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IDENTIFICATION OF MOLECULAR MEDIATORS OF SIGNALLING FROM INTESTINE TO INSULIN SENSITIZATION AND THE BROWNING OF ADIPOSE TISSUE: ROLE OF LBP AND FGF15/19

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1. Summary of the project

The aim of this study was to investigate the relationship between obesity-associated metabolic disorders, mainly insulin resistance, and intestinal secretion of fibroblast growth factor 15/19 (FGF15/19) and lipopolysaccharide binding protein (LBP), and to determine the impact of FGF15/19 and LBP on insulin sensitivity, metabolic homeostasis and browning of adipose tissue, as potential therapeutic intervention systems.

Human studies indicated that circulating levels of FGF19 are decreased in patients with morbid obesity whereas levels of LBP are increased, especially in those with non-alcoholic fatty liver disease (NAFLD). Using the FGF15-null mouse model and gain-of-function approaches based on mouse FGF15 or human FGF19 overexpression, FGF15/19 was identified as a molecular mechanism that facilitates the communication of the intestine to adipose tissue, thus regulating the adaptive browning of white adipose tissue. We also evaluated the potential use of LBP as a therapeutic target. Specific adipose tissue LBP gene knock-down prevented obesity-associated fat accretion and induced adipose tissue browning. Moreover, liver specific LBP gene knock-down using siRNA lipid nanoparticles reduced hepatic steatosis and improved insulin resistance in diet-induced obesity mouse models. Altogether current results substantiate tissue-specific targeting of LBP as a therapeutic strategy in the prevention and therapy of obesity associated liver steatosis and adipose disturbances. Likewise, FGF15/19-mediated induction of adipose tissue browning appears as an intestine-originating process that contributes to metabolic health improvement by promoting energy expenditure and protecting against hyperglycemia and hyperlipidemia.

2. Results

FGF15 (and its human orthologue, FGF19) is an enterokine (a hormone secreted by the intestine) and liver is its main recognized target. Our findings indicated a negative association between circulating FGF19 levels and BMI, adiposity, insulin, and HOMA-IR levels in a cohort of morbidly obese patients. In contrast, plasma FGF19 levels positively correlated with the expression of the brown/beige marker gene UCP1 in human subcutaneous adipose tissue. This prompted us to use mouse models to study

the role of FGF15/19 as a potential mediator of intestine originating signaling to control the browning of adipose tissues. Experimental increases in FGF15 or FGF19 induced white fat browning in mice, including increased UCP1 protein levels. Mice lacking FGF15 showed markedly impaired white adipose tissue browning and a mild reduction in parameters indicative of BAT activity in response to cold-induced environmental thermogenic challenges. This was concomitant with signs of altered systemic metabolism, such as reduced glucose tolerance and impaired cold-induced insulin sensitization. Browning of white adipose tissue was also impaired in mice with reduced β-klotho (KLB), the obligatory co-receptor for FGF15/19 (and FGF21) action on target tissues. In conclusion, FGF15/19 is a molecular mechanism that facilitates the communication of the intestine to adipose tissues, thus promoting energy expenditure and improving metabolic health. The intestinal origin of FGF15/19 shows promise to be explored for being targeted through dietary interventions to profit its potential beneficial effects on obesity and/or diabetes.

Our transcriptomic studies of the human jejunum showed that expression of genes related to the immune response, and in particular the antiviral response, is significantly associated with insulin sensitivity and basal blood glucose levels. The negative association between fat mass and the expression of genes related to the catabolism of fatty acids in the jejunum would indicate that inhibition of these genes could have an obesogenic effect, enhancing the accumulation of body fat. The expression in jejunum of lysozyme (marker of Paneth cell functionality) correlates significantly (and negatively) with age, suggesting that aging would reduce the functionality of Paneth cells. However, there was no association with other metabolic parameters. In contrast, serum/plasma and adipose tissue lysozyme levels were significantly associated with obesity and insulin resistance. In addition, our pre-clinical studies (in mice) showed that lysozyme silencing in adipose tissue reduced weight gain and improved adipose tissue physiology.

LPS-binding protein (LBP) is a relevant component of innate immunity response, thought to be mainly produced by the liver but also synthesized in adipose tissues and small intestine. Circulating LBP levels were found to be increased with obesity and, in patients with morbid obesity, in those with non-alcoholic fatty liver disease (NAFLD). Mice deficient in LBP showed decreased liver lipid accumulation in the context of obesity. LBP-null mice also showed decreased weight gain, induction of adipose tissue

browning and protection against inflammatory responses induced by a high-fat diet. We further evaluated the use of LBP as a therapeutic target using diet-induced obesity mouse models. Liver specific LBP gene knock-down using lipid encapsulated unlocked nucleic acid modified siRNA nanoparticles reduced hepatic steatosis and improved insulin resistance in diet-induced obesity mouse models. Moreover, specific LBP gene knockdown in adipose tissue through lentiviral shRNA-LBP vectors prevented diet-induced body weight and induced adipose tissue browning. In conclusion, LBP is both a biomarker and an active actor in determining the inflammation associated with insulin resistance and hepatic steatosis in obesity. Our results support that intervention upon LBP is a promising therapeutic strategy, via tissue-specific silencing in the liver and/or adipose tissue, for improvement of obesity/diabetes related metabolic disease.

3. Relevance with possible future implications

Our data and others have revealed a negative correlation between circulating FGF19 levels and obesity and type II diabetes in humans. We propose plasma FGF19 levels as a biomarker and regulatory actor in relation to obesity and associated metabolic disorders

The endocrine factor FGF15/19 is a signaling component of intestinal origin (enterokine) that participates in the control of adipose tissue plasticity during thermogenic adaptations. Considering that browning of adipose tissue protects against metabolic diseases in rodent models and possibly also in humans, FGF15/19 is proposed as a molecular mechanism mediating intestine to adipose tissue communication, and as a therapeutic target for promoting energy expenditure and metabolic homeostasis.

Serum/plasma lysozyme and adipose tissue levels are significantly associated with obesity and insulin resistance. In addition, preclinical studies (in mice) performed show that lysozyme silencing in adipose tissue reduces weight gain and improves adipose tissue physiology. In future projects, we propose to evaluate the impact of lysozyme modulation on obesity and insulin resistance in humans.

Inhibition of hepatic LBP expression using lipid nanoparticles is a safe way to reduce circulating LBP levels, and has a therapeutic effect, preventing and reducing liver fat accumulation and improving adipose tissue physiology and sensitivity to insulin. These findings have led to the development of a patent. The next step would be to plan preclinical experiments in nonhuman primates to confirm these beneficial effects before proposing clinical trials in humans.

Silencing of LBP in adipose tissue would confirm the importance of LBP in adipose tissue expansion and hypertrophy.

In summary, and taking into account our data, several gene therapy approaches can be envisaged: silencing of hepatic LBP using of siRNA nanoparticles can be a very useful tool to prevent hepatic steatosis and improve insulin sensitivity, while silencing of LBP and lysozyme in adipose tissue could improve obesity-associated metabolic disorders.

4. Generated scientific bibliography

Moure R, Cairó M, Morón-Ros S, Quesada-López T, Campderrós L, Cereijo R, Hernáez A, Villarroya F, Giralt M.
Levels of β-klotho determine the thermogenic responsiveness of adipose tissues: involvement of the autocrine action of FGF21.
Am J Physiol Endocrinol Metab. 2021 Apr 1;320(4):E822-E834.
doi: 10.1152/ajpendo.00270.2020. Epub 2021 Feb 22. PMID: 33615874.

Latorre J, Lluch A, Ortega FJ, Gavaldà-Navarro A, Comas F, Morón-Ros S, Rodríguez A, Becerril S, Villarroya F, Frühbeck G, Ricart W, Giralt M, Fernández-Real JM, Moreno-Navarrete JM.
Adipose tissue knockdown of lysozyme reduces local inflammation and improves adipogenesis in high-fat diet-fed mice.
Pharmacol Res. 2021 Apr;166:105486.
doi: 10.1016/j.phrs.2021.105486. Epub 2021 Feb 5. PMID: 33556481.

Morón-Ros S, Uriarte I, Berasain C, Avila MA, Sabater-Masdeu M, Moreno-Navarrete JM, Fernández-Real JM, Giralt M, Villarroya F, Gavaldà-Navarro A.
FGF15/19 is required for adipose tissue plasticity in response to thermogenic adaptations.
Mol Metab. 2021 Jan;43:101113.
doi: 10.1016/j.molmet.2020.101113. Epub 2020 Nov 7. PMID: 33171307.

Moreno-Navarrete JM, Latorre J, Lluch A, Ortega FJ, Comas F, Arnoriaga-Rodríguez M, Ricart W, Fernández-Real JM.
Lysozyme is a component of the innate immune system linked to obesity associated-chronic low-grade inflammation and altered glucose tolerance.
Clin Nutr. 2021 Mar;40(3):1420-1429.
doi: 10.1016/j.clnu.2020.08.036. Epub 2020 Sep 4. PMID: 32943240

Villarroya F, Gavaldà-Navarro A, Peyrou M, Villarroya J, Giralt M.
Brown Adipokines.
Handb Exp Pharmacol. 2019;251:239-256.
doi: 10.1007/164_2018_119. PMID: 29675584. Springer Nature. ISBN: 978-3-030-10512-9

Villarroya F, Gavaldà-Navarro A, Peyrou M, Villarroya J, Giralt M.
The Lives and Times of Brown Adipokines.
Trends Endocrinol Metab. 2017 Dec;28(12):855-867.
doi: 10.1016/j.tem.2017.10.005. PMID: 29113711.

SUBMITTED
Comas F, Díaz-Trelles R, Latorre J, Gavaldà-Navarro A, Morón-Ros S, Mukthavaram R, Ortega F, Castells-Nobau A, Mayneris-Perxachs J, Milbank E, Dragano N, Oliveras-Cañellas N, Ricart W, Federici M, Burcelin R, López M, Karmali PP, Tachikawa K, Chivukula P, Villarroya F, Giralt M, Fernández-Real JM, Moreno-Navarrete JM.
The targeting of LBP through lipid nanoparticles containing unlocked nucleic acid modified siRNAs improves metabolic-associated fatty liver disease in mice.
Nat Commun. (under Review, positive 1st round).

Latorre J, Ortega F, Oliveras-Cañellas N, Comas F, Lluch A, Gavaldà-Navarro A, Morón-Ros S, Ricart W, Villarroya F, Giralt M, Fernández-Real JM, Moreno-Navarrete JM.
Specific adipose tissue Lbp gene knockdown prevents diet-induced body weight gain, impacting on fat accretion-related gene expression.
Mol Ther Nucleic Acids (under Review, positive 1st round).

IN PREPARATION
Comas F, Díaz-Trelles R, Gavaldà-Navarro A, Milbank E, Dragano N, Morón-Ros S, Mukthavaram R, Latorre J, Ortega F, Arnoriaga-Rodriguez M, Oliveras-Cañellas N, Ricart W, Karmali PP, Tachikawa K, Chivukula P, Villarroya F, Giralt M, López M, Fernández-Real JM, Moreno-Navarrete JM.
Plasma lipopolysaccharide-binding protein is involved in gender-related differences in food consumption, fat accretion and adipose tissue dysfunction.
(Manuscript in preparation).