530. Transcriptome profile of liver from pigs fed with two different levels of soybean oil revealed the NF-κB1 modulation

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Abstract

Nuclear factor-kappa light chain enhancer of activated B-cells (*NF-κB*) is a transcription factor (TF) considered to be a crucial element in several physiological processes, including fatty acid (FA) metabolism. This study aimed to identify differentially expressed genes (DEG) in liver of pigs fed with two different proportions of the soybean oil (SOY), which could be related to the *NF-κB* network. A total of 281 DEG between SOY (1.5%) and SOY (3.0%) diets (log2fold-change ≥1 or ≤-1; FDR-corrected *P*-value<0.1) were identified, in which 129 were down-regulated and 152 were up-regulated in SOY1.5 group. The gene-network enriched from DEG in response to an increase in the SOY in the liver transcriptomic profile provides us mechanisms to understand the regulatory effects of FAs, which may alter the behaviour of TFs and be modulated according to diet.

Introduction

Nuclear factor-kappa light chain enhancer of activated B-cells (*NF-κB*) is a protein complex of transcription factors, which plays critical roles in immunological processes, inflammation, proliferation, survival, and cell differentiation (Chen *et al.,* 1999). Moreover, *NF-κB* has been demonstrated to be involved in several physiological and pathological processes as well as in cell survival processes (Oeckinghaus and Ghosh, 2009).

The NF-κB protein complex are considered fundamental regulator of inflammation in liver cell populations and are critical for hepatocyte survival (Patterson and Georgel, 2014). Although not directly involved in fatty acid (FA) metabolism, *NF-κB* may act by activating the transcription of a series of enzymes that are related to inflammatory processes and signalling molecules synthesis (Patterson and Georgel, 2014). For instance, cyclooxygenases (COX) and lipoxygenase (LOX), which act in the conversion of members of omega-3 (n-3) and omega-6 (n-6) fatty acid families into signalling molecules that may activate or inhibit pathways related to inflammation processes (Schmitz and Ecker 2008). Then, the use of n-3 and n-6 fatty acids rich oils, such as soybean oil, in the diet reduce the levels of inflammatory mediators (Calder, 2010).

Therefore, maintaining an adequate regulation of this complex of transcription factors is considered by the literature as a critical factor for the maintenance of immune homeostasis of several tissues, besides regulating the expression of several genes related to immunological, inflammatory, as well as cell differentiation and survival processes. However, the transcription factors involved in key processes through the modification of gene expression in pigs are still poorly understood. This study aimed to identify differentially expressed genes (DEGs) in liver of immunocastrated male Large White pigs fed different levels of soybean oil, which could be related to the *NF-κB* network.

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Materials & methods

All animal procedures were approved by the 'Luiz de Queiroz' College of Agriculture Animal Care and Use Committee (University of São Paulo, Piracicaba, Brazil, protocol number 2018.1787.11.6 and number CEUA 2018-28). In a 98-day study, 35 immunocastrated male pigs (halothane homozygous-negative (NN); average initial body weight of 28.44±2.95 kg) were assigned to one of two treatments in a randomized complete block design with six replicate pens per treatment. Dietary treatments consisted of corn-soybean meal diets supplemented with 1.5% SOY (SOY1.5) or 3% SOY (SOY3.0). There were 17 pigs in the SOY1.5 group and 18 in SOY3.0 group. Diets were formulated to meet or exceed Rostagno *et al.* (2011) recommendations for growing-finishing pigs, comprising 3.28 and 3.36 Mcal of metabolizable energy/kg of diet (as-fed basis) for the SOY1.5 and SOY3.0 groups, respectively. The details of animal housing and experimental diet compositions were mentioned by Almeida *et al.* (2021). All pigs were slaughtered with average final body weight of 133.9±9.4 kg and liver samples were collected, snap-frozen in liquid nitrogen, and then stored at -80 °C until analysis. Total RNA was extracted from liver tissue samples using commercial RNA extraction kits (RNeasy® Mini Kit, Qiagen), according to the manufacturer instructions. The mRNA library preparation and sequencing (RNA-Seq) were described in Fanalli *et al.* (2021). The RNA-Sequencing (RNA-Seq) data quality was checked using the FastQC, v. 0.11.8 software [http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc/]. Adapters and bases with low PHRED score were removed using the Trim Galore v. 0.6.5. Reads with a minimum length of 70 bases were aligned and mapped to the reference pig genome (*Sus Scrofa*11.1) using the assembly available at Ensembl Release 102 [http://www.ensembl.org/Sus_scrofa/Info/Index]. Alignment and mapping were performed using the STAR v. 2.7.6a. The differential expressed genes (DEG) were identified using the DESeq2 package available at Bioconductor open-source software for bioinformatics (Love *et al.*, 2014), using multi-factor design. The functional enrichment analysis of DEG was performed to obtain (P<0.01) comparative networks by analyzing the single network (transcription factor) and process networks using the default parameters of MetaCore software (Clarivate Analytics) v. 21.3 build 70600, which were filtered for liver tissue and *Homo sapiens* species dataset to show activating and inhibiting effects. For the functional analysis to identify important transcription factor (TF) related to lipid homeostasis and immune response, the construction of the gene network by the TF mechanism was performed. For each TF of the master list, the algorithm generated a subnetwork with all the shortest paths to which the TF from the nearest recipient with direct ligands on the list.

