FGF signal regulates gastrulation cell movements and morphology through its target *NRH*

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Abstract

We used cDNA microarray analysis to screen for FGF target genes in *Xenopus* embryos treated with the FGFR1 inhibitor SU5402, and identified neurotrophin receptor homolog (*NRH*) as an FGF target. Causing gain of NRH function by *NRH* mRNA or loss of NRH function using a Morpholino antisense-oligonucleotide (Mo) led to gastrulation defects without affecting mesoderm differentiation. Depletion of NRH by the Mo perturbed the polarization of cells in the dorsal marginal zone (DMZ), thereby inhibiting the intercalation of the cells during convergent extension as well as the filopodia formation on DMZ cells. Deletion analysis showed that the carboxyl-terminal region of NRH, which includes the "death domain," was necessary and sufficient to rescue gastrulation defects and to induce the protrusive cell morphology. Furthermore, we found that the FGF signal was both capable of inducing filopodia in animal cap cells, where they do not normally form, and necessary for filopodia formation in DMZ cells. Finally, we demonstrated that FGF required NRH function to induce normal DMZ cell morphology. This study is the first to identify an in vivo role for FGF in the regulation of cell morphology, and we have linked this function to the control of gastrulation cell movements via NRH.
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Introduction

Morphogenesis during early development, involving cell proliferation, differentiation, and migration, is regulated