P-31

Lin28 Inhibition by a Small Molecule Led to Insulin Resistance and Increased Ketogenesis

E. Lekka[1], G. Civenni[2], C. Berk[1], S. Schmidli[1], A. Kokanovic[2], C. Catapano[2], J. Hall[1]

[1] *Institute of Pharmaceutical Sciences, ETH Zurich, 8093 Zurich, Switzerland*
[2] *Institute of Oncology Research, 6500 Bellinzona, Switzerland*

Introduction: The RNA-binding protein Lin28 and its paralogue Lin28b play critical roles in embryonic development, tumorigenesis, pluripotency, and energy utilization. Lin28 proteins bind to the terminal loops of most let-7 precursors and block their processing into mature miRNAs. Thus, Lin28 proteins de-repress the expression of let-7 target genes. Lin28 proteins also have let-7 independent roles. They bind directly to multiple mRNAs, amplifying their translation. Aberrant expression of Lin28 and let-7 has been observed in many human malignancies. Increasing evidence suggests that the Lin28/let-7 axis is physiologically required for normal glucose homeostasis [1]. Lin28 and Lin28b can increase the expression and sensitivity of components of the insulin-PI3K-mTOR signalling pathway and numerous metabolic genes are direct let-7 targets [1].
Aims: In the current project, we sought to examine the effect of the small molecule Lin28 inhibitor 1632 [2] in a mouse model of prostate cancer and to characterize the pharmacological properties of this compound.
Methods: Xenograft mice of human prostate cancer were intraperitoneally injected with 1632 and *in vivo* measurements (tumour growth/weight, glycaemia) were performed. Myoblast C2C12 and hepatoblastoma HepG2 cells were used as relevant *in vitro* systems. Transfections with plasmids or siRNAs were done with Lipofectamine 2000. Immunoblotting and qPCR were used to study the protein and mRNA levels of genes of interest. A ketone body assay was used to measure the concentration of 3-OH butyrate in mouse serum, and cell growth medium. A lactate assay was used to measure lactate concentration in cell growth medium.
Results: 1632-treated mice did not display any statistically significant change in their tumour growth and weight, but they showed elevated blood glucose levels. In light of the role of Lin28 in glucose metabolism, we analysed the protein levels of Lin28b, as well as some central components of the insulin-PI3K-mTOR pathway (IRb, P-Akt/Total Akt) and we found that they are down-regulated in skeletal muscle from 1632-treated mice. In the C2C12 cell culture system, gain and loss of function of Lin28 confirmed a role in fine-tuning the PI3K-mTOR pathway. Subsequent treatment of mice with 1632 showed a 3-fold increase in the serum concentration of 3-OH butyrate in 1632-treated mice over their mock-treated littermates. To investigate the potential role of Lin28 in ketogenesis, we have treated HepG2 cells with siLin28b and 1632. 1632-treated HepG2 cells displayed increased 3-OH butyrate secretion in their growth medium, along with decreased Lin28b and Insulin Receptor β levels.
Conclusions: Lin28b inhibition by a small molecule led to increased glycaemia, accompanied by repression of the insulin-PI3K-mTOR pathway in xenograft mice of prostate cancer. Non-tumour bearing mice treated with the same compound exhibited increased ketogenesis. This project offers the opportunity to gain a better understanding of how Lin28 participates in crucial metabolic processes, such as insulin sensitivity, glucose metabolism and ketogenesis.

Keywords: Lin28, glucose metabolism, ketogenesis

References:
[1] Zhu H et al. Cell 2011; 147: 81-94.
[2] Roos M et al. ACS Chem Biol 2016; 11: 2773-2781.