miR-22 inhibition as GLP1 agonist orthogonal mechanism for NAFLD and NASH treatment

Panella R[1,2,3], Tomasini S[1], Hansen HH[4], Feigh M[4], Kauppinen S[1]

[1]Center for RNA Medicine, Department of Clinical Medicine, Aalborg University, Copenhagen, Denmark; [2]Cancer Research Institute & Harvard Medical School Initiative for RNA Medicine, Department of Pathology, Beth Israel Deaconess Medical Center / Harvard Medical School, Boston, MA; [3]Resalis Therapeutics Srl, Via Nizza 52, 10126, Torino, Italy, [4]Gubra Aps, Hørsholm, Denmark

1 INTRODUCTION

Obesity is a growing public health problem. It is associated with several comorbidities like type 2 diabetes, cardiovascular disease, non-alcoholic fatty liver disease (NAFLD) and cancer.

NAFLD is characterized by a strong liver steatosis that over time evolves with chronic inflammation and fibrosis into non-alcoholic steatohepatitis (NASH). So far, the only therapeutic intervention in advanced clinical stage is the use of GLP1 agonist. These agonists that reduce appetite and thus food intake only have an indirect effect on hepatic steatosis. Furthermore, GLP1 effects are not stable over time and patients tend to show rebound in weight gain. Thus, both NAFLD and NASH have a high unmeet medical need and require alternative treatment options.

Here we identified microRNA-22 (miR-22) as an essential rheostat involved in the control of lipid and energy metabolism and present an effective Locked Nucleic Acid (LNA) oligonucleotide-based therapy for the treatment of obesity. The pharmacological inhibition of miR-22 reverses both obesity and NAFLD in different diet-induced murine models, without affecting food consumption. This approach provides a potential alternative to GLP1 agonist for the therapeutic intervention of obesity, NAFLD and NASH.

2 MATERIALS & METHODS

All experiments were carried out on female wild-type (C57BL/6J) obtained from The Jackson Laboratory (mouse strain Cat. #000664). The mice were given *ad libitum* access to normal chow, 60% high fat diet (HFD) or Gubra-Amylin-NASH (GAN) diet high in fat, fructose and cholesterol and drinking water for the entire duration of the experiment.

Mice were kept on HFD and treated with vehicle, a scramble LNA (SCR) or an LNA anti-miR-22 once a week with s.c. injection. All tissues were harvested, washed in PBS and stored on ice or fixed in 4% PFA overnight.

The anti-miR-22 oligos were designed using a mix-mer strategy to target the seed region of hsa-miR-22-3p, evolutionary conserved between mouse and human.

5 ACKNOWLEDGEMENTS & DISCLOSURES

This project is part of an international teamwork supported by Novo Nordisk Foundation, thought the "Challenge Program" NNF18OC0033438; PR, NAM, KS are beneficiaries of this Grant. PR and KS are founders and shareholders of "Resalis Therapeutics"; PR is also a Board Member at "Resalis Therapeutics".

3 RESULTS

We designed and tested a therapeutic strategy to pharmacologically inhibit miR-22 *in-vivo* and protect or cure from obesity/NAFLD.

(a) Mice treated with LNA against miR-22, contrary to mice treated with a scramble oligo or with just vehicle, were protected from becoming obese when fed with HFD.

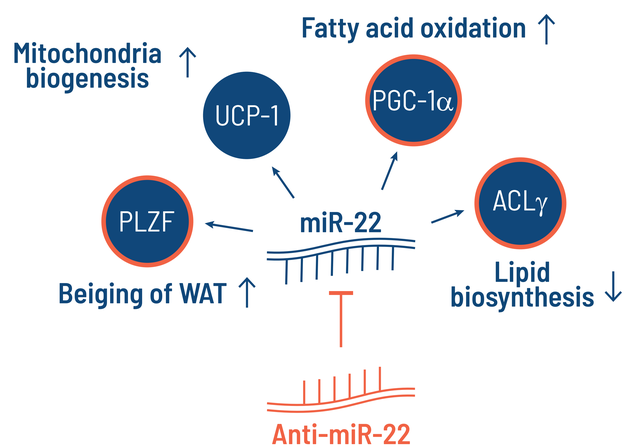
(a/b) Anti-miR-22 therapy was effective in reducing mouse weight in mice already obese and reverting liver steatosis.

