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Gene expression pattern

Expression of CD44 during early development of the chick embryo

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Abstract

CD44, the major cell-surface receptor for hyaluronate, is expressed on many cell types to mediate different functions including cell activation, homing and adhesion. The early pattern of CD44 expression was determined in the avian embryo by using a specific monoclonal antibody in whole-mount and tissue sections. CD44 was first expressed on cephalic neural fold cells and later on by subpopulations of pre-and migratory cranial neural crest cells. Trunk neural crest cells did not express CD44. At the 18–20 somite stage, CD44 expressing cells were also localized in the caudal region of the embryo, in the mesoderm of the remaining primitive streak and in the caudal ectoderm and above the secondary neural tube during the process of cavitation. In addition, some hemopoietic cells present in the blood stream were also CD44 positive.

Keywords: CD44; Hyaluronate receptor; Chick embryo; Neural crest; Primitive streak; Hemopoietic cells

1. Results and discussion

CD44 is a membrane glycoprotein which exists in a variety of isoforms that constitute a family of adhesion molecules: it is the major cell-surface receptor for hyaluronate (Aruffo et al., 1990). In this study, we used a monoclonal antibody against the chicken homologue of CD44 to investigate the expression of this integrin during early chick development when migration of different cell lineages occurs, including the cells of the neural crest (NC).

In vertebrates, NC cells originate in the neural folds and produce sensory and autonomic ganglia at both cranial and truncal levels. Only cranial NC cells derived from the rhombencephalon, mesencephalon and posterior diencephalon (Couly and Le Douarin, 1987; Le Douarin et al., 1993) give rise to ectomesenchymal derivatives such as the connective tissue of the face, cartilage and bones (Le Lièvre and Le Douarin, 1975). Cranial NC cells migrate superficially, between ectoderm and paraxial cephalic mesoderm, in a hyaluronate-rich matrix (Pratt et al., 1975). Hyaluronate is secreted by the ectoderm and is required for NC cell migration (Pratt et al., 1976). It is well known that during migration NC cells contact the extra-cellular matrix via adhesion molecules (Perris, 1997).

CD44 expression was analyzed in detail by whole-mount and section immunohistochemistry in chick embryos from the 3 to 20 somite stages (ss).

CD44 was first expressed at the 5 ss, rostrally, in the tips of the neural folds at the prosencephalic and anterior midbrain levels (Fig. 1A–C). At this stage, CD44 expression was intense in the superficial layer of the neural folds that yield NC, as well as in the anterior-most region which does not produce NC cells and remains epithelial (Couly and Le Douarin, 1987). It should be noted that, while NC arise in the dorsal neural tube just after its closure, CD44 expressing cells were seen within the ectodermal part of the prosencephalic neural folds (Fig. 1B,C).

Between the 8 and 14 ss, only subpopulations of cephalic NC cells expressed CD44 (Fig. 1D–K). No CD44 was detected in the trunk region. At these stages, strong expression of the CD44 antigen was restricted to the rhombencephalic region.

Examination of transverse sections at the 8 ss showed that CD44 positive cells were present in the dorsal midbrain (Fig. 1D). These pre-migratory NC cells expressing CD44 were HNK1 negative as expected (data not shown).

At the 11 ss, the caudal limit of CD44 expression by migrating NC cells approximately corresponded to rhombomere 5 (Fig. 1E). Transverse sections of the embryos showed the emergence and migration of CD44 positive NC cells from the dorsal rhombencephalic neuroepithelium

