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Aortic carboxypeptidase-like protein is expressed in collagen-rich tissues during mouse embryonic development

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Abstract

Aortic carboxypeptidase-like protein (ACLP) was originally identified in vascular smooth muscle cells and contains discoidin and catalytically inactive metallocarboxypeptidase domains. ACLP is a secreted protein that associates with the extracellular matrix and is essential for abdominal wall development and contributes to dermal wound healing. Because of these developmental and adult phenotypes, we examined the expression of ACLP by immunohistochemistry throughout mouse embryonic development. ACLP was not detected in 7.5 days post-coitum (dpc) embryos, however at 9.5 dpc low levels of expression were detected in the somites and dorsal aorta. Expression was detected in both the yolk sac and embryonic vasculature at 10.5 dpc. ACLP expression increased in both large and small blood vessels at 11.5 and 13.5 dpc and intense expression was detected within the vascular smooth muscle layer in 16.5 dpc embryos. At later developmental time points, discrete areas of ACLP expression were detected in the mesenchymal cells in the dermal layer, developing skeletal structures, connective tissue, and in the umbilical ring and vessels. The predominance of ACLP immunoreactivity localized with collagen-rich regions including tendons and basement membranes. Overall, the developmental expression pattern is consistent with a regulatory or structural role in the abdominal wall, vasculature, and dermis.

Keywords: ACLP; Discoidin; Vascular smooth muscle; Collagen; Mouse embryo

Aortic carboxypeptidase-like protein (ACLP) is secreted by vascular smooth muscle cells and associates with the extracellular matrix (ECM) (Layne et al., 2001), however, its function has not been elucidated entirely. ACLP contains a signal peptide at the N-terminus, an extensin domain, a discoidin domain, and a catalytically inactive metallocarboxypeptidase domain at its carboxyl terminus (Layne et al., 1998). A C-terminal cDNA fragment of ACLP, termed AEBP1 has been identified in mouse adipocytes (He et al., 1995; Ro et al., 2001). However, several studies have not been able to detect the AEBP1 mRNA or protein in cells or tissues (Layne et al., 1998, 2001; Gagnon et al., 2002; Abderrahim-Ferkoune et al., 2004). ACLP is structurally related to CPX-1 and CPX-2, which also have signal peptides, discoidin, and metallocarboxypeptidase domains (Lei et al., 1999; Reznik and Fricker, 2001; Xin et al., 1998). Most ACLP-null mice die perinatally from the abdominal wall defect gastroschisis (Layne et al., 2001). In addition, adult ACLP-null mice exhibit dermal wound healing deficiencies due to reduced dermal fibroblast proliferation (Layne et al., 2001). Consistent with a role in tissue repair, ACLP is highly expressed in vascular smooth muscle cells and is also induced in settings of vascular injury and neointima formation (Layne et al., 2002). Although the mechanisms by which ACLP exerts its effects are unknown, a recent study indicated that overexpression of ACLP was sufficient to induce smooth muscle cell gene expression (Abderrahim-Ferkoune et al., 2004). Because of the importance of ACLP in abdominal wall development, wound healing, and potentially the vasculature, the goal of the present study was to characterize the expression pattern of ACLP throughout mouse embryonic development.

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1. Results and discussion

To evaluate the expression of ACLP during mouse embryonic development we performed immunohistochemistry on embryos of various gestational stages using a rabbit polyclonal antibody directed against the C-terminus of ACLP. ACLP was not detected in 7.5 dpc mouse embryos (Fig. 1A). Although the embryos did not express ACLP, significant ACLP protein was detected in the maternal tissues at 7.5 dpc including the uterine blood vessels (data not shown) and in reticular fibers in the endometrium (Fig. 1B). ACLP was first detected at 9.5 dpc in the developing dorsal aorta (Fig. 1C), ectoderm (Fig. 1D), and on the basal side of the dermomyotome in the somites

