Journal of Animal and Veterinary Advances 10 (3): 311-315, 2011
ISSN: 1680-5593

Expression Characters of IGF2 and IGFBP3 in Eight Tissues of Wuzhishan Pig

[1]Hou Guanyu, [1]Wang Dongjin, [1]Zhou Hanlin, [1]Zeng Hongpu, [1]Guan Song and [2]Ma Yuehui
[1]Tropical Crops Genetic Resources Institute, CATAS, 571737 Danzhou Hainan, P.R. China
[2]Institute of Animal Science, Chinese Academy of Agricultural Sciences, 100193 Beijing, China

Abstract: The expression of IGF2 and IGFBP3 mRNA in the heart, liver, lung, kidney, spleen, intestine muscle and stomach of wuzhishan pigs from 30-150 days of age was investigated with fluorescent quantitative PCR assay. Results showed that IGF2 expression showed a significant reduction trend ($p<0.05$) in all of 8 tissues from the highest level at 30 days of age to the lowest at 150 days of age; IGFBP3 expression showed a similar reduction trend among 6 tissues (heart, lung, spleen, kidney, intestine muscle and stomach) from 30-150 days of age while a trend of increase during early development (until 90 days of age in liver or until 60 days in skeletal muscle) then decrease thereafter was seen with lowest at 150 days of age.

Key words: Wuzhishan pig, expression character, real time quantitative PCR, IGF2, IGFBP3, skeletal muscle

INTRODUCTION

Insulin-like growth factors (IGFs including IGF1 and IGF2) are multifunctional cell proliferation regulators named after their structural resemblance to insulin. Studies have shown that IGFs precisely regulate skeletal muscles development, myofibril generation and hypertrophy (Barton, 2006; Rosenblatt *et al*., 1994; Scandinavica, 1999; Florini *et al*., 1991a, b). IGF2 is composed of 67 amino acid residues. It plays an very important regulatory functions in fetal development, tumor proliferation and muscle growth (De Chiara *et al*., 1991; Gerrard *et al*., 1998; Florini *et al*., 1986).

In myocyte culture, IGF2 is required for the differentiation of satellite cells (Florini *et al*., 1991a, b). Because of its essential function, IGF2 has been a focus of research for a long time. However, the mechanism by which it functions is still unclear (Erbay *et al*., 2003). IGF2 functions by binding with IGF2 receptor (IGFR). Few IGFs exist unbound in plasma. Most IGFs (97%) are compounded with one of the 6 IGF binding proteins (IGFBP1-6). These IGFBPs serve as an IGF reservoir, increase its half life and regulate its activities (Neely and Rosenfeld, 1992). Among the 6 IGFBPs, IGFBP3 is the predominant binding protein.

The wuzhishan pig breed is endangered, precious and lightest pig breed in China. It has high tolerance, stable inheritance, low metabolic rate, thin skin and backfat. Because its anatomy, physiology and pathology are similar to humans, wuzhishan pigs have been used as a model in pharmacological, veterinary and comparative medicine studies (Haitao and Hong, 2007). Therefore, it is very important for the development of human society to understand the special gene expression and developmental changes. The ontogeny of IGF2 and IGFBP3 has investigated in 8 tissues of wuzhishan pigs with real time quantitative PCR method in order to provide new data for further research of the molecular mechanism of developmental regulation.

MATERIALS AND METHODS

Materials: Wuzhishan pigs were obtained from China Tropical Agriculture Research Institute. Ten dams which had delivered 2 or 3 L before were selected for pure breeding and their 3rd or 4th L was used in this study. The piglets were raised with conventional standard and weaned 30 days old. Males were castrated 5 day old. Three castrated males and three females were sacrificed at 30, 60, 90, 120 and 150 days of age. Heart, liver, lung, spleen, kidney, muscle, stomach and small intestine were collected and frozen briefly in liquid nitrogen and stored at -80°C.

Major equipment and reagent: Maxwell 16 total RNA purification kit and AMVRTase were purchased from Promega (USA). SYBR PremixEx Taq was purchased from Dalian Bao Biotech Co. Nucleic acid extraction device (Maxwell16) was from Promega. PCR equipment was from Biometra (Germany). Gel image system 120 was from Kodak.

Quantitative PCR

Total RNA extraction and reverse transcription: Total RNA was extracted according to the instruction of the kit manufacturer examined with agarose gel electrophoresis. The concentration was determined with ultraviolet spectrophotometer and diluted to 1 μg μL^{-1}.

Corresponding Author: Hou Guanyu, Tropical Crops Genetic Resources Institute, CATAS, 571737 Danzhou Hainan, P.R. China

Table 1: Real time RT-PCR primer information

Genes	Primers (5'→3')	Product size (bp)	Annealing temperature (°C)	GenBank accession No.
ACTB	F: TCTGGCACCACACCTTCT			
	R:TGATCTGGGTCATCTTCTCAC	114	62.0	DQ178122
IGF2	F: CACCCTCCAGTTTGTCTGCG			
	R: AGCTACGGAAGCAGCACTCT	108	61.0	X56094
IGFBP3	F: AGCGCTACAAGGTCGACTAC			
	R: TCTCGCGCTTGGACTCAGA	73	61.0	AF085482

ACTB (β-actin) was the internal reference

Reverse transcription: Reaction mix containing 1 μL of total RNA, 1 μL of oligo (dT) 18 (50 μmol L^{-1}), 5 μL of dd H_2O (RNase free) was incubated at 70°C for 10 min chilled rapidly on ice. Then, 1 μL of 5X buffer (containing Mg^{2+}), 5 μL of 10 mmol L^{-1} dNTP, 1 μL of Rnase inhibitor (40 U μL^{-1}) 1 μL of M-MLV RTase (200 U μL^{-1}), 2 μL of dd H_2O (RNase-free) were added to the mix which was subsequently incubated at 42°C for 60 min followed by inactivation for 5 min at 99°C. Finally the reaction mix was chilled on ice and stored at -20°C until use.

Primer design: Primers were designed using Primer 3.0 (http://frodo.wi.mit.edu/) and synthesized by Dalian Bao Biotech Co. Relevant information was shown in Table 1 (Erkens *et al*., 2006; Tilley *et al*., 2007).

Real time PCR: Reactions were performed with Reaplex[4] quantitative PCR equipment (Eppendorf). The reaction mix (25 μL) contained 12.5 μL of SYBR Premix ExTaq, 1 μL of primers (10 μmol L^{-1}), 2 μL of DNA template, 9.5 μL of ddH_2O. The thermal cycle parameter was as the following: preheat at 95°C for 30 sec; 45 cycles of denaturation at 95°C for 5 sec; annealing/extension 20 sec (Table 1) and extension at 72°C for 15 sec. During melting curve analysis, temperature was gradually raised from 60-95°C at a rate of 0.875°C/30 sec. Each time, internal reference and unknown samples were assessed with triplicates and one No-Template Control (NTC) was used.

Data analysis: Relative quantification method was used to measure mRNA quantity and ACTB served as an internal reference as described by Winer *et al*. (1999). The expression levels at 30 days were set as one unit of expression. Variance analysis was used to test significance of difference. Multiple comparisons were done with SAS 9.01 software.

RESULTS AND DISCUSSION

Expression character of IGF2 mRNA: As shown in Table 2, levels of IGF2 mRNA decreased in each tissue during animals developing. The highest mRNA levels were observed at 90 days of age in each tissue while the lowest were observed at 150 days of age. The differences were very significant ($p<0.01$). Levels of IGF2 mRNA in heart, liver, lung, spleen and kidney were higher at 30, 60 and 90 days of age than at 120 and 150 days of age ($p<0.01$). No significant differences were seen in IGF2 mRNA levels between 120 and 150 days of age ($p>0.05$). At 150 days of age, liver and lung mRNA levels were significantly reduced comparing with those at 120 days of age. In muscle and stomach, the levels at 30 and 60 days of age were significantly higher than those at 90, 120 and 150 days of age ($p<0.01$).

