EXPRESSION NOTE

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Expression of SC1 is associated with the migration of myotomes along the dermomyotome during somitogenesis in early mouse embryos

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Abstract SC1 is a secreted glycoprotein with a high amino acid sequence similarity to SPARC (Secreted Protein, Acidic, Rich in Cysteine). SC1 transcripts were first detected in mouse embryos after day 8.5 post coitus (p.c.) in somites at the medial lip of the dermomyotome. Expression of SC1 transcripts by the progenitor cells continued as they began involuting under the dermomyotome and during their migration along the lateral wall of the dermomyotome. After myotome migration was completed, SC1 mRNA expression was downregulated in the trunk region. The data indicate that SC1 expression is restricted to the initial stages of epaxial myotome differentiation and migration, undergoing rapid downregulation prior to myotome emigration from the somitic environment.

Key words SC1 · Myotome expression · Matrix glycoprotein

Materials and methods

For whole-mount in situ hybridization, cDNAs coding for a 300-bp domain 5' to the SPARC-like domain of rat SC1 (Accession number U27562) and mouse SC1 (Accession number U77330) were used to generate digoxigenin-labelled sense and anti-sense cRNA probes. The mouse SC1 cDNA was a gift of Dr. E. Helene Sage, University of Washington, Seattle, WA. Whole-mount in situ hybridization using CD-1 mice (Charles River) was carried out according to Conlon and Rossant (1992).

Mounting evidence indicates that extracellular matrix macromolecules play important roles in modulating the complex series of cellular events associated with somito-

genesis. For example, fibronectin (FN) is expressed at high levels by mesenchymal cells prior to their condensation into compact somitomeres. Cultured chicken segmental plates incubated with a synthetic peptide corresponding to the integrin binding sequence motif of FN (Gly-Arg-Gly-Asp-Ser) fail to undergo segmentation (Lash et al. 1987). During the chondrogenic pathway of somite differentiation, complex changes are observed in the types of proteoglycans secreted by sclerotome cells (Vasan 1987).

We now report the expression of SC1 mRNA, encoding a secreted acidic glycoprotein related to SPARC (Secreted Protein, Acidic, Rich in Cysteine; Lane and Sage 1990) during early mouse embryonic development. During somite differentiation, SC1 mRNA expression is restricted to the epaxial myogenic pathway. SC1 was originally isolated from a brain expression library and found to be expressed during the postnatal development of the brain and at high levels in adult brain (Johnston et al. 1990). Northern blot analysis of RNA isolated from embryonic and adult mouse tissues demonstrated that rat and mouse antisense cRNA probes hybridized to 2.8-kb transcripts (data not shown), the length reported for both rat (Johnston et al. 1990) and mouse (Soderling et al. 1997) SC1 mRNA. Moreover, whole-mount in situ hybridization staining patterns obtained were also found to be identical using either SC1 cRNA sources.

Hybridization signal was not detected in embryos with anti-sense SC1 cRNA at day 8.5 post coitus (p.c.) by which time embryos had formed 8–10 pairs of somites (data not shown). However, expression became visible by day 9–9.5 p.c. in the dorsal axis shortly after rosette-like epithelial somites in the anterior region of the trunk had adopted a cuboidal appearance (black and white arrow, Fig. 1A). Morphological studies have shown that the adoption of a more cuboidal appearance coincides with the segregation of the dermomyotome from the sclerotome. Transverse sections through these somites (Fig. 1C) revealed hybridization was restricted to the medial lip of the dermomyotome (large arrow), a region fated to initiate the myogenic pathway of somite differentiation (Rawls and Olson 1997). These cells

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elongate and rotate towards the neural tube before their migration along the lateral surface of the dermomyotome. Moreover, SC1 RNA accumulation was concentrated at the basal surface of the rotating cells, suggesting a vectorial secretion of SC1 protein towards the migrating edge. Sections through epithelial somites in the posterior region of embryos showed no expression of SC1 RNA (Fig. 1F). SC1 expression was also absent from trunk structures such as the notochord and neural tube.

