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RESEARCH ARTICLE

SFRP2 affects prenatal muscle development and is regulated by microRNA-1/206 in pigs

MA Yan-jiao[1, 2*], YANG Ya-lan[2*], SUN Wei[3], ZHOU Rong[2*], LI Kui[2], TANG Zhong-lin[2]

[1] *Jilin Provincial Key Laboratory, College of Animal Science, Jilin University, Changchun 130012, P.R.China*
[2] *Key Laboratory of Farm Animal Genetic Resources and Germplasm Innovation, Ministry of Agriculture/Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing 100193, P.R.China*
[3] *College of Animal Science and Technology, Northwest A&F University, Yangling 712100, P.R.China*

Abstract

Secreted frizzled-related protein 2 (*SFRP2*), a member of the *SFRPs* family, is associated with cell growth and differentiation in myogenesis. Our previous study suggested that *SFRP2* was a potential target of microRNA (miRNA)-1/206, which was considered as myomiRs. To further explore the biological function and regulation mechanisms of the *SFRP2* gene in porcine skeletal muscle development, we first analyzed the sequence structure of the porcine *SFRP2* gene. Subsequently, we detected its tissue distribution in adult Tongcheng pigs (a Chinese indigenous breed) and investigated its dynamic expression in developmental skeletal muscle (13 prenatal and 7 postnatal time points) in Tongcheng pigs. An interaction analysis between *SFRP2* and myomiRs was also performed. The results showed that the expression pattern of the *SFRP2* varied greatly across diverse tissues. It exhibited abundant expression in prenatal skeletal muscle and peaked at 55 days post coitus (E55), and had a lower expression in postnatal skeletal muscle, indicating that the *SFRP2* gene might affect porcine embryonic skeletal muscle development. Co-expression analysis revealed that the expression levels of *SFRP2* correlated negatively with miRNA-1 (r=–0.570, P-value=0.009) and miRNA-206 (r=–0.546, P-value=0.013), but positively with *SFRP1* (r=0.613, P-value=0.004). The bioinformatics analysis and dual luciferase assay verified that the *SFRP2* was a putative target of miRNA-1/206 in pigs. Therefore, this study is helpful for understanding the biological function and molecular regulation of the *SFRP2* gene during porcine skeletal muscle development.

Keywords: *SFRP2*, miRNA-206, miRNA-1, skeletal muscle, development, pig

1. Introduction

The *SFRP*s gene family has five members (*SFRP1–5*) in the mouse and human genomes (Jones and Jomary 2002), which possesses a domain that is similar to one in the Wnt-receptor frizzled proteins and inhibits Wnt receptor binding to down-regulate pathway signaling during development (Xu *et al.* 1998). The Wnt signaling pathways consist of complex network of proteins that are involved in the control of many physiological processes in mammals, including

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MA Yan-jiao, E-mail: 252076890@qq.com;
Correspondence ZHOU Rong, Tel/Fax: +86-10-62813339, E-mail: zhourong03@caas.cn
* These authors contributed equally to this study.

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cell proliferation, migration, differentiation, and inflammation (Reya and Clevers 2005; George 2008). *SFRP2* is a Wnt antagonist that participates in many molecular functions and biological processes, such as cardiac progenitor cell differentiation (He *et al.* 2010) and myogenesis (Levin *et al.* 2001). In chickens, *SFRP2* plays an active role in embryogenesis, especially in the development of the muscles, eyes, and other tissues (Lin *et al.* 2007). Additionally, *SFPR2* can prevent myoblasts from entering the terminal differentiation process and inhibit myoblast differentiation (Descamps *et al.* 2008). By inhibiting a positive transcriptional autofeedback loop of Wnt3a, *SFRP2* may inhibit Wnt3a transcription and regulate cardiomyogenic differentiation (Deb *et al.* 2008). Moreover, *SFRP2* gene is also a target of the Pax2 transcription factor which participates in cell differentiation, survival, and tissue remodeling (Brophy *et al.* 2003).

