MECHANISMS OF AMH RESCUE OF OVARIAN FOLLICLES FROM THE ACUTE EFFECTS OF CYCLOPHOSPHAMIDE

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Abstract Body

Objective: Cyclophosphamide (Cp), a gonadotoxic Alkylating agent, causes ovarian damage, but administration of exogenous anti-Mullerian hormone (AMH) before Cp has been shown to protect the ovarian reserve in mice [1]. We have shown that modified RNA encoding AMH (modRNA-AMH) similarly improves primordial follicle (PrF) retention in wild-type mice ovaries and human xenografted ovarian tissue exposed to Cp, but the mechanism underlying this effect is not understood.

Materials and methods: 8-9-week-old C57/B6 females underwent intraovarian injection (IO) of buffer or modRNA-AMH (25 mcg) followed by intraperitoneal (IP) injection 12 hours later of saline or Cp (150 mg/kg); n= four experimental groups, three mice in each group. Ovaries were harvested 12 hours after IP Cp (24 hours after IO modRNA-AMH). All right ovaries were flash frozen, processed, and analyzed by bulk RNA sequencing. All left ones were fixed in 4% paraformaldehyde and underwent histologic and immunofluorescent (IF) imaging.

Results: Comparison of ovaries exposed to CP versus control treatments revealed transcriptional upregulation of genes related to follicular growth and steroidogenesis (mki67, top2a, ccnb1, amhr2, gdf9, bmp15, inha, inhbb, foxo1, hsd17b1, amh, apoa1/4, fshr, Hist2h2ac, Hist1h1b/c/d), and downregulation of transcripts implicated in cellular/follicle growth arrest (fosb, jun, junb, sfrp4, grem1). Remarkably, pretreatment with modRNA-AMH induced restoration of the gene expression levels observed in control ovaries for a majority of transcripts.

Conclusion: Transcriptomic analysis at 12 hours post-Cp sheds light on the molecular mechanisms of both its acute effect within the ovary and the ferto-protective influence that modRNA-AMH confers. Based on these data, we hypothesize that Cp elicits a phenotype suggestive of global follicular mobilization/growth, and that modRNA-AMH provides a surrogate for endogenous AMH to mitigate this influence. Further studies interrogating the downstream targets of Cp influence and AMH-mediated rescue may provide a means of protecting ovarian reserve from ablative chemotherapy.

Kano, M. et al. AMH/MIS as a contraceptive that protects the ovarian reserve during chemotherapy. Proc Natl Acad Sci U S A 2017, 114, E1688-E1697.

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