Available online at www.sciencedirect.com
ScienceDirect
Developmental Biology 304 (2007) 127–140
DEVELOPMENTAL BIOLOGY
www.elsevier.com/locate/ydbio

Wnt11-R signaling regulates a calcium sensitive EMT event essential for dorsal fin development of Xenopus

Robert J. Garriock, Paul A. Krieg *

Department of Cell Biology and Anatomy, University of Arizona Health Sciences Center, 1501 N. Campbell Avenue, P.O. Box 245044, Tucson, AZ 85724, USA

Received for publication 14 April 2006; revised 23 November 2006; accepted 11 December 2006
Available online 15 December 2006

Abstract

In the frog embryo, a sub-population of trunk neural crest (NC) cells undergoes a dorsal route of migration to contribute to the mesenchyme in the core of the dorsal fin. Here we show that a second population of cells, originally located in the dorsomedial region of the somite, also contributes to the fin mesenchyme. We find that the frog orthologue of Wnt11 (Wnt11-R) is expressed in both the NC and somite cell populations that migrate into the fin matrix. Wnt11-R is expressed prior to migration and persists in the mesenchymal cells after they have distributed throughout the fin. Loss of function studies demonstrate that Wnt11-R activity is required for an epithelial to mesenchymal transformation (EMT) event that precedes migration of cells into the fin matrix. In Wnt11-R depleted embryos, the absence of fin core cells leads to defective dorsal fin development and to collapse of the fin structure. Experiments using small molecule inhibitors indicate that dorsal migration of fin core cells depends on calcium signaling through calcium/calmodulin-dependent kinase II (CaMKII). In Wnt11-R depleted embryos, normal migration of NC cells and dorsal somite cells into the fin and normal fin development can be rescued by stimulation of calcium release. These studies are consistent with a model in which Wnt11-R signaling, via a downstream calcium pathway, regulates fin cell migration and, more generally, indicates a role for non-canonical Wnt signaling in regulation of EMT.

Keywords: Wnt11; Wnt11-R; *Xenopus*; Trunk neural crest; Fine core cells; CaMKII; Non-canonical Wnt signaling

Introduction

The dorsal fin is a prominent feature of the Amphibian embryo that extends along the dorsal surface of the animal, from behind the head to the tip of the tail. The mature dorsal fin is a simple keel-shaped out-pocketing of the dorsal epidermis that is supported by mesenchymal cells and extracellular matrix (Tucker, 1986). Development of the fin begins during the early tail bud stage and the structure remains present until metamorphosis when both the fin and tail regress. The dorsal fin develops as a result of inductive signals from the neural crest (NC) (DuShane, 1935; Bodenstein, 1952; Tucker and Slack, 2004). At first, epidermis of the nascent fin undergoes cell proliferation and extends dorsally, accompanied by an accumulation of extracellular matrix, but the interior of the fin is largely acellular. During tailbud stages, non-pigmented NC cells migrate into the fin matrix (Twitty and Bodenstein, 1941; Tucker and Erickson, 1986; Collazo et al., 1993), followed by a later migration of pigmented melanocytes occurring during the early tadpole stages. In contrast, the ventral fin of Xenopus is induced by mesoderm, not neural crest, and is populated primarily by mesoderm-derived cells (Tucker and Slack, 2004), although at least some neural crest cells are also present (Collazo et al., 1993). Recent studies of dorsal fin development in the axolotl indicate that, in addition to the well-characterized NC population, cells originally located in the somite also contribute to the fin mesenchyme (Sobkow et al., 2006).

Expression studies in Xenopus have shown that a Wnt11 related sequence, Wnt11-R, is expressed in the heart, the neural tube, the dorsal somite and mesenchymal cells within the dorsal fin (Garriock et al., 2005). Xenopus contains two Wnt11 genes and Wnt11-R is the orthologue of avian and mammalian Wnt11 (Garriock et al., 2005 and data not shown). Wnt11 proteins are members of the non-canonical family of Wnt-ligands which bind to cysteine-rich frizzled and ROR receptors and LRP5/6 coreceptors (Bhanot et al., 1996; Wang et al., 1996; Wehrli et al., 2000; Hikasa et al., 2002). After ligand binding, Wnt11 and

* Corresponding author. Fax: +1 520 626 2097.
E-mail address: pkrieg@email.arizona.edu (P.A. Krieg).