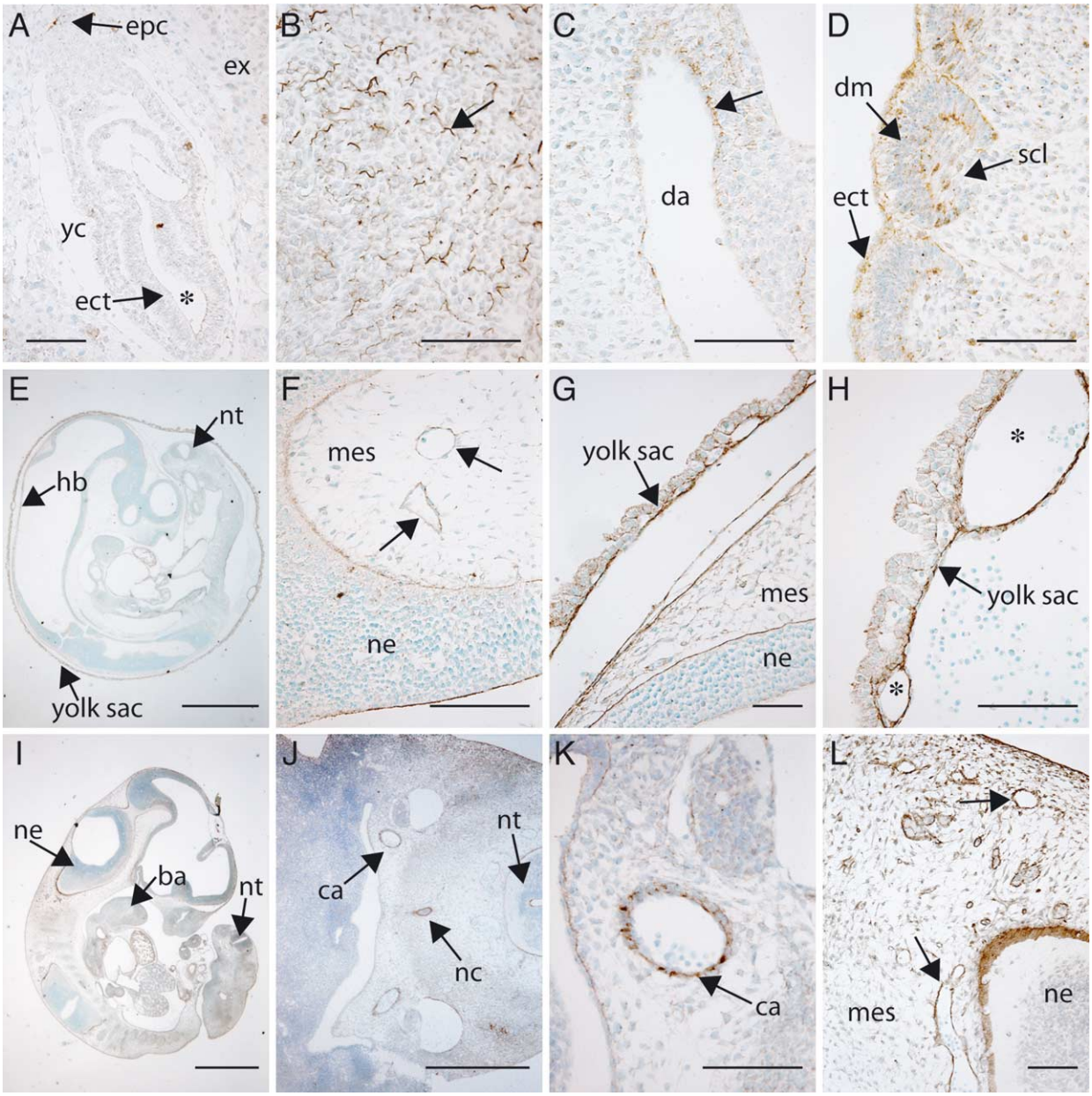

Fig. 1. ACLP expression in early embryogenesis. (A) ACLP is minimally expressed in 7.5 dpc mouse embryos (approximate sagittal section). Ectoplacental cone (epc) is at top of panel, yolk sac cavity (yc), ectoderm (ect), extraembryonic tissue (ex), and amniotic cavity are indicated (*). (B) Focal ACLP expression in reticular fibers in endometrial tissue at 7.5 dpc. (C) ACLP is detected in dorsal aorta (da) in sagittally sectioned 9.5 dpc embryo. (D) ACLP expression in somites at 9.5 dpc, ectoderm (ect), dermomyotome (dm), sclerotome (scl). (E) 10.5 dpc embryo within yolk sac (approximate sagittal section), neural tube (nt) in caudal region, roof of hindbrain (hb). (F) ACLP expression in early embryonic blood vessels at 10.5 dpc (arrows) in cephalic mesenchyme (mes), neuroepithelium (ne). (G) Diffuse ACLP in mesenchyme (mes), but not in neuroepithelium (ne) at 10.5 dpc. (H) Expression in yolk sac blood vessels (*) at 10.5 dpc. I-L ACLP expression in 11.5 dpc embryos. (I) Sagittal section of 11.5 dpc embryo, neuroepithelium (ne), mandibular component of first branchial arch (ba), neural tube in caudal region (nt). (J) Transverse section (rostral) of 11.5 dpc embryo with ACLP expression in carotid artery (ca) and notochord (nc), neural tube (nt) is at right side of panel. (K) Higher magnification of panel J, showing ACLP expression in carotid artery. (L) ACLP expression in numerous smaller caliber blood vessels (arrows) in head mesenchyme (mes), neuroepithelium (ne). Scale bars A–D, F–H, K, L 100 μm; E, I 1 mm; J 500 μm.