Gene 560 (2015) 96–106

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Transcriptomic analysis of Rongchang pig brains and livers

Ling Gan [a], Liwei Xie [b], Fuyuan Zuo [c], Zhonghuai Xiang [d], Ningjia He [d,*]

[a] The Department of Veterinary Medicine, Rongchang Campus, Southwest University, Rongchang, Chongqing 402460, China
[b] Center of Molecular Medicine, University of Georgia, Athens, GA 30602, USA
[c] The Department of Animal Husbandry, Rongchang Campus, Southwest University, Rongchang, Chongqing 402460, China
[d] State Key Laboratory of Silkworm Genome Biology, Southwest University, Beibei, Chongqing 400715, China

ARTICLE INFO

Article history:
Received 9 October 2014
Received in revised form 31 December 2014
Accepted 26 January 2015
Available online 28 January 2015

Keywords:
RNA sequence
Transcriptome profiles
Pigs
Neuropeptide
Neuropeptide receptor

ABSTRACT

Recent developments in high-throughput RNA sequencing (RNA-seq) technology have led to a dramatic impact on our understanding of the structure and expression profiles of the mammalian transcriptome. To gain insights into the usefulness of swine production and biomedical model, the transcriptome profiling of Rongchang pig brains and livers was characterized using RNA-seq technology to uncover functional candidate molecules. In the study, total RNAs from brains and livers of Rongchang pig were sequenced and 8.6 Gb sequencing data was obtained. This analysis revealed tissue specificity through the identification of 5575 and 4600 differentially expressed genes (DEGs) in brains and livers, respectively and the functional analysis of DEGs. Furthermore, 83 neuropeptide gene transcripts, 69 neuropeptide receptor gene transcripts, 10 pro-neuropeptide convertase gene transcripts and many other neuropeptide related protein gene transcripts were identified. Totally, the major characteristics of the transcriptional profiles of Rongchang pig brains and livers were present.

1. Introduction

Over the past several decades, pigs have been widely studied for their agriculture relevance and used as a potential animal model for biomedical research due to genomic, anatomic and physiologic similarities with humans (Douglas, 1972). Economically, meat quality and growth performance have been the major focus of swine studies, meanwhile this model system has been applied to study a wide range of human health and disease related topics, such as cancer, reproductive health, drug metabolism, wound healing, and cardiovascular disease (Anzenbacherova et al., 2003; Sullivan et al., 2001; Kurahashi et al., 2004). In addition, the similarities between swine and human brains were well documented, with swine models used to study a wide range of human neurological diseases (Traystman, 2003). Swine was also utilized to study extracorporeal liver perfusion (ECLP) as it relates to patients of acute liver failure (Horslen et al., 2000). From above, swine brain and liver are the important targeted tissues in the biomedical model system. Currently, although various swine tissue transcriptomic data have been obtained, more studies focused on muscle to gain insights into improving meat quality from the perspective of agricultural economy (Monego et al., 2005; Ferraz et al., 2008), the transcriptomic analysis of brains and livers in pig remains limited.

The brain and liver are complementary and at the crossroads of many key neuroendocrine, metabolic and behavioral regulations. The relationship of the brain and liver had been analyzed (de Groote, 1963). Furthermore, many studies discovered the reflection of brain function in the liver and the remote regulation of the brain to liver,