The developmental changes of IGF2 mRNA levels were shown in Fig. 1. Results showed that the patterns of IGF2 expression were similar among eight tissues. The mRNA levels were high during early development and gradually decreased. The tendency of reduction was the smallest in liver and greatest in spleen.

Expression character of IGFBP3 mRNA: Levels of IGFBP3 mRNA were shown in Table 3. Results showed that IGFBP3 mRNA levels showed a trend of reduction in heart, lung, spleen, kidney, intestine and stomach as the pigs grew older. The highest IGFBP3 mRNA levels were seen at 30 days of age and the lowest were seen at 150 days of age. The differences between the highest and lowest were statistically significant ($p<0.01$). In the liver, IGFBP3 mRNA level increased from 30-90 days of age then declined to the lowest at 150 days of age. In muscle, IGFBP3 mRNA level was highest at 60 days of age and lowest at 150 days of age. The difference was not statistically significant between 30 and 60 days of age but very significant among 90, 120 and 150 days of age ($p<0.01$). Developmental changes of IGFBP3 mRNA levels were shown in Fig. 2. Levels of IGFBP3 mRNA exhibited similar trend among heart, lung, spleen, kidney and stomach: high during early stage then decrease continuously.

The levels in muscle and liver were similarly increased during early stage until 60 or 90 days of age then started to decrease gradually to the lowest at 150 days of age. IGF2 is a typical paternal imprinting gene regulating animal growth. Studies have shown that loss of paternal IGF2 expression resulted in the reduction in body weight by 40% (De Chiara *et al*., 1991). Other reports showed that IGF2 is associated with growth rate and

Table 2: IGF2 mRNA levels in 8 tissues of wuzhishan pig

	Age (days)				
Tissues	30	60	90	120	150
Heart	1.0000Aa	0.3717±0.0187Bb	0.2857±0.0280Bc	0.1873±0.0272Cd	0.1737±0.0291Cd
Liver	1.0000Aa	0.7060±0.0779Bb	0.5507±0.1003BCc	0.4762±0.0274Cc	0.1828±0.0198Dd
Lung	1.0000Aa	0.4158±0.0266Bb	0.3606±0.0477Bb	0.2284±0.0299Cc	0.1253±0.0110Dd
Spleen	1.0000Aa	0.2706±0.0418Bbc	0.2888±0.0133Bb	0.1873±0.0363BCc	0.0927±0.0104Cd
Kidney	1.0000Aa	0.3122±0.0495Bb	0.2635±0.0474BCbc	0.1954±0.0309Cd	0.1999±0.0419Ccd
Muscle	1.0000Aa	0.5221±0.0515Bb	0.3547±0.0470Cc	0.1937±0.0146Dd	0.0916±0.0112De
Stomach	1.0000Aa	0.4644±0.0334Bb	0.2358±0.0249Cc	0.2067±0.0161Ccd	0.1565±0.0260Cd
Intestine	1.0000Aa	0.5747±0.0562Bb	0.3929±0.0408Cc	0.2279±0.0252Dd	0.1765±0.0103Dd

Table 3: Levels of IGFBP3 mRNA in 7 tissues of wuzhishan pigs

	Age (days)				
Tissues	30	60	90	120	150
Heart	1Aa	0.7563±0.0984Bb	0.6743±0.1017BCb	0.6092±0.1006BCb	0.4677±0.0693Cc
Liver	1Bb	1.1238±0.1667Bb	1.7795±0.3702Aa	1.0151±0.0497Bb	0.7053±0.0615Bb
Lung	1Aa	0.6916±0.0545Bb	0.6393±0.0846Bbc	0.4896±0.0920BCcd	0.3399±0.0696Cd
Spleen	1Aa	0.7302±0.0479Bb	0.5936±0.0640Cc	0.4192±0.0646Dd	0.2411±0.0143Ee
Kidney	1Aa	0.4120±0.0206Bb	0.3298±0.0361Cc	0.2014±0.0086Dd	0.1271±0.0085De
Muscle	1ABa	1.1476±0.0816Aa	0.8080±0.1130Bb	0.4207±0.1038Cc	0.1969±0.0348Dd
Stomach	1Aa	0.4216±0.0169Bb	0.2881±0.0182Cc	0.2530±0.0653Cc	0.1157±0.0330Dd
Intestine	1Aa	0.5888±0.1178Bb	0.3018±0.0540Ccd	0.3787±0.0971Cc	0.2087±0.0285Cd

