Research progress of the correlation between porcine Insulin-like growth factor 1 (IGF-I) and growth

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Abstract. Insulin-like growth factor 1 (IGF-I) is an important factor which plays the roles in regulating animal growth, development and metabolism. IGF-I also called somatomedin C (SM-C) mainly mediate growth hormone that plays the roles of promoting growth and is a kind of polypeptide regulating cell proliferation and differentiation. The paper review influences of IGFBPs, SNPs of porcine IGF-I gene, IGF-I signaling pathway and feed level on IGF-I, moreover, the correlation between porcine insulin-like growth factor IGF-I and growth.

1. Introduction
Insulin-like growth factors (IGFs) belong to a family of growth factors with structural homology to proinsulin [1]. Salmon and Daughaday first injected growth hormone into hypophysectomized rats, found that serum can stimulate 35S sulfate combined with cartilage tissue which cultured in vitro, but there is no such phenomenon that directly add growth hormone (GH) to the tissue culture medium, inferred that GH plays an indirect role in promoting growth by inducing the body output a number of factors, and these factors named “vulcanization factor.” Further appraisal by scholars found that such intermediate growth factors have two different molecules, and they can play the kind of role of growth hormone in vitro cartilage transplant process, later this factor is named somatomedin. Utilize the sequence found that this molecule has a high homology with insulin precursor, and which named as insulin-like growth factor-I (IGF-I).

IGF-I have endocrine, autocrine and paracrine characteristics of polypeptide molecules [2], it is expressed in various organs of animal, and the highest expression is in the liver. The IGF-I synthesis and secretion in liver are regulated by growth hormone, protein level and dietary energy, affecting animal growth rate [3]. IGF-I as a multifunctional growth factor not only promotes cellular uptake of amino acids and glucose, increases the synthesis of protein, fat and glycogen, stimulates DNA replication, cell proliferation and differentiation, but also stimulates the gonad to secrete hormones, promotes lactation and animal small intestine development. And our team expressed the IGF-I mature peptide of Tibetan pig in the Pichia pastoris GS115, and found that the expression products have biological activity to promote cell proliferation [4]. IGF-I is the most complex and diverse growth factor at present, it is a major growth factor to promote rodent cells mitosis. The early evolutionary origin of the IGF-like peptides and the highly conserved amino acid sequences among vertebrate species emphasize the important role of IGF-I [5].
IGF-I gene structure and expression have a direct impact on the growth and development of animals, humans, pigs, rats and foxes etc. Previous studies have shown that the plasma IGF-I level is positively correlated with body weight and weight increase and IGF-I gene has been used as a candidate gene for regulate growth traits of pig [6]. The paper summarize the role of promoting growth mechanisms and the research results of swine IGF-I.

2. Molecules and the genetic structure and characteristics of IGF-I gene

Insulin-like growth factors (IGFs) belong to a family of growth factors with structural homology to proinsulin. IGFs are widely expressed in animal tissues, organs, and especially in liver and involved in numerous functions at various stages of development. IGF-I is a single chain alkaline protein composed of 70 amino acids. IGF-I displays a complex interaction scheme, as the hormone has a key function in the regulation of cellular proliferation and differentiation. The solution structure of IGF-I has been determined from NMR spectroscopy data, IGF-I shares the unique insulin-like fold with insulin [7]. Mature IGF-I is a peptide composed of 70 amino acids, with the molecular weight of 7 649 U and the isoelectric point pI of 8.2.

The IGF-I amino acid sequence is highly conserved in different mammals and invertebrates, it is evolved from the same gene prototype, and has important functions. Sequence of human, swine and cattle is exactly the same, while there is an amino acid difference between human and sheep, 3-4 amino acid difference between human and mouse in IGF-I protein. Our teams study and carefully analyze the IGF-I gene of Tibetan pig and Yanan pig, in addition, constructing phylogenetic tree with other 10 species (Figure 1) [8]. The two IGF-I gene sequence shared high homology with the porcine IGF-I gene reported by Leroith et al. [9], which were an identity of 100% and 99.61%. It shared 99.61% homology between the Tibetan pig and Yanan pig IGF-I gene and all above 90% homology among the cows, sheep, people, dogs, deer IGF-I genes, which further conformed that it is highly conserved.

