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Corneal keratocytes retain neural crest progenitor cell properties

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Abstract

Corneal keratocytes have a remarkable ability to heal the cornea throughout life. Given their developmental origin from the cranial neural crest, we asked whether this regenerative ability was related to the stem cell-like properties of their neural crest precursors. To this end, we challenged corneal stromal keratocytes by injecting them into a new environment along cranial neural crest migratory pathways. The results show that injected stromal keratocytes change their phenotype, proliferate and migrate ventrally adjacent to host neural crest cells. They then contribute to the corneal endothelial and stromal layers, the musculature of the eye, mandibular process, blood vessels and cardiac cushion tissue of the host. However, they fail to form neurons in cranial ganglia or branchial arch cartilage, illustrating that they are at least partially restricted progenitors rather than stem cells. The data show that, even at late embryonic stages, corneal keratocytes are not terminally differentiated, but maintain plasticity and multipotentiality, contributing to non-neuronal cranial neural crest derivatives.

Keywords: Cornea; Neural crest; Keratocyte; Differentiation

Introduction

The cornea is a transparent tissue located at the anterior-most surface of the eye that transmits and refracts light to the retina. Corneal keratocytes are able to heal wounds and, throughout life, can regenerate the cornea after injury or surgery. However, little is known about the relationship between the early development of the cornea and its subsequent plasticity and ability to regenerate after wounding.

In all vertebrate embryos, the cornea is initially comprised of a layer of ectoderm overlying the lens. In the chick embryo, development of the cornea begins at embryonic day 3 (E3) when the optic cup and lens induce the overlying ectoderm to synthesize an acellular primary stroma consisting of collagen fibrils (Hay and Revel, 1969; Hendrix et al., 1982; Fitch et al., 1988). This is followed at E4 by a wave of invasion of neural crest mesenchyme that form an endothelial layer on the inner surface of the corneal primary stroma, adjacent to the lens. Shortly thereafter, a second wave of neural crest cells invades and contributes to the primary stroma (Hay, 1980; Hay and Revel, 1969).

Within the primary stroma, mesenchymal neural crest differentiate into keratocytes by E6 and begin to synthesize and secrete an extracellular matrix composed of collagens I, V and VI and proteoglycans (Hart, 1976; Hay et al., 1979; Linsenmayer et al., 1983, 1986; Funderburgh et al., 1986). As maturation proceeds, the stroma dehydrates becoming thin and transparent, containing flattened and interconnected keratocyes (Jester et al., 1994). In normal corneas, keratocytes appear quiescent but can resume migration, mitosis, wound healing and repair after injury.

A major problem in corneal repair is that improper healing can result in formation of scar tissue (Rawe et al., 1992; Melles et al., 1995), suggesting that wound repair does not necessarily reiterate the normal process of development. Therefore, understanding the developmental potential of differentiated corneal stromal cells is important for understanding the mechanisms of repair. However, little is known about the relationship between corneal stromal cells and the neural crest precursors from which they are derived. One interesting possibility is that the regenerative ability of the cornea is related to the stem cell-like properties of neural crest cells.

To examine this relationship, we have utilized quail/chick chimeric grafts to follow the invasion of the cornea by neural crest precursors and their differentiation over time.

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We then asked whether these cells retain multipotentiality following overt differentiation as keratocytes. To this end, we challenged the developmental potential of neural crest-derived corneal stromal keratocytes by transplanting them onto early neural crest migratory pathways. In their new environment, these cells appeared to migrate but failed to intermix with nascent neural crest cells. Moreover, the stromal keratocytes down regulated keratan sulfate proteoglycan (KSPG) expression and differentiated into multipotent progenitors with the ability to form some neural crest derivatives such as smooth muscle and myofibrils in addition to corneal keratocytes and endothelial cells. However, they failed to form all neural crest derivatives and did not give rise to tissues of other origin. The results show that corneal keratocytes are highly plastic even after overt differentiation and this may contribute to their ability to efficiently heal wounds throughout life.

Materials and methods

Embryos

Fertilized White Leghorn chick (*Gallus gallus domesticus*) and Japanese quail (*Coturnix coturnix japonica*) eggs were obtained from commercial sources. Chick eggs were incubated at 38°C for 30 h to obtain 5- to 7-somite (HH stages 8–9, Hamburger and Hamilton, 1951) embryos. Quail eggs were incubated at 38°C for 27 h to obtain 7-somite (HH stage 9) embryos. Embryos were prepared as previously described (Lwigale, 2001). Briefly, chick eggs were windowed and the vitelline membrane was removed from the region of surgery using pulled glass needles. Quail-donor embryos of similar developmental stages were lifted from the eggs using filter paper rings, rinsed and kept at room temperature in sterile Ringer's solution until needed.