(c) Therapeutic approach was effective in reducing weight gain also in the Gubra-Amylin mouse model and (d) improved NAFLD, lobular inflammation and steatosis score.

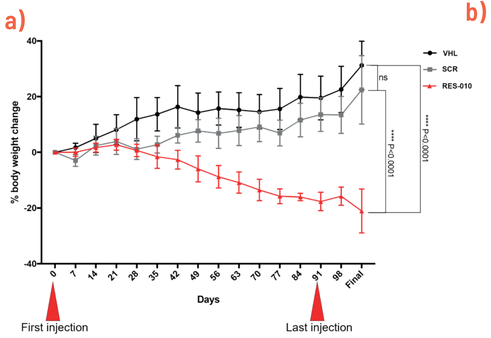
(e) Food intake was measured on a weekly basis in the DIO model or (f) as longitudinal value over time in the GAN model.

In both cases the observed effect was independent of food consumption, showing that our therapy is acting on pathways and molecular mechanisms that are orthogonal to GLP1 agonist.

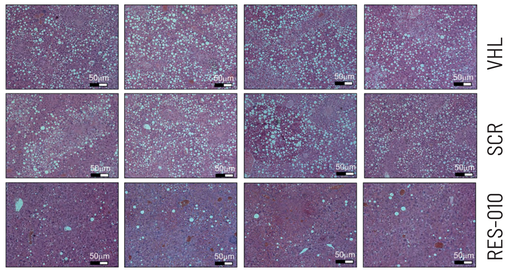
We identifyed 3 major metabolic players that are under miR-22 control and that can control lipid biosynthesis, mitochondrial biogenesis and beiging of white adipose tissue.



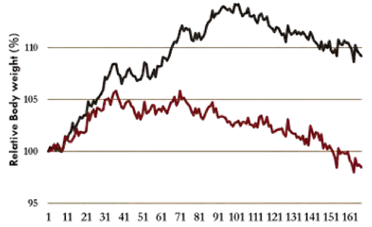
a)



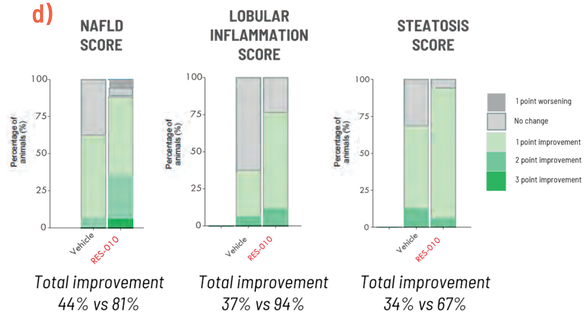
b)



c)



d)

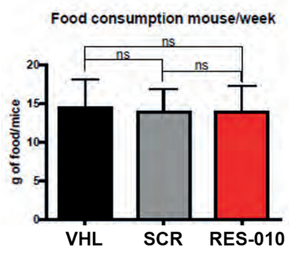


Total improvement
44% vs 81%

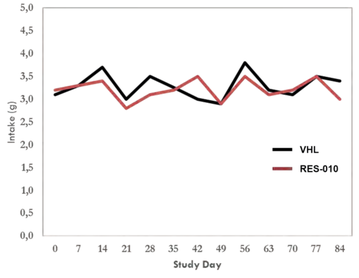
Total improvement
37% vs 94%

Total improvement
34% vs 67%

e)



f)



4 CONCLUSION

Our data provides evidence that miR-22 is a potential therapeutic target for the treatment of obesity and NAFLD due to its ability of controlling key metabolic pathways that all converge on obesity.