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The floor plate is sufficient for development of the sclerotome and spine without the notochord

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ABSTRACT

Danforth's short-tail (*Sd*) mouse is a semi-dominant mutation affecting the development of the vertebral column. Although the notochord degenerates completely by embryonic day 9.5, the vertebral column exists up to the lumber region, suggesting that the floor plate can substitute for notochord function. We previously established the mutant mouse line, Skt^{Gt}, through gene trap mutagenesis and identified the novel gene, *Skt*, which was mapped 0.95 cM distal to the *Sd* locus. Taking advantage of the fact that monitoring notochordal development and genotyping of the *Sd* locus can be performed using the Skt^{Gt} allele, we assessed the development of the vertebra, notochord, somite, floor plate and sclerotome in +-+/+-Skt^{Gt}, *Sd*-+/+-+, *Sd*-Skt^{Gt}/+-+, *Sd*-Skt^{Gt}/+-Skt^{Gt}, *Sd*-+/*Sd*-+ and *Sd*-Skt^{Gt}/*Sd*-Skt^{Gt} embryos. In *Sd* homozygous mutants with a C57BL/6 genetic background, the vertebral column was truncated in the 6th thoracic vertebra, which was more severe than previously reported. The floor plate and sclerotome developed to the level of somite before notochord degeneration and the number of remaining vertebrae corresponded well with the level of development of the floor plate and sclerotome. Defects to the sclerotome and subsequent vertebral development were not due to failure of somitogenesis. Taken together, these results suggest that the notochord induced floor plate development before degeneration, and that the remaining floor plate is sufficient for maintenance of differentiation of the somite into the sclerotome and vertebra in the absence of the notochord.

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1. Introduction

The central skeletal element of vertebrates, the vertebral column, combines solidity, stability, elasticity and mobility. The mechanical properties of the vertebral column are conferred by the metameric array of vertebrae, which are separated by intervertebral discs in higher vertebrates and are directly articulated in the lower vertebrate (Romer and Parsons, 1986). Despite these differences, the principles of vertebrate axial skeleton formation are conserved. The source for the vertebral column components is the paraxial mesoderm. Laid down during gastrulation as a mesenchyme flanking notochord and neuroectoderm, the tissue segments when discrete somites detach by epithelialization. The somite produces a second tissue, the sclerotome, the skeletogeneous part of the somite, via mesenchymal transformation in the ventromedial regions. This mesenchyme condenses in a metameric fashion, first laterally to form the neural arches, and later medially, giving rise to the vertebral bodies and the intervertebral discs. Thus, the ventromedial differentiation of the somite is a crucial step in the formation of the axial skeleton as a whole (Gossler and Tam, 2002; Humzah and Soames, 1988; Langman, 1969; Theiler, 1988).

The role of the notochord or floor plate in the differentiation of the sclerotome was demonstrated by implantation experiments. Implantation of an ectopic notochord or floor plate induced the differentiation of somitic derivatives into cartilage, inhibiting the development of the dorsal muscle or dermis (Pourquie et al., 1993; Yamada et al., 1991). Later, it was shown that sclerotome differentiation from the somite and differentiation of the floor plate are induced by Sonic hedgehog (Shh), a signaling protein secreted from the notochord (Ang and Rossant, 1994; Chiang et al., 1996; Roelink et al., 1995; Wilson et al., 1995). Shh signals emanating from the notochord and floor plate were shown to be essential for sclerotome differentiation and subsequent formation of the vertebral column (Chiang et al., 1996; Fan and Tessier-Lavigne, 1994; Roelink et al., 1995; Teillet et al., 1998). McMahon et al. (1998) demonstrated that the activity of notochord-derived Noggin was required for sclerotome differentiation, because Noggin-deficient embryos display defective sclerotome formation. On the other hand, bone morphogenic protein (BMP)2 or BMP4 can inhibit Shh-mediated induction of *Pax1* in the somitic mesenchyme and thus inhibit sclerotomal cell growth and differentiation into cartilage (McMahon et al., 1998; Monsoro-Burq et al., 1996). Noggin binds tightly to BMP2 and BMP4, thus preventing BMP2 and BMP4 from binding to their receptors (Zimmerman et al., 1996). In any case, it is evident that the sclerotome is generated in response to a common signal from the floor plate and notochord.

Danforth's short tail (*Sd*) mouse is a semi-dominant spontaneous mutant characterized by a short kinky tail, urogenital defects and anorectal malformations (Dunn et al., 1940; Favre et al., 1999; Gruneberg, 1958). Although this mutant locus was mapped to the proximal part of mouse chromosome 2, the gene has not been identified yet (Alfred et al., 1997; Lane and Birkenmeier, 1993). *Sd* homozygotes have similar but more severe abnormalities than *Sd* heterozygotes. In *Sd*/*Sd* homozygotes, a notochord is formed but degenerates completely between day 9.5 and 11.0 post-coitum (pc) (Dietrich et al., 1993; Gruneberg, 1958). The *Sd* homozygous embryos showed premature termination of the vertebral column in the lumber or sacral vertebrate depending on the genetic background (Dietrich et al., 1993; Dunn et al., 1940; Theiler, 1988). Dietrich et al. (1993) demonstrated that both the vertebral column and floor plate are unaffected in the anterior region, while embryos lacked both the vertebral column and floor plate in their posterior region. Based on this finding, they proposed that the notochord is no longer necessary after the floor plate is established. However, there is no clear evidence that the floor plate can substitute for notochord function. This is because the gene for the *Sd* mutation has not been identified yet and the genotype of the *Sd* allele is only possible by external inspection. Analysis of the embryo before embryonic day 10.5 (E10.5) is essential to address the role of the floor plate in vertebrate formation. However, *Sd* mutant embryos show no apparent phenotype by external inspection before E10.5. We have previously established the mutant mouse line, Skt^{Gt}, through gene trap mutagenesis in embryonic stem cells. The novel gene identified was termed the *Sickle tail (Skt)*, which was mapped at 0.95 cM distal to the *Sd* locus (Semba et al., 2006). By crossing *Sd* and Skt^{Gt} mutants, we obtained the double mutant which carried *Sd* and Skt^{Gt} in the *cis* configuration (*Sd*-Skt^{Gt}/+-+). The *lacZ* gene was inserted into the *Skt* locus and served as a marker to genotype embryos for the *Sd* mutation (Semba et al., 2006). In addition, as the *lacZ* gene in the Skt^{Gt} allele is expressed in the notochordal cells during embryogenesis, notochordal formation and degeneration can be monitored by *lacZ* expression. Furthermore, the Skt^{Gt} homozygous mutant mice can survive to adulthood and show characteristics of a kinky tail due to abnormalities of intervertebral disc formation only in the caudal spine. Thus, the Skt^{Gt} allele is quite useful to address the question whether the floor plate can substitute for notochord function.

In this study, we first established the *Sd* mutant strain in a C57BL/6 background to address the effect of genetic background on vertebrate phenotype. We examined the role of the notochord and floor plate on the differentiation of vertebral bodies using molecular markers, Skt^{Gt} as a notochord marker, *Uncx* and *Paraxis* as somite markers, *Shh* as a floor plate marker and *Pax1* as a sclerotome marker (Burgess et al., 1995; Furumoto et al., 1999; Mansouri et al., 1997; Roelink et al., 1995; Semba et al., 2006). Our results showed that the floor plate is sufficient for differentiation of the somite into a sclerotome and axial skeletal development in the absence of the notochord.

2. Materials and methods

2.1. Generation and genotyping of mutant mice

We have previously established the mutant mouse line, B6;CB-$Skt^{GtAyu8021IMEG}$ (Skt^{Gt}), in which the *lacZ* gene was inserted into the *Skt* locus (Semba et al., 2006). *Sd* mice