0012-1606/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2006.12.020

related non-canonical Wnt-proteins signal through the Wnt/calcium pathway and the planar cell polarity (PCP) pathway, both of which involve the intracellular protein Dishevelled (Dsh) (Sokol, 1996; Sheldahl et al., 2003; reviewed in Fanto and McNeill, 2004; Kohn and Moon, 2005). Through the Wnt/calcium pathway, Wnt11 can elicit intracellular calcium fluctuations resulting in the activation of PKC, calmodulin and CaMKII (Sheldahl et al., 1999; Kuhl et al., 2000). The PCP pathway signals through RhoA and can result in the activation of JNK (Li et al., 1999; Yamanaka et al., 2002; Kim and Han, 2005). Non-canonical Wnt-signaling through the PCP and Wnt/calcium pathways modulates a variety of cell behaviors including convergent extension movements during gastrulation (Heisenberg et al., 2000; Tada and Smith, 2000; Choi and Han, 2002; Yamanaka et al., 2002, Wallingford et al., 2000; Wallingford and Harland, 2001), neural tube closure (Wallingford and Harland, 2002), dendritic outgrowth (Rosso et al., 2005), heart tube morphogenesis (Garriock et al., 2005) and cranial neural crest migration (De Calisto et al., 2005).

In mouse, chicken, frog and zebrafish embryos, Wnt11/Wnt11-R expression marks populations of cells that undergo morphogenetic movements and cell shape change (Ku and Melton, 1993; Kispert et al., 1996; Heisenberg et al., 2000; Olivera-Martinez et al., 2002; De Calisto et al., 2005; Garriock et al., 2005). In this report we show that Wnt11-R expression marks a subset of somite cells and of trunk NC cells that will migrate dorsally to occupy the core of the Xenopus dorsal fin. The function of Wnt11-R is initially required for an EMT event that precedes migration of cells into the fin and ultimately for maintenance of fin structure. These Wnt11-R activities are mediated through a calcium sensitive pathway involving CamKII.

Materials and methods

Embryology and microinjection

Xenopus laevis embryos were staged according to Nieuwkoop and Faber (1994) and cultured in 0.2× MMR. Microinjections occurred in 4% Ficoll in 0.4xMMR and embryos were maintained in this medium for the first 12 h. Embryos were then cultured in 0.2xMMR until harvested. A morpholino oligo (MO) complementary to sequences in the 5′ UTR and shared by both pseudo-tetraploid copies of the Wnt11-R transcript has previously been shown to inhibit translation in vivo (Garriock et al., 2005). The sequence of the Wnt11-R MO1 is (5′-CTTCATCTTCAAAACCCAATAACAA-3′) and control mismatched MO is (5′-CTTGTACTTCTATAGCCTATAAGAA-3′). MOs were stored in 50 mM HEPES pH 8.0, diluted in water and heated to 65 °C for 10 min prior to injection. For neural crest targeting and somite targeting, 15–30 ng of MO was injected at the 8 and 16 cell stage targeted to the D1.2 and V1.2 blastomeres (Dale and Slack, 1987; Moody, 1987). KN-93, CaMKII inhibitor, was prepared as 10 mM stocks in DMSO and used immediately at 10 μM concentrations diluted in 0.2× MMR media (KN-93 Cat# S-2022, A. G. Scientific Inc). The media containing inhibitor was exchanged every 3 h. Calcium signaling was activated with 100 nM thapsigargin (Sigma) or 10 nM A23187 ionophore (Sigma) for 1 h.

Whole-mount in situ hybridization

Whole-mount in situ hybridization was carried out using a modification of the protocol by Harland (1991), with antisense digoxigenin-labeled probes for Wnt11-R (Garriock et al., 2005). Plasmids were linearized with Not1 and transcribed with T7 RNA polymerase using the MEGAscript kit (Ambion). For serial sections, embryos were post-fixed in 4% paraformaldehyde, embedded in Paraplast and 10 μm transverse sections were prepared. DAPI was used to stain nuclei for cell counts.

