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Mouse model of human infertility: Transient and local inhibition of endometrial STAT-3 activation results in implantation failure

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Abstract **Embryo implantation involves a series of biochemical reactions and its failure is an important therapeutic target of infertility treatment. We established an infertile mouse model using transient and local suppression of signal transducer and activator of transcription-3 (STAT-3) activity by STAT-3 decoy transfer into the uterine cavity during implantation, resulting in <30% implantation. This infertility is caused by suppression of decidualization, which is indispensable for implantation, and independent of progesterone. These conditions may mimic clinically unexplained infertility. Our results suggest that STAT-3 could be a useful target for diagnosis and therapy of human implantation failure.**

Keywords: STAT-3; Implantation failure; Infertility; In vivo gene transfer; Molecular therapy; HVJ-E vector

1. Introduction

Approximately 10–15% of all couples experience infertility during their reproductive lives [1]. Implantation failure is considered to be a major reason for their infertility. One third of implantation failures might be attributed to the embryo itself. The remaining 2/3 of failures appear to be the result of inadequate uterine receptivity [2]. It is thus important to improve uterine receptivity in infertility treatment. However, the pathophysiology of implantation failure is unexplained as is the molecular mechanism of implantation. Uterine receptivity has been clinically evaluated via serum progesterone levels, ultrasonographic endometrial thickness, and histological dating by endometrial biopsy [3]. A discrepancy between the hormonal milieu and endometrial morphology and/or function could be an important cause of implantation failure [4], although there is no consensus description for the diagnosis to date. We hypothesize that a few multi-potential transcriptional factors might initiate the regulatory cascade and have pivotal roles in generating the implantation window. Therefore, we devised a local and transient in vivo gene transfer system to modulate the function of endometrial signaling molecules during the implantation window, without disturbing the course of pregnancy [5]. STATs are a family of latent cytoplasmic proteins involved in transmitting extra-cellular signals to the nucleus [6]. Signal transducer and activator of transcription-3 (STAT-3) is activated by gp130, a subunit of the receptor for the interleukin-6 (IL-6) family of cytokines, including leukemia inhibitory factor (LIF) and IL-11. As gene targeting analysis indicated LIF and IL-11 play crucial roles in implantation [7,8], the physiological function of STAT-3 activation in implantation is of great interest. However, STAT-3 deficiency in embryos is lethal, and obtaining adult STAT-3 null female mice impossible [9]. Here, we perturbed STAT-3 activation in the mouse uterus during the implantation period using a STAT3-decoy/transient in vivo DNA transfer system to establish an implantation failure model.

2. Materials and methods

2.1. Animals

Female ICR mice (SLC, Shizuoka, Japan) aged 8–10 weeks in estrus were bred with male ICR mice, and the morning when vaginal plugging was observed was designated as day 0.5 post coitus (p.c.). Animal experiments were performed according to the guidelines for animal use approved by the Institutional Animal Care and Use Committee of Osaka University Graduate School of Medicine.

2.2. STAT-3 decoy transfer

A STAT-3 binding consensus sequence which acts as a decoy *cis*-element (5′-CCTTCCGGGAATTCCTTCCGGGAATTC-3′; underlines indicate consensus element) [10] and a scramble sequence (5′-AGTCCATTCGGCAGGCCTCTGCTCTAT-3′), were synthesized as phosphorotioate-modified oligodeoxynucleotides (ODNs), and purified by HPLC (Hokkaido System Science, Sapporo, Japan). Decoy ODNs were prepared by annealing of sense and antisense ODNs. Double stranded decoy ODNs were transferred to the murine uterus by HVJ-E vector (GenomONE-NEO®, Ishihara Sangyo Co. Ltd. Osaka, Japan) as described previously [5,11].

2.3. Tissue sampling and analysis of implantation/course of pregnancy

The mice on day 5.0 p.c., after in vivo gene transfer, were sacrificed by administration of excess anesthetic. The uteri were removed and frozen in liquid nitrogen. Day 5.0 p.c. pregnant animals received an

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Abbreviations: STAT-3, signal transducer and activator of transcription-3; LIF, leukemia inhibitory factor; IL, interleukin; HVJ-E, (hemagglutinating virus of Japan, also known as Sendai virus)-envelope; HTF, human tubal fluid medium; ODN, oligodeoxynucleotide

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i.v. injection of 0.5% Evans Blue 15 min prior to sacrifice to visualize implantation sites [12]. The serum progesterone level on day 5.0 p.c. was measured using a DPC progesterone RIA kit (Diagnostic Production Co. Ltd., LA, USA).

2.4. Activity assay of active STAT-3

Nuclear proteins from uterine tissue were prepared as described [13]. Nuclear extract was aliquoted, frozen in liquid nitrogen and stored at −80 °C. Protein concentration was determined using a protein assay kit (Bio-Rad Laboratories, Inc. CA) and the amount of activated STAT-3 was assayed using intracellular DuoSet® IC (R&D systems, Inc. MN), according to the manufacturer's instructions.

2.5. Detection of transfected DNA in embryos

The cDNA of murine STAT-3 Y705 F, a dominant negative mutant of STAT-3, was distributed from RIKEN BRC DNA Bank (RDB & 2354). The open reading frame of STAT-3 Y705F was excised and introduced into pcDNA3 (Invitrogen, San Diego, CA) (pcDNA3-STAT-3 Y705F). Plasmid DNA was purified using a Qiagen column (Tokyo, Japan). Transfection of pcDNA3-STAT-3 Y705F with HVJ-E vector was preformed as previously described [11] and blastocysts were flushed out from the uterus on day 4.0 p.c. The DNA was extracted from each single blastocyst and PCR was performed as described previously [11].