![Molecular phylogenetic tree by Neighbor-Joining method.](image)

In addition, Bayne found that the highly conserved IGF-I sequence was remained in vertebrate evolution 300 million years ago. The cDNA sequence of IGF-I encodes a prepropeptide of 153 amino acid residues which contains a signal peptide and for structural domains (B, C, A, D), the crystal structure shown in Figure 2. The signal peptide and the E domain are cleaved, while the mature peptide with B, C, A, and D domains are kept [10]. The highly identical B and A domains are thought to be important in the binding of IGF-I with its receptor and Insulin-like growth factor-binding protein (IGFBP), while less conservative in the C and D domains, C domain region is shorter, hydrophilicity, separating B, A to two domains zone; D domain is a prominent area that contains a hairpin structure. The IGF-I structure comprises a central R-helix spanning residues 8-17 in the B-region between cysteine residues 6 and 18 and two smaller R-helical regions spanning amino acids 44-49 and 54-59, respectively, in the A-region of the molecule[11].
Figure 2. Primary structure of IGF-I with regions B, C, A, and D.

The crystal structure of IGF-I is similar to insulin (Figure 3), the structure of the disulfide bond can be degraded by reductase, thioredoxin can make the IGF-I produce the same degradation like the insulin, analyzing the secondary structure and tertiary structure of IGF-I by signal IP, found that 1-25 aa of IGF-I gene encoding protein for the signal peptide, after the translation process, leaving only 49-118 aa mature protein, a the mature protein to retain only one IGF-I protein binding sites in 74-81 aa, and retains the most major a-helical region.

Figure 3. The crystal structure of IGF-I and insulin. (A: Crystal Structure of IGF-I; B: Crystal Structure of insulin)

The full length of Porcine IGF-I gene is 80kb, which consists six exons and five introns and located on the q23–24 of pig chromosome 5. Rodent and human IGF-I gene also include six exons. Exons 1 and 2 exist the transcription initiation site of IGF-I, which also the leader exons, may encode multiple 5’-UTR and a variety of signal peptides, of which, there are 4 transcription initiation sites in exon 1, 3 in exon 2; exon 3 and exon 4 coding for the mature protein peptide chain; exon 5 and exon 6 as the selective spliced exons, responsible for encoding the carboxy-terminal extension E peptide 16 amino acids and the adjacent 3’-UTR, exon 6 containing RNase hypersensitive sites and A/T rich region. The study found that during transcription, IGF-I mRNA molecule capable of covalently link polyadenylation produce polyA protect the mRNA from exonuclease cleavage; IGF-I gene can be generated by alternative splicing of different mRNA isoforms, which may affect different species IGF-I protein level and lead to differences in their phenotypes.

3. The influence of Insulin-like growth factor binding protein to IGF-I

IGFBPs are a group of proteins that have high affinity with IGF. The potency of IGF-I is regulated through the interaction with six homologous circulating serum binding proteins, IGFBP-1 to IGFBP-6, the protein family mainly involves two aspects in the body, IGF dependent and independent of IGF, IGF-dependent effects mainly reflected IGFBPs that can regulate bioavailability of IGF, IGFBPs independent of the IGF effects mainly reflected in their regulation of cell proliferation differentiation, IGFBPs independent of IGF of molecular network get more and more people's attention. The hepatic IGF-I circulates almost entirely in the IGFBP-bound form. IGFBP-3, a major IGFBP species in the serum, binding more than 90% of the circulating IGF-I in a large ternary complex consisting of IGFBP-3, subsequent studies demonstrated that IGFBP 3 modulated IGF-I cellular actions and that they had effects that were believed to be IGF-I-independent, IGFBP-3 also has been shown to
stimulate cell growth and carry out other functions in an IGF-I dependent manner in a variety of cell types. IGFBP3 as carriers of IGFs, which plays an important role on regulate IGFs release and extend the half-life of IGFs and regulate IGFs biological activity. Therefore, IGF-I and IGF-IR, IGFBP3 and its gene expression in the pig that play an important role in growth and development.

