Developmental Biology 319 (2008) 346–358

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/developmentalbiology

Wise promotes coalescence of cells of neural crest and placode origins in the trigeminal region during head development

Yasuyo Shigetani [a], Sara Howard [a,1], Sonia Guidato [a,1], Kenryo Furushima [b], Takaya Abe [b], Nobue Itasaki [a,*]

[a] Division of Developmental Neurobiology, MRC National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK
[b] Laboratory for Animal Resources and Genetic Engineering, Center for Developmental Biology (CDB), RIKEN Kobe, 2-2-3 Minatojima Minamimachi, Chuo-ku, Kobe 650-0046, Japan

ARTICLE INFO

Article history:
Received for publication 1 December 2007
Received 24 April 2008
Accepted 25 April 2008
Available online 7 May 2008

Keywords:
Wnt signaling
Wise
Chick
Mouse
Trigeminal region
Neural crest cells
Placodal cells
Wnt6

ABSTRACT

While most cranial ganglia contain neurons of either neural crest or placodal origin, neurons of the trigeminal ganglion derive from both populations. The Wnt signaling pathway is known to be required for the development of neural crest cells and for trigeminal ganglion formation, however, migrating neural crest cells do not express any known Wnt ligands. Here we demonstrate that Wise, a Wnt modulator expressed in the surface ectoderm overlying the trigeminal ganglion, play a role in promoting the assembly of placodal and neural crest cells. When overexpressed in chick, Wise causes delamination of ectodermal cells and attracts migrating neural crest cells. Overexpression of Wise is thus sufficient to ectopically induce ganglion-like structures consisting of both origins. The function of Wise is likely synergized with *Wnt6*, expressed in an overlapping manner with *Wise* in the surface ectoderm. Electroporation of morpholino antisense oligonucleotides against *Wise* and *Wnt6* causes decrease in the contact of neural crest cells with the delaminated placode-derived cells. In addition, targeted deletion of *Wise* in mouse causes phenotypes that can be explained by a decrease in the contribution of neural crest cells to the ophthalmic lobe of the trigeminal ganglion. These data suggest that Wise is able to function cell non-autonomously on neural crest cells and promote trigeminal ganglion formation.

Introduction

The trigeminal nerve is the largest cranial nerve, containing both sensory and motor neurons responsible primarily for sensation in the face and movement for mastication. The trigeminal ganglion consists of two lobes, the ophthalmic and maxillomandibular lobes, the latter of which further bifurcates to upper and lower jaws. The ganglion comprises cells which derive from three distinct origins: two different ectodermal placodes – the ophthalmic trigeminal (opV) and maxillomandibular trigeminal (mmV) placodes – which contribute cutaneous sensory neurons to the distal regions of their respective ganglionic lobes and; the neural crest, which contributes cutaneous and some proprioceptive neurons to the proximal region of the ganglion, as well as all satellite glial cells (D'Amico-Martel and Noden, 1983; Hamburger, 1961). Placodal cells delaminate from the ectodermal layer and migrate into the mesenchymal space, where they meet neural crest cells that have migrated from the dorsal neural tube.

Importance of the coalescence of the neural crest and placodal populations for trigeminal ganglion formation has been shown by experimental removal of each of the populations in the chick embryo (Hamburger, 1961; Moody and Heaton, 1983b; Stark et al., 1997). Removal of neural crest cells results in ganglia positioned deep under the ectoderm in which the ophthalmic and maxillomandibular lobes have become separated, and there is a lack or a delay in the formation of their connections with the hindbrain (Hamburger, 1961; Moody and Heaton, 1983b; Stark et al., 1997). This suggests that while neural crest cells are not required for the delamination and migration of placodal cells, they serve as an aggregation centre for the fusion of the two placode-derived cell populations, and assist in the formation of the neuronal connection between the ganglion and hindbrain. Ablation of placodal cells, on the other hand, although difficult to achieve completely because of placode regeneration, results in the formation of trigeminal ganglia in normal positions and with connections to the hindbrain, but of variable sizes and with peripheral projection defects (Hamburger, 1961). These defects, including absence of ophthalmic and/or maxillary branches, suggest that placodal cells function as pioneers for axonal path-finding and projections (Baker and Bronner-Fraser, 2001; Hamburger, 1961; Moody and Heaton, 1983a; Moody and Heaton, 1983b). Thus, both the neural crest population and the placodal population have distinct roles to play in the formation of the trigeminal ganglion. Supporting their instructive role in the assembly of the two populations, trigeminal placodal cells have a strong tendency to coalesce with neural crest cells; even in ectopic locations at the trunk level, neurons derived from grafted ophthalmic placode contribute to dorsal root ganglia, which are normally formed solely by neural crest-derived cells (Baker et al., 2002).

