IPD Project Details

Project ID: IPD7164

Project Title: Delineating the role of human ?-microseminoprotein in male reproduction

Description: Background: Beta-microseminoprotein (MSMB, ?-MSP) is a non-glycosylated, cysteine rich protein secreted by the epithelial cells of the prostate. ?-MSP is found in abundance in human seminal plasma and is also present on the spermatozoa. It is introduced into the semen during ejaculation and found to be absent in post-capacitated spermatozoa. Its abundance in semen along with the fact that it is present on the pre-capacitated spermatozoa suggests that it may have a function which has not yet been elucidated. b) Novelty: Many functions have been attributed to ?-MSP but its exact role in human reproduction is unclear. The difference between ?-MSP levels of fertile and infertile individuals will be delineated. Identification and characterization of novel binding partners of ?-MSP on spermatozoa of fertile versus infertile men will be accomplished. The mechanism by which ?-MSP exerts its action will be elucidated. Sperm capacitation which is essential for successful fertilization is associated with modification of protein composition of spermatozoa. The mechanism by which ?-MSP is lost during this process will be deciphered. c) Objectives: To investigate the difference in ?-MSP levels and identify its novel binding partners in fertile.

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Sample Preparation: 25?g protein sample was first reduced with 5 mM TCEP and subsequently alkylated with 50 mM iodoacetamide. The protein was then digested with Trypsin at a 1:50 Trypsin-to-lysate ratio for 16 hours at 37°C. After digestion, the mixture was purified using a C18 silica cartridge and then concentrated by drying in a speed vac. The resulting dried pellet was resuspended in buffer A, which consists of 2% acetonitrile and 0.1% formic acid.

Peptide Separation: For mass spectrometric analysis, all the experiments were performed on an Easy-nLC-1000 system (Thermo Fisher Scientific) coupled with an Orbitrap Exploris 240 mass spectrometer (Thermo Fisher Scientific) and equipped with a nano electrospray ion source. 1ug of phosphopeptides sample was dissolved in buffer A containing 2% acetonitrile/0.1% formic acid and resolved using Picofrit column (1.8-micron resin, 15cm length). Gradient elution was performed with a 0–38% gradient

of buffer B (80% acetonitrile, 0.1% formic acid) at a flow rate of 500nl/min for 96mins, followed by 90% of buffer B for 11 min and finally column equilibration for 3 minutes. Orbitrap Exploris 240 was used to acquire MS spectra under the following conditions: Max IT = 60ms, AGC target = 300%; RF Lens = 70%; R = 60K, mass range = 375?1500. MS2 data was collected using the following conditions: Max IT= 60ms, R= 15K, AGC target 100%. MS/MS data was acquired using a data-dependent top20 method dynamically choosing the most abundant precursor ions from the survey scan, wherein dynamic exclusion was employed for 30s.

Protein Characterization: Sample was processed and RAW files generated was analyzed with Proteome Discoverer (v2.5) against Uniprot reference database. For dual Sequest and Amanda search, the precursor and fragment mass tolerances were set at 10 ppm and 0.02 Da, respectively. The protease used to generate peptides, i.e. enzyme specificity was set for trypsin/P (cleavage at the C terminus of “K/R: unless followed by “P”). Carbamidomethyl on cysteine as fixed modification and oxidation of methionine and N-terminal acetylation were considered as variable modifications for database search. Both peptide spectrum match and protein false discovery rate were set to 0.01 FDR.

Experiment Type: Bottom-up

Species: Homo sapiens No Data

Tissue: Spermatozoa No Data

Cell Type: Spermatozoa No Data

Disease: Normozoospermic Individuals No Data

Instrument Details: Data in instrument_details Data in instrument_details

Protein Modifications: No

PubMed ID: