SILENCING PROGESTERONE-INDUCED BLOCKING FACTOR (PIBF) IN PRIMARY MOUSE EMBRYO CELLS

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Introduction: The immunomodulatory effects of progesterone are mediated by a protein named Progesterone-Induced Blocking Factor (PIBF). During pregnancy a 34 kDa PIBF isoform is secreted by the placenta, decidua and activated maternal lymphocytes and supports local immune-tolerance by inhibiting the activity of natural killer cells, inducing Th1 to Th2 type cytokine shift as well as asymmetric antibody production and thereby exerting an anti-abortive effect in mice. The full-length (90 kDa) PIBF isoform is associated with the centrosome and regulates spindle pole integrity in mitotic cells. PIBF1 gene in mice map on chromosome14, it contains 23 different introns and transcription produces 16 different messenger RNAs. Previously we established that PIBF is expressed by the oocyte as well as by pre-implantation embryo and we plan to examine the role of embryonic PIBF in implantation by implanting PIBF-deficient embryos. The present study aims to identify PIBF in embryonic cells and to select the siRNA and determine the concentration that most efficiently depletes PIBF from embryonic cells.

Methods: Since mouse embryos are available in limited amount and their protein contents is very low we created primary cell culture from 13 days old whole CD-1 mouse embryos for setting the most effective gene scilencing protocol. We examined the expression of PIBF in the cultured cells by immunohistochemistry, using polyclonal anti-PIBF antibody. The embryonic cells were treated by small interferating RNAs targeted to different regions of PIBF. The efficiency of scilencing was investigated by densitometric analysis following western blot technique.

Results: PIBF was detectable in primary mouse embryo cell culture and we were able to reduce its expression with siRNA technology. Four different siRNAs were tested with diverse sequence targets on PIBF mRNA. Treatments were performed in several concentrations using one siRNA at a time and combined them to select the most efficiently functioning siRNA which will be used to silence successfully the PIBF expression in pre-implantation embryo.

Conclusion: Our results indicate that - in addition to pregnancy associated tissues and maternal lymphocytes – the embryo can also produce PIBF, which might regulate local maternal anti-fetal immune response during implantation. After selecting the siRNA which reduces the expression of PIBF protein most efficiently we will examine its significance in implantation and early embryo development.

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