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A transcriptional cofactor YAP regulates IFNT expression via transcription factor TEAD in bovine conceptuses

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ABSTRACT

Interferon tau (IFNT) is the pregnancy recognition protein in all ruminants, and its expression is restricted to trophoblast cells. Interferon tau production increases as the conceptus elongates; however, its expression is downregulated soon after the initiation of conceptus attachment to the uterine epithelium. Our previous study identified that among 8 bovine *IFNT* genes, only 2 forms of *IFNTs*, *IFNT2* and *IFN-tau-c1*, were expressed by the conceptuses during the peri-attachment period. To characterize whether Hippo signaling including a transcription cofactor yes-associated protein (YAP) was involved in the IFNT regulation, we examined the expression and effects of YAP and/or TEAD in human choriocarcinoma JEG3 and bovine trophoblast CT-1 cells, and in bovine conceptuses obtained from day 17, 20 or 22 pregnant animals (pregnant day 19.5 = day of conceptus attachment to the endometrium). YAP was expressed in bovine conceptuses and transfection of YAP or TEAD4, a transcription factor partner of YAP, expression plasmid increased the luciferase activity of *IFNT2* and *IFN-tau-c1* reporter plasmids in JEG3 cells. In the presence of *YAP* expression plasmid, *TEAD2* or *TEAD4* expression plasmid further upregulated transcriptional activity of *IFNT2* or *IFN-tau-c1* constructs, which were substantially reduced in the absence of the TEAD-binding site on *IFNT2* or *IFN-tau-c1* promoter region in JEG3 cells. In CT-1 cells, treatment with *TEAD2*, *TEAD4*, or *YAP* small-interfering RNA down-regulated endogenous *IFNT* expression. It should be noted that TEAD2 and TEAD4 were predominantly localized in the nuclei of trophectoderm of Day 17 conceptuses, but nuclear localization appeared to be lower in those cells of conceptuses on days 20 and 22 of pregnancy. Moreover, the binding of TEAD4 to the TEAD-binding site of the *IFN-tau-c1* promoter region in day 17 conceptuses was less in day 20 and 22 conceptuses. Furthermore, the level of YAP phosphorylation increased in day 20 and 22 conceptuses. These results indicated that although YAP/TEAD had the ability to up-regulate *IFNT* gene transcription on day 17, *IFNT2* or *IFN-tau-c1* was down-regulated following changes in the localization of TEAD2 and TEAD4 from the nucleus to the cytoplasm and increases in phosphorylation and degradation of YAP. These data suggest that TEAD relocation and/or YAP degradation following its phosphorylation down-regulates *IFNT* gene transcription after conceptus attachment to the uterine endometrium.

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1. Introduction

Embryonic losses during the period of conceptus attachment to the maternal endometrium reach nearly 50% [1,2]. Attachment of conceptus to the endometrium is an initial step toward the establishment of pregnancy, although the molecular mechanisms associated with physical and biochemical communications between conceptus and endometrium that result in proper conceptus-endometrial attachment are still far from complete understanding. Interferon tau (IFNT) is the pregnancy recognition protein in all ruminants, and its expression is restricted to trophoblast cells during the periattachment period [3,4]. Interferon tau maintains the corpus luteum function via inhibition of luteolytic pulses of endometrial prostaglandin F2α [5–7]. Bovine IFNT increases on day 15 of pregnancy (day 0 = day of estrus), peaks on days 19 to 20 and is downregulated soon after the initiation of conceptus attachment to the uterine epithelium [8–10]. Despite the fact that the expression and effects of IFNT are extensively studied, transcriptional regulation, in particular downregulation, of *IFNT* genes has not been fully elucidated.

