Fundació La Marató de TV3
22nd SYMPOSIUM
Diabetes and Obesity

ANALYSES OF THE GLOBAL KINASE ACTIVITIES IN ADIPOSE TISSUE TO FIND MOLECULAR DIFFERENCES RESULTING FROM INSULIN RESISTANCE ACROSS DIFFERENT CLINICAL PHENOTYPES FROM LEAN TO OBESE AND TYPE 2 DIABETIC PATIENTS. DISCOVERY OF NEW TARGETS FOR THERAPY

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1. Summary

Insulin resistance (IR) is a central pathophysiological contributor to the development of many cardiometabolic complications associated with obesity and is an essential effector in type 2 diabetes (T2DM). However, it is becoming increasingly apparent that IR does not necessarily develop in every obese patient but can also occur in lean individuals. The broad aim of this collaborative project was to define the global activity kinase map in adipose tissue in the setting of insulin resistance and to identify new candidates as potential targets for treating its associated metabolic disturbances.

We performed a global study of the kinase activity, using a new technology (PamGene's activity-based kinase assay) in adipose tissue of a human well-characterized cohort, and selected the best panel of kinases based on different clinical phenotypes according to their IR status, ranging from lean to obese and T2DM patients. In this context, we first validated the panel of differentially activated kinases using subcutaneous and visceral adipose tissue from the same subjects. Second, in a representative subset of these subjects, mature adipocytes and pre-adipocytes were isolated and the panel of kinases selected were tested. Finally, the candidate pathways or signalling cascades were validated by further in vitro and in vivo studies in rodent models and in human adipocytes.

We intended to provide a deeper understanding of the molecular mechanisms involved in metabolic homeostasis that occurs in obese and T2DM patients. Altogether, the project execution has allowed us to find new therapeutic targets to treat IR before irreversible consequences develop.

2. Results

The main objective of this coordinated project was to define the global map of kinase activity in adipose tissue in the context of IR, and thus identify new candidates for potential use in the treatment of metabolic associated disorders. Kinases are a family of proteins that play a critical role in signal transduction which underlies many cellular processes. The functional PamGene kinase assay is activity-based and detects protein

kinase activity directly in cellular and tissue lysates, through measuring peptide phosphorylation by protein kinases.

A multidisciplinary team including hospital and research staff was involved in this project, magnifying its relevance and clinical applicability. The first and most essential part for the elaboration of the project was the characterization of a human cohort that included normo-weight patients, obese patients and patients with DMT2 classified according to their IR, and the extraction of adipose tissue samples. At this time point, and applying the assays of kinase activity in the human samples obtained, we were able to identify: 1) a subset of new kinases potentially involved in the pro-inflammatory polarization of macrophages, and 2) a group of new kinases potentially involved in the IR of adipose tissue.

Particularly, we were able to demonstrate the key role of PIM1 kinase (Pim-1 proto-oncogene, serine/threonine kinase) in inflammation related to macrophage biology and adipose tissue IR, specifically promoting IR in diabetic patients. In addition, in vivo inhibition of PIM1 in diabetic mice (chemical inhibitor SGI-1776) leads to a decrease in the inflammatory profile of adipose tissue as well as an increase in its insulin sensitivity. This study has validated a new role of PIM1 in promoting the pro-inflammatory polarization of macrophages in the context of obesity, relating their already identified role in cancer and inflammation to metabolism and IR. This paves the way for the implementation of PIM1 inhibitors not only as a therapeutic treatment for cancer but also for T2DM.

Thanks to the La Marató de TV3 funding, PIM1 was identified as a candidate kinase, which has been studied in depth but has also opened the door to the study of other important kinases in this process which are still being investigated.

3. Relevance with possible future implications

The overall aim of our study was to discover new kinases whose activities are deregulated during the development of IR in obese subjects independently of adiposity. To implement our strategy, we took advantage of a population of obese subjects protected from the development of IR and other obesity-derived pathologies.

Comparing the global kinase activities in the adipose tissue of these subjects with the kinase activities of obese diabetic patients allowed us to discover new kinases, specifically active in the adipose tissue of the pathological group. This strategy allowed us to overcome confounding factors that may be related to obesity per se and not to IR. In addition, our results shed light on the mechanism behind the ability of a certain population of obese subjects to maintain metabolic health and elude metabolic complications.

Despite the role of inflammation in maintaining the organism's homeostasis in conditions of injury/wound healing and infection, systemic chronic inflammation is a key component of several diseases including T2DM and metabolic syndrome, both characterized by systemic IR. To provide an additional understanding of the onset of IR, a process highly linked to an increase in proinflammatory macrophages in adipose tissue, we have taken advantage of a novel technology/method allowing for a comparison between the global kinase activities between different biological samples (PamGene). This approach identified PIM1 as a candidate kinase, which was studied in depth, but it revealed many other candidates that are still being investigated.

The importance of the identification of PIM1 as a new player in adipose tissue inflammation and IR is threefold. First, PIM1 kinase was upregulated in human and mouse adipose tissue in conditions of IR. Second, a specific kinase inhibitor was available for further preclinical testing (SGI-1776). And third, a role for the identified kinase in diabetes had not been previously described. This paves the road to implement PIM1 inhibitors not only as a therapeutic treatment for cancer but also for T2DM. But most importantly, this study will serve as a basis for the development of new specific pharmaceutical therapies in the field of diabetes, which is a global epidemic, but our findings are also of high clinical relevance to other pathologies caused/promoted by local or systemic inflammation.

4. Scientific bibliography generated

Article in review at JCI insights: IF: 6.205 Q1 Medicine, research & experimental

The activity of PIM1 in macrophages promotes inflammation and can be targeted to treat insulin resistance in obesity

Doctoral thesis: **The role of novel kinases in adipose tissue biology**
Date of defence: 28/05/2019
Doctorand: Anita Nasrallah
University of Lausanne