2.6. Induction of decidualization

To induce experimental decidualization, pseudopregnant female mice mated with vasectomized male mice received an intraluminal infusion of 10 μl sesame oil in one uterine horn on day 3.5 p.c. and were sacrificed after 72 h as described by Rider et al. [14].

2.7. RT-PCR

Total RNA was extracted by using TRIZOL® (Invitrogen). Single-stranded cDNA was synthesized from 3 μg of total RNA using SuperScript™II (Invitrogen). The PCR for progesterone receptor-A was performed using rTaq DNA polymerase (Toyobo, Osaka, Japan) as described by Clemens et al. [15].

2.8. Statistical evaluation of results

Statistical analysis was performed using the Mann–Whitney *U* test.

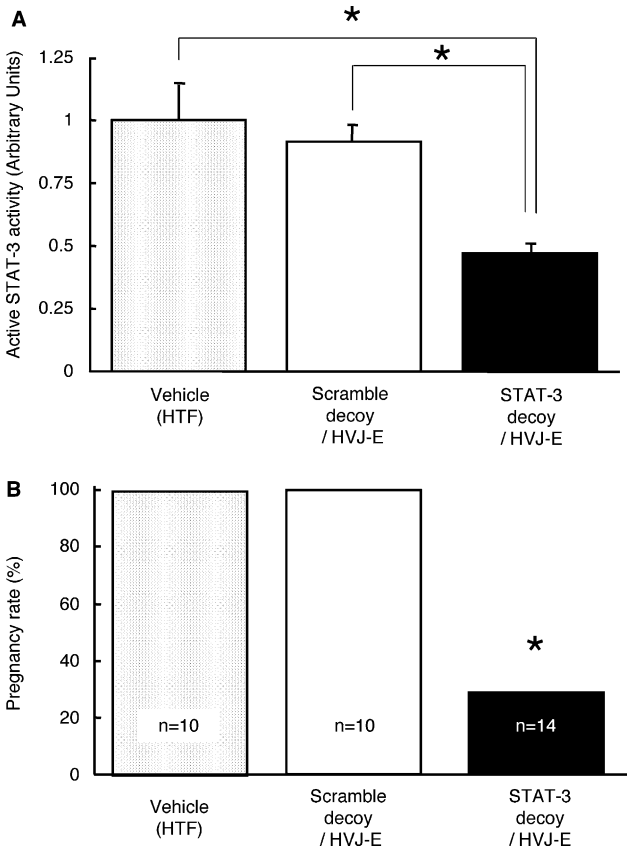

Fig. 1. Effect of STAT-3 decoy transfer in mouse uterus. The mice had vehicle (HTF), scramble or STAT-3 decoy transferred on day 1.5 p.c. (A) STAT-3 activity in nuclear extract from whole uterus at 5.0 p.c. Each group consisted of at least 5 mice. All bars represent the mean ± S.E.M. $^{*}P < 0.0005$ by the Mann–Whitney *U* test. (B) Pregnancy rate on day 15.5 p.c. $^{*}P < 0.05$ by the Mann–Whitney *U* test.

3. Results and discussion

3.1. Transfection of STAT-3 decoy suppressed STAT-3 activity and pregnancy

STAT-3 activity in the uterine nuclear extract on day 5.0 p.c. after STAT-3 decoy transfection was significantly suppressed compared to control uteri (Fig. 1A). On day 15.5 p.c., we examined viable fetuses in utero. In the group treated with vehicle (human tubal fluid medium, HTF) and scramble decoy, all mice became pregnant. However, in mice treated with the STAT-3 decoy, approximately 70% of mice lacked viable fetuses (Fig. 1B). An alternative STAT-3 decoy with the single cis-binding sequence, (5′-GATCCTTCTGGGAATTCCTAGATC-3′, 3′-CTAGGAAGACCCTTAAGGATCTAG-5′, consensus sequences) failed to suppress pregnancy (100% of mice became pregnant after transfection; data not shown). Using this gene transfer method, we previously showed that the transferred gene did not affect embryos and the course of pregnancy, i.e., in luciferase cDNA transferred mice, embryos developed and were delivered normally [11] and IκBαM cDNA, a dominant negative mutant of IκB to suppress the activation of nuclear factor κB, transferred mice delivered their pups 1 day after the normal due date [5]. We flushed out normal blastocysts on 4.0 p.c. from STAT-3 decoy transfer uteri (Fig. 2A), although no embryo was recovered from scramble decoy or pcDNA3 transferred uterus on 4.0 p.c. The transferred gene after pcDNA3-STAT-3 Y705F was undetectable in flushed out embryos by PCR (Fig. 2B). These previous and present observations showed that STAT-3 decoy transfer had neither killed the blastocysts nor washed them out through the uterus.

In the STAT-3 decoy transfer group, 75% of mice did not exhibit implantation sites on day 5.0 p.c. detected by Evans Blue. In the scramble decoy transfer group, implantation sites were evident in all mice (Fig. 3A and B). On day 8.0 p.c., in control uteri, embryos had already formed primitive somites. However, decidualization was apparently disturbed in the STAT-3 decoy treated uterus (Fig. 3C), indicating that decreased implantation sites on day 5.0 p.c. were not due to delayed implantation of dormant embryos. Therefore, STAT-3 suppression led infertility was caused by inadequate uterine receptivity, i.e., implantation failure.

3.2. Decidualization response

As decidualization was totally inhibited in the STAT-3 decoy transfected uterus on day 8.0 p.c., we examined the effect of STAT-3 suppression on mechanical decidualization. The decidual response was obviously suppressed in the STAT-3 decoy transfected uterus (Fig. 4A and B).