TARGETING THE AKT/MTOR PATHWAYS DURING OVARIAN TISSUE CRYOPRESERVATION AND TRANSPLANTATION WITH PHARMACOLOGICAL INHIBITORS TO PRESERVE THE PRIMORDIAL FOLLICLE RESERVE

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Abstract Body

Cryopreservation of ovarian tissue followed by autotransplantation (OTCTP) is currently the only fertility preservation option for prepubertal patients or patients requiring urgent therapy for malignancies. Once in remission, autotransplantation of slow-frozen (SF)/thawed tissue is performed when patients want to conceive. A major issue of the procedure is follicular loss directly after grafting, mainly due to ischemia, apoptosis, and primordial follicle activation. To improve follicular survival during the OTCTP procedure, we tested several inhibitors of follicle activation pathways (Akt/mTOR), first in whole ovary organotypic in vitro culture with/without prior exposure to gonadotoxic therapies. We next evaluated the efficacy of these inhibitors in vivo using an ovarian transplantation model, the widely used kidney capsule model. In vitro, 4-week-old mice ovaries were cultured with the active metabolite of cyclophosphamide (4-hydroperoxycyclophosphamide (4-HC)), either with/without LY294002 (PI3K inhibitor), rapamycin (mTOR inhibitor), or BEZ235 (PI3K and mTOR inhibitor). Western blotting revealed all inhibitors were able to inhibit mTOR pathway activation enhanced by 4-HC, whereas only LY294002 and BEZ235 counteracted 4-HC-induced Akt activation. We next tested, in vivo, the effects of rapamycin on OTCTP-induced follicle activation. 4-week-old mice ovaries, either fresh, SF, or SF with rapamycin, were autotransplanted under the kidney capsule of mice and recovered 3 weeks later. Immunohistochemical analyses of ovarian grafts showed rapamycin counteracted SF/transplantation-induced follicle proliferation and Akt/mTOR pathway activation. Our results indicate that addition of Akt/mTOR pathway inhibitors during OTCTP to transiently maintain the follicle pool in a quiescent state is a promising way to improve grafting for further fertility restoration by limiting the rapid “burn out” of the primordial follicle pool. Ultimately, this will extend life of ovarian grafts.