Molecular and Cellular Endocrinology 384 (2014) 175–184

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

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

Insulin-like growth factor I induces proliferation and migration of porcine trophectoderm cells through multiple cell signaling pathways, including protooncogenic protein kinase 1 and mitogen-activated protein kinase

Wooyoung Jeong [a,1], Gwonhwa Song [a,1], Fuller W. Bazer [b], Jinyoung Kim [c,*]

[a] Division of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul, Republic of Korea
[b] Center for Animal Biotechnology and Genomics and Department of Animal Science, Texas A&M University, College Station, TX, USA
[c] Department of Animal Resources Science, Dankook University, Cheonan, Republic of Korea

ARTICLE INFO

Article history:
Received 13 November 2013
Received in revised form 8 January 2014
Accepted 31 January 2014
Available online 6 February 2014

Keywords:
Pig
Insulin-like growth factor
Trophoblast
Proliferation
Migration

ABSTRACT

During early pregnancy, the developing conceptus is dependent upon a wide range of growth factors and nutrients that are secreted by or transported by uterine epithelia into the uterus at the maternal–conceptus interface for successful implantation and placentation. Among these factors, insulin-like growth factor-I (IGF-I) is known to play an important role in development of the early embryo and uterine endometrium. However, few studies have been conducted with pigs to determine IGF-I-induced functional effects on peri-implantation embryos such as activation of cell signaling cascades responsible for growth, proliferation and differentiation of cells of the conceptus. Therefore, the aim of this study was to analyze mRNA expression of endometrial IGF-I and its receptor, to examine the functional role of IGF-I on primary porcine trophectoderm (pTr) cells and to assess potential signaling pathways responsible for biological activities of IGF-1. In the present study, expression of endometrial *type I IGF receptor* (*IGF-IR*) mRNA increased significantly from Day 10 to Day 12 of pregnancy and the increase was greater for pregnant than cyclic gilts. Both *IGF-I* and *IGF-IR* mRNAs were abundant in endometrial luminal-, glandular epithelia, and stratum compactum stroma on Day 12 of pregnancy. In addition, IGF-I significantly induced phosphorylation of AKT1, ERK1/2 and RPS6 in a time- and concentration-dependent manner in pTr cells. Immunofluorescence microscopy revealed that IGF-I treated pTr cells exhibited increased abundance of phosphorylated (p)-AKT1 and p-ERK1/2 MAPK proteins in the nucleus and cytoplasm, and p-RPS6 proteins in the cytosol as compared to non-treated pTr cells. In the presence of the ERK1/2 MAPK inhibitor (U0126), IGF-I-induced AKT1 phosphorylation was not affected, whereas the PI3K inhibitor (LY294002) decreased IGF-I-induced phosphorylation of ERK1/2 and AKT1 proteins, and both the PI3K-AKT1 and ERK1/2 MAPK pathways were blocked by LY294002. Furthermore, IGF-I significantly stimulated both proliferation and migration of pTr cells, but these effects were blocked by P38 inhibitor (SB203580), U0126, MTOR inhibitor (rapamycin) and LY294002. Taken together, these results indicate that IGF-I coordinately regulates multiple cell signaling pathways including PI3K-AKT1-RPS6 and ERK1/2 MAPK signaling pathways that are critical to proliferation, migration and survival of trophectoderm cells during early pregnancy in pigs.

1. Introduction

As in any eutherian mammal, successful establishment and maintenance of pregnancy in the pig follows a well-organized reciprocal communication between the developing conceptus and maternal uterus to support pregnancy recognition, implantation, placentation and exchange of nutrients and gases (Bazer et al., 2012). The peri-implantation period is one of the most critical stages of pregnancy for initiation of the maternal–conceptus dialogue, and it is also the period when the majority of conceptus mortality and reduction in litter size occurs in pigs (Stroband and Van der Lende, 1990). During this period, the spherical porcine blastocyst dramatically elongates to tubular and filamentous forms between Days 10 and 12 of pregnancy, and then comes in close

* Corresponding author. Address: Department of Animal Resources Science, Dankook University, Cheonan 330-714, Republic of Korea. Tel.: +82 41 550 3657; fax: +82 41 565 2949.
E-mail address: jinyoungkim@dankook.ac.kr (J. Kim).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.mce.2014.01.023
0303-7207/

proximity and begins to make attachment to the uterine luminal epithelium (LE) by Day 13 of pregnancy to establish a diffuse epitheliochorial type of placenta in which uterine LE is not destroyed during placental invasion as in other species (Geisert et al., 1982a; Keys and King, 1990; Geisert and Yelich, 1997; Leiser and Kaufmann, 1994; Burghardt et al., 1997). This process is especially important in pigs as it maximizes placenta–uterine surface area of contact for the efficient transfer of nutrients and gases between the maternal and conceptus vascular system (Stroband and Van der Lende, 1990; Song et al., 2011).

During the peri-implantation period of pregnancy, the free-floating conceptus develops in the absence of direct contact with the uterine LE, so uterine secretions are essential for growth and development of the conceptus. Many factors including growth factors, cytokines, enzymes, hormones, ions and nutrients in uterine secretions make up what is known as histotroph. Histotroph is implicated in regulation of functional local interactions at the maternal–conceptus interface during the peri-implantation period of pregnancy (Carson et al., 2000; Lim et al., 2002; Spencer et al., 2007; Spencer and Bazer, 2004; Gray et al., 2001a,b). Histotroph includes important growth factors for conceptus development and implantation that are expressed by uterine epithelia in a temporal and cell-specific manner, as well as secretions from the peri-implantation conceptus (Kane et al., 1997).

The insulin-like growth factors (IGFs), IGF-I and IGF-II, are single chain polypeptides which have structural homology with proinsulin (Heyner et al., 1989; Kaye et al., 1992). IGF-I and IGF-II have mitogenic and insulin-like metabolic effects that regulate a variety of fundamental cell properties by binding to type I IGF receptors (IGF-IR) and type II IGF receptors (IGF-IIR), respectively on the cell surface (Rechler and Nissley, 1985; Jones and Clemmons, 1995). The IGFs are significant regulators of conceptus and placental development during the peri-implantation period of pregnancy in humans, rodents, and domestic animals (Irwin et al., 1999; Wathes et al., 1998; Watson et al., 1999; Gluckman, 1986; Binoux, 1995; Kim et al., 2008). Results from studies of humans and mice suggest that IGF-I and IGF-II play important roles in stimulating protein synthesis, growth and development of the conceptus by regulating migration and proliferation of trophectoderm cells (Harvey and Kaye, 1992a,b,c, 1990, 1988, 1991; Gardner and Kaye, 1991; DeChiara et al., 1990; Zwijsen et al., 2000). In domestic mammals such as cattle and sheep, IGFs and their receptors are expressed in the uterus, oviduct and conceptus during the peri-implantation period of pregnancy and regulate growth and development of blastocysts, as well as endometrial gland morphogenesis for implantation and placentation (Watson et al., 1992; Robinson et al., 2000; Ko et al., 1991). Furthermore, our previous study with ovine trophectoderm cells revealed that IGF-II stimulates migration via multiple cell signaling pathways (Kim et al., 2008).

In pigs, IGF-I, IGF-II and their specific receptors are expressed in the endometrium throughout the peri-implantation period of conceptus development (Green et al., 1995; Simmen et al., 1990). Endometrial *IGF-II* transcripts increase as pregnancy progresses, whereas *IGF-I* transcripts in the uterus and secretory IGF-I protein in uterine flushings are greatest on Day 12 of pregnancy, coinciding with the time of conceptus elongation and secretion of estrogen for pregnancy recognition signaling (Simmen et al., 1992; Geisert et al., 2001; Miese-Looy et al., 2012). Also, enhanced release of porcine IGF-I stimulates embryonic protein synthesis, and expression of genes such as cytochrome P450 aromatase for secretion of estrogens by the conceptus (Green et al., 1995; Estrada et al., 1991; Ko et al., 1994; Hofig et al., 1991). Available evidence suggests that IGF-I is highly relevant to peri-implantation events during early pregnancy in pigs, nevertheless, potential novel functions of IGF-I during early pregnancy and molecular mechanisms that link the IGF-I system to peri-implantation processes are poorly understood. The present study provides detailed analyses of temporal and spatial expression of endometrial *IGF-I* and *IGF-I* receptor mRNAs throughout the estrous cycle and peri-implantation period of early pregnancy, as well as establishment of direct links between IGF-I and cell signaling pathways in pTr cells.

This study tested the hypothesis that IGF-I from the endometrium and/or conceptus functions through multiple cell signaling pathways critical to proliferation, migration and survival of porcine trophectoderm (pTr) cells during implantation and placentation. Therefore, the specific objectives of this study were to determine: (1) the temporal and cell-specific expression of IGF-I and IGF-IR in porcine endometrium during the estrous cycle and early pregnancy; (2) effects of IGF-I on transactivation of PI3K-AKT1 and ERK1/2 MAPK signaling pathways in pTr cells; and (3) functional effects of IGF-I on proliferation and migration of pTr cells.

2. Materials and methods

2.1. Experimental animals and animal care

Sexually mature gilts of similar age, weight, and genetic background were observed daily for estrus (Day 0) and exhibited at least two estrous cycles of normal duration (18–21 days) before being used in this study. All experimental and surgical procedures were in compliance with the Guide for Care and Use of Agricultural Animals in Teaching and Research and approved by the Institutional Animal Care and Use Committee of Texas A&M University.

2.2. Experimental design and tissue collection

Gilts were assigned randomly to either cyclic or pregnant status. Those in the pregnant group were bred when detected in estrus and 12 and 24 h later. Gilts were ovariohysterectomized on either Day 9, 12, or 15 of the estrous cycle or on Day 9, 10, 12, 13, 14 or 20 of pregnancy (n = 3–4 pigs per day per status). For confirmation of pregnancy prior to implantation, the lumen of each uterine horn was flushed with 20 ml of physiological saline and examined for the presence of morphologically normal conceptuses. Uteri from cyclic and pregnant gilts were processed to obtain several sections (~0.5 cm) from the entire uterine wall in the middle of each uterine horn. The uterine tissue was fixed in fresh 4% paraformaldehyde in PBS (pH 7.2) and embedded in Paraplast-Plus (Oxford Laboratory, St. Louis, MO).

2.3. Cell culture

Mononuclear porcine trophectoderm (pTr) cells from Day 12 pig conceptuses were cultured and used in the present *in vitro* studies as described previously (Ka et al., 2001). For experiments, monolayer cultures of pTr cells were grown in culture medium to 80% confluence in 100-mm tissue culture dishes. Cells were serum starved for 24 h, and then treated with recombinant human IGF-I (100 ng/ml; R&D Systems, Inc., Minneapolis, MN) for 0, 5, 15, 30, 60 or 120 min. Based on preliminary dose–response experiments, 100 ng/ml IGF-I was selected for use in all experiments in the present study. This design was replicated in three independent experiments.

2.4. RNA Isolation

Total cellular RNA was isolated from endometrium from cyclic and pregnant gilts using Trizol reagent (Invitrogen, Carlsbad, CA) and purified using an RNeasy Mini Kit (Qiagen) according to the manufacturer's recommendations. The quantity and quality of to-