Previously, we have shown that transcription factor caudal type homeobox 2 (CDX2), expressed in the ovine trophoblasts during the conceptus elongation period, is one of the transcription factors required for trophoblast specific expression of IFNT [11]. We also reported that CDX2 and chromatin structure could be the key elements that determine trophoblast cell-specific activation of *IFNT* gene expression [12]. In addition to CDX2, several transcription factors have been identified to regulate *IFNT* gene expression, of which v-ets avian erythroblastosis virus E26 oncogene homolog 2 (ETS2) and distal-less 3 (DLX3) bind to the promoter of *IFNT* gene, and activator protein 1 (AP1) bind to the enhancer region [13–17]. Moreover, IFNT expression is upregulated by cyclic adenosine 3′,5-monophosphate-response element binding protein-binding protein (CREBBP), a histone acetyltransferase, and also transcription factors GATA2 and GATA3 [18–20]. In the previous study, we identified 2 *IFNT* isoforms, *IFNT2* (NM_001015511.3), and *IFN-tau-c1* (NM_001031765.1), in utero and showed that the expression of *IFNT2* transcript was 5-fold higher than that of *IFN-tau-c1*. The transcription factors CDX2, ETS2, AP1, and CREBBP have upregulated *IFN-tau-c1* and *IFNT2*, whereas AP1 site mutation was effective in the downregulation of *IFNT2*, but not *IFN-tau-c1* transcription [9].

Although several transcription factors that upregulate the expression of *IFNT* genes have been identified, the mechanism by which IFNTs level is downregulated after the initiation of conceptus attachment to the endometrium has not been well characterized. We reported that the degree of *IFNT* gene transcription is reflective of the level of *CDX2* gene transcription in the bovine trophectoderm [21], which coincides with the initiation of conceptus attachment to the uterine epithelium. After conceptus attachment, T-box protein eomesodermin (EOMES) expression increased in the trophectoderm, which partially downregulated IFNT gene expression [22]. These observations have provided the notion in which there must be another molecular mechanism associated with *IFNT* gene downregulation.

It has been reported that a transcriptional factor yes-associated protein (YAP) regulates gene expressions in the mouse trophectoderm [23]. Yes-associated protein is inactivated by cell-cell contact, adherence junction and tight junction, whereas the disruption of cell-cell contact results in an increase in transcriptional activity of *YAP* gene [24]. The mechanism of *YAP* gene inhibition involves phosphorylation of Ser 127, which promotes translocation of YAP from nucleus to cytoplasmic region and subsequent binding to a cytoplasmic scaffold protein 14-3-3, resulting in YAP degradation [23–25]. Yes-associated protein binds to TEA domain family transcription factors (TEADs) [26], which are conserved transcription factors consisting of TEAD1 to TEAD4, and share the conserved TEA DNA binding domain at the N-terminal region in humans and mice [27]. However, it has not been characterized whether YAP and TEADs play a regulatory role on *IFNT* transcription in the bovine trophectoderm during periattachment period. In this study, we examined whether conceptus attachment regulated YAP and/or TEADs activity and subsequent changes in IFNT expression in the bovine trophectoderm.

2. Materials and methods

2.1. Animals and tissue collection

All animal procedures in this study were approved by the Committee for Experimental Animals at Zennoh Embryo Transfer (ET) Center and the University of Tokyo. For ET processes, 7-day embryos (day 0 = day of estrus) were collected from superovulated and artificially inseminated cattle. Twenty-four blastocysts derived from the superovulation/AI procedure were transferred nonsurgically into the uterine horn of 12 Holstein heifers (2 blastocysts/recipient), ipsilateral to the corpus luteum, on day 7 of the estrous cycle [28]. Elongated conceptuses on day 17, 20, or 22 (P17, P20, or P22, respectively; 3 animals each) were collected nonsurgically by uterine flushing with PBS and snap-frozen in liquid nitrogen.

2.2. RNA extraction and quantitative real-time polymerase chain reaction (RT-PCR)

Using ISOGEN reagent (Nippon gene, Tokyo, Japan), total RNAs were extracted from conceptuses or cultured cells. For RT-PCR analyses, isolated RNA (total 0.5 μg) was reverse transcribed to cDNA using the ReverTra Ace qPCR RT Kit (Toyobo, Osaka, Japan) according to the manufacturer's instructions. The cDNA reaction mixture was subjected to RT-PCR amplification using the Thunderbird SYBR qPCR Mix Kit (Toyobo) with primers listed in Table 1, and PCR amplification was carried out on a Step One Plus real-time PCR System (Applied Biosystems, Foster City, CA, USA). Amplification efficiencies of each target and the reference gene, bovine beta-actin (*ACTB*), were examined through their calibration curves and found to be comparable. The thermal profile for qPCR consisted of 40 cycles at 95°C for 15 s, and annealing and extension at 60°C for 60 s. Average threshold (Ct) values for each target were determined by Sequence Detection System software v2.2 (Applied