YW1-13 Decreased expression of cold-shock protein (Cirp, Rbm3) in testes at elevated temperature

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Physiological scrotal hypothermia is necessary for normal spermatogenesis and fertility in mammals. Cirp is identified as a cold-inducible RNA-binding protein in mouse cells *in vitro*. Human RNA binding motif protein 3 (RBM3) is structurally highly similar to the Cirp, and both mRNAs are induced in human cells at the scrotal temperature (32°C). Cirp and Rbm3 are constitutively expressed in the testis of mouse and structurally highly similar to RBM1, a candidate for the human azoospermia factor. In the mouse testis, Cirp mRNA was detected in the germ cells, and a high level of Cirp protein was detected in the nucleus of primary spermatocytes. Rbm3 mRNA and protein were detected in Sertoli cells of seminiferous tubules at all stages. When mouse testis was exposed to heat stress by experimental cryptorchidism, the expression of Cirp was decreased in germ cells and Rbm3 was decreased in Sertoli cells. In human testis with varicocele analyzed immunohistochemically, germ cells expressed less Cirp protein than those in the testis without varicocele. These results demonstrated that Cirp expression is down-regulated at elevated temperature in male germ cells of mice and humans. Rbm3 may play important roles distinct from those played by Cirp in spermatogenesis and cryptorchidism by regulating the gene expression in Sertoli cells. Analysis of Cirp and Rbm3 expression in the testes will help elucidate the molecular mechanisms leading to male infertility.

YW1-14 Role of germ cell apoptosis in testicular pathophysiology

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The process of spermatogenesis is vulnerable to various stresses. We investigated the cellular dynamics, in special reference to germ cell apoptosis, in experimental testicular torsion, vasectomy and cryptorchidism using rat and mouse. Massive apoptotic cell death induced by testicular torsion was inhibited by caspase or nitric oxide inhibitors, while inhibition of apoptosis did not contribute the improvement in spermatogenesis. Calcium-activated protein kinase: calpain, triggered both apoptosis and necrosis of the germ cells and only calpain inhibition ameliorated the impaired spermatogenesis. Vasectomy induced germ cell apoptosis through activations of mitogen-activated protein kinases and overexpression of p53 in early and delayed phase, respectively. In the early phase after vasectomy, hyperdynamic cellular turn over, in other words; active cell death via apoptosis mainly in primary and secondary spermatocyte and enhanced DNA synthesis mainly in primary spermatocyte and spermatogonia were simultaneously observed. In experimental cryptorchidism, wild-type mice showed greater number of apoptotic cells compared to heat shock transcription factor 1 (HSF1) - null mice. Basically, HSF1, which senses protein denaturation and acts as molecular chaperone, prevents cell death from various stresses, while it promoted apoptosis of germ cells in thermal stress. Different from other organs, germ cells actively die, but not survive, through apoptosis to maintain testicular homeostasis against many stresses.

YW1-15 Implication of phagocytic clearance of apoptotic cells for spermatogenesis

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Many differentiating spermatogenic cells undergo apoptotic death during normal spermatogenic pathway in mammals. However, spermatogenic cell apoptosis is hardly detectable by histologically examining the testes, probably due to rapid elimination of dying spermatogenic cells from the seminiferous tubules by Sertoli cells. Previous *in vitro* studies showed that Sertoli cells selectively phagocytose dying spermatogenic cells by recognizing the membrane phospholipid phosphatidylserine (PS) exposed to the surface of spermatogenic cells during apoptosis. To examine whether this is true *in vivo*, the PS-binding protein annexin V was microinjected into the mouse seminiferous tubules, and the testes were histologically examined. Annexin V injection caused no histological changes in the testes, but brought about a significant increase in the number of dead or dying spermatogenic cells as assessed by TUNEL staining. The number of Sertoli cells did not change, and annexin V itself did not induce apoptosis in primary cultured spermatogenic cells. These results indicate that annexin V inhibited clearance of apoptotic spermatogenic cells and suggest that PS-mediated phagocytosis of those cells by Sertoli cells occurs *in vivo*. Finally, the injection of annexin V caused a significant reduction of sperm in the epididymis under certain experimental conditions. This suggests that the elimination of apoptotic cells is required for the production of sperm.

YW1-16 VEGF RESTORES ERECTILE FUNCTION THROUGH MODULATION OF THE SEX HORMONE RECEPTORS IN DIABETIC RAT PENIS

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Introduction and Objective: We hypothesize that intracavernous injection of vascular endothelial growth factor (VEGF) may reverse diabetes-related erectile dysfunction through modulation of sex hormone receptor genes.

Methods: Group 1 served as a control group. In group 2 and group 3 diabetes was induced by streptozotocin. Group 2 rats were sacrificed 12-weeks after induction of diabetes. Group 3 received intracavernosal VEGF injection 6-weeks after induction of diabetic status and sacrificed 6-weeks after injection of VEGF. Erectile function of each rat was analyzed by the measurement of intracavernous pressure (ICP). Gene and protein expression of androgen receptor (AR), estrogen receptor-alpha (ER- α), estrogen receptor-beta (ER- β), and progesterone receptor (PR) was analyzed by RT-PCR and immunostaining, respectively.

Results: Mean ICP was lower in diabetic rats than controls. After VEGF injection, ICP was higher than in untreated diabetic rats. ER- β and PR mRNA and protein expression were lower in the diabetic rat crura than controls. After VEGF injections, ER- β and PR gene and protein expression levels were restored to near normal levels. The AR and ER- α gene and protein expression were similar among all groups.

Conclusions: These findings suggest that injection of VEGF may reverse diabetes-related erectile dysfunction through modulation of ER- β and PR.