CLINICAL STUDY

Do corneal endothelial dystrophies represent ocular neurocristopathies? A discussion on the available evidence

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Abstract

Current thinking on the embryological origin of human corneal endothelium has it that this cellular population stems from neural crest precursors. There are arguments in support of this assumption but equally persuasive ones against it. We present a discussion of the evidence for and against a neural crest origin for human corneal endothelial cells. In suggesting that caution be applied to extrapolating from non-human work, we would question the fallibility of the current thinking on this subject. To denote human corneal dystrophies as neurocristopathies may be a premature assumption.

Key words Corneal dystrophies, Corneal endothelium, Neural crest, Ocular embryology

Neural crest cells (NCC) are a sub-population of neuroectoderm cells that arise along the dorsolateral aspects of the neural folds. Budding and invagination of the cephalic end of the newly closed neural tube create the optic vesicles and cups that go on to develop into the embryonic eye. Around day 22 of gestation, as the neural plate folds, NCC are liberated into the embryonic mesoderm. As the neuroectodermal modelling creates the optic vesicles, NCC migrate to infiltrate the encompassing primary mesenchyme. Primary mesenchyme becomes secondary mesenchyme when the mesoderm is admixed with NCC.[1]

There is universal agreement that corneal endothelium derives from mesenchyme. This paper discusses the arguments regarding whether mesenchyme is primary or secondary, i.e. whether there is sufficient evidence to establish that *human* corneal endothelium derives from NCC. In other words do corneal endothelial dystrophies represent ocular neurocristopathies?

How can you establish what structures derive from NCC?

The easiest method for structuring embryological experimentation is to ablate the potential sub-population to be studied. A thin slice of neural tube or folds is resected from an experimental animal at an appropriate stage of development. The problems with this technique are that interpretation is difficult as some embryos may idiosyncratically regenerate the ablated NCC. It is also possible that in removing NCC the function of an intermediary, important for differentiation, may be compromised. It may be this latter sub-population, rather than the NCC themselves, that is the crucial cell type teleologically.[1] The possibility that ablated NCC can be regenerated from pluripotent tubal neuroectoderm will further compromise the validity of this method of study.

An infinitely more precise and subtle approach is to label donor NCC and transplant these cells into an embryonic recipient. One technique used was to radiolabel the whole donor embryo with tritiated thymidine and transplant NCC from this animal into an unlabelled recipient.[2] The problem was that radio-dilution occurs at each cell division, making tracing beyond a very limited number of generations impossible. A recent variation on the labelling theme includes the use of wheat germ agglutinin–gold conjugate as the cell marker.[3] The most elegant extension of this transplantation approach has been the development of the chick/quail chimera system. Most quail cells, unlike chick cells, have prominent nucleoli. This facilitates a tracing technique that is not affected by dilutional complications. Using this system it has been possible to show that avian corneal endothelial cells and stromal fibroblasts are derived from NCC.[4,5] Because melanocytes do not have this prominent chromatin clump, further cellular marking systems have been designed to trace the lineage of this population.[6]

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Because there is no obvious phenotypic difference between prospective NCC and neural tube cells, tracing single cell lineages is the only definitive method to determine future ontogeny. Individual cells can be labelled by the intracellular injection of a large membrane-impermeant dye that can only be passed on by cell division (lysinated rhodamine dextran).[7] In a similar way, individual neural tube cells can be labelled with replication-incompetent retroviruses carrying a marker gene.[8]

Whilst these latter techniques should afford a more detailed examination of NCC ontogeny and its contribution to the tissues of the mammalian eye, we are unaware of any published reports. To date, the quail/chick chimera system offers the only proven evidence of NCC lineage in ocular development, although recent work with this system has concentrated on cerebral development and the neural crest origin of the skull and facial bones.[9] In birds, the corneal endothelium is derived from secondary mesenchyme, i.e. it is of NCC origin. The question remains as to whether all avian corneal endothelial cells derive from NCC or whether some develop from mesoderm, allowing for the fact that secondary mesenchyme is an admixture of NCC and mesoderm. The appearance of posterior polymorphous dystrophy (PPD) in humans and dogs (American cocker spaniels), with islets of affected tissue in a sea of normality, would argue for such a mosaiform origin.[10,11]

Can the evidence in birds be extrapolated to man? To suggest that the lining of an internal body cavity, such as the eye, is derived from NCC goes against accepted embryological wisdom. In general, mesodermal epithelium, of which the corneal endothelium is an example, should derive from primary, neural-crest-free, mesenchyme.[12]

In most species, the corneal endothelium develops before the corneal stroma is infiltrated by primordial fibroblasts.[12] The quail/chick experiments suggested that avian corneal endothelium *and* corneal fibroblasts develop from NCC lines. If that were the case there should not be this temporal discordance between endothelium and fibroblasts – unless a dual-wave theory of NCC migration is proposed. If the theory that NCC are the embryological derivation of both cell types is accepted, should we think of corneal *stromal* dystrophies (granular, macular and lattice) as ocular neurocristopathies? Although there are no reports of true associations between endothelial and stromal dystrophies, endothelial cells in some patients with stromal dystrophies *do* show abnormalities with the production of atypical mucopolysaccharide. This does not seem to happen when the stromal changes recur in a graft, implying that the endothelium may be involved in the initial degenerative process. These endothelial changes may be secondary to the stromal disease or may represent primary pathology in their own right. Do stromal fibroblasts and endothelial cells have a common ontogenic origin? If this is the case one would need to devise an explanation for a migrational or terminal induction failure of NCC at the target organ, i.e. the cornea. Is this complicated hypothesis the answer or do endothelial cells and stromal fibroblasts have different cell lineages? Investigation of this conundrum has not, to our knowledge, been undertaken.

Word of mouth

Existing evidence thus suggests that at least part of the avian corneal endothelium is derived from NCC. The first line of one of the most widely quoted papers on the embryology of human endothelial disorders states 'The corneal endothelium is derived from the neural crest.'[13] The authors draw principally from the quail/chick experiments but extrapolate these findings to humans although there is no direct evidence in mammals, let alone man, to do so. They further add that 'most authors accept a neural crest origin of the corneal endothelium in man'. 'Most authors' turn out to be three other groups of workers who have extrapolated from the same avian experiments! Although there is no direct evidence that mammalian corneal endothelium derives from NCC it seems that this suggestion has become core-teaching fact. So deeply embedded is this teleology that a case report has been published on the association of congenital hereditary endothelial dystrophy (CHED) and nail fold hypoplasia suggesting a systemic neurocristopathy![14]

The timing's not right

Anatomically, Descemet's membrane, the basement membrane of the endothelium, is composed of an anterior banded and a posterior non-banded layer. The embryonic endothelium secretes anterior banded material until around term, when production switches completely to the production of non-banded substance.[15]

Patients with CHED have a normal, fetal, anterior banded Descemet's membrane but an abnormal, perinatal, posterior non-banded portion.[16] It would suggest, therefore, that endothelial cells in CHED produce normal Descemet's membrane material up to term but then malfunction in producing abnormal posterior collagen. NCC migration to the developing eye occurs at around week 4–5. If NCC *were* the origin of the corneal endothelium, why do supposedly abnormal cells produce a normal basement membrane up to term, a further 35 weeks or so later, and *then* suddenly begin pathological production? To explain this finding it has been suggested that there could be four possible types of neural crest disorders: deficient production, abnormal migration (explaining Reiger's and Peter's anomalies), abnormal proliferation (iridocorneal epitheliopathies) and abnormal terminal induction.[13] The last sub-type is suggested as the cause of CHED, Fuchs' endothelial dystrophy and PPD. It should be pointed out that this classification is an entirely theoretical one based on presumption rather than experimentation. To suggest that CHED and Fuchs' endothelial dystrophy could be caused by the same embryological problem, when the diseases are separated by around 60 years chronologically, stretches the imagination, or rather a

theory, to fit clinical fact. It would be difficult to think of a common inducing environment or substance that would cause the malfunction of endothelial cells in neonates and septuagenarians.

Other established systemic neurocristopathies have *congenital* or *neonatal* effects, e.g. Hirschsprung's disease. In some cases other neural crest systems are also affected, associating Hirschsprung's with congenital patchy hypopigmentation, deafness and stenosis of the pulmonary trunk.[17] In established neurocristopathies there has been no need to hypothesise about a failure of terminal induction. Why should corneal endothelial cells be unique in this respect?

Melanocytes derive from neural crest

Other quail/chick studies have shown that NCC give rise to dermal melanocytes.[1] In the avian embryo, truncal neural crest cells that migrate ventrally go on to develop into neurons and glial cells whereas those migrating dorsally become dermal melanocytes. It has been suggested that NCC need to differentiate into melanocytes *before* being able to migrate dorsally.[6] Even though the origin of *iris* melanocytes is unknown, attention has been drawn to the metaplastic activity of 'transplanted' iris melanocytes. If, through an act of sufficient trauma, these cells are driven into the cornea, they can proliferate on the posterior corneal surface and acquire the characteristics of endothelial cells.[18] They may secrete a collagenous layer posterior to Descemet's membrane similar to that secreted by damaged endothelial cells that have undergone a reactive fibrous transformation.[19] Some authors have used this evidence to suggest a common embryological origin for melanocytes and corneal endothelium (i.e. NCC). The implication seems to be that melanocytes retain a degree of pluripotency, which would appear misguided as melanocytes, as opposed to melanoblasts, are terminally differentiated cells.

By immunising mice with truncal NCC differentiated into melanocytes, monoclonal antibodies to melanocytes and melanoblasts can be raised.[20] This may allow mammalian tracing experiments to be performed in the future.

Summary

There is undoubtedly embryological evidence that at least a percentage of avian corneal endothelial cells derive from neural crest cells. Congenital developmental abnormalities of the avian eye are rare. Such a defect of the cornea has not been reported.[21] There is no refuting evidence that the human corneal endothelium is not of neural crest origin, but again there is no supportive evidence that it is. To take the next logical step in classifying corneal dystrophies as neurocristopathies may well be premature. Our arguments above suggest that we should not accept the extrapolation from bird to human without appropriate consideration. Further recombinant DNA work may show that the hypothesis is correct, but before that time we respectfully suggest that a theory remain a theory until proven otherwise!

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