Gene Expression Patterns 6 (2006) 935–940

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Expression analysis of the novel gene collagen triple helix repeat containing-1 (*Cthrc1*)

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Received 9 February 2006; received in revised form 2 March 2006; accepted 14 March 2006
Available online 17 March 2006

Abstract

We recently identified collagen triple helix repeat containing-1 (*Cthrc1*) as a novel gene induced in adventitial fibroblasts after arterial injury. Cthrc1 is a 30 kDa secreted protein that has the ability to inhibit collagen matrix synthesis. Cthrc1 is also glycosylated and retains a signal sequence consistent with the presence of Cthrc1 in the extracellular space. In injured arteries and skin wounds, we have found Cthrc1 expression to be associated with myofibroblasts and sites of collagen matrix deposition. Furthermore, we demonstrated that Cthrc1 inhibits collagen matrix deposition in vitro. Using in situ hybridization and immunohistochemistry, we characterized the expression domains of Cthrc1 during murine embryonic development and in postnatal tissues. In mouse embryos, Cthrc1 was expressed in the visceral endoderm, notochord, neural tube, developing kidney, and heart. Abundant expression of Cthrc1 was observed in the developing skeleton, i.e., in cartilage primordia, in growth plate cartilage with exclusion of the hypertrophic zone, in the bone matrix and periostium. Bones from adults showed expression of Cthrc1 only in the bone matrix and periostium while the articular cartilage lacked expression. Cthrc1 is typically expressed at epithelial–mesenchymal interfaces that include the epidermis and dermis, basal corneal epithelium, airway epithelium, esophagus epithelium, choroid plexus epithelium, and meninges. In the adult kidney, collecting ducts and distal tubuli expressed Cthrc1. Collectively, the sites of Cthrc1 expression overlap considerably with those reported for TGF-β family members and interstitial collagens. The present study provides useful information towards the understanding of potential Cthrc1 functions.

Keywords: Bone; Cartilage; Osteocyte; Osteoblast; Tooth; Dentin line; Skin; Epidermis; Notochord; Cardiac muscle; Neural tube; Purkinje cells; Choroid plexus; Kidney; Collecting duct; Distal tubules; Cornea; Bone marrow; Alveolar epithelium; Neurons; Megakaryocyte; Parafollicular cells; Thyroid

Cthrc1 is both novel and highly conserved among vertebrates only (Pyagay et al., 2005). Database searches failed to reveal other Cthrc family members. The presence of a *Cthrc1* homolog in sea squirt, one of the first organisms to form a notochord during the larval stage, indicates that the evolution of the Cthrc1 gene dates back at least 550 million years. Since there is currently no other publication on Cthrc1, information about Cthrc1 expression in vivo is presently non-existent. For the identification of potential sites of Cthrc1 activity in tissues we have performed a comprehensive expression analysis in developing mouse embryos and postnatal tissues.

1. Results and discussion

1.1. Expression of Cthrc1 mRNA during mouse embryonic development

Whole mount in situ hybridization was used to examine Cthrc1 mRNA expression during early mouse development. At 8.5 dpc Cthrc1 mRNA was prominently expressed in the notochord with a caudal to rostral gradient (Fig. 1A, nc). Expression was also seen in the floor plate of the anterior ventral neural tube (Fig. 1B, fp). Somitic expression became evident at 9.5 dpc (Fig. 1C, s)

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doi:10.1016/j.modgep.2006.03.008

