Macrophage colony-stimulating factor (M-CSF) intermediately participating in the process of luteinizing hormone-induced natriuretic peptide receptor 2 (NPR2) decrease and oocyte meiotic resumption

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Problem statement: Luteinizing hormone (LH) regulation of the ligand natriuretic peptide precursor type C and its receptor natriuretic peptide receptor 2 (NPR2) is critical for oocyte maturation. But the mechanism is not fully understood. Macrophage colony-stimulating factor (M-CSF) has recently been demonstrated to be involved in the processes of oocyte maturation and ovulation.

Methods: We explored the effect of M-CSF in the LH-induced regulation of oocyte meiosis using immature female C57BL/6 mice. Mice were injected i.p. with 5 IU of equine chorionic gonadotropin (eCG) to stimulate follicle development. After 44-48 h, the eCG-stimulated mice were next injected i.p. with an ovulatory dose of 5 IU human chorionic gonadotropin (hCG). Ovaries were excised at selected times. Pre-ovulatory follicles (POFs) and cumulus-oocyte complexes were cultured in different medium. Immunohistochemical analysis and quantitative real-time PCR analysis were used to assess the expression of M-CSF, M-CSF receptor (M-CSF-R), and NPR2. The presence of germinal vesicle breakdown (GVBD) was examined under a stereomicroscope to morphologically evaluate the meiosis resumption of oocytes.

Results: NPR2 was mainly expressed in cumulus cells of POFs, while M-CSF and M-CSF-R were expressed in both mural granulosa cells and cumulus cells. The levels of M-CSF/M-CSF-R and NPR2 decreased within 4 h after hCG treatment. M-CSF not only reduced the expression of NPR2 mRNA via its receptor M-CSF-R, but also increased the proportion of GVBD of oocyte.

Figure 1. Expression patterns of M-CSF, M-CSF-R and NPR2 in the ovaries of eCG-stimulated mice. Localization of M-CSF (A and B), M-CSF-R (C and D) and NPR2 (E and F) was analyzed using immunohistochemistry. White arrows, periantral mural GCs. Scale bar, 100 μm.

Conclusion: M-CSF plays the role of an intermediate signal, inducing a vital decrease in NPR2 levels in cumulus cells, and regulates the process of LH-induced meiotic resumption.

Figure 2. Gonadotropin control of M-CSF, M-CSF-R and NPR2 expression in ovaries in vivo. (A) Localization of M-CSF, M-CSF-R and NPR2 in ovarian follicles shown with immunohistochemistry. Red box represents the time point of 48 h after eCG treatment. Scale bars, 100 μm. (B) Mean optical density of ovarian slides. (C) Expression of M-CSF, M-CSF-R and NPR2 mRNA by quantitative RT-PCR. *, $P < .05$ compared with the corresponding control.

Figure 3. Effect of hCG on NPR2 mRNA expression in POFs. (A) The kinetics of hCG-induced NPR2 mRNA levels in POFs. *, $P < .05$ compared with corresponding control. (B) The image of cultured POFs. Scale bar, 100 μm.

Figure 4. Effect of M-CSF on NPR2 mRNA expression and oocyte maturation. COCs isolated from eCG-stimulated mice were cultured in MEM-α in the existence of 30 nM NPPC (control) added with 200ng/ml M-CSF and/or 1μmol/L GW2580 for 2 h.