As development progressed (Fig. 1B, day 9.5–10 p.c.), hybridization signals were visible from the dorsal to ventral edges of midtrunk and anterior somites. In addition, SC1 signals were apparent in the limb bud and the first branchial arch. Transverse sections through these somites revealed SC1 expression was highest in the developing myotome (Fig. 1D). The expression pattern also indicated that SC1 expression by myotome cells continued throughout their migration along the dermomyotome. As migration of myotome cells progressed, a weak signal was observed within the overlying dermomyotome. Sagittal sections through the trunk (Fig. 1E) confirmed that SC1 RNA expression was significantly higher in the myotome compared to the dermomyotome. In contrast to SC1, SPARC was found to be expressed at high levels in all somitic cells at this stage of development (data not shown). The apparent SC1 signals in the head (Fig. 1A) and the ventral region of the trunk (Fig. 1B) were due to probe trapping in cavities since the same staining pattern was observed in sense controls. The signal in somites was only observed with the anti-sense probe.

Fig. 1A–F SC1 expression is restricted to cells forming the myotome compartment of somites in early mouse embryos. **A** The trunk region of a day 9–9.5 embryo (X45) showed that signal was restricted to the dorsal aspect of developing trunk somite pairs (*black and white arrow*). Signal was not visible in the newly formed epithelialized somites anterior to the presegmental plate (*white arrow*). **B** SC1 expression was found to increase in a posterior to anterior direction in a day 9.5–10 embryo (X28.5). Within individual somites, expression was also observed to increase from the ventral to dorsal edge. **C** A transverse section through a somite of a day 9 embryo at a level where the neural tube had not closed and the dermomyotome and sclerotome had separated. SC1 expression was highest in cells that formed the medial lip of the dermomyotome (*large arrow*). These cells are fated to migrate under the dermomyotome to form the myotome. Staining was absent from the neural tube, sclerotome and the central to lateral region of the dermomyotome. SC1 RNA was localized to the basal surface of the elongating and rotating cells. **D** Transverse section through a mid-trunk somite of a day 9.5 embryo. Myotome cells continued to express SC1 RNA as they migrated along the lateral edge of the dermomyotome. As the myotome cells migrated, weak expression of SC1 RNA was detected in the overlying dermomyotome. Expression of SC1 RNA was not detected in the notochord or neural tube. **E** A sagittal section of a day 9.5 embryo. SC1 expression was significantly higher within the myotome compared to the dermomyotome. **F** Expression of SC1 was not detected in epithelial somites at the posterior end of a day 9 embryo (*dm* dermomyotome, *es* epithelial somites, *m* myotome, *n* notochord, *nt* neural tube, *s* sclerotome *Bar* 26.4 μ m in **C**, 55 μ m in **D**, **E** and **F**)

By day 10–10.5 p.c. SC1 transcripts were no longer detected in the trunk (data not shown). The data therefore suggest that SC1 plays a restricted role in promoting migration of myotome cells along the lateral wall of the dermomyotome. Expression was turned-off by these cells before they began emigrating from their somitic environment to form back muscles. Consistent with the absence of SC1 expression by emigrating myotome cells was the observation that SC1 expression was not detected at the lateral lip of the dermomyotome, a region containing precursor cells that give rise to hypaxial (limb and body wall) muscles. Unlike their dorsal counterpart, these progenitor cells elongate and begin emigration before initiating a myogenic differentiation pathway (Rawls and Olson 1997). Recent *in situ* hybridization experiments using tissue sections have shown that SC1 is expressed throughout the central and peripheral nervous system by day 12 in mouse embryos (Soderling et al. 1997). As development progressed, expression also became more abundant in developing vasculature, indicating that SC1 has a much broader pattern of expression in day 12 than day 9–10.5 embryos.

Human endothelial cells that form the specialized postcapillary vascular structures called high endothelial venules express high levels of hevin, the human homolog of rat SC1 (Johnston et al. 1990; Girard and Springer 1995). These venules serve to promote lymphocyte adherence and transendothelial migration to and from the blood vesicles. Recent studies have demonstrated that, like SPARC, hevin inhibits adhesion of endothelial cells to the underlying substratum (Sage et al. 1989; Girard and Springer 1996). Moreover, in developing cerebellum, SC1 transcripts are expressed by Bergman glial cells and the resultant protein is deposited along the length of their radial fibers when the radial fibers serve as migratory tracks for granule cells (Mendis et al. 1994, 1996a,b). Therefore, a possible explanation for the expression pattern reported for SC1 in this study is that SC1 makes use of its antiadhesive potential to promote epaxial myogenic precursor cell rotation and migration during myotome formation. The timing of SC1 expression also raises the possibility that SC1 may play a role in promoting the epithelial to mesenchyme transformation of the myogenic precursor cells prior to their migration.

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