Identifying and Screening Targets for Contraceptive Impact – *Herr*

The University of Virginia and Merck recently announced that they have entered into a strategic research collaboration to characterize reproductive enzymes and develop novel drugs for female contraception. The new collaboration focuses on non-steroidal small molecule drugs that would target reproductive cells more selectively than steroids, with possibly fewer side effects. In this seminar Dr. Herr outlined the reproductive cell and molecular biology of an oocyte-specific contraceptive target, SAS1R. Although this target is likely not suitable for a single-dose sterilant due to its selective expression during later stages of folliculogenesis, the characterization of SAS1R serves as a model for the types of questions important to discover and validate a sterilant target.

The Herr lab has a long interest in sperm proteins that may mediate fertilization. Although several sperm proteins that bind to mammalian oocytes have been identified, there has been less success in identifying oolemmal receptors for sperm ligands. Following the acrosome reaction (AR) the plasma membrane of the mammalian spermatozoa is remodeled and the anterior 2/3 of the sperm head becomes limited by the inner acrosomal membrane (IAM), while the equatorial segment (ES), the site of oocyte fusion, is retained. Proteins localized on the IAM and ES are candidates for mediating fertilization events. The testis-specific c lysozyme-like, intra-acrosomal protein SLLP1, encoded by the human gene SPACA3 at locus 17q11.2, localizes on the IAM and ES following the AR. SLLP1 possesses conserved substrate-binding sites for N-acetylglucosamines while lacking bacteriolytic activity.[1] SLLP1 was also localized in mouse sperm acrosomal membranes and was demonstrated to have oolemma-binding properties in this species.[2] Antiserum to SLLP1 and recombinant SLLP1 (rSLLP1) blocked binding and fusion of sperm with oocytes in mice, suggesting that the protein may play a key role in sperm/egg adhesion.[2] SLLP1 bound to the microvillar domain of the oocyte, suggesting the presence of oolemmal receptor(s) for SLLP1 in this region.

To search for egg receptors, affinity panning with mSLLP1 as bait was performed with mouse oocyte lysates employing surface plasmon resonance followed by mass spectrometric microsequencing. An oocyte-specific membrane metalloproteinase, SAS1R (Sperm Acrosomal SLLP1 Receptor), which is conserved among mammals, was identified as a SLLP1 receptor. cDNA cloning revealed six SAS1R variants, including signal peptide, zinc binding active site signature sequence and a putative transmembrane domain. Immunofluorescence localized SAS1R to secondary and Graafian follicles, to the cell periphery in germinal vesicle stage oocytes, and to the plasma membrane overlying the microvillar domain of oocytes arrested at metaphase II. SAS1R was subsequently detected in the perivitelline space and in punctuate regions of the oolemma in the zygote and early embryo, consistent with the interpretation that SAS1R is shed into the perivitelline space, becoming virtually undetectable in blastocysts. Full length SAS1R transfected in CHO-K1 cells localized at the surface of non-permeabilized cells, indicating that newly synthesized SAS1R was translocated to the cell surface. mSLLP1 and SAS1R co-localized to the microvillar domain in unfertilized M2 oocytes and to the acrosomes of acrosome-reacted sperm. Molecular interactions between mouse SLLP1 and SAS1R were demonstrated by surface plasmon resonance method, far-western analysis, yeast two-hybrid analyses under stringent selection conditions, and by immunoprecipitation of SAS1R by anti-mSLLP1, as well as the converse. Purified recombinant SAS1R was also found to have active protease activity against synthetic peptide in a fluorescence resonance energy transfer based assay and to inhibit fertilization in-vitro. SAS1R appears to be the first oocyte-specific oolemmal metalloprotease and sperm ligand receptor identified to date.

An analysis of the ontogeny of SAS1R during oogenesis in the neonatal, pubertal and adult mouse ovary (0, 1.5, 2, 3, 4, 7, 14, 28 and 56 days) was undertaken using guinea-pig anti-recSAS1R serum on Bouin's fixed paraffin embedded sections using the HRP method. Staging defined primary and secondary follicles, respectively, as oocytes surrounded with a single, cuboidal granulosa layer, or with two or more layers. SAS1R protein was first noted in oocytes in bilaminar secondary follicles beginning with day 3 and in all larger secondary, antral and Graafian follicles. SAS1R was not detected in resting naked oocytes or in oocytes of primordial or primary follicles, nor in non-germ cells of any stage. Since SAS1R protein expression was initiated in follicles with two or more layers of granulosa cells, the protein might be useful as a biomarker for follicular staging, particularly granulosa proliferation, or as a target for a reversible contraceptive that might act selectively on developing oocytes while sparing primary oocytes within primordial and primary follicles.[3]

References

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