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Gene targeting and expression analysis of mouse *Tem1/endosialin* using a *lacZ* reporter

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ABSTRACT

TEM1 (*endosialin*) expression is increased in the stroma and tumor vasculature of several common human cancers. The exact physiological role of TEM1 is still unknown since *Tem1*-deficient mice are viable and show only a lower rate of abdominal site-specific tumor invasion in tumor transplantation experiments. Previous studies have reported *Tem1* expression in mouse embryos and adults, but did not determine the timing or location of the earliest expression, and did not examine all organ systems. Using the highly sensitive Bluo-Gal staining method for detecting temporal and spatial *Tem1-lacZ* activity in *lacZ* knock-in (*+/lacZ*) mice, we found that *Tem1* gene expression was initially detectable in the dorsal aortic wall, the heart, the umbilical vessels, the first branchial arch, and the cephalic mesenchyme at E9.5. From E10.5 to E14.5, *Tem1* gene expression was additionally seen mainly in the genital tubercle, the mesonephros, the whisker follicles, the mesenchymal tissues around the eye, and the lung. Remarkably, the kidney expressed abundant *Tem1-lacZ* starting from E16.5. Postnatally, *Tem1* expression decreased in most organs but elevated expression persisted in the renal glomerulus and the uterus, where the expression pattern varied at different estrous cycle stages. Co-localization studies indicated that most vimentin-positive cells co-expressed Tem1-lacZ, while a large portion of CD31- or desmin-positive cells were also positive for Tem1-lacZ. Taken together, our observations suggest that *Tem1* is expressed throughout embryonic and adult development in several types of mesenchymal cells closely related to blood vessels.

Tumor endothelial marker 1 (TEM1), also known as endosialin or CD248, is a type I transmembrane glycoprotein initially identified by a monoclonal antibody against human fetal fibroblasts (Rettig et al., 1992). *TEM1* is expressed in the stroma of common epithelial cancers but is absent or expressed at low levels in normal tissues and benign epithelial tumors (Rettig et al., 1992). It was also isolated as one of the most differentially expressed genes in a serial analysis of gene expression that compared RNA from human colon cancer epithelia to normal colon mucosa (St Croix et al., 2000). Upregulation of *TEM1* expression in tumor vasculature has been confirmed (Brady et al., 2004; Dolznig et al., 2005; Huber et al., 2006; MacFadyen et al., 2005). The exact localization of TEM1 protein has been claimed in pericytes but not in endothelial cells (MacFadyen et al., 2005).

Tem1-deficient mice develop normally and their angiogenesis and wound healing is comparable to wild-type mice (Nanda et al., 2006). When tumor cells are transplanted into abdominal sites of *Tem1*-deficient mice, a lower rate of tumor growth, peritoneal carcinomatosis, and liver metastasis is observed compared to wild-type mice. Tumor cells transplanted to subcutaneous tissues do not show the same effects. This suggests that abdominal site-specific tumor invasion requires Tem1, probably via the interaction of Tem1 with tumor cell proteins. However, whether *Tem1*-deficiency induces other phenotypes in other mouse tissues remain to be clarified.

Previous studies using immunohistochemistry or in situ hybridization have demonstrated that *Tem1* expression changes dramatically in selected mouse embryo stages and in adult mouse tissues (Carson-Walter et al., 2001; MacFadyen et al., 2007; Opavsky et al.,

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2001; Rupp et al., 2006). In mouse embryos, *Tem1* is expressed abundantly in the stromal fibroblasts surrounding epithelial tissues and in the developing vasculature of several organs. The expression is quickly downregulated after birth and is restricted to only a few organs in adult mice, such as the kidney and spleen. However, previous studies assayed only a limited number of stages and embryonic organs, and therefore did not fully determine when and where *Tem1* is first expressed during development. In addition, whether embryonic cells other than stromal fibroblasts also express *Tem1* during development remains to be determined. A more sensitive and straightforward method for determining *Tem1* expression, and a more comprehensive analysis that extends to earlier embryonic stages are, therefore, required. Recently, we knocked the *lacZ* gene into the exon of the mouse *Tem1* gene (*Tem1-lacZ*) and generated viable heterozygous (*+/lacZ*) and homozygous (*lacZ/lacZ*) mice. In this study, we used these mice to characterize the *Tem1* ontogeny. By comparing temporal and spatial expression patterns at different stages in embryonic and postnatal development, we found that *Tem1* expression was upregulated very early in tissues involved in angiogenesis during embryonic development but postnatal expression was limited to specific organs.

1. Results and discussion

1.1. Generation of Tem1-lacZ knock-in mouse lines

We disrupted the *Tem1* gene by replacing its single exon with the *lacZ* reporter gene in embryonic stem (ES) cells (Fig. 1A). Correctly targeted clones were confirmed by Southern blot analysis (Fig. 1B) and injected into blastocytes to obtain chimeric mice. Chimeras were mated to C57B/6 mice to generate germline transmission and heterozygous (*+/lacZ*) mice. Southern blot (Fig. 1C) and PCR (Fig. 1D) were used to genotype wild-type, heterozygous (*+/lacZ*), and homozygous (*lacZ/lacZ* or knockout) mice. We used F2 and F3 generations of *Tem1-lacZ* mice in this study. Homozygous (*lacZ/lacZ*) mice were viable and fertile, without any obvious congenital anomalies during development or after birth, consistent with previous studies (Nanda et al., 2006).

1.2. Tem1-lacZ was initially expressed in the E9.5 mouse embryo

To determine when *Tem1* (*endosialin*) was first expressed during mouse development, we stained E8.5 heterozygous (*+/lacZ*) *Tem1-lacZ* embryos with Bluo-Gal and found an absence of specific

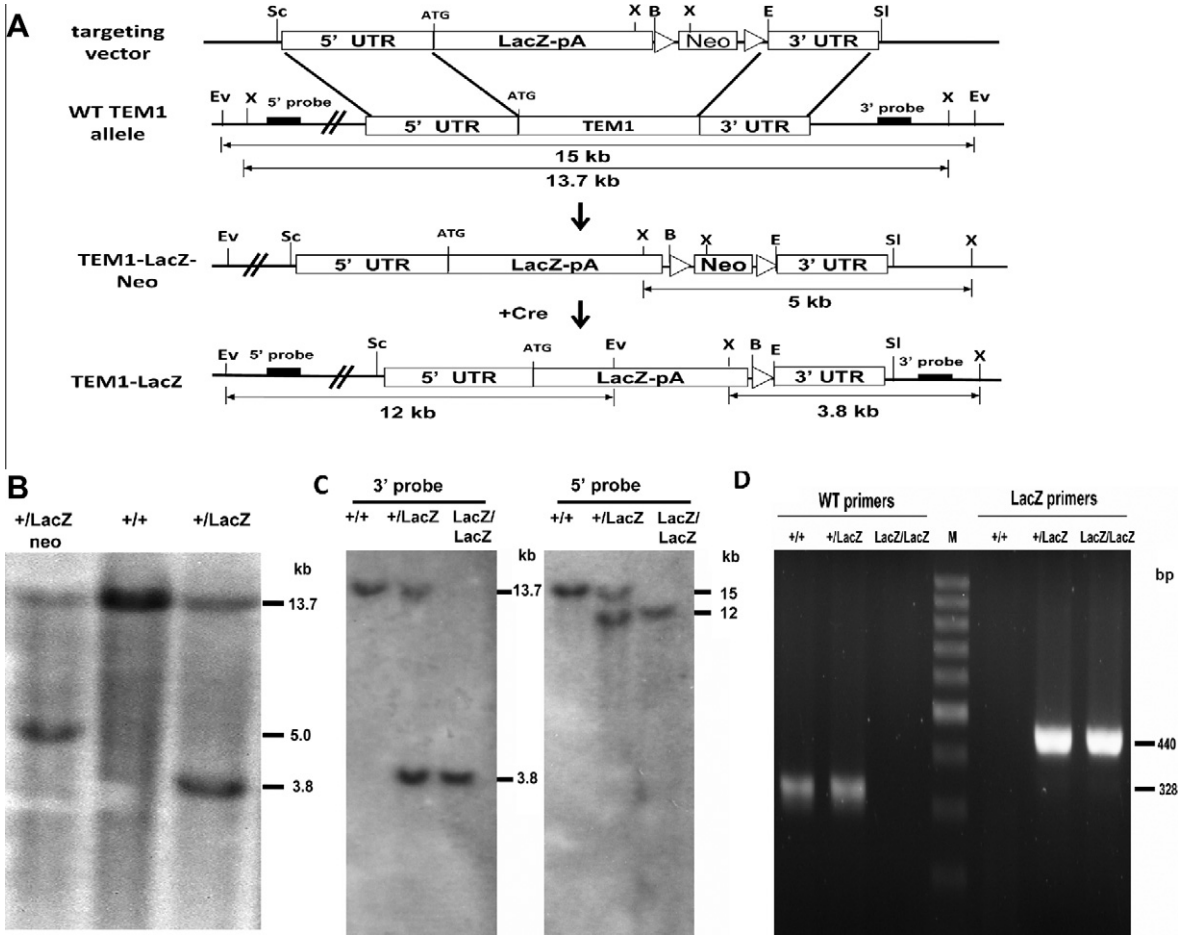

Fig. 1. Targeted disruption of the *Tem1* gene and genotype analysis. (A) Maps of the targeting vector, wild-type *Tem1* allele, recombinant allele with *Tem1* exon replaced by *the lacZ/neo gene*, and final recombinant allele with *lacZ* but without *neo*, which was removed by Cre recombinase. The positions of 5′ and 3′ probes for Southern blotting are labeled as black boxes. Wild-type or mutant allele-specific restriction fragments are indicated as lines, with expected sizes. B, *Bam*HI; E, *Eco*RI; Ev, *Eco*RV; Sc, *Sac*II; Sl, *Sal*I; X, *Xba*I. (B) Southern blot analysis of XbaI-digested DNA from wild-type and recombinant ES clones using the 3′ probe. Wild-type (*+/+*), 13.7 kb; *Tem1-lacZ-neo*, 5 kb; *Tem1-lacZ* 3.8 kb. (C) Southern blot analysis of DNA from a litter from a *+/lacZ*. *Xba*I- or *Eco*RV-digested DNAs were hybridized with 3′ or 5′ probes. The 3′ probe generated a 13.7-kb fragment from the wild-type allele and a 3.8 kb fragment in the mutant allele; the 5′ probe generated a 15-kb fragment from the wild-type allele and a 12-kb fragment from the mutant allele. (D) PCR-based genotyping of *+/+*, *+/lacZ*, and *lacZ/lacZ* mice. Wild-type allele, 328 bp fragment with wild-type-specific primers; *Tem1-lacZ* allele, 440-bp fragment with targeting vector-specific primers.