Implantation in eutherians: Which came first, the inflammatory reaction or attachment?

Ji-Long Liu[a,1]

In PNAS, Griffith et al. (1) examine the transcriptome changes in the opossum uterus during pregnancy. The authors find that term pregnancy, which is correlated with the loss of the eggshell in the opossum, is characterized by an inflammatory response consistent with implantation in humans and mice. They suggest that implantation in eutherians was derived from an ancestral inflammatory attachment reaction. This is a very interesting hypothesis. However, from the aspect of mouse and human implantation, it seems that inflammatory reaction occurs before attachment.

L-selectin expressed by trophoblast cells of the blastocyst is a crucial mediator of embryo attachment (2). It is well-known that L-selectin plays a key role in leukocyte capture from the bloodstream, raising the possibility that embryo implantation is a mimicry of the leukocyte–endothelium interaction. In humans and monkeys, the inflammatory marker prostaglandin-endoperoxide synthase 2 (PTGS2) is significantly elevated in receptive endometrium compared with prereceptive endometrium (3, 4). In mice, leukemia inhibitory factor (LIF) transiently increases in mouse uterus before implantation (5). LIF is an IL-6 class cytokine with proinflammatory potential (6). These data indicate that the endometrium before implantation is already in an inflammatory state under the control of ovarian steroids. Hence, by acting like a leukocyte, the embryo sticks and migrates into the "inflamed" endometrium.

Does the preimplantation floating embryo contribute to the uterine inflammatory reaction? To answer this question, mouse uterine tissues were collected on the morning of day 4 pseudopregnancy (PP) and natural pregnancy (NP), respectively. In the PP model, female mice were mated with vasectomized males and thus no embryo exists in the uterus. Through RNA-seq, I identified a total of 223 differentially expressed genes, of which 146 genes were up-regulated and 77 genes were down-regulated in NP compared with PP (Fig. 1*A*). Based on an enrichment test (7), immune response was the only gene ontology term that was significantly enriched among differentially expressed genes [false-discovery rate (FDR) < 0.05]. Strikingly, the PTGS2 gene was significantly up-regulated in NP compared with PP (fold-change = 6.29, FDR = 0.0312). This result was further confirmed by quantitative RT-PCR (Fig. 1*B*) and Western blot (Fig. 1*C*). My data suggest that the preimplantation embryo may educate the endometrium into an inflammatory state.

In conclusion, inflammatory reaction happens before attachment during implantation. The preimplantation inflammatory reaction in the uterus is at least partially caused by the embryo. Therefore, I propose an alternative hypothesis that implantation in eutherians is derived from ancestral inflammatory reaction by mimicking the leukocyte capture in blood vessels. This hypothesis can better explain the data obtained from mouse and human implantation.

Acknowledgments

This work was funded by National Natural Science Foundation of China Grants 31771665 and 31271602 (to J.-L.L.).

[a]College of Veterinary Medicine, South China Agricultural University, 510642 Guangzhou, China

Author contributions: J.-L.L. performed research, analyzed data, and wrote the paper.

The author declares no conflict of interest.

[1]Email: jilongliu@scau.edu.cn.

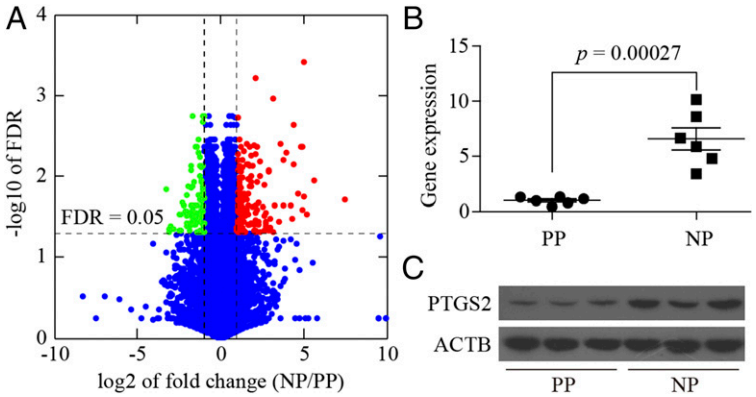

Fig. 1. (*A*) Volcano plot for the comparison of RNA-seq data between PP and NP. Nonchanged genes are shown in blue, while differently expressed genes (fold-change > 2 and FDR < 0.05) are denoted in red or green. *n* = 3. (*B*) Validation of PTGS2 gene expression by quantitative RT-PCR. Data are presented as the mean ± SEM. Primer sequences: PTGS2-Forward: cagatgactgcccaactccc, PTGS2-Reverse: tgaacccaggtcctcgctta; RPL7-Forward: gcagatgtaccgcactgagattc, RPL7-Reverse: acctttgggcttactccattgata. (*C*) Western blot validation. Antibodies for PTGS2 (D5H5, 74 kDa) and ACTB (D6A8, 45 kDa) were obtained from Cell Signaling Technology.

1 Griffith OW, et al. (2017) Embryo implantation evolved from an ancestral inflammatory attachment reaction. *Proc Natl Acad Sci USA* 114:E6566–E6575.
2 Genbacev OD, et al. (2003) Trophoblast L-selectin-mediated adhesion at the maternal-fetal interface. *Science* 299:405–408.
3 Marions L, Danielsson KG (1999) Expression of cyclo-oxygenase in human endometrium during the implantation period. *Mol Hum Reprod* 5:961–965.
4 Sun T, et al. (2004) Cyclooxygenases and prostaglandin E synthases in the endometrium of the rhesus monkey during the menstrual cycle. *Reproduction* 127:465–473.
5 Stewart CL, et al. (1992) Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. *Nature* 359:76–79.
6 Gadient RA, Patterson PH (1999) Leukemia inhibitory factor, interleukin 6, and other cytokines using the GP130 transducing receptor: Roles in inflammation and injury. *Stem Cells* 17:127–137.
7 Huang DW, et al. (2007) DAVID bioinformatics resources: Expanded annotation database and novel algorithms to better extract biology from large gene lists. *Nucleic Acids Res* 35:W169–W175.