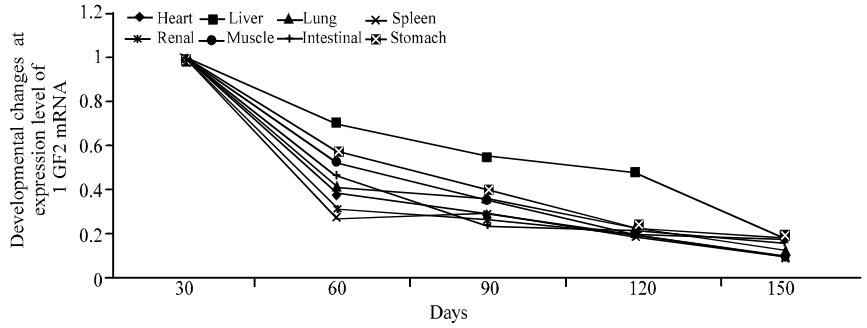

Fig. 1: Developmental changes of IGE3 mRNA in 8 tissues of wuzhishan pigs

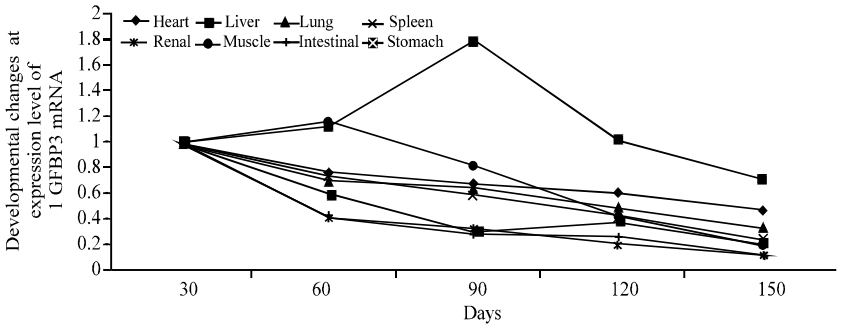

Fig. 2: Ontogeny of IGE3 mRNA in 7 pig tissues

backfat thickness(Jeon *et al*., 1999; Knoll *et al*., 2000). Wuzhishan pig is the smallest breed among the medium sized pigs that can be used in medical studies (Haitao and Hong, 2007). Therefore, an understanding of the relationship between IGF2 expression and pig growth is important for veterinary and comparative medicine and human health. Tilley *et al*. (2007) studied IGF2 expression in muscle tissue of swine fetuses at 45, 65 and 100 days of pregnancy. They demonstrated that IGF2 mRNA level decreased from 65-100 days of pregnancy (Tilley *et al*., 2007). Gerrard *et al*. (1998) studied IGF2 expression in skeletal muscle in fetus (30, 44, 59, 68, 75, 89 and 109 days of pregnancy) and young pig (21 days and 6 months of age). They showed that fetal IGF2 mRNA reached a peak

level at 59 days of pregnancy and decreased thereafter (Gerrard *et al*., 1998). Tang studied IGF2 mRNA levels in Tongcheng and large white fetuses at 30, 65 and 90 days of pregnancy. They found that skeletal muscle IGF2 mRNA levels were the highest at 65 days of pregnancy (Zhonglin *et al*., 2008). These studies demonstrated that IGF2 is a fetal growth factor participating in early physiological process.

