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Cre/*loxP*-Mediated Inactivation of the Murine *Pten* Tumor Suppressor Gene

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PTEN (or *MMAC1/TEP1*) tumor suppressor gene is frequently mutated in a variety of human cancers and in three cancer predisposition syndromes (Eng and Peacocke, 1998; Dahia, 2000). PTEN negatively regulates the phosphatidylinositol 3-kinase (PI3 kinase) signaling pathway by dephosphorylating PIP3, the product of PI3 kinase (for review, see Cantley and Neel, 1999). Inactivation of *Pten* (chromosome 19) in mouse models confirmed PTEN to be a bona fide tumor suppressor (Di Cristofano *et al.,* 1998; Podsypanina *et al.,* 1998; Suzuki *et al.,* 1998; Lesche *et al.,* submitted): $Pten^{+/-}$ mice developed tumors in multiple organs and $Pten^{-/-}$ mice died during embryogenesis before midgestation. To overcome the early lethal phenotype in $Pten^{-/-}$ mice and to study the roles of PTEN in embryonic development, adult tissue function, and tumorigenesis, we have generated a conditional *Pten* knockout mouse strain.

LoxP sequences were inserted into the endogenous *Pten* locus flanking exon 5 as illustrated in Figure 1. Exon 5 encodes the phosphatase domain of PTEN in which many tumor-associated mutations have been detected. $Pten^{loxP/+}$ ES cells were injected into either C57/B6 or Balb/c blastocysts. Chimeric mice were backcrossed to either C57/B6 or Balb/c mice and germ-line transmission of the $Pten^{loxP/+}$ allele was confirmed by Southern blot and PCR genotyping (not shown). In contrast to the embryonic lethal phenotype observed in $Pten^{-/-}$ mice, $Pten^{loxP/loxP}$ animals were viable. Normal PTEN level and function were detected in $Pten^{loxP/loxP}$ MEF cells and no spontaneous tumor formations were observed up to two years, suggesting that introducing the *loxP* sites into the *Pten* locus does not perturb the normal function of PTEN.

To demonstrate Cre-mediated exon 5 deletion, we crossed $Pten^{loxP/loxP}$ animals with the GFAP-Cre transgenic mice (Zhuo *et al.,* 2001) aimed for brain-specific deletion. As shown in Figure 2c, Cre expression in the $Pten^{loxP/+}$; $GFAP\text{-}Cre^{+/-}$ mice resulted in neural-specific excision of exon 5 (lanes 1–5). In contrast, very low or no excision could be detected in other nonneural tissues (lanes 6–9). Finally, we showed that no PTEN protein could be detected in conditional deleted tissue and the known downstream signaling molecule AKT/PKB was hyperphosphorylated (Fig. 2c). Thus, the $Pten^{loxP/loxP}$ mouse line generated will be valuable for studying the function of PTEN in animal development and tumorigenesis.

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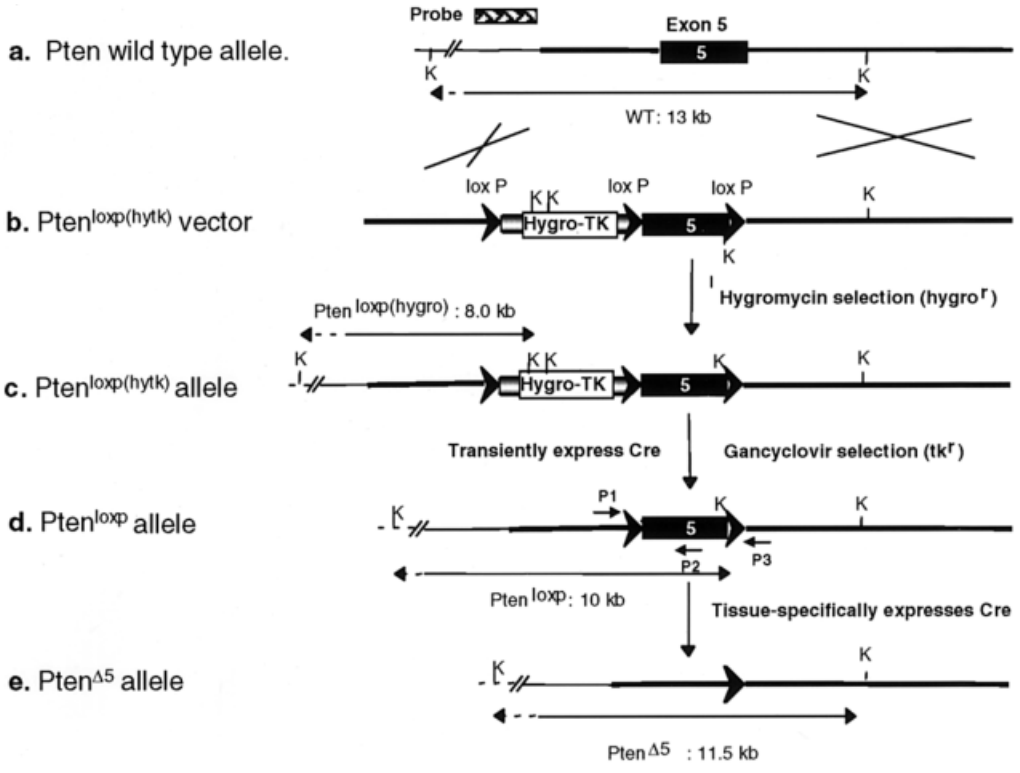

FIG. 1. Generation of *Pten*$^{loxP/+}$ allele. **(a)** Genomic structure of *Pten* locus with exon 5 boxed. **(b)** *Pten*$^{loxP(hytk)}$ targeting vector. **(c)** After electroporation (50 μg linearized DNA/10^7 LW1 ES cells; 400 V/25 μF) and hygromycin (80 μg/ml) selection, homologous recombinants were identified by Southern blot analysis using an external probe indicated in (a) (not shown). **(d)** Targeted ES cells were transiently transfected with Cre-expressing vector and selected with gancyclovir (1 μM/ml). Surviving clones with flanked-exon 5 were used to generate *Pten*$^{loxP/+}$ mouse strains according to standard procedure. P1–P3, primers used for PCR genotyping. **(e)** Exon 5 flanked by the *loxP* sites can be deleted upon Cre expression. This event can be monitored by Southern blot or PCR analysis. K: KonI site

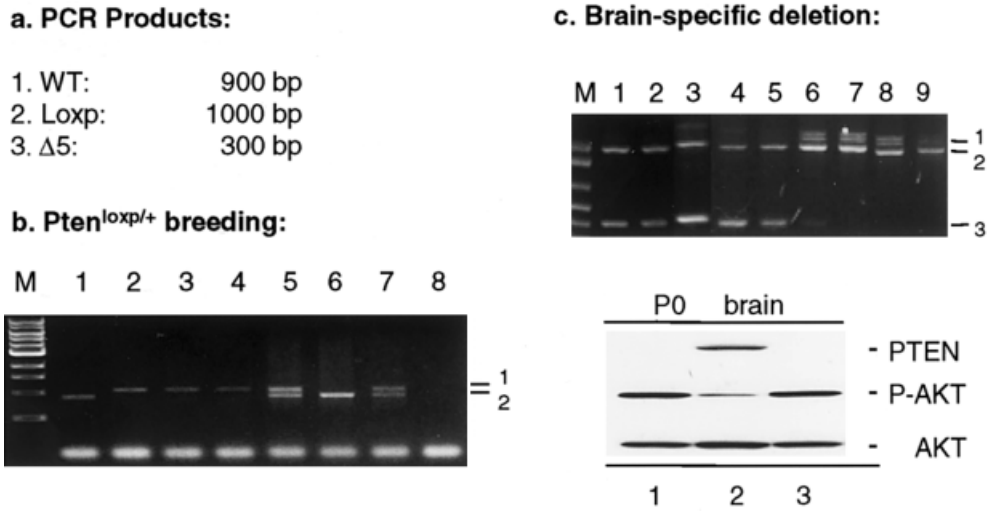

FIG. 2. Conditional inactivation of *Pten* gene. **(a)** Predicted PCR products. Primers used are forward primer P1, 5′-ACTCAAGGCAGG-GATGAGC-3′, and two reverse primers, P2 5′-AATCTAGGGCCTCTTGTG CC-3′ and P3 5′-GCTTGATATCGAATTCCTGCAGC-3′. **(b)** An example of PCR genotyping. Lanes 1 and 6, WT; lanes 5 and 7, heterozygous; lanes 2–4, homozygous for *loxP* alleles; lane 8, no DNA added. (c) GFAP-Cre-mediated *Pten* deletion in *Pten*$^{loxP/+}$;*Cre*$^{+/-}$ mice (upper panel). Lanes 1–5, neural tissues: cortex, hippocampus, cerebellum, brain stem, and spinal cord, respectively; lanes 6–9, nonneural tissues: thymus, heart, kidney, skin, respectively. Western blot analysis (lower panels) using P0 *Pten*$^{loxP/loxP}$;*Cre*$^{+/-}$ brain samples. Lanes 1 and 3, mutant; lane 2, WT control. Antibodies used were α-PTEN, NEB; α-P-AKT, and α-AKT (NEB).