Results

On average 78% of total paired reads aligned against the reference genome. After filtering, 19,250 genes were considered for differential gene expression analysis between the SOY1.5 and SOY3.0 group. A total of 281 DEG (log2fold-change ≥1 or ≤-1; FDR-corrected P-value<0.1) were identified, where 129 were down-regulated (log2FC ranging from -3.0 to -0.20) and 152 up-regulated (log2FC ranging from 4.8 to 0.24) in the SOY1.5 group by DESeq2 (Fanalli *et al.*,2022). We detected in the functional enrichment the networks related to inflammation such as Inflammation_IL-6 signaling with DEG *ORM1*, *CDKN1A* and Inflammation_Kallikrein-kinin system with DEG *KNG1* both networks involved with the *NF-κB* transcription factor (P<0.1). Among the total of DEG identified, we showed in the Figure 1 those 23 down-regulated (blue circles) and 21 up-regulated (red circles) in the SOY1.5 group, where the color shade represented the log2fold-change value. These DEG are target genes for the main TF network detected by MetaCore, which identified the *E2F1, SOX9, SMAD3, STAT3, ETS1, Tcf, PR (nuclear), Androgen receptor, TSHZ1, NF-κB and NF-κB1 (p150)* as TF (Figure 1).

Discussion

Previously, we demonstrated that feeding pig diets with SOY3.0 increased the proportion of oleic acid (OA, C18:1 n-9) and decreased the percentages of linoleic acid (LA, C18: 2 n-6) and alpha-linoleic acid (ALA, C18:3 n-3) in the loin intramuscular fat compared to those fed SOY1.5 (Almeida *et al.*, 2021). These

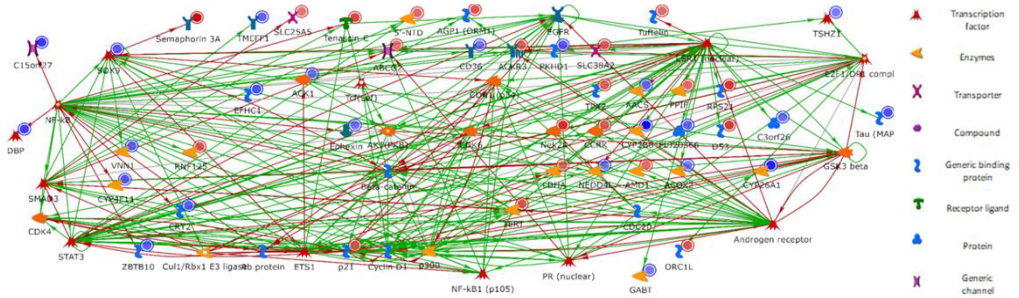

Figure 1. Network built based on the analysed networks (transcription factor) from the list of differentially expressed genes in the liver samples from pig fed with diets with 1.5 and 3.0% of soybean oil groups. Red circles represent up-regulated genes, whereas down-regulated genes are marked with blue circles. The color shade of the circles represents the log2fold-change value. Image created by MetaCore (Clarivate Analytics) [https://portal.genego.com/].

FA have been associated with the lower occurrence of some inflammatory diseases such as obesity, type 2 diabetes, and atherosclerosis (Jung *et al.*, 2008; Poudyal *et al.,* 2011). In the current study, we observed a significant (FDR 10%) number of DEGs in liver of pigs fed SOY1.5 vs SOY3.0, which were related to the network of important transcription factors linked to inflammatory response in liver tissue, being identified the *NF-κB*. *NF-κB* is related as a mediator in the process of vascular inflammation and one of the process networks enriched with DEGs is Inflammation_IL-6 signaling. Vascular inflammation is detected by the increase in circulating Interleukin-6 (IL-6), produced by inflammatory cells and secreted during inflammation by macrophages, endothelial cells, and fibroblasts, important in inducing B lymphocytes to differentiate into antibody-forming cells (plasma cells), in addition, IL6 signaling can lead to anti-apoptotic effects (Brasier, 2010). Another process network that *NF-κB* together DEG *KNG1* has been enriched with is the Inflammation_Kallikrein-kinin system related to inflammation, blood pressure control, coagulation (Peyrou, 2020).

A previous study carried out by Cai *et al.* (2005) reported that *NF-κB* is activated by genetic obesity and may be induced by diet (Chen *et al.,* 1999). The authors also observed that the lipid accumulation in the liver results in liver inflammation through *NF-κB* activation and cytokine production, causing insulin resistance both locally in the liver and systemically. The TF *NF-κB* has an important relationship to immunological and inflammation processes and can be activated by various stimuli, such as oxidizing free radicals and cytokines (Schmitz and Ecker 2008). As a key element of classical immunity, the proinflammatory *IKKβ / NF-κB* pathway modulates a wide range of cellular processes, including proliferation, immunity, inflammation, and apoptosis, from transcription of target genes. Furthermore, pro-inflammatory cytokine responses to dietary changes are induced via signalling pathways that mediate *NF-κB* activation (Bu *et al.*, 2021).

According to Rizzo and Laganà (2020), *NF-kB*, nuclear factor erythroid 2-related factor 2 (Nrf2), peroxisome proliferator-activated receptor gamma *(PPARγ)* and heat shock proteins (HSP), are affected by electrophilic long-chain FA n-3 derivatives and show effects in reducing inflammatory processes and oxidative stress. Using rat L6 myoblasts, Poletto *et al.* (2015) reported that FAs stimulated *NF-kB* expression. The authors demonstrated that gene expression may be regulated by OA and AL and 200 μM of either FA (OA or LA) is enough to increase *Rela* expression and protein binding to the *Slc2a4 – NF-kB* binding site in the skeletal muscle (Poletto *et al.,* 2015).

The network enriched from DEG in response to an increase in the soybean oil in the liver transcriptomic profile provides us with mechanisms to help understand the regulatory effects of fatty acids that can be altering the behaviour of transcription factors, which could be modulated according to diet. Further study to evaluate the effect of the two diets on the inflammatory response of the liver of the pigs used herein is necessary. Immunoassay tests from proteins extracted of the liver of the samples of this study are being performed by our group.

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