Quail-chick grafts

To track neural crest contribution to the cornea at various stages of development, dorsal neural tube explants were ablated from the region between caudal diencephalon and rhombomere 1 of chick hosts and replaced with quail dorsal neural tubes of similar region (Fig. 1A). Eggs were sealed with Scotch tape and chimeric embryos were reincubated for an additional 3–6 days.

Stromal cell isolation and injections

Corneas were dissected under sterile conditions from E7 to E16 (period after formation of the secondary stroma until just before hatching) quail embryos. On average, 100 corneas were used for each cell preparation. Trimmed corneas were digested with dispase (Roche, IN) for 15 min then rinsed in Ringers solution with 0.1% bovine serum albumin (BSA). The corneal epithelial cell layer was peeled away using fine forceps then the endothelium was gently scraped away leaving only the stroma. Stromal keratocytes were isolated as described by Conrad (1970). Briefly, stromal tissues were digested with 0.25% collagenase (Worthington, NJ) in Ringer's solution for approximately 0.5–2 h (depending on tissue age) until all the collagen matrix encasing

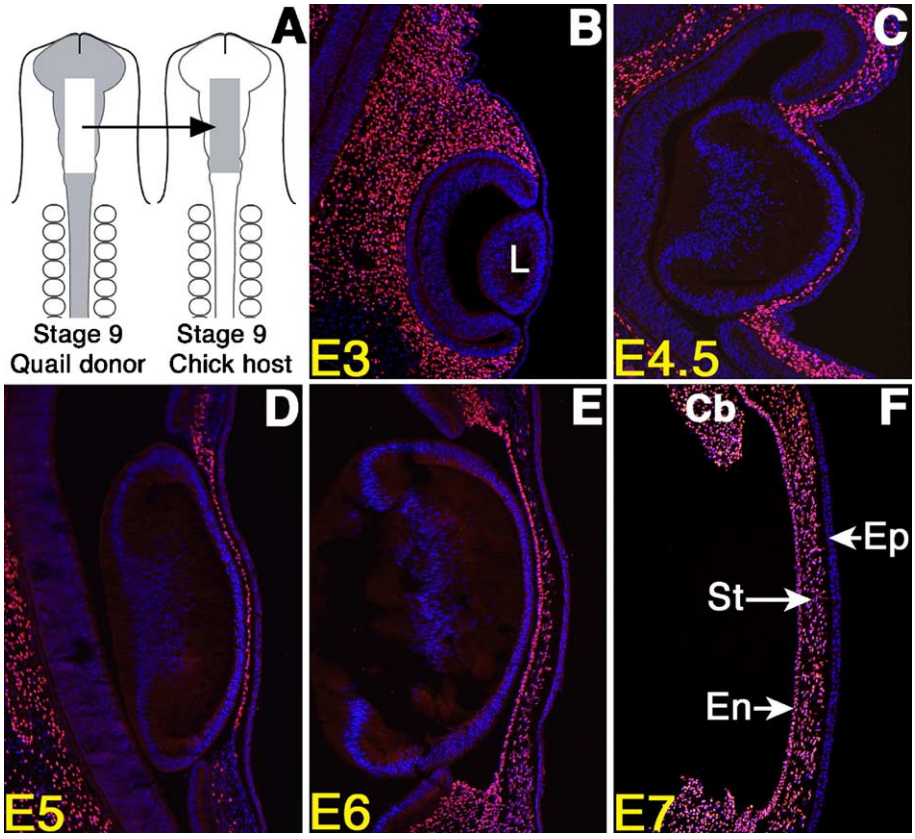

Fig. 1. Schematic diagram (A) illustrating the methods of dorsal neural tube transplantation from quail to chick embryos and sections through chimeras (B–F) showing the resultant contribution of QCPN-positive quail-derived neural crest cells (red) to the cornea at various developmental stages. At E3 (B), quail neural crest are located in the periocular region surrounding the primitive lens. At E4.5 (C), neural crest cells have begun migrating between the lens and epithelium such that by E5 (D), they have formed an inner layer called the endothelium. By E6 (E), neural crest are migrating between the epithelium and endothelium and by E7 (F), all layers of the cornea are formed with the endothelium and stroma being of neural crest descent. L, lens; Ep, epithelium; St, stroma; Cb, ciliary body; En, endothelium.