Shehab et al. [12] found that treat human breast epithelial cells by IGF-I in vitro culture that can promote cell growth, while after joined IGFBP-3 that inhibited the growth. The IGFBP-I initially proposed as inert carriers of IGF-I whose primary function were to limit IGF-I access to receptors and regulate IGF-I positioned on specific tissues and cells that determine tissue-specific of IGF-I. At the same time it modulates the ability to binding with IGFR, affecting the signal intensity of IGFR downstream signal transduction pathway, extending the half-life of IGF-I, inhibiting cell metabolism and cell proliferation, which were believed to be IGF-I-independent. These effects further regulates IGF-I biological activities in the organization, and the growth and development of the animal. Assefa et al. [13] found that IGFBP-I can inhibit or promote IGF-I in different cells, it is depend on the phosphorylation status of IGFBP-I.

Insulin-like growth factors (IGFs) and IGF binding proteins (IGFBPs) are paracrine regulators of tissue growth and development, and are expressed at the sites of biological action. Although it is clear that IGFs and their receptors play a central role in the regulation of cell growth, the role of IGFBPs is far less well understood. In the circulation, these proteins, due to their high affinity for IGFs, may limit access of the growth factors to the tissues. IGFBPs could both inhibit and promote the actions of IGFs. Additionally, proteases acting on IGFBPs in the tissues could reduce their affinity for IGFs causing local release of these peptides to the tissues.

We believe that IGF binding proteins interact at the tissue level to regulate cell growth and proliferation. Circulating levels of IGFs and IGFBPs reflect production by many tissues within an animal and play a less important role in developmental processes. Exogenous IGFBP-I injections have been shown to cause reductions in free IGF-I. The most abundant IGFBP in circulation, may play a role in cell growth and its metabolic effects are largely opposite to those of IGF-I. IGFBP-3 inhibits the biological activity of IGF-I by sequestrating IGF-I into a circulating reservoir, thereby reducing levels of free IGF-I in circulation. Studies on transgenic animal showed that overexpression of IGFBP-3 was associated with inhibition of cell growth. These findings suggest that IGFBPs are important regulators of maternal tissues by modulating the IGF-IIIGF-I receptor interactions or by exerting IGF-I dependent actions.

4. The influence of IGF-I gene SNP loci on the growth capability of pigs
IGF-I is a kind of polypeptide, many scholars at home and abroad to study the polymorphism of IGF-I gene, found the polymorphic loci of IGF-I gene, and it has been proved to have a close relationship with the growth and development of skeletal muscle. The study found that the polymorphism of short tandem repeat sequence (CA) of IGF-I gene 5 regulatory region was significantly affected by the Jinhua pig birth weight and weight; and the IGF-I SNP of partial small and large-scale pigs was studied, exon 3 of IGF-I gene was detected in a SNP site, which contained 3 genotypes and PIC was generally 0.20~0.34. Exon 4 was detected in 2 SNPs, which contained 6 genotypes and PIC was 0.30~0.60. By PIC can better reflect the value of a small pigs are a low degree of polymorphism and large pigs are highly polymorphic, and each SNP loci statistics show G201A, A440G, and T455C loci. A and AT are the advantages of large pig allelic genes, and above two sites small pigs are no common general features. It is explained that growth performance and SNP loci of IGF-I are closely related.

Gu et al. [14] reported the TC genotype of the mini-pigs in Tibet, which have a faster growth rate and smaller size than those of the TT genotype, that shows IGF-I gene has a certain effect on the growth traits (micro) in Tibetan pigs. SNP loci of IGF-I gene in Guangxi bama mini-pigs had an advantage on AA genotype, and GG of Landrace was the dominant genes, increase in the body weight and body length of crossbred F2 generations of two (AG) were lower than homozygous genes (AA or GG) in the average value basically, SNP loci of IGF-I was closely related to the growth performance of pigs. Research reported the gene frequency statistics of the SNP loci of IGF-I showed IGF-I gene
G2 CC sites in Wuzhishan pig, Diannan small-ear pigs, Fragrant pigs, Large white pigs, Meishan pigs whose advantage gene type were GG genotype with high consistency. G89A site in Wuzhishan pigs, Large White pigs, Meishan pigs were AA type, and in the Diannan small-ear pig, Fragrant pigs, Large White were GG type. The same SNP genotype and allele types between different pig breeds have difference, but no difference between the miniature pigs and large pigs. It was showed IGF-I SNP could be used as a potential genetic marker of pig muscle growth.