Cell lineage studies and neural tube transplants

Cell lineage studies utilized 2.5 mg/ml DiI (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate, Molecular probes, Cat# D282). Lineage tracer prepared and stored using standard methods (Collazo et al., 1993) was microinjected in a volume of 2.6 pl into the somite or the neural tube at St 19 and location of labeled cells was then followed directly by fluorescence. Neural tubes from St 19–20 embryos (i.e. prior to NC migration) were excised from donor embryos injected with 750 pg of synthetic GFP mRNA alone or in combination with 15 ng of Wnt11-R morpholino or 15 ng control morpholino. Tissue was implanted into the region of the dorsal somite, beneath the epidermis, of a similarly staged uninjected host embryo in 0.2× MMR. Embryos were cultured until St 37 and the dorsal fins of those embryos were examined for the presence of GFP-expressing cells within the fin. For neural tube ablations, a segment of the neural tube was carefully excised from St 19 embryos, which were then allowed to develop until St 27 when they were assayed.

Results

Wnt11-R marks neural crest cells, dorsal somite cells and fin mesenchyme

We have carried out in situ hybridization analysis of Wnt11-R expression in the trunk region of the embryo, encompassing

Fig. 1. In situ hybridization analysis of Wnt11-R expression during Xenopus fin development. (A) Dorsal view of St 22 embryo showing Wnt11-R transcripts in the neural tube and in cells at the medial border of the somite. (B) Transverse section through the embryo in panel A showing Wnt11-R expression in the dorsal neural tube. (C) Dorsal view of St 24 embryo showing expression of Wnt11-R in the neural tube and in the somite. (D) Dorsal view of St 26 embryo with Wnt11-R expression in the neural tube and more extensively in the somite. (E) Lateral view of St 26 embryo showing expression of Wnt11-R in the pharyngeal arches and in dorsal tissues of the embryo. (F) Enlarged view of panel E showing expression of Wnt11-R in the dorsal somite. (G) Transverse section showing Wnt11-R transcripts in dorsal neural tube and the dorsal region of the somites. (H) Lateral view of St 27 embryo showing expression of Wnt11-R in dorsal tissues and in cranial NC migrating into the pharyngeal arch region. (I) Enlarged view of panel H showing expression of Wnt11-R in the somite and in individual cells at the base of the dorsal fin (arrow). (J) Transverse section through St 27 embryo showing expression of Wnt11-R in detached cells at the base of the dorsal fin (white arrow). We sometimes observe a small number of cells within the fin that at not expressing Wnt11-R (black arrow). (K) Lateral view of St 34 embryo showing expression of Wnt11-R in the heart, cranial neural crest cells and dorsal tissues. (L) Enlarged view of panel K showing numerous separate stained cells within the fin. (M) Transverse section through St 34 embryo showing Wnt11-R expressing cells dispersed within the dorsal fin core and along the dorsal and lateral surface of the somite. (N, O) Transverse sections through the trunk of a St 25 embryo from which a region of the neural tube has been ablated. For reference, the notochord is outlined in yellow. The section in panel N is located anterior to the region where the neural tube has been removed. Note Wnt11-R expression in the dorsal neural tube and the dorsomedial region of the somite. The section in panel O shows the region where the neural tube is missing. Wnt11-R expression in the dorsal somite (arrow) is equivalent to that in the control section. (P) Transverse section through St 34 embryo at the level indicated by the arrowhead in panel K showing stained cells dorsal to the neural tube. (Q) Lateral view of the tail of St 37 embryo showing Wnt11-R expressing cells within the dorsal fin but not the ventral fin. (R) Magnified view of the dorsal fin of an unbleached St 37 embryo showing Wnt11-R expressing cells, plus a large pigmented melanocyte (arrow). (S) Identical region of the fin shown in panel R after bleaching. Note that the melanocyte does not express detectable levels of Wnt11-R. Abbreviations: nt, neural tube; s, somite.