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Grant Title: Roles for N-Glycans in Spermatogenesis

Abstract

The aim of this project was to identify roles for N-glycans in spermatogenesis by investigations of a physiological inhibitor of MGAT1 (GlcNAcT-I), the enzyme that initiates the synthesis of complex N-glycans. The inhibitor, termed GlcNAcT-I Inhibitory Protein long isoform, (GnT1IP-L), blocks the synthesis of complex N-glycans, leaving an immature oligomannosyl N-glycan at each complex site. The GnT1IP gene is highly expressed in testis, and is poorly expressed in men with certain fertility problems. Expression of GnT1IP-L, leading to inhibition of MGAT1 activity, markedly enhances cell adhesion to Sertoli cell lines. The **objectives** of the experiments were two-fold: 1) To investigate the intracellular site of action of GnT1IP-L, and the specificity of GnT1IP-L activity for MGAT1; and 2) To test the hypothesis that GnT1IP-L inhibits MGAT1 during spermatogenesis and is important for spermatogenesis. The **methods** used for the first aim were to test inhibition of endogenous MGAT1 in Chinese hamster ovary (CHO) cells by chimeric constructs of GnT1IP-L targeted to different compartments of the secretory pathway, and to analyze the specificity of GnT1IP-L interactions with other GlcNAc-transferases of the medial Golgi using fluorescence energy transfer (FRET) in collaboration with Dr. Sakari Kellokumpu's laboratory. For the second aim, we generated transgenic mice expressing an *Mgat1* cDNA under control of the *Ldhc* promoter in spermatocytes or the *Stra8* promoter in spermatogonia, with the aim of overwhelming the GnT1IP-L inhibitor, and we also generated mice with an inactivating mutation in the GnT1IP gene. One mouse strain lacked GnT1IP in all mouse tissues while in the other, the GnT1IP gene was inactivated solely in spermatogonia, and therefore was absent from germ cells during spermatogenesis. The **results** of these experiments showed that GnT1IP-L acts to inhibit MGAT1 only when it is localized to early/medial Golgi compartments. In addition, FRET experiments revealed that GnT1IP-L interacts specifically with MGAT1 and does not interact with MGAT2, MGAT3, MGAT4b or MGAT5. When GnT1IP was deleted in FVB mice, no obvious effects on mouse development were observed, and male homozygous mutant mice were fertile. Similar results were obtained when GnT1IP was deleted in spermatogonia. When MGAT1-HA was overexpressed under either the *Stra8* or *Ldhc* promoter, one founder was obtained for each that transmitted through the germline. Experiments are ongoing to identify roles for GnT1IP in spermatogenesis.