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Short Communication

Differential expression of genes linked to the leukemia inhibitor factor signaling pathway during the estrus cycle and early pregnancy in the porcine endometrium

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ABSTRACT

The objective of this study was to determine the expression profiles of leukemia inhibitory factor (*LIF*) and its receptor (*LIFR*), interleukin 6 receptor (*IL6R*), tumor protein p53 (*TP53*) and B-cell CLL/lymphoma 2 (*BCL2*) in the porcine endometrium on selected days of the estrous cycle and pregnancy. Time- and reproductive status (estrous cycle/pregnancy)-specific patterns of expression were identified for all investigated genes. The most pronounced changes were seen on Days 12 and 14 of pregnancy when maternal recognition of pregnancy and implantation, respectively, occurs in pigs.

1. Introduction

A number of studies suggest a substantial role of IL (interleukin)6-like cytokine family members in mammalian reproduction. This family comprises several molecules, including IL6, IL11, leukemia inhibitory factor (LIF) and ciliary neurotrophic factor (CNTF) [for review see: 1]. LIF is a cytokine which seems to play a pivotal role in the regulation of endometrial functions and embryo implantation and has been

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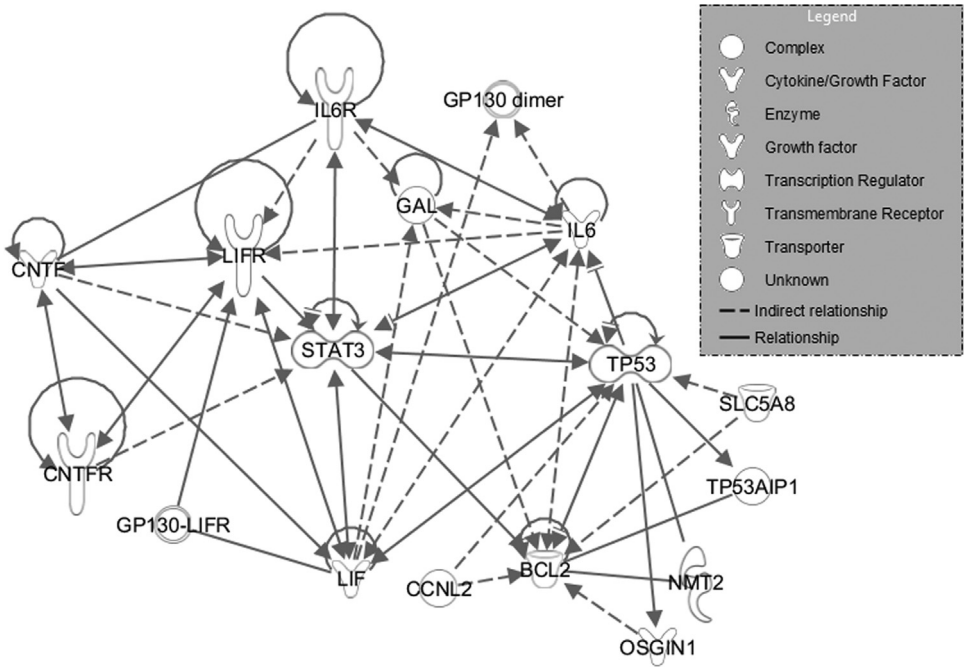

Fig. 1 – A scheme presenting the LIF signaling pathway involving interactions between examined genes. Ingenuity Pathway Analysis tool (Ingenuity® Systems, www.ingenuity.com) was used to denote relationships between the genes. CCNL2, cyclin L2; CNTFR, ciliary neurotrophic factor receptor; GAL, galanin; NMT2, N-myristoyltransferase 2; OSGIN1, oxidative stress induced growth inhibitor 1; SLC5A8, solute carrier family 5 member 8; TP53AIP1, tumor protein p53 regulated apoptosis inducing protein 1.

identified in the uterus of many species, such as humans [2] and pigs [3,4]. Both LIF and IL6 are present in the uterine environment during early pregnancy of the pig, affecting endometrial function as well as the attachment and proliferation of trophoblast cells [4]. Although LIF is mainly recognized for its regulatory functions of inflammatory cell responses, it also controls uterine receptivity for blastocyst implantation and influences trophoblast proliferation, invasion and differentiation in mice and humans [5–7].

LIF triggers its effects by the induction of a signaling heterodimer receptor consisting of the specific LIF receptor (LIFR) and its subunit GP130. This activates the RAS/mitogen activated protein kinase (RAS/MAPK) and Janus kinase/signal transducer and activator of transcription (JAK/STAT) cascades. Substantial crosstalk exists between the intracellular signaling pathways of IL6-like cytokine family members [for review see: 8]. The JAK/STAT pathway is crucial in embryo implantation as shown by the embryonic lethality of STAT3-deficient mice [9]. On the other hand, IL6 receptor (IL6R)-mediated STAT3 activation and translocation into the nucleus are essential for mediating the invasion promoting effects of LIF and IL6 in trophoblast and choriocarcinoma cells [7,10]. Interestingly, LIF was identified as a tumor protein p53 (tp53)-regulated gene. A mutation of the *tp53* gene (apoptotic effector) in mice results in a reduction of *LIF* mRNA, a cytokine necessary for the uterine receptivity and implantation [11]. Since LIF is involved in the regulation of reproductive processes, we tested the expression of genes linked to the LIF signaling pathway in the porcine endometrium during the estrous cycle and early pregnancy. The lack of comprehensive studies showing the expression of genes involved in LIF signaling in pigs forced us to investigate the expression of five (*LIF*, *LIFR*, *IL6R*, *TP53*, and *BCL2*) selected genes, for which literature-based relationships were found in the Ingenuity Pathway Analysis application (Fig. 1).

2. Materials and methods

Endometrial samples were collected from crossbred gilts (Hampshire × Duroc) of similar age (7–8 months), weight (140–145.5 kg) and genetic background from one commercial herd. After exhibiting two natural estrous cycles, gilts were randomly assigned to the group of cyclic or pregnant animals. Gilts assigned to the latter group were artificially inseminated 12 h after onset of estrus (Day 0) and 24 h later. Samples were collected on Days 10 ($n = 5$), 12 ($n = 5$), 14 ($n = 8$), or 16 ($n = 5$) of the estrous cycle and Days 10 ($n = 5$), 12 ($n = 5$), 14 ($n = 7$), or 16 ($n = 5$) of pregnancy. Reproductive status was verified by the examination of utero-ovarian morphology and the presence of at least four morphologically normal conceptuses. Endometrial tissue samples were snap-frozen in liquid nitrogen and stored at −80 °C until further use.

Endometrial tissues were homogenized in Lysis/Binding Buffer (Ambion, Austin, TX, USA) with a FastPrep-24 instrument (MP Biomedicals, Solon, OH, USA). Total RNA was extracted using a mirVana™ miRNA Isolation Kit (Ambion) according to the manufacturer's instructions. The purity and quantity of extracted RNA was estimated using NanoDrop 1000 (Thermo Scientific, Wilmington, DE, USA). Real-time RT-PCR (qRT-PCR) was performed with an ABI Prism 7900 HT sequence detection