A HUMAN HEPATOCYTE-LIKE CELL BASED *IN VITRO* MODEL FOR HEPATIC INSULIN-DRIVEN *DE NOVO* LIPOGENESIS

Karolien Buyl[1], Martine Vrints[2], Ruani Fernando[1], Thomas Van Eeckhoutte[2], Mia Jans[2], Joost Boeckmans[1], Robim M. Rodrigues[1], Vera Rogiers[1], Jan Van Der Schueren[2,] Joery De Kock[1], Tamara Vanhaecke[1]*, Filip Beirinckx [2]*

[1] Department of *In Vitro* Toxicology and Dermato-Cosmetology (IVTD), Faculty of Medicine and Pharmacy, Vrije Universiteit Brussel (VUB), Laarbeeklaan 103, B-1090 Brussels, Belgium

[2] Galapagos NV, Industriepark Mechelen Noord, Generaal De Wittelaan L11 A3, B-2880 Mechelen, Belgium

* Equally contributing senior author

Steatosis, marked by increased intra hepatic triglyceride accumulation, is a hallmark of non-alcoholic fatty liver disease (NAFLD) and precedes the progression to non-alcoholic steatohepatitis (NASH) and liver fibrosis. Hepatic *de novo* lipogenesis (DNL), activated by glucose and insulin, is a major pathway in the development of steatosis and contributes to 38% of the intrahepatic triglyceride-palmitate content in NAFLD patients (Smith et al., 2020). Recent studies in both animal models and NAFLD patients demonstrated that a reduction in steatosis is associated with an improvement of NASH and hepatic fibrosis, indicating the therapeutic potential of drugs acting on hepatic steatosis (Harrison et al., 2019; Gapp et al., 2020). Currently there is a lack of human *in vitro* hepatocyte models that can support the identification of novel drugs inhibiting hepatic DNL. None of the existing models are described to be sensitive for insulin driven DNL, while the available rodent hepatocyte models (*ex vivo* or precision-cut liver slices) have insufficient throughput for effective drug discovery (Prins et al., 2019). In collaboration with the lab of *In Vitro* Toxicology and Dermato-Cosmetology of the Vrije Universiteit Brussel (VUB), we identified that the human hepatocyte-like cells (HLCs) (Natale et al.,

2018), derived from skin precursor cells (hSKP), are uniquely sensitive to insulin driven DNL, shown by both gene expression and lipid accumulation readouts. We demonstrated that the sensitive HLCs showed an increased SREBP-1C expression, a key transcription factor for DNL, upon insulin stimulation. Moreover, inhibition and activation of the DNL pathway could be demonstrated using reference inhibitors (ACCi and AKTi) and activators (LXRa). After miniaturization of the lipid accumulation assay to a 384-well plate format, a library of publicly available mode-of-action chemical substances was screened to validate the relevance of the model and to identify novel targets involved in the DNL.

KEY WORDS

Non-alcoholic fatty liver disease (NAFLD)

De novo lipogenesis (DNL)

High throughput assay

Human hepatocyte-like cells (hSKP-HPC)

Drug discovery

REFERENCES

1. Smith, G., Shankaran, M., Yoshino, M *et al.* (2020) J Clin Invest. 130, 1453-1460. doi:10.1172/JCI134165.
2. Harrison, S., Bashir, M., Guy, C., *et al*. (2019) Lancet 2019; 394: 2012–24.dio: 10.1016/S0140-6736(19)32517-6
3. Gapp, B., Jourdain, M., Bringe, P., *et al*. (2020) Hepatology Communications 4, 109-125. dio: 10.1002/hep4.1443
4. Prins, G., Luangmonkong, T., Oosterhuis, D., *et al*. (2019) Nutrients 11, 507. doi: 10.3390/nu11030507

5. Natale, A., Boeckmans, J., Desmae, T. *et al.* (2018) Toxicology Letters 284, 184–194. doi: 10.1016/j.toxlet.2017.11.014