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09 The role carbohydrate moieties of ZIF-1 in inhibiting spermatozoa-zona pellucida binding

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Objective: ZIF-1 is a glycoprotein in human follicular fluid that suppresses spermatozoa-zona binding, a critical step during fertilization. It is well accepted that mammalian gamete binding requires the appropriate recognition of specific carbohydrate sequences expressed on the zona pellucida. This study determines the role of carbohydrate moieties of ZIF-1 in inhibiting spermatozoa-zona pellucida binding.
Methods: Spermatozoa samples were obtained from men attending our infertility clinics. ZIF-1 was purified from human follicular fluid obtained from women during oocyte retrieval for assisted reproduction treatment. N-Glycosidase F Deglycosylation Kit (Roche) was used to deglycosylate the glycoprotein. The resulting carbohydrate moieties were purified by gel filtration. The effect of the carbohydrate moieties on spermatozoa-zona binding was determined by hemizona binding assay. The effect of various monosaccharides on the binding of ZIF-1 to spermatozoa was studied by competition-binding assay.
Results: Deglycosylated ZIF-1 did not inhibit the spermatozoa-zona pellucida binding. In fact, deglycosylated ZIF-1 did not bind to the treated spermatozoa. Among the various monosaccharides studied, mannose, fucose, acetylgalactosomine and acetylglucosamine compete with iodinated ZIF-1 for binding to human spermatozoa.
Conclusion: The carbodydrate moieties of ZIF-1 were important for the biological activity of the glycoprotein. Mannose, fucose, acetylgalactosomine and acetylglucosamine are likely to play a role in binding of ZIF-1 to the sperm membrane.
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010 DOWN-REGULATION OF A NOVEL TUMOR SUPPRESSOR GENE, PROTEIN TYROSINE PHOSPHATASE GAMMA (PTPγ), IN HUMAN BREAST BY ESTROGENICALLY ACTIVE AGENTS

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PTPγ is implicated as tumor suppressor gene. Zeranol (Z), a nonsteroidal estrogenically active growth promoter, is used in U.S. beef. **Objectives:** Investigating PTPγ expression by estradiol (E2) and Z; Examining the function of PTPγ. **Methods:** RT-PCR for evaluation of PTPγ mRNA in normal and cancerous human breast (nHBT, cHBT) and E2- and Z-treated nHBT; Immunohistochemistry for PTPγ in HBT; ^{3}H-thymidine incorporation for growth of MCF-7 stably transfected with PTPγ. **Results:** PTPγ mRNA was 50–60% lower in cHBT than in nHBT, and was suppressed by E2 and Z (30nM; 24h) in cultured nHBT by ~80%; PTPγ was immunolocalized to nHBT epithelium, and E2 or Z diminished PTPγ staining; PTPγ-transfected MCF-7 grew slower than mock-transfected cells. **Conclusions:** PTPγ is down-regulated by estrogenically active agents, and may be a biomarker for health risk from endocrine disruptors. PTPγ is a tumor suppressor gene in human breast. (Dept. of Defense Breast Cancer Res. grants DAMD8140 & DAMD0391)