IGF2 mRNA level reduces during postnatal development in other pig breeds. This trend is a similar in wuzhishan pigs. However, the prenatal IGF2 expression in wuzhishan pigs requires further study. Guo *et al*. (2008) determined IGF2 mRNA in large white and Taihu pigs of 30, 60, 90 120 and 150 days of age. They discovered that IGF2 mRNA level in large white was the highest at 30 days of age ($p<0.01$).

After that the level decreased, reaching the lowest at 120 days of age (Guo *et al*., 2008). Stinckens *et al*. (2007) investigated developmental changes of IGF2 mRNA in muscles tissue of Landrace at 4, 8, 16 and 26 weeks of age. Pigs of all genotype showed a decrease in IGF2 mRNA level (Stinckens *et al*., 2007). These reports are consistent with the results. The study has also shown the temporal reduction of IGF2 mRNA in heart, liver, lung, spleen, kidney, muscle, small intestine and stomach. The levels are different among different tissue. The liver IGF2 mRNA was most abundant all the time suggesting that liver is the major IGF2 synthesis organ. This result is similar to those of Peng *et al*. (1998).

CONCLUSION

IGFs exert their biological action through binding with their respective receptors. The biological availability of IGFs is modulated by IGFBPs. The expression of IGF2 and IGFBP3 were coordinated in most tissues of wuzhishan pig, suggesting that IGFBP3 expression affect development through an IGF2-dependent mechanism. IGFBP3 expression has similar tissue differences with the liver expressing the highest amount all the time. Other studies showed similar results (Bar *et al*., 1987; Dewu *et al*., 2008).

Different from IGF2 expression, hepatic IGFBP3 was the highest at 90 days of age while muscle IGFBP3 peaked at 60 days of age. Mohan and Baylink (2002) reported that IGFBP3 was abundant in infant peaked in adolescent and decreased thereafter (Mohan and Baylink, 2002). Guo *et al*. (2008) showed the highest level of IGFBP3 appeared in liver at 90 days of age in Lantang pigs. These studies indicate that IGFBP3 expression may peak after birth suggesting that IGFBP3 may have other functions in addition to binding with IGF2. Whether it is related to development awaits further study.

ACKNOWLEDGEMENTS

This research was supported by Basic Commonweal Research Fund for National Academic Institution of Chinese Academy of Tropical Agricultural (grant PZS-013) and Communion Platform Constructional Project for Livestock and Poultry Germplasm Resource (grant 2005DKA21101).

REFERENCES

Bar, R.S., L.C. Harrison, R.C. Baxter, M. Boes, B.L. Dake, B. Booth and A. Cox, 1987. Production of IGF-binding proteins by vascular endothelial cells. Biochem. Biophys. Res. Commun., 148: 734-739.

Barton, E.R., 2006. Viral expression of insulin-like growth factor-I isoforms promotes different responses in skeletal muscle. J. Applied Physiol., 100: 1778-1784.

De Chiara, T.M., E.J. Robertson and A. Efstratiadis, 1991. Parental imprinting of the mouse insulin-like growth factor II gene. Cell, 64: 849-859.

Dewu, L., Z. Yunxiang, R. Guangcai, Z. Hao and W. Zhenfang, 2008. Developmental differential expression of IGF-?, IGF-?R and IGFBP3 genes in liver and muscle of lantang and landrace pigs. Acta Veterinaria et Zootechnica Sinica, 39: 866-872.

Erbay, E., I.H. Park, P.D. Nuzzi, C.J. Schoenherr and J. Chen 2003. IGF-II transcription in skeletal myogenesis is controlled by mTOR and nutrients. J. Cell Biol., 163: 931-931.

Erkens, T., M. van Poucke, J. Vandesompele, K. Goossens, A. van Zeveren and L.J. Peelman, 2006. Development of a new set of reference genes for normalization of real-time RT-PCR data of porcine backfat and longissimus dorsi muscle and evaluation with PPARGC 1 A. BMC Biotechnol., 6: 41-41.

Florini, J.R., D.Z. Ewton and K.A. Magri, 1991a. Hormones, growth factors and myogenic differentiation. Ann. Rev. Physiol., 53: 201-216.

Florini, J.R., D.Z. Ewton, S.L. Falen and J.J. van Wyk, 1986. Biphasic concentration dependency of stimulation of myoblast differentiation by somatomedins. Am. J. Physiol.-Cell Physiol., 250: 771-778.

Florini, J.R., K.A. Magri, D.Z. Ewton, P.L. James, K. Grindstaff and P.S. Rotwein, 1991b. Spontaneous differentiation of skeletal myoblasts is dependent upon autocrine secretion of insulin-like growth factor-II. J. Biol. Chem., 266: 15917-15923.

Gerrard, D.E., C.S. Okamura, M.A. Ranalletta and A.L. Grant, 1998. Developmental expression and location of IGF-I and IGF-II mRNA and protein in skeletal muscle. Am. Soc. Anim. Sci., 76: 1004-1011.

Guo, Y.J., G.Q. Tang, X.W. Li, L. Zhu and M.Z. Li, 2008. Developmental expression changes of IGF2 and IGFBP3 genes in adipose tissue of two pig breeds. Hereditas, 30: 602-606.

Haitao, S. and W. Hong, 2007. Preliminary analysis of chinese miniature pig strains and resources. Acta Laboratorium Animalis Scientia Sinica, 15: 70-75.

Jeon, J.T., O. Carlborg, A. Tornsten, E. Giuffra and C.P. Amarger *et al*., 1999. A paternally expressed QTL affecting skeletal and cardiac muscle mass in pigs maps to the IGF-2 locus. Nat. Genet., 21: 157-158.

Knoll, A., L. Putnova and S. Cepica, 2000. A ncil PCR-RFLP within intron 2 of the porcine insulin-like growth factor 2 (IGF2) gene. Anim. Genet., 31: 150-151.

Mohan, S. and D.J. Baylink, 2002. IGF-binding proteins are multifunctional and act via IGF-dependent and-independent mechanisms. J. Endocrinol., 175: 19-31.

Neely, E.K. and R.G. Rosenfeld, 1992. Insulin-like growth factors (IGFs) reduce IGF-binding protein-4 (IGFBP-4) concentration and stimulate IGFBP-3 independently of IGF receptors in human fibroblasts and epidermal cells. Endocrinology, 130: 985-993.

Peng, M., T. Abribat, E. Calvo, D. LeBel and M.F. Palin *et al*., 1998. Ontogeny of insulin-like growth factors (IGF), IGF binding proteins, IGF receptors and growth hormone receptor mRNA levels in porcine pancreas. J. Anim. Sci., 76: 1178-1188.

Rosenblatt, J.D., D. Yong and D.J. Parry, 1994. Satellite cell activity is required for hypertrophy of overloaded adult rat muscle. Muscle Nerve, 17: 608-613.

Scandinavica, A.P., 1999. Contribution of satellite cells to IGF-I induced hypertrophy of skeletal muscle. Acta Physiol. Scand., 167: 301-305.

Stinckens, A., K. van den Maagdenberg, T. Luyten, M. Georges, S. de Smet and N. Buys, 2007. The RYR1 g. 1843C> T mutation is associated with the effect of the IGF2 intron3-g. 3072G> A mutation on muscle hypertrophy. Anim. Genet., 38: 67-71.

Tilley, R.E., C.J. McNeil, C.J. Ashworth, K.R. Page and H.J. McArdle, 2007. Altered muscle development and expression of the insulin-like growth factor system in growth retarded fetal pigs. Domestic Anim. Endocrinol., 32: 167-177.

Winer, J., C.K.S. Jung, P.M. Williams and I. Shacke, 1999. Development and validation of real-time quantitative reverse transcriptase-polymerase chain reaction for monitoring gene expression in cardiac myocytes *in vitro*. Anal. Biochem., 270: 41-49.

Zhonglin, T., L. Yong, D. Hong, Y. Shulin and M. Yulian *et al*., 2008. Establishment of Q-PCR method of IGF2 gene and its expression in fetus skeletal muscle from chinese indigenous and exotic breed pigs. J. Agric. Biotechnol., 16: 202-207.