Abbreviations: RNA-seq, RNA sequencing; DEGs, differentially expressed genes; NMS, neuromedin S; MSH, melanocyte-stimulating hormone; ECLP, extracorporeal liver perfusion; SAGE, serial analysis of gene expression; IGFBPs, insulin-like growth factor binding proteins; qRT-PCR, quantitative real-time PCR; CDS, coding sequences; Tus, transcriptional units; RPKM, the number of reads per kilobase per million mapped reads; GO, gene ontology; KEGG, Kyoto encyclopedia of genes and genomes; ATP, adenosine triphosphate; GADPH, glyceraldehyde-3-phosphate dehydrogenase; SYN1, synapsin Ib; DPP4, dipeptidyl peptidase 4; NPY, neuropeptide Y; CCK, cholecystokinin; CART, cocaine- and amphetamine-regulated transcript; CHGA, chromogranin A; CHGB, chromogranin B; CRF, corticoliberin; PENK, proenkephalin; RESP18, regulated endocrine-specific protein 18; SCG, secretogranin; SS, somatostatin; TAC1, protachykinin 1; VIP, vasoactive intestinal peptide; IGF2, insulin-like growth factor 2 (Somatomedin A); HCRT, hypocretin (orexin) neuropeptide precursor; AGRP, agouti-related peptide homolog; BNP, brain natriuretic peptide; ANF, atrial natriuretic factor; NPS, neuropeptide S; CRSP1, calcitonin receptor-stimulating peptide 1; EDN2, endothelin 2; GIP, gastric inhibitory polypeptide; GRP, gastrin-releasing peptide; INSL5, insulin-like 5; NPB, neuropeptide B; NPFF, neuropeptide FF; NPS, neuropeptide S; NPW, neuropeptide W; PROK2, prokineticin2; TRH, prothyroliberin; UCN, urocortin; VGF, neurosecretory protein VGF; NMUR2, neuromedin U receptor 2; NPYR, neuropeptide Y receptor; CCKAR, cholecystokinin A receptor; CCKBR, cholecystokinin B receptor; HCRTR, orexin receptor; PCSK, pro-neuropeptide convertase subtilisin/kexin; 7B2, the pro-neuropeptide convertase facilitator gene; QT, stringent quality threshold; CNS, central nervous system; HPA, hypothalamic–pituitary–adrenal; POMC, pro-opiomelanocortin; NMU, neuromedin U; ARC, the actuate nucleus; PVN, paraventricular nucleus; MCHR1, melanin-concentrating hormone receptor 1; MCHR2, melanin-concentrating hormone receptor 2; MC4R, melanocortin receptor 4; MC5R, melanocortin receptor 5; GH, growth hormone; GHRH, growth-hormone-releasing hormone; NPSR, neuropeptide S receptor.

* Corresponding author.
E-mail addresses: gl9089@sina.com (L. Gan), liweixie@uga.edu (L. Xie), zfuyuan@163.com (F. Zuo), xbxzh@swu.edu.cn (Z. Xiang), hejia@swu.edu.cn (N. He).

http://dx.doi.org/10.1016/j.gene.2015.01.051
0378-1119/

such as the suggested hypothalamo–pituitary–liver axis (Gustafsson et al., 1981) and the study of mutual relationship between liver tryptophan pyrrolase activity and brain 5-hydroxytryptamine (5-HT) in rat (Hillier et al., 1975). All of these suggested that there is a biologically active regulatory network between brains and livers, which plays an important role in the health and growth of mammals. This context encouraged us to investigate the transcriptomic profile of swine brains and livers to enrich the knowledge about the relationship between the two tissues.

The brain is the central regulator of the nervous system and houses a large number of neuropeptide genes. However, there are limited literatures on the expression of neuropeptide genes in the liver (Goehler and Sternini, 1991). Neuropeptides are important regulatory molecules for cellular communication and thus physiologically relevant to the regulation of the growth and development, including breeding, feeding, stress, memory and susceptibility to substance abuse (Hoyer and Bartfai, 2012). For example, Yang et al. reported the inhibitory effect of neuromedin S (NMS) on luteinizing hormone secretion mediated via melanocyte-stimulating hormone (MSH) neurons in the hypothalamus–pituitary axis of ovariectomized swine (Yang et al., 2010). Additional research also approved that the hypothalamic peptides, orexin A and orexin B, are involved in the control of food intake, sleep patterns, autonomic and neuroendocrine systems, and may also affect reproductive functions via the porcine hypothalamic–pituitary–ovarian axis (Kaminski et al., 2010). Characterization of various swine neuropeptides also has offered insights into cell transplantation, nervous system diseases, and injury (Schwartz et al., 2005). So profiling the expression levels of neuropeptide and neuropeptide-related genes in brains and livers of pigs will shed insights into the swine production and medical study.

Deep RNA-seq is a high throughput sequencing technology, allowing the rapid identification and analysis of the vast majority of the transcriptome. This platform provides the means to expand our comprehension of transcriptome, expression patterns and enables the elucidation of process specific gene functioning to ultimately identify regulatory candidate genes. Furthermore, RNA-seq is even more sensitive at detecting low abundance RNAs, such as the expression of neuropeptide or neuropeptide receptor genes, compared to traditional SAGE (serial analysis of gene expression) or microarray platforms (Mortazavi et al., 2008; Sultan et al., 2008; Wilhelm et al., 2008), which grants us better chance to exploit the important regulatory molecules at low expressed levels. Recently, the RNA-seq platform has been employed to characterize the transcriptome in humans and some model species (Mortazavi et al., 2008; Sultan et al., 2008; Wen et al., 2010), with the analysis of the transcriptome of Rongchang pig brains and livers not yet reported.