Gene expression is meditated by various regulatory factors, such as microRNA (Mukherji *et al.* 2011), methylation (Jaenisch and Bird 2003), and histone modification (Heintzman *et al.* 2009). MicroRNAs are a class of small, single-stranded, non-coding RNAs (~21–24 nt in length) that regulate gene expression at the post-transcriptional level by binding to the 3′-untranslated regions (3′-UTRs) of messenger RNAs (mRNAs) in mammals (Bartel 2004; He and Hannon 2004). miRNAs play a vital role in the development and growth of skeletal muscle (Kloosterman and Plasterk 2006). For example, microRNA (miRNA)-148a promotes myogenic differentiation of both C2C12 myoblasts and primary muscle cells by down-regulating the Rho-associated, coiled-coil containing protein kinase 1 (*ROCK1*) gene (Zhang *et al.* 2012). miRNA-127 (Yang *et al.* 2014) and miRNA-155 (Zhao *et al.* 2012) are also identified as microRNAs associated with skeletal muscle development in pigs. miRNA-1/206, which are considered as myomiRs, are specifically expressed in skeletal muscle (McCarthy and Esser 2007; McCarthy 2008). Our recent study showed that miRNA-1/206 were abundantly expressed in porcine *longissimus dorsi* muscle (Hou *et al.* 2012; Tang *et al.* 2014).

Although many studies have focused on the interaction of *SFRP2* with Wnt signaling pathways (Katoh and Katoh 2007; Suzuki *et al.* 2008) in humans and mice, there have been no reports on the function and molecular regulation mechanism of *SFRP2* in pigs. In the present study, to shed more light on the function of *SFRP2* in porcine skeletal muscle development, we first analyzed the sequence structure of the *SFRP2* gene and investigated its spatial distribution and dynamic expression using real-time polymerase chain reaction (qPCR). Subsequently, co-expression analyses were performed to measure the correlation between *SFRP1*, miRNA-1/206 and *SFRP2*. The bioinformatics analysis and dual luciferase reporter assay were utilized to explore the interaction between the *SFRP2* and miRNA-1/206. Our results suggested that the *SFRP2* gene might affect prenatal skeletal muscle development and was regulated by miRNA-1/206 in pigs.

2. Results

2.1. Sequence structure analysis of the porcine *SFRP2* gene

The protein sequence of the porcine *SFRP2* was retrieved from the GenBank database (accession no. NM_001244395). It showed that the porcine *SFRP2* gene encoded a polypeptide of 294 amino acid residues. The calculated molecular mass and isoelectric point of *SFRP2* are 33.31 kD and 7.41, respectively. We compared the amino acid sequence of porcine *SFRP2* with other *SFPR*s genes. The alignments results show that the precursor protein of *SFRP*s genes contain a frizzled domain and a netrin C-terminal domain (Fig. 1). The molecular phylogenetic tree of the porcine *SFRP*s gene family showed that *SFRP1* and *SFRP5* are firstly clustered together, and then clustered with *SFRP2*. *SFRP3* was clustered with *SFRP4* to another subgroup (Fig. 2). We used the PSORT program to predict the SFRP2 protein localization sites in cells. The results showed that the localization sites of SFRP2 protein had a 44.4% possibility of being located in extracellular, 22.2% possibility in cytoplasmic and 22.2% in the endoplasmic reticulum based on the K-NN prediction.

2.2. Tissue distribution of the porcine *SFRP2* gene

Quantitative real-time PCR was performed to measure the relative expression levels of *SFRP2* in different tissues of adult Tongcheng pigs. According to the standard curve, the amplification efficiency value of *SFRP2* and *GAPDH* gene was about 99 and 98%, respectively (Table 1), indicating that our qPCR experiment was reliable. The expression pattern of porcine *SFRP2* varied greatly in diverse tissues at the mRNA level. As shown in Fig. 3, *SFRP2* was abundantly expressed in the stomach, lung, and large and small intestine, moderately expressed in the *longissimus dorsi* muscle and leg muscle, and weakly expressed in the heart and spleen. The expression level of *SFRP2* was significantly higher in the stomach ($P<0.05$) and significantly lower in heart ($P<0.05$) than in the other tissues (Fig. 3).

2.3. Dynamic expression of the porcine *SFRP2* gene during skeletal muscle development

We gathered the *longissimus dorsi* muscle samples at 20 prenatal and postnatal developmental stages in Tongcheng pigs. Quantitative real-time PCR analysis showed that