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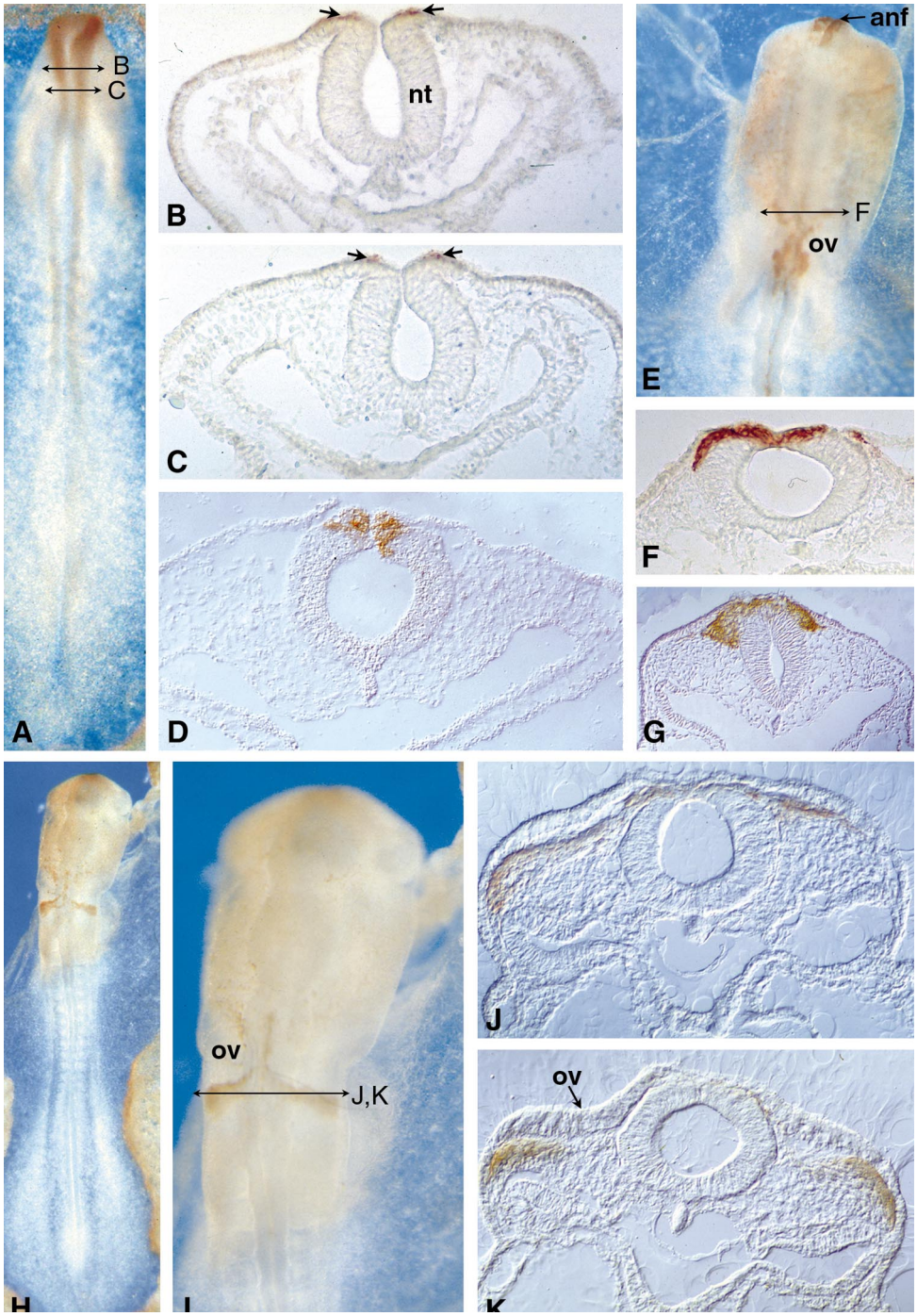

Fig. 1. Expression of CD44 prior and during neural crest cells migration. (A–C) Whole-mount immunohistochemistry in a 5 ss embryo (A) and transverse sections (B,C) cut at the levels indicated in (A). (D) Transverse section of a 8 ss embryo, incubated with antibody against CD44. Rhombencephalic level. (E,F) Whole-mount immunochemistry, dorsal view of the cephalic region of a 11 ss embryo (E) and subsequent transverse section (F) at the anterio rhombencephalic level indicated in (E). (G) Transverse section at the anterio rhombencephalic level of a 12 ss embryo, immunostained with anti-CD44 antibody. (H–K) Whole-mount immunohistochemistry in a 14 ss embryo (H,I) and transverse sections (J,K) at the level indicated in (I). anf, anterior neural fold; nt, neural tube; ov, otic vesicle.

laterally underneath the ectoderm (Fig. 1F). CD44 was also present in the anterior neural folds (Fig. 1E).

At the 12 ss, CD44-migrating NC cells had grown in number (Fig. 1G).

At the 14 ss, a bilateral stream of CD44-positive NC cells was expanding caudally to the otic vesicles (Fig. 1H–K). These NC cells migrated from the dorsal neural tube, laterally, under the ectoderm (Fig. 1J,K).

At the 18–20 ss, CD44 positive cells were still present at those cephalic locations described in younger embryos (Fig.