Wu Dan studied found that serum IGF-I levels in Landrace pigs were higher than those in Guangxi Bama minipig at different growth stages, and the SNP locus was found in exon 2 of IGF-1 gene [15]. The polymorphism of IGF-I gene was studied by PCR-RELP, 92 Nanchang white pigs and 170 Yorkshire pigs were selected as the research objects. The results showed that in Nanchang white pigs, the weight of AA genotype was significantly higher than that of AB pigs (P<0.05). In 6 month old Yorkshire pigs, the weight of BB type pigs was bigger than that of AB pigs. The researchers used PCR-RFCP, and found that there were 2 HhaI restriction sites in the PCR products of IGF-I genes from 6 pig breeds, one of which restriction site produces a genetic polymorphism. The results suggested that IGF-I gene is potential genetic markers in the study on porcine muscle growth and could be used as markers and a candidate gene in marker assisted selection to improve the efficiency and accuracy selection. Regulation zone and coding the genetic variation of IGF-I gene can affect the individual's growth and carcass composition.

5. Research of the correlation between porcine Insulin-like growth factor IGF-I signaling pathway and growth
IGF-1 signaling pathway is a complex system. IGF-I has three signaling pathways (Figure 4): the Ras/Raf/MEK/ERK signal transduction pathways, PI3K/AKT signal transduction pathways, and protein/14-3-3 Raf signal transduction pathways. The Ras/Raf/MEK/ERK and PI3K/AKT signal transduction pathways associated with cell proliferation, PI3K is activated to form phosphorylation phosphoinositide (PIP3), PIP3 is cell growth signals, and the PI3K pathway is the most classic way to inhibit cell growth. The 14-3-3 protein/RAF signal transduction pathways mainly through passivating apoptin to inhibit apoptosis. Conditioning enzyme (ERK), ERK as a member of Mitogen-activated protein kinase family, the basic signal transmission steps to follow the mitogen-activated protein kinase kinase 3 enzymatic cascade, namely the Ras-Raf-MEK-ERK pathway, when ERK transmit signal to the cell, mitosis process starts.

![IGF-I signaling pathways](image)

Figure 4. IGF-I signaling pathways. [16] Ras-Raf-MEK-ERK is a set of cascade signal transduction from the cell surface receptors to transcription factor to regulate gene expression. Raf-MEK-ERK pathway acts on the apoptosis such as Bad, Bim, Mcl-1, (caspase-9) and Bcl-2 remains controversial. After Raf is activated, its C-terminal catalytic domain can binding with MEK and catalyze two arginine sites of region VIII to phosphorylation. Ras/Raf/MEK/ERK signaling pathway is one of the three mitogen-activated protein kinases signal transduction pathways, The highly conserved mitogen-activated protein kinase (MAPK)
family is one of the major kinase families that regulate cells by transducing extracellular into cellular responses, which include PI3K/Akt mediated by IRS-1 and MAPI signal transduction pathways. In PI3K/Akt mediated by IRS-1 pathway, after *IGF-I* ligand binding with the *IGF-I* R, it activates downstream signaling pathways by IRS, CRL, SHC and other proteins. At last, it induces metabolic activity through the downstream PI3I inositol phospholipid 3-kinase/PKB (protein kinase B) pathway. MAPI way also mediated by the IRS, primarily regulate the proliferation and differentiation of tissue cells. And Rizzoli et al. [17] reported a reduction in *IGF-I* production and activity will result in a reduction of age-related bone mass and low levels of bone formation, *IGF-I* signaling pathway plays important role in anabolic bone growth and development. In addition, some cytokine receptors can activate ERK by activating Janus kinase (JAK), thereby promoting muscle growth and development in animals.

6. Feed level affect the *IGF-I* gene to the growth of pig

Shan et al. [18] found that add copper to diet can significantly improve the concentration of serum *IGF-I*, liver ghrelin (GHR) mRNA level and liver cell membrane GHR specific binding activity, liver *IGF-I* mRNA, the growth mechanism of the high copper do instead of GH, GHR regulation. O'Neill et al. [19] found that dietary cysteamine (CS) supplementation modulates the growth rate, serum *IGF-I* concentrations, and the gene expression of GHR, *IGF-I*, *IGF-I* R, IGFBP-3, and IR in a dose-dependent manner. CS supplementation has tissue-specific regulation of GHR, *IGF-I*, *IGF-I* R, and IGFBP-3 mRNA levels. Moreover, the results also imply the possible physiologic role of the GH-IGF axis in mediating the dietary CS supplementation-supported growth of finishing pigs.