The Rongchang pig is one of the Chinese indigenous breeds which produces high quality meat, in addition to being used as the ideal model to study the heredity of deafness in China, since the inner ear of Rongchang pig is similar to humans in structure and function and the deafness phenotype of albino Rongchang pig is consistent with that of human Waardenburg syndrome type 2A caused by microphthalmia-associated transcription factor M (Ren, 2013). So the transcriptomic analysis of Rongchang pig brains and livers will not only enrich genetic resources of pigs and gain insights into the usefulness of production but also give some clues for biomedical model. In this study, RNA-seq analysis was carried out to generate transcriptional profiles of Rongchang pig brains and livers to identify differentially expressed genes potentially involved in tissue specific functioning. These identified genes included but not limited to gene-encoding neuropeptides, neuropeptide receptors, pro-neuropeptide convertase, insulin-like growth factors binding proteins (IGFBPs), and other neuropeptide related proteins. Collectively, our results further expand our knowledge of Rongchang pig brain and liver gene expression profile and the relationship between two tissues, provide a useful resource for swine biomedical study and further understand production traits such as development, fatness and growth performance through identifying some regulatory candidate genes.

2. Materials and methods

2.1. Animal material

Six purebred Rongchang pigs (3 females and 3 males) at 7 days of age were housed and slaughtered. The whole brains and livers were simultaneously collected and frozen in liquid nitrogen and stored at −80 °C until use. The experimental procedures followed the actual law of animal protection that was approved by the Animal Care Advisory Committee of Southwest University, China.

2.2. Library preparation

Total RNA from the liver or brain of each pig was extracted as previously described (Esteve-Codina et al., 2011). Extractions from 6 individuals were pooled as tissue samples. Total RNA was treated with RQ1 DNase (Promega) to remove DNA. RNA quality and quantity were assessed using a SmartSpec Plus (BioRad, USA) at an absorbance of 260 nm/280 nm, and RNA integrity was further verified via 1.5% agarose gel electrophoresis. For each sample 10 g of total RNA was processed, with polyadenylated mRNAs purified and concentrated with oligo (dT)-conjugated magnetic beads (Invitrogen) prior to directional RNA-seq library preparation. Purified mRNAs were iron fragmented at 95 °C followed by end repair and 5′ adaptor ligation. Reverse transcription was performed utilizing primers harboring 3′ adaptor sequence in conjunction with random hexamers The resulting cDNAs were purified and amplified, with PCR products 200–500 bp purified, quantified and stored at −80 °C. Libraries were prepared according to the manufacturer's instructions and sequenced using the Hiseq 2000 system (BGI, Inc., Shenzhen, China) for 90 nt pair-end sequencing.

2.3. RNA-Seq raw data clean-up and alignment statistics

Raw reads were discarded if containing more than 2 unknown (N) bases or if too short (less than 20 nt), with the remaining reads processed by clipping the adaptors and removing low quality bases. FASTX-Tool kit (Version 0.0.13) (http://hannonlab.cshl.edu/fastx_toolkit/) was used to get the clean reads. After that, clean reads were aligned to the reference genome by TopHat (Trapnell et al., 2009). Based on gene annotation, aligned reads with more than one genomic location were discarded to eliminate location ambiguity. RNA-seq reads were analyzed on the basis of the coverage length of the transcriptional units. Based on the 5′ end transcriptional unit standard, the cDNA was equally divided into 100 parts and each dubbed a single bin, with the average number per bin computed. Unique reads were used to calculate read number, RPKM value for each gene, gene coverage and depth, and read distribution around start and stop codons. The RPKM values are calculated using unique-mapped reads and mappable transcript regions. The analysis of the gene expression correlation was made by Pearson correlation coefficient.

2.4. DEGs between the two tissues and functional enrichment analysis

Differentially expressed genes resulted from the comparison of the transcriptome of the brain and liver samples. The genes with fold change of more than 2 in brains relative to livers or less than 0.5 represent up- and down-regulated expression, respectively. DEGs between the two samples were analyzed using the R package edgeR (Robinson et al., 2010). For each gene, the p-value was computed and the significance threshold necessary to control the false discovery rate (FDR) at a given value was calculated. Fold changes were also determined with edgeR and differentially expressed genes were submitted to the DAVID server (http://david.abcc.ncifcrf.gov/) for enrichment analysis (Huang et al., 2009) and enrichment clusters were sorted by the enrichment score. Categories for each cluster were sorted by p-value with the