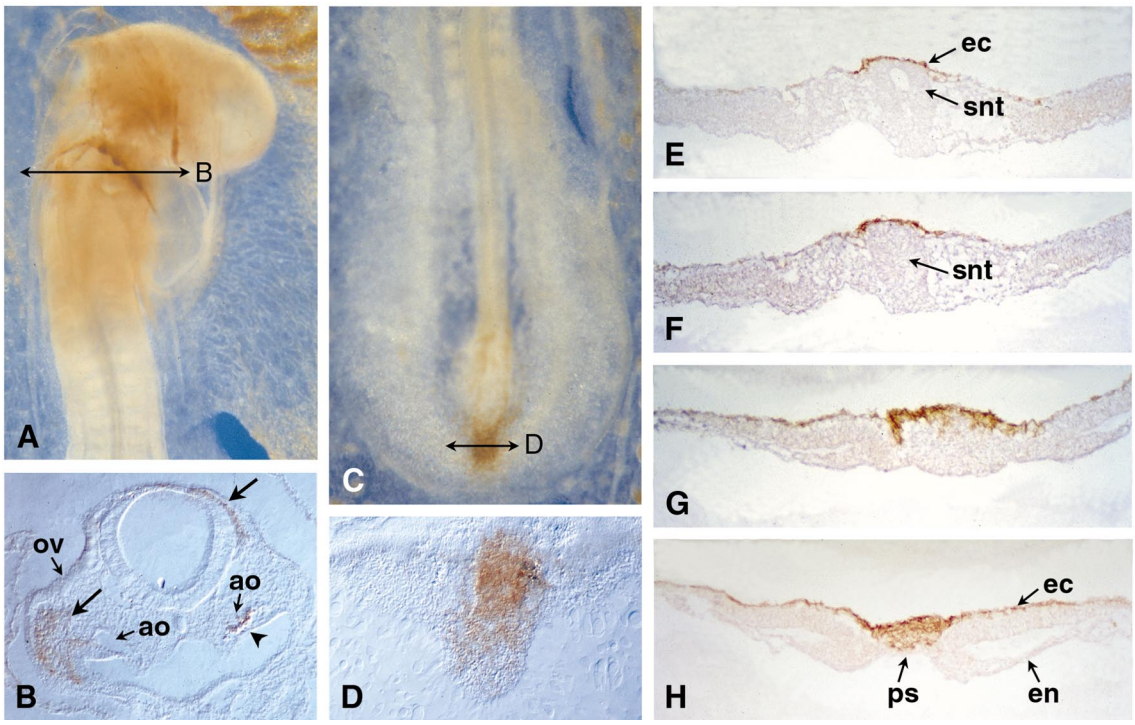

Fig. 2. Expression of CD44 at the tail bud stage (18–20 ss) (A–D) whole-mount immunocytochemistry in a 18 ss embryo. Dorsolateral view of the head and anterior trunk (A) and transverse section (B) at the level indicated in (A). View of the caudal region of the same embryo (C) and transverse section (D) at the level indicated in (C). (E–H) Transverse sections in a 20 ss embryo, done according a rostrocaudal sequence in the tail region and immunostained with anti-CD44 antibody. ec, ectoderm; en, endoderm; mes, mesoderm; ps, primitive streak; ov, otic vesicles; snt, secondary neural tube; ao, aorta. Arrow head: hemopoietic cells; arrow: NC cells.

2A,B). Moreover, CD44 expressing cells began to be detected in other tissues. Some hemopoietic cells in the blood were CD44 positive as observed in the lumen of the aorta (Fig. 2B). Interestingly, a mass of CD44 expressing cells was located caudally to the tail bud (Fig. 2C–H). These cells were HNK1 negative (data not shown) and corresponded to the remaining primitive streak (Fig. 2D,H). The ectoderm above the secondary neural tube (see Catala et al., 1995 for references) also expressed CD44 (Fig. 2E–H).

In this study, we showed that avian CD44 expressing cells were present during early development in two pluripotent structures, the neural crest (cephalic) and the tail bud mesenchyme. The former plays a critical role in the construction of the vertebrate head, the latter is an active morphogenic site. Moreover, CD44 is expressed by cellular types which belong to two main systems, neural and hemopoietic. Both types of cells are migratory and require integrins to interact with the appropriate environment.

2. Material and methods

Embryonic stages were set according to the number of somites and embryos were fixed in 4% (w/v) paraformaldehyde.

For whole-mount staining, embryos were immersed in a detergent solution after being treated with graded amount of methanol. They were washed and stained in PBS containing 2% gelatin and 0.25% triton. Avidin-biotin peroxidase complex was used. Embryos were embedded in gelatin-sucrose, frozen in isopentane at −70°C and 20 μm sections were done.

Antibody staining and immunoperoxidase tissue analysis were also performed on 15 μm cryostat sections as previously described (Corbel et al., 1990).

Two monoclonal antibodies were used: Av6 produced by Davison et al. (personal communication) which recognizes the chick homologue of CD44 and HNK-1 which recognizes CD57 (Vincent and Thierry, 1984).

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