Wei et al. [20] found that add n-3 polyunsaturated fatty acids in the dietary can enhance the expression of *IGF-I* gene and promote muscle growth. Liu and other research has shown that add GSH in the dietary can improve the concentration of pig blood *IGF-I*, and the liver expression of *IGF-I*, inhibit the expression of *IGF-I* R of fat tissue, accelerate the growth of piglets. Zhou et al. [21] studied shows that short-term feeding weaned piglets 1% of potassium formate can significantly increase the expression of *IGF-I* and *IGF-I* R. Youreva et al. [22] found that *IGF-I* plays an important role in the digestive tract development of newborn animals, when use in low doses of *IGF-I* fed newborn pig, it can improve the small intestinal villus of brush border enzyme activity, while higher doses of *IGF-I* can stimulate the growth of the small intestine tissue, thus promotes the growth and development of piglets.

Research shows that feeding formula milk to pig can increase the level of serum *IGF-I* than the normal lactation piglets, and *IGF-I* is an important factor of regulation of the prenatal and postnatal growth. Different scholars have reported, *IGF-I* mRNA expression in the liver is significant decline with low protein or protein diet fed mice. In addition, the protein and energy ratio under the different diet of sheep, the expression quantity of *IGF-I* mRNA of liver is different. Bruchim et al. [23] researched shows that the pig amount of mRNA expression of the *IGF-I* in swine adipose tissue as the diet increases with the rising of protein levels. This series of studies have shown that low protein diet to slow pig growth and development, promotion of GH secretion, reduce the secretion of *IGF-I*, lower abundance of *IGF-I* gene expression; high-protein diet growth of pigs, reduce GH secretion, increase the secretion of *IGF-I*, increase abundance of *IGF-I* gene expression. These results indicate that nutritional state differentially regulates GH-IGF-I system components in a tissue-specific manner and that such alterations disable the growth-promoting actions of GH and promote the lipid-mobilizing actions of the hormone.

7. Discussion

Growth traits are an important part of economic traits in pigs. Study confirmed that *IGF-I* is a major endocrine hormone closely related to animal growth and development. Plasma *IGF-I* level is positively correlated with body weight and weight increase. Tibetan pig was originally found exclusively on the Tibetan plateau, a natural and harsh environment with an average altitude of, 4268 m above sea level. This species survived a wild state and only recently attracted attention because of increased interest in meat production at high altitudes. Tibetan pig is also an ideal experimental animal.
as the model of dwarf animals in biology and the comparative medicine, because of the physiological characteristics and metabolism with those human. This research reviews the correlation between porcine Insulin-like growth factor IGF-I and growth that can potentially shed light on pig growth. IGF-I gene can be used as a candidate gene for regulate growth traits of pig, playing a role in a variety of tissues and organs’ development, which also has a wide range of physiological effects in the treatment of many diseases. Therefore, the regulation in vivo expression levels of IGF-I in the growth and development of the pig, thus contributing to the growth of pigs have great potentiality, which has great significance in animal husbandry, especially in pig industry.

IGF-I has an important implications for improving pig performance, IGF-I expression in vivo directly affected by swine IGFBPS, IGF-I gene SNP loci, IGF-I receptor, IGF-I signaling pathway and fodder and other factors, affecting the growth of pigs, and other factors (such as the environment, etc.) also regulate the growth of pigs. However growth, development, reproduction is an extremely complex physiological process, in addition to being influenced by genetic, nutritional, environmental and many other factors, the growth of animals are ultimately regulated by the neuroendocrine growth axis (GHRH-GU-IGF). Pig IGFBP, IGF-I gene SNP loci, IGF-I receptor, IGF-I signaling pathway and fodder and other factors affecting the level of expression of IGF-I molecular mechanism is unclear, the study also scattered, did not form a complete access, and present research, mostly concentrated in differences breeds IGF-I gene and regulation of IGF-I on growth.

In future studies, we need to refine the influence of different factors on the expression levels of IGF-I and molecular mechanisms, explore other external (environmental) factors such as the regulation of the relationship between growth and development, as well as more in-depth study of the molecular system regulatory mechanism, the formation of a complete path for a comprehensive understanding of pig IGFBPS, affect IGF-I gene SNP loci, IGF-I receptor, IGF-I signaling pathway and feed levels and other factors on the expression of IGF-I levels and improve pig the growth performance provides strong evidence.

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