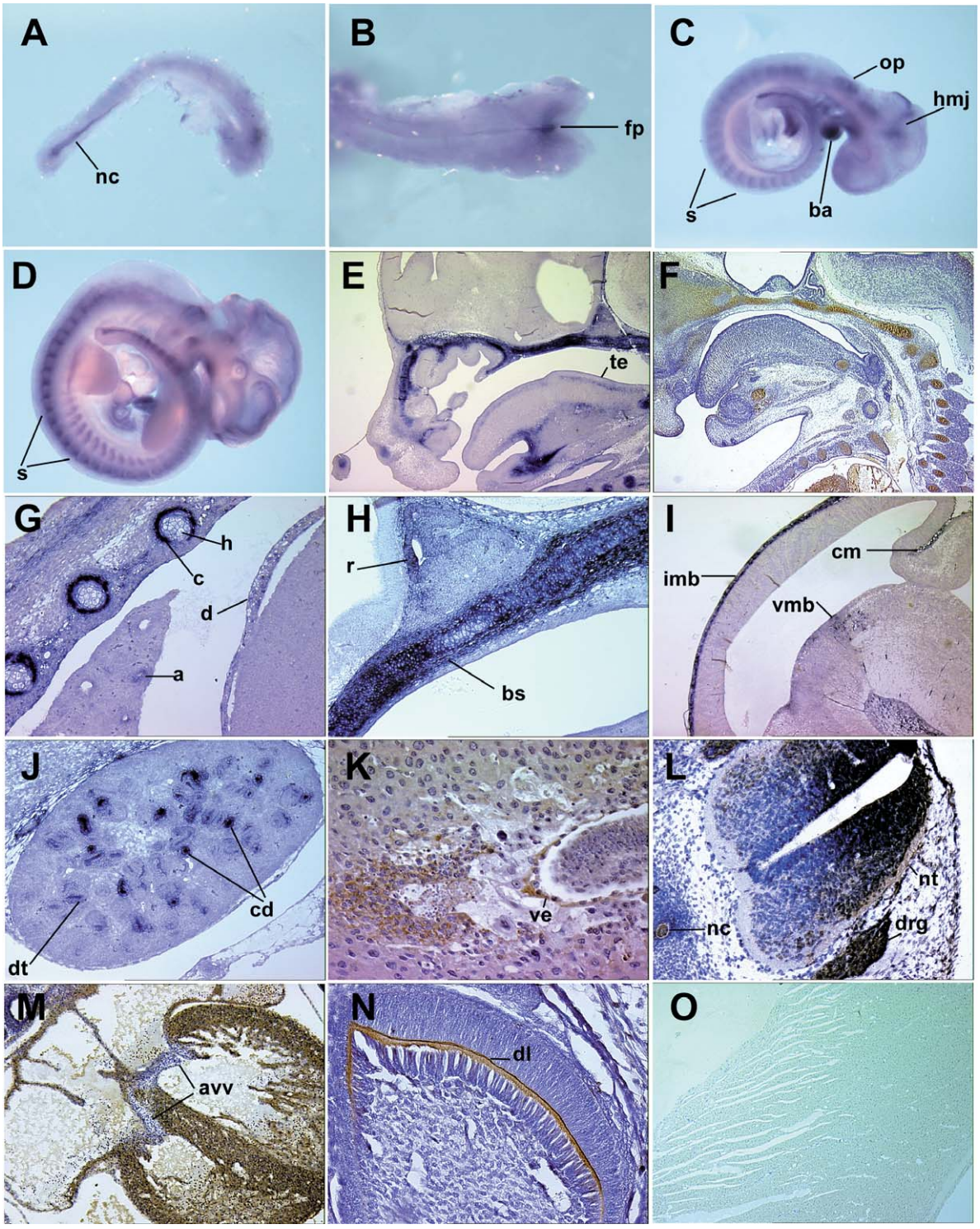

Fig. 1. In situ hybridization with digoxigenin-UTP labeled antisense Cthrc1 riboprobe was performed on wholemount mouse embryos at 8.5 dpc (A and B), 9.5 dpc (C), and 10.5 dpc (D). In situ hybridization on 14.5 dpc sagitally sectioned mouse embryos showing the skull (E), chestwall with developing rib cartilage (G), Rathke's pouch and underlying cartilage primordial (H), midbrain (I), and kidney (J). Immunostaining with an anti-Cthrc1 antidody was performed on 14.5 dpc mouse embryo (F), 6.5 dpc mouse embryos (K), 12.5 dpc neural tube (L), 14.5 dpc heart (M), and 18.5 dpc incisor tooth (N). Incubation of a sectioned cardiac muscle with preimmune serum (O) serves as a control for immunostaining demonstrating the absence of nonspecific staining. Definition of labels: nc, notochord; fp, floor plate; s, somites; op, otic placode; ba, branchial arches; hmj, hindbrain–midbrain junction; te, tongue epithelium; c, chondrocytes; h, hypertrophic chondrocytes; a, airways; d, diaphragm; r, Rathke's pouch; bs, basisphenoid bone; imb, intramembranous bone formation; vmb, ventral midbrain; cm, cephalic mesenchyme; dt, distal tubules; cd, collecting ducts; ve, visceral endoderm; nt, neural tube; drg, dorsal root ganglia; avv, atrioventricular valve; dl, dentin line. Original magnification: (A–D) 10×; (E and F) 25×; (H and O) 50×; (G, I, L) 100×; (J, K, M, N) 200×. Nuclear stain with hematoxylin in (F) and (K–O), immunoreactivity seen in brown, hybridized antisense Cthrc1 probe seen in blue.