* Corresponding author.
E-mail address: nitasak@nimr.mrc.ac.uk (N. Itasaki).
[1] These authors contributed equally to this work.

0012-1606/$ – see front matter.
doi:10.1016/j.ydbio.2008.04.033

The Wnt signaling pathway is involved in multiple steps of the development of neural crest cells, with both the Wnt/β-catenin and Wnt/planar cell polarity (PCP) pathways being implicated in these processes. For example, while Wnt/β-catenin is involved in the specification, proliferation and fate determination of neural crest cells (Brault et al., 2001; Burstyn-Cohen et al., 2004; De Calisto et al., 2005; García-Castro et al., 2002; Hari et al., 2002; Ikeya et al., 1997; Lewis et al., 2004; Saint-Jeannet et al., 1997; Taneyhill and Bronner-Fraser, 2005), the Wnt/PCP pathway is required for their migration (De Calisto et al., 2005). Little is currently known about the molecular mechanisms underlying the induction of the trigeminal placodes, although it has been recently shown that opV placode cells require the Wnt/β-catenin pathway in order to adopt or maintain an opV fate (Lassiter et al., 2007).

Despite the repeated requirement of Wnt pathway activation during their development, neural crest cells do not express Wnt ligands after migrating out of the dorsal neural tube. The lack of any known Wnt mRNA expressed in the migrating neural crest cells suggests that the ligand may be provided cell non-autonomously by other tissues. Wise is a secreted Wnt signal modulator expressed in many regions including ectodermal derivatives. In the cranial region of 3-day chick embryos, *Wise* is expressed strongly in the surface ectoderm of the trigeminal region, including the periocular region, the maxillary prominence and the mandibular arch (Shigetani and Itasaki, 2007). Wise functions in multiple ways: by binding to LRP6, Wise either enhances or inhibits the function of Wnt ligands depending on the partner Wnt ligand (Beaudoin et al., 2005; Guidato and Itasaki, 2007; Yanagita et al., 2004). Wise is also able to affect the Wnt/PCP pathway (Itasaki et al., 2003), possibly by interacting with LRP6, which has also been suggested to be involved in the pathway (Tahinci et al., 2007). As Wise alone does not activate Wnt receptors (Guidato and Itasaki, 2007), exerting its function requires an accompanying Wnt ligand. A candidate for the Wise partner in the chick cranial region is Wnt6, which is co-expressed with Wise in the head ectoderm on the trajectories of the trigeminal nerves at ganglionic stages (Schubert et al., 2002). In this study we demonstrate that Wise promotes coalescence of neural crest cells and placodal cells to form ganglion-like structures. This is likely to be achieved by delamination of ectodermal cells and the attraction of neural crest cells to them. Loss of function analyses in chick and mouse support the idea that the coalescence of neural crest cells to the placode-derived cells may have been decreased when Wise expression is compromised. These data suggest that Wise is able to function cell non-autonomously on neural crest cells and promote trigeminal ganglion formation.

Materials and methods

Virus infection

Chick *Wise* cDNA (Itasaki et al., 2003) was subcloned into RCAS(A) vector (Petropoulos and Hughes, 1991). As a control, human placental alkaline phosphatase (ALP) in the RCAS(A) vector (Fekete and Cepko, 1993) was used. Retrovirus was made as described previously (Itasaki and Nakamura, 1996). Viral solution was injected into the neural canal and the space underneath the vitelline membrane of SPF chick embryos (Institute for Animal Health, England) at Hamburger and Hamilton (HH) stages 8 to 9 (Hamburger and Hamilton, 1951), and the embryos were incubated for up to two days before harvesting.

Chick electroporation

In ovo electroporation in chick embryos was performed at HH stages 9 to 10 as described previously (Itasaki et al., 1999). For electroporation in the neural crest, plasmid DNA was injected into the lumen of the neural tube and electrodes were positioned bilaterally. For electroporating into the surface ectoderm, DNA was injected in the space between the vitelline membrane and the embryo, and the cathode was placed on the vitelline membrane while the anode was in the yolk beneath the endoderm. Chick *Wise* cDNA was subcloned into pCAGGS (Momose et al., 1999) with IRES-GFP. To make a *Wnt6* expression construct in the same vector, a chick EST clone was obtained from GeneService (899f16). Since this clone misses the first 9 amino acid coding region (a part of the signal sequence), the signal sequence of mouse *Kremen2*, combined to a myc tag, was attached to the signal sequence cleavage site of chick *Wnt6*, as predicted from mouse *Wnt6*. The expression of electroporated genes was monitored by the GFP expression and detected by anti-GFP antibody (Molecular Probes).

