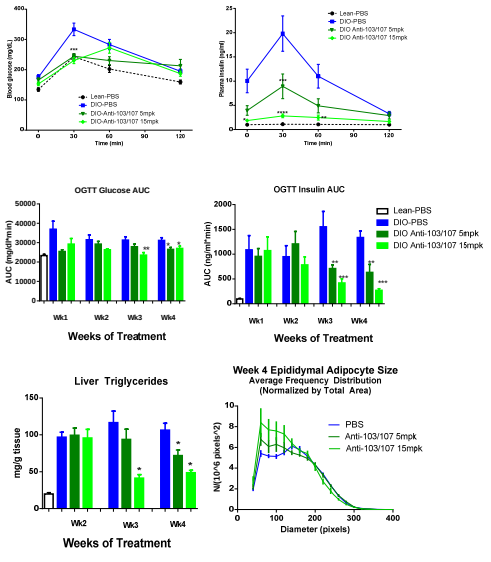


RESULTS: DIO Mice

Anti-miR-103/107 treatment improves glucose excursion in diet-induced obese (DIO) mice in response to oral or IP glucose challenge

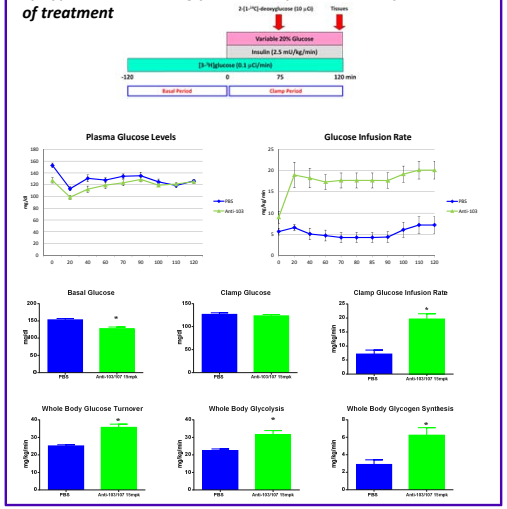


Anti-miR-103/107 mediated improvements in glucose homeostasis and liver triglycerides occur starting at 3 weeks of treatment. Significant changes in adipocyte size were detected after 4 weeks of treatment.

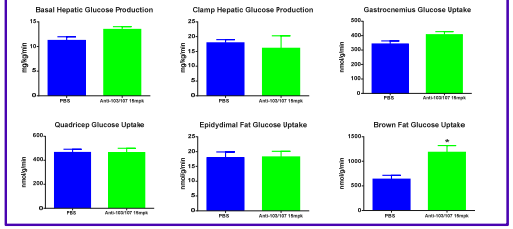


RESULTS: DIO Clamp

Anti-miR-103/107 treatment improves insulin sensitivity as shown by hyperinsulinemic euglycemic clamp in DIO mice after 3 weeks of treatment



Analysis of radiolabeled glucose tracers administered during the clamp procedure suggest anti-miR-103/107 leads to brown fat activation



CONCLUSIONS

- Inhibition of miR-103/107 decreases fasting blood glucose and plasma insulin and improves glucose tolerance as measured by oral and intraperitoneal glucose tolerance tests
- Time course studies in DIO mice suggest improvements in glucose homeostasis occur at the same time as decreases in hepatic triglycerides
- Significant increase in the number of small adipocytes was not evident until after 4 weeks of anti-miR-103/107 treatment, suggesting changes in adipocyte size distribution may be downstream of improved insulin sensitivity or lipid handling
- Results of the hyperinsulinemic euglycemic clamp confirm anti-miR-103/107 improves glucose homeostasis by increasing insulin sensitivity and suggest this may be mediated by brown fat activation