Generation of Goats Lacking Prion Protein

The conformation change of the normal cellular prion protein (PrP) is central to prion disease. It has been reported that mice or cattle lacking PrP are without apparent development or behavioral abnormality (Büeler et al., 1992; Richt et al., 2007). Yet, ablating PrP in sheep or goats has never been achieved. Here, we report the successful generation of two live PrP null goats.

Five heterozygous PrP (PRNP+/-) founder female goats (Yu et al., 2006) were mated with wild-type (PRNP+/+) male goats. A total of five F1 goats were delivered. Genotyping showed that one male and one female of F1 goats were PRNP+/-, while the other three were wild-type (PRNP+/+).

In the second round animal breeding, the heterozygous PrP (PRNP+/-) F1 male goat was mated with the heterozygous PrP (PRNP+/-) F1 female goat and five founder female goats. Three of them became pregnant and delivered a total of five F2 goats.

The genotype of F2 goats was determined by PCR analysis. Genomic DNA of ear fibroblasts isolated from F2 goats was amplified by PCR using P1/P4 primers (Yu et al., 2006). A 2.8 kb PCR product is expected from the normal PRNP locus and a 3.5 kb fragment is expected from the disrupted PRNP locus (Yu et al., 2006). As shown in Figure 1A, a single 3.5 kb product was amplified from genomic DNA isolated from two homozygous PrP null (PRNP-/-) goats, while genomic DNA PCR of wild-type (PRNP+/+) or heterozygous (PRNP+/-) goats produced either a 2.8 kb fragment or both 2.8 and 3.5 kb fragments, respectively. Two of the five F2 goats were found to be homozygous PrP null (PRNP-/-) goats.

The complete disruption of PRNP gene in these two goats was further confirmed at mRNA and protein levels. We isolated mRNA and protein from ear fibroblasts of two PrP null (PRNP-/-) goats. The control mRNA and protein were isolated from ear fibroblasts of a wild-type (PRNP+/+) goat. RT-PCR analysis with P6/P4 primers (Yu et al., 2006) confirmed that no PrP mRNA was expressed in PrP null goats (Fig. 1B). Using western blot analysis with mouse anti-PrP monoclonal antibody 4C6 to detect PrPC expression or with a mouse anti-actin monoclonal antibody as internal control, two PrP-/- goats at about 2 months of age (+/-, PRNP+/-; +/-, PRNP+/-, -/-, PRNP+/-, -/-, PRNP+/-). Genomic PCR, RT-PCR and Western blot analysis were mainly performed as described previously (Yu et al., 2006).

Figure 1. PrP null goats. A: Genomic PCR analysis of DNA sample of the ear fibroblasts of one PRNP+/-, one PRNP+/- and two PRNP-/- goats with P1/P4 primers. B: RT-PCR analysis of the RNA sample of the ear fibroblasts of one PRNP+/- and two PRNP-/- goats with P6/P4 primers. C: Western blot analysis of the protein sample of the ear fibroblasts of one PRNP+/- and two PRNP-/- goats with a mouse anti-PrP monoclonal antibody 4C6 to detect PrPC expression or with a mouse anti-actin monoclonal antibody as internal control. D: Two PRNP-/- goats at about 2 months of age (+/-, PRNP+/-; +/-, PRNP+/-, -/-, PRNP+/-, -/-, PRNP+/-). Genomic PCR, RT-PCR and Western blot analysis were mainly performed as described previously (Yu et al., 2006).

Acknowledgments

This work was supported by grants from Shanghai Rising-Star Program (07QB14022) and China Postdoctoral Science Foundation (20060400174).

References