Embryo staining

In situ hybridization and immunostaining were performed following standard protocol (Ogasawara et al., 2000). Antibodies used are: HNK1 (BD Biosciences); anti-neurofilament-associated protein antibody, 3A10 (DSHB); anti-GFP antibody (Molecular Probes); anti-viral gag antibody, 3C2 (DSHB); anti-Islet-1/2 antibody, 4D5 (DSHB). For the staining of actin filaments, phalloidin-FITC (Sigma) was used. For double staining of *in situ* hybridization and immunostaining, embryos were first processed for *in situ* hybridization detecting digoxigenin labeled probes for *Pax3* or *Brn3a* with anti-digoxigenin alkaline phosphatase with NBT and BCIP (purple), followed by incubation with HNK1 antibody, which was detected by HRP conjugated secondary antibody and diaminobenzidine (brown), or using anti-GFP-antibody and FITC-conjugated secondary antibody. When required, embryos were cryosectioned at 12 μm before immunostaining.

Cell lineage analyses

For cell lineage analyses (Figs. 2E,F), embryos were electroporated with *GFP* in the neural tube at stages between HH 9$^-$ and 10 and infected with *Wise* virus. Once the neural tube had become closed, requiring 3–8 h of incubation, the head ectoderm was labeled by injecting DiI solution into the space between the vitelline membrane and the embryo (Stark et al., 1997). Embryos were incubated for a further 1.5 days and analyzed under an epifluorescent microscope.

Implantation of cell aggregates

For quantitative analyses of neural crest cell migration into fibroblast cell aggregates (Figs. 3A–G), chick fibroblasts infected with virus encoding either *ALP* or *Wise* were aggregated *in vitro* by a hanging-drop in DMEM. Aggregates were labeled with CellTracker (Molecular Probes) in red and grafted into embryos that had been electroporated with GFP in the neural fold. The aggregates were positioned adjacent to the midbrain underneath the surface ectoderm at HH stages 9$^-$ to 9$^+$. Embryos were cultured until they reached HH stage 11. Embryos from each group with comparable aggregate size and comparable expression of GFP in the neural tube were processed for cryosectioning. In each embryo, serial transverse sections were made at 12 μm thickness. Typically, the grafted cell aggregates were spanned in 9 serial sections, among which the section containing the largest number of neural crest cells was used for quantification. The number of GFP-positive neural crest cells in the aggregate was counted. The area of the cell aggregate was also measured. Cases where largest surface area of the aggregate was less than 1000 μm^2 were excluded from the analysis. If the neural crest cells were on the edge of the aggregate, only the cells that were surrounded by the aggregate cells by more than half of the edge were counted. The number of neural crest cells in each case was normalized to the average surface area of the cell aggregate for comparison (See Supplementary Table 1).

In vitro culture of neural crest cells

To examine the effect of Wise on neural crest cell morphology (Figs. 3H–R), the midbrain was explanted from HH stages 8$^+$ to 9 embryos on coverslips that had been coated with a sheet of chick fibroblast cells expressing either *ALP* or *Wise*. The explant was covered with thin collagen and cultured for two days before processing for staining with either 3C2 antibody (Figs. 3I,K) or phalloidin-FITC (Figs. 3M,N,P,Q) and with HNK1 antibody. The longest stretch of HNK1-positive cells was measured using confocal microscopy for 56 cells in each group, from 6 independent explants. Fig. 3R shows the average length and the standard deviation.

Electroporation of morpholino antisense oligonucleotides

Morpholino oligonucleotides against chick *Wnt6* (5′-TGAGGCCGGACCTTAC**CCTG-CTGCA**-3′, spanning the boundary between the second exon and the second intron; bold is the end of the second exon) and *Wise* (5′-GAATGGCGGAGAGAAG**CAT**GATTGG-3′, beginning of the coding; bold indicates the complementary sequence to the first methionine codon) were custom-made and conjugated with Fluorescein (Gene Tools). A 1:1 mix of these morpholinos were used at the concentration of 100 μM dissolved in H_2O. For a control, a standard control morpholino oligo conjugated with fluorescein provided by the supplier was used. Based on a weak positive charge of fluorescein and no charge of morpholino oligonucleotides, the morpholino was electroporated on the surface ectoderm by placing the anode on the dorsal side of embryos and the cathode on the ventral side. Embryos were incubated until HH stages 14 to 15, and only the embryos showing green fluorescence in the trigeminal region were harvested. Embryos were stained for *Brn3a* and HNK1 in whole mounts, followed by cryosectioning. The inhibitory effect of morpholinos was evaluated either by RT-PCR of electroporated embryos with *Wnt6* splice blocking morpholino, or by *in vitro* translation for *Wise* translation initiation blocking morpholino (Supplementary Fig. 4).

Targeted deletion of Wise in mouse

The *Wise* mutant mice (Accession number: CDB0048K) were generated with TT2 embryonic stem cells as described (Murata et al., 2004; Yagi et al., 1993). *Wise* genomic