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Sparc (Osteonectin) functions in morphogenesis of the pharyngeal skeleton and inner ear

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Abstract

Sparc (Osteonectin), a matricellular glycoprotein expressed by many differentiated cells, is a major non-collagenous constituent of vertebrate bones. Recent studies indicate that Sparc expression appears early in development, although its function and regulation during embryogenesis are largely unknown. We cloned zebrafish *sparc* and investigated its role during development, using a mo rpholino antisense oligonucleotide-based knockdown approach. Consistent with its strong expression in the otic vesicle and developing pharyngeal cartilages, knockdown of Sparc function resulted in specific inner ear and cartilage defects that are highlighted by changes in gene expression, morphology and behavior. We rescued the knockdown phenotypes by co-injecting *sparc* mRNA, providing evidence that the knockdown phenotype is due specifically to impairment of Sparc function. A comparison of the phenotypes of Sparc knockdown and known zebrafish mutants with similar defects places Sparc downstream of *sox9* in the genetic network that regulates development of the pharyngeal skeleton and inner ear of vertebrates.

Keywords: Cartilage; Col2a1a; Osteonectin; Otic vesicle; Otx1; Sox9; Sparc

1. Introduction

Sparc (secreted protein, acidic and rich in cysteine), also known as Osteonectin or BM-40 (basement membrane tumor matrix component), is a secreted calcium-binding glycoprotein that belongs to the matricellular protein family (Yan and Sage, 1999). Rather than acting as structural components of the extracellular matrix, members of this family mediate cell–matrix interactions (Bornstein, 1995). Sparc is a multifunctional protein with a high affinity for cations and hydroxyapatite that provides support to extracellular matrix and mediates the activities of a wide range of growth factors (Brekken and Sage, 2001). Phenotypic abnormalities revealed by loss-of-function studies also support the interpretation that Sparc mainly functions in cell–matrix interactions (Gilmour et al., 1998; Delany et al., 2003; Bradshaw et al., 2002; Bradshaw et al., 2003; Brekken et al., 2003; Eckfeldt et al., 2005).

Initially purified from bovine bone matrix (Termine et al., 1981), Sparc is also found in embryonic and adult tissues that undergo active proliferation and dynamic morphogenesis (Holland et al., 1987; Sage et al., 1989; Damjanovski et al., 1994; Yan and Sage, 1999; Damjanovski et al., 1998; Bradshaw and Sage, 2001; Padhi et al., 2004; Renn et al., 2006a,b). With few exceptions, most animal species have a single Sparc-encoding gene (Laize et al., 2005). Sequence analysis reveals more than 70% amino acid identity among vertebrate Sparcs, whereas vertebrate and invertebrate Sparcs share only 38% identity (Yan and Sage, 1999; Laize et al., 2005). Surprisingly, no mutations in this gene have been identified in humans, although mouse *Sparc* mutants display various phenotypic abnormalities, such as cataracts,

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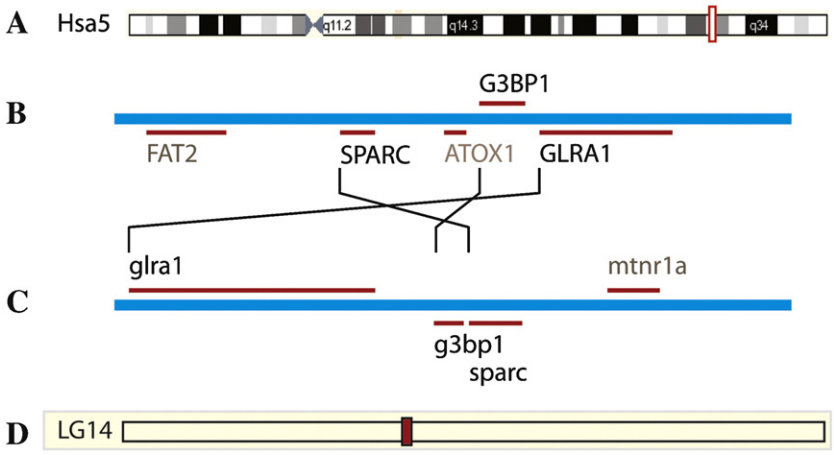

Fig. 1. Conserved syntenies confirm the orthology of zebrafish *sparc* and human *SPARC* genes. (A) human chromosome 5 (Hsa5), with the location of *SPARC* boxed in red and expanded in (B). (C) the *sparc* containing region of the zebrafish genome, which resides in linkage group (LG) 14, in the red boxed region (D). In both species, *sparc* or *SPARC* and two of the three closest neighbors on one side are arranged in the same order, thus demonstrating conservation of this chromosome segment in both lineages from the last common ancestor of zebrafish and humans. The human *ATOX1* gene does not appear to have an ortholog in the zebrafish genome (Zv7) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

osteopenia, accelerated closure of dermal wounds, increased adiposity and enhanced growth of tumors (Gilmour et al., 1998, Delany et al., 2003, Bradshaw et al., 2002, Bradshaw et al., 2003, Brekken et al., 2003,). Knockout mice deficient for Sparc are normal when born, but develop severe eye pathology including cataracts, as well as tail defects and other bone-related defects after birth. The late onset of defects is likely due to severely reduced numbers of differentiated osteoblasts (Delany et al., 2003). However, studies carried out in other organisms suggest an important role for Sparc in early development. Inappropriate expression of Sparc in *Xenopus* embryos has dramatic affects on tissue morphogenesis (Damjanovski et al., 1998), whereas inactivation of Sparc in *C. elegans* by RNA interference suggests that Sparc is required for embryo viability (Fitzgerald and Schwarzbauer, 1998).

It has been hypothesized that the presence of other members of the SPARC family functionally compensate for the lack of SPARC expression (Brekken and Sage, 2001), leading to mild defects in SPARC-null mice. Therefore, in other organisms, such as *C. elegans*, where there is less redundancy, reduction in SPARC produces much more significant defects including embryonic lethality (Fitzgerald and Schwarzbauer, 1998).

Phylogenetic analyses of genome data indicate that gene duplication of SPARC gave rise to SPARCL1 (SPARC like-1), which in turn served as the common ancestral gene for the secretory calcium-binding phosphoprotein family (SCPPs), after the divergence of cartilaginous fish and bony fish but before the divergence of zfish and mammalian lineages. This implies that early vertebrate mineralization did not use SCPPs and that SPARC may be critical for initial mineralization in all bony fish (osteoichthyes). Consistent with this inference, no genes orthologous to mammalian enamel proteins, constituents of the secretory calcium-binding phosphoprotein (SCPP) family, have been identified in teleosts. Therefore, mammals apparently have more SPARC or SPARCL1 functional homologs than teleosts. Consequently, our observations in zebrafish likely uncover the significant roles of sparc.

Thus, the precise function of Sparc, especially during early embryogenesis, remains largely unknown. A recent report indicates that expression of the medaka *sparc* gene can serve as an early marker for skeletal and extracellular matrix (Renn et al., 2006a,b). Its dynamic expression pattern during embryogenesis, similar to other vertebrates, suggests a conserved function of Sparc in vertebrates (Renn et al., 2006a,b).

Here, we provide the first functional study of Sparc in zebrafish. We found that the zebrafish *sparc* gene is expressed in a temporally and spatially specific manner, with strong expression in the developing inner ear and pharyngeal cartilage. Knockdown of Sparc function by morpholino antisense oligonucleotides (MOs) produced a developmental phenotype that corresponds well to its expression pattern. We have further shown that Sparc interacts with genes in known genetic networks, unveiling its novel functions in regulating pharyngeal cartilage and inner ear development.

2. Results

2.1. Conserved syntenies confirm that sparc is the zebrafish ortholog of human SPARC

Although sequences called *sparc* have been deposited in Genbank (Renn et al., 2006a,b; Lien et al., 2006; Whitehead et al., 2005; Padhi et al., 2004; Kawasaki et al., 2004), there has as yet been no analysis of conserved syntenies of the zebrafish *sparc* gene. *SPARC* is on the long arm of human chromosome 5 (Hsa5) (Fig. 1A), and immediately adjacent are *ATOX1, G3BP1,* and *GLRA1* (Fig. 1B). The best reciprocal blast hits (RBH) for two of these human genes lie as immediate neighbors of zebrafish *sparc* (Fig. 1C) on linkage group 14 (LG14) (Fig. 1D). Because RBH is a generally recognized method for