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MECHANISMS RESPONSIBLE FOR THE DEFECTIVE DIFFERENTIATION CAPACITY OF ADIPOSE-DERIVED STEM CELLS IN OBESITY: IDENTIFICATION OF NEW REGULATORS AMENABLE FOR THE DEVELOPMENT OF INNOVATIVE THERAPIES

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1. Summary

Adipose tissue contains a pool of multipotent stem cells, designated as adipose-derived stem cells (ASCs) that are able to replicate or to develop as mature adipocytes, which are important players in adipose tissue remodeling during obesity. Obesity is linked to defective cellular turnover but the mechanisms implicated in the recruitment of ASCs to adipogenesis are unknown. The identification of the factors responsible of the recruitment and subsequent adipogenic differentiation of hASCs is essential to identify novel targets to fight against obesity. The global aim of this proposal was to elucidate the mechanisms involved in the altered biology of human ASCs during obesity and to validate key regulators of adipogenic differentiation. Thus, we have set out an integrated, multidisciplinary approach at different but complementary levels including omics, as well as cellular and animal models. Furthermore, we have studied in depth the role of the autophagy protein TP53INP2 as a regulator of adipogenesis in humans and in mice. We document through the use of cultured cells, mouse models, and human biopsies that TP53INP2 is a key regulator of adipogenesis. In white precursor cells, TP53INP2 is a repressor of adipogenesis, whereas it operates as an activator of brown adipogenesis.

The information obtained in this coordinated project is useful to validate new drug targets and therapeutical approaches for the treatment of obesity and related comorbidities such as type 2 diabetes, and it makes it possible to propose that TP53INP2 represents a novel target for the treatment of metabolic disorders.

2. Results

Multilevel analysis of hASC cells allowed us to obtain a great deal of data that will feed our research activities during the next years. At present this has made it possible to document the following concepts: a) adipocyte precursors from obese patients have a characteristic epigenetic signature, which has a significant impact on the metabolic phenotype of derived mature adipocytes; b) TBX15 is a key metabolic regulator of adipocytes; c) type 2 diabetes promotes the release of proteins into the extracellular space, which are potential biomarkers of the pathophysiological events underlying this disease. Specifically, we have identified some new proteins with therapeutic potential

in the treatment of obesity and associated comorbidities (e.g. cancer); d) the ASCs from visceral origin show a mesothelial origin, which might explain some of their immunomodulatory properties. As mentioned before, this analysis has made possible the identification of multiple candidates, which opens the door for future studies.

We have also precisely explored the role of TP53INP2 on the physiology of adipose tissue. We have documented that TP53INP2 is regulated during obesity, and obese subjects showed reduced TP53INP2 mRNA levels compared with the control group in both subcutaneous and visceral fat. We also tested in vitro the adipogenic capacity of hASCs in the presence or absence of TP53INP2. TP53INP2 overexpression blocked human adipogenic differentiation and reduced PPARγ expression in human adipocytes. In mouse 3T3-L1 pre-adipocytes, TP53INP2 also negatively regulated adipogenesis. In contrast to those studies, TP53INP2 deficient brown preadipocytes lost their brown adipogenic capacity compared to control cells. The mechanisms by which TP53INP2 inhibits white adipogenesis involves β-catenin, and we have demonstrated that TP53INP2-deficient 3T3-L1 preadipocytes showed reduced levels of total β-catenin and of active non-phosphorylated β-catenin. In contrast, TP53INP2 gain-of-function caused an increase in the abundance of total and active β-catenin in mouse 3T3-L1 and in human preadipocytes. Moreover, we have documented that TP53INP2 modulates the levels of β-catenin trough an autophagy dependent mechanism. Specifically, TP53INP2 promotes the sequestering of GSK3-β inside multivesicular endosomes to facilitate the accumulation of β-catenin and the subsequent blockage of adipogenesis. Finally, we have also provided evidence for a role of TP53INP2 in adipose tissue physiology. Thus, we have demonstrated that TP53INP2 ablation causes adipose hyperplasia in different white adipose depots, whereas brown adipose becomes inactivated. Based on these data we propose the search of mechanisms of TP53INP2 activation that may lead to anti-obesity therapies.

