

Chemistry and Molecular Biology Department
and NIH Center for Protease Research
Candidate Seminar
December 20, 2007
4:00 pm in Dunbar 152

**Expression of cellular prion protein (PrPC) in sheep uteroplacental tissues:
Implications for scrapie transmission**

ABSTRACT

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Our long term goal was to study the eradication of ovine scrapie at NDSU and to develop improved methods for genotyping the single nucleotide polymorphisms (SNP) associated with scrapie susceptibility. Scrapie is believed to be transmitted at birth via the infected placenta. However, the normal function(s) of PrPC in reproductive biology are still not clearly defined and what causes PrPC to change to the pathogenic form, PrPSc, which accumulates and is believed to be the cause of the disease are unknown. The quantity of PrPC expressed may be related to how much PrPSc accumulates. Thus, we evaluated PrPC expression in several experiments to localize and quantify PrPC expression in uteroplacental tissues.

Experiment 1: The sheep prion gene contains two SNPs that may mediate resistance to developing scrapie, one at codon 136, alanine (A) or valine (V), and another at codon 171, arginine (R) or glutamine (Q). The R allele is thought to confer resistance to scrapie, with the AA₁₃₆ RR₁₇₁ genotype the most resistant to scrapie and QR₁₇₁ only rarely infected. The objective of this study was to develop a reliable assay to genotype sheep for scrapie susceptibility and then use the information for breeding purposes. The Assays by Design protocol was used for development of probes and primers for codon 136 and Primer Express[®] for codon 171 (Applied Biosystems; Foster City, CA). To perform the analysis, genomic DNA was isolated from blood or muscle using commercially available kits. For validation, 70 SNP determinations for each codon were conducted without prior knowledge of genotype and compared to commercial testing (GeneCheck, Inc.; Ft. Collins, CO). Error rate determined by comparisons to the reported genotype was less than 1%. A total of 935 samples from blood (n = 818) and muscle (n = 117) were tested for both codons with 928 successful determinations and only 7 samples (<1% of total samples) that could not be determined. Eighty-six percent of the sheep tested (n = 798) contained an R at codon 171 and were substantially scrapie-resistant. Our SNP assay was cost-efficient, simple, and reliable.

Experiment 2: To determine the effects of nutrition and genotype on PrPC mRNA expression, singleton pregnancies from a single sire were established by embryo transfer to adolescent dams, which then were offered a moderate (M, n=22), high (H, n=20), or high-low (HL, with high to low switch at day [d]90 of gestation, n=10) diet intake. Whole placentomes, collected at d90 or 130 of gestation, were separated into maternal, (caruncle, CAR) and fetal (cotyledon, COT) tissues for quantitative real-time RT-PCR determination of PrPC mRNA expression. PrPC genotypes were determined for codons 136 and 171 using SNP assay. In CAR, across all treatments, PrPC mRNA was greater (P<0.001) on d90 than on d130, but in COT it was similar on both days. On d90, PrPC mRNA tended to be greater (P<0.08) in CAR than in COT, but on d130, PrPC mRNA was greater (P<0.001) in COT than in CAR. There were no effects of nutrition on d90 of pregnancy. However, on d130, for CAR and COT, PrPC mRNA was reduced (P<0.01) in H compared to M, but was similar in M and HL. PrPC mRNA expression was unaffected by codon 136 genotype. In CAR on d90 and in COT on d130, PrPC mRNA was greater (P<0.06) in ewes with an R at codon 171 than in those with QQ. Thus, differences in PrPC mRNA expression in fetal and maternal placental tissues were influenced by nutrition, day of pregnancy, and genotype.

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Experiment 3: PrP-C mRNA expression levels were evaluated in endometrial caruncular (CAR) and intercaruncular (ICAR) tissues (n=4-6/group) obtained at 0, 2, 4, 8, 16, and 24 h after E2 treatment of OVX ewes. PrP-C mRNA expression in OVX E2-treated ewes was greater ($P < 0.01$) in CAR than in ICAR tissues. Compared to 0 h, expression of PrP-C mRNA in CAR was increased ($P < 0.04$) at 2 h and tended to be increased ($P < 0.10$) at 8 h after E2. However, in ICAR, PrP-C mRNA was unchanged after E2 treatment. It appears that the hormonal environment of the uterus may be influencing levels of expression of PrP-C.

Experiment 4: PrPC protein was localized in developing embryos and uteroplacental tissues and PrPC mRNA and protein were quantified in non-pregnant (NP, day 10 of the estrous cycle) uterus and in placental CAR and COT, on days 20, 22, 24, 26, 28, or 30 of early pregnancy. In embryos, PrPC protein was present in the mesonephros, heart, ectoderm, and in areas around the spinal cord and intestinal tract. In the NP uterus, PrPC protein was present at the apical borders of surface luminal epithelium and epithelia of a few luminal glands, outer muscular layer of large blood vessels and in scattered stromal cells of the deep ICAR areas of the uterus. In placental CAR, PrPC protein was found in the cytoplasm of flattened luminal epithelial cells apposed to the fetal membranes, whereas in COT, PrPC protein was localized to mononucleate, binucleate, and syncytial trophoblast cells. PrPC mRNA expression in CAR was unchanged from NP samples, but increased ($P < 0.01$) in COT from days 20 to 28 of early pregnancy and then decreased ($P < 0.01$) on day 30 of pregnancy compared with day 28. The localization of PrPC to specific cells and structures within embryos, uterine tissues, and placentomes suggests that PrPC has a specific role in these developmental processes.

Experiment 5: The effects of fetal genotype ($AA_{136}QQ_{171}$ vs. $AA_{136}QR_{171}$) on expression of PrPC mRNA and protein were compared in day 60 pregnant ewes (n=16; 3 singleton and 13 twin pregnancies). Across all genotypes, expression of PrPC mRNA was greater ($P < 0.01$) in COT than in CAR. There was no effect of genotype on expression of PrPC mRNA in either CAR or COT. However, PrPC protein expression in CAR was less ($P < 0.05$) in $AA_{136}QR_{171}$ than in $AA_{136}QQ_{171}$ fetal genotype. By contrast, in COT, PrPC protein expression was greater ($P < 0.06$) in $AA_{136}QR_{171}$ than in $AA_{136}QQ_{171}$ fetal genotype. Thus, levels of PrPC expression in each tissue were influenced by genotype differently.

Our studies have localized PrPC to specific uteroplacental cells and have shown that levels of PrPC expression are influenced by estradiol, nutrition, day of pregnancy, and genotype. Further research is needed to determine the function of PrPC in uteroplacental development and what factors cause the conversion of PrPC to PrPSc and subsequent transmission of scrapie.