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Transient postnatal over nutrition induces long-term alterations in cardiac NLRP3-inflammasome pathway

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Abstract *Background and aims:* The prevalence of obesity is increasing worldwide at an alarming rate. Altered early nutrition, in particular postnatal overfeeding (PNOF), is a risk factor for impaired cardiac function in adulthood. In the understanding of the initiation or progression of heart diseases, NLRP3 inflammasome and non-coding RNAs have been proposed as key players. In this context, the aim of this study was to decipher the role of NLRP3 inflammasome and its post transcriptional control by micro-RNAs in the regulation of cardiac metabolic function induced by PNOF in mice.
Methods and results: Based on a model of mice exposed to PNOF through litter size reduction, we observed increased cardiac protein expression levels of NLRP3 and ETS-1 associated with alterations in insulin signaling. Additionally, miR-193b levels were down-regulated in the adult hearts of overfed animals. In a cardiomyocyte cell line, transfection with miR-193b induced down-regulation of ETS-1 and NLRP3 and improved insulin signaling.
Conclusions: These findings suggest that the miR-193b could be involved in cardiac phenotypic changes observed in adulthood induced by PNOF likely through the regulation of ETS-1 and NLRP3 expression, and through this of insulin signaling.

Introduction

Environmental exposures during early life, such as parental lifestyle, exposure to toxicants, imbalance diet increase the susceptibility of offspring to develop chronic diseases such as obesity, diabetes, and cardiovascular diseases [1]. Gravely, the prevalence of child obesity has been recently increasing at an alarming rate [2]. Maternal over-nutrition during lactation and postnatal overfeeding (PNOF) have been shown to program post-weaning obesity and cardio-vascular complications in rat pups [3]. Given that turnover of human cardiomyocytes is limited over a lifetime, the heart may be particularly sensitive to early events, such as exposure to nutritional excess.

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Indeed, PNOF induces excessive lipid accumulation in myocardial cells [4], defects in cardiac insulin signaling, increased heart rate and left ventricular wall thickness, hypertrophy and fibrosis [5]. A major challenge in developing targeted therapeutics for this type of heart disease is to understand how the molecular and genetic changes induced by PNOF lead to altered cardiac functions. Inflammasomes are attractive candidates to understand these mechanisms, given that they are strongly implicated in the initiation or progression of chronic diseases, including heart disease. The inflammasomes are key signaling platforms that detect sterile stressors as well as transduce signals triggering an inflammatory cascade reaction. The specific sensor, nucleoside-triphosphatase domain (NACHT), leucine-rich repeat (LRR), and pyrin domain (PYD) domains-containing protein 3 (NLRP3), has been widely studied due to its ability to sense a large number of endogenous activators, including metabolic substrates, such as glucose, ATP and fatty acids. NLRP3 connects to CASPASE-1 via the adapter Apoptosis-associated speck-like protein containing a CARD domain (ASC) and its activation leads to CASPASE-1 self-cleavage and activation. Active CASPASE-1 proteolytically activates proinflammatory cytokines, including pro-Interleukin-1β (pro-IL-1b). In addition to its role in inflammation, the inflammasome regulates metabolism [6], lipid deposition [7], and insulin resistance [8]. In the early origins of chronic diseases, epigenetic mechanisms are likely key players. These mechanisms regulate every steps of heart development [9], lipid metabolism, glucose homeostasis and insulin signaling. The major epigenetic features include DNA methylation, histone modifications and non-coding RNA. Importantly, approximately 60% of the human genes are under the control of micro-RNAs, which can drive cardiac and metabolic disorders. In this context, the inflammasome and its regulation through micro-RNAs represent a potential candidate in the understanding of the long-term effects induced by PNOF on cardiac function. The aim of the present study was to delineate the role of the NLRP3 inflammasome in a model of cardio-vascular alteration induced by PNOF, and to determine the role of upstream micro-RNAs regulators.

Methods

Reagents and detailed protocol are described in supplementary data file.

In vivo experimental studies

All animals received humane care, and the study protocol complied with the institution's guidelines. Adult female C57BL/6 mice were mated with male mice and given a standard diet *ad libitum* during pregnancy and lactation. On the third day of life, the litter size was adjusted to 9 male pups (normal fed, NF) or reduced to 3 male pups to induce PNOF. After weaning (day 24), mice of both groups had free access to a standard diet and water. Male offspring was sacrificed at weaning (PND24) or at 7 month.

Cell cultures and transfection

H9C2 cardiomyoblast cell line were transfected with 50 pmol rno-pre-miR-193b or a scramble pre-miR-control. 48 h following transfection, cells were harvested for protein analyses.

Protein analyses

Frozen cardiac tissue and H9C2 cells were homogenized in lysis buffer and proteins were analyzed by Western blot as previously described [10].

mRNA and micro-RNAs measurement

Total RNA was isolated from frozen heart as previously described [11]. mRNAs and micro-RNAs were reverse transcribed, and measured by real-time quantitative polymerase chain reaction. The relative expression levels of mRNAs and micro-RNAs were calculated using the comparative ΔΔCt method by normalizing to β-Actin and to miR-16 levels.

Circulating cytokines

IL-6plasma levels were measured using a magnetic Luminex screening assay.

Data analysis

The data were analyzed with GraphPad Prism software version 6.05 (GraphPad Software, Inc.). The values were expressed as the mean ± SEM to account for sample and animal variation within a dataset. The Student's *t* test was performed to determine whether there were differences between all groups ($p < 0.05$).

Results

PNOF induces inflammation and alters cardiac NLRP3 inflammasome (Fig. 1)

The heart dysfunction and the metabolic phenotype induced by PNOF have been previously reported by Li et al. [12]. In the same set of overfed animals which were maintained *ad libitum* on a standard diet after weaning until sacrifice, we investigated the expression pattern of pro-inflammatory cytokines, and inflammatory biomarkers. No modification was detected in systemic concentration of C-reactive protein (CRP), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α) with PNOF (data not shown). In contrast, IL-6 levels were significantly increased in overfed animals indicating a global low inflammatory status in overfed animals (Fig. 1A). To delineate the role of the NLRP3 inflammasome in adult cardiac dysfunctions induced by PNOF, we compared the expression levels of the inflammasome components in cardiac tissues from adult mice exposed to PNOF through litter size reduction with normal fed mice (NF) raised in litters of

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