3. Relevance with possible future implications

Obesity constitutes a global pandemic with devastating consequences that affect more than two billion people. About 40% of the world's population has obesity or overweight. Obesity plays a central role in morbidity and mortality of diseases of multiple organs and systems, and it is a major contributor to the growing incidence of type 2 diabetes,

and some forms of cancer. In turn, these diseases have a great impact in the biology of adipose tissue cells, including ASCs, which possess a central role in maintaining adipose tissue homeostasis by regulating adipocyte turnover. Beyond their role as adipocyte precursors, the study of ASCs is gaining popularity since emerging evidence also point to ASCs as potential key drivers in cancer progression. Moreover, ASCs also represent an excellent cellular-based tool for clinical applications in regenerative medicine due to their immunomodulatory and wound healing capabilities. Comprehensive knowledge of ASCs biology might have several clinical implications, namely:

a) it might help the development of new and more efficient therapeutic strategies to combat obesity and its related comorbidities (specially type 2 diabetes) targeting adipose tissue expansion, which could have an impact in both prevention and progression;

b) it may contribute in the knowledge of the obesity-cancer link and in the design of new therapies;

c) It will assist in the improvement of the ASCs use in regenerative medicine.
More specifically, we have robustly documented that TP53INP2 is a key regulator of white adipogenesis through a mechanism that entails activation of beta-catenin. These studies have revealed a novel mechanism of regulation of adipogenesis, and this will permit the future design of novel therapeutic strategies based on the modulation of TP53INP2 to fight against metabolic diseases.

In all, this proposal has generated a wealth of molecular information that is relevant in the identification of novel targets for the treatment of metabolic diseases. In addition, the mining of data generated through this workflow will drive future research and projects in ASCs thanks to the identification of new targets.

4. Publications

Ejarque M, Ceperuelo-Mallafré V, Serena C, Maymo-Masip E, Duran X, Díaz-Ramos A, Millan-Scheiding M, Núñez-Álvarez Y, Núñez-Roa C, Gama P, Garcia-

Roves PM, Peinado MA, Gimble JM, Zorzano A, Vendrell J, Fernández-Veledo S. Adipose tissue mitochondrial dysfunction in human obesity is linked to a specific DNA methylation signature in adipose-derived stem cells. **Int J Obes (Lond)**. 2019. doi: 10.1038/s41366-018-0219-6.

Romero M, Zorzano A. Role of autophagy in the regulation of adipose tissue biology. **Cell Cycle**. 18, 1435-1445, 2019.

Ivanova S, Polajnar M, Hernandez Alvarez MI, Slobodnyuk K, Plana N, Nebreda AR, Palacin M, Gomis R, Behrends C., Zorzano A. Autophagic protein TP53INP2 regulates death receptor-induced apoptosis by scaffolding TRAF6 and caspase-8. **EMBO J**. 38, pii: e99300, 2019.

Ivanova S, Zorzano A. TP53INP2 at the crossroad of apoptosis and autophagy in death receptor signaling. **Mol. Cell. Oncol**. 6, e1632687, 2019.

Gordaliza-Alaguero I, Cantó C, Zorzano A. Metabolic implications of organelle-mitochondria communication. **EMBO Rep**. 20, e47928, 2019.

Sun-Wang JL, Ivanova S, Zorzano A. The dialogue between the ubiquitin-proteasome system and autophagy: implications in ageing. **Ageing Res Rev**. 64, 2020. doi: 10.1016/j.arr.2020.101203.

Madeira A, Serena C, Ejarque M, Maymó-Masip E, Millán M, Navarro-Ruiz MC, Guzmán-Ruiz R, Malagón MM, Espin E, Martí M, Menach M, Megía A, Vendrell J, Fernández-Veledo S. Crohn's Disease Increases the Mesothelial Properties of Adipocyte Progenitors in the Creeping Fat. ***Int. J. Mol. Sci.*** 2021, *22*, x. https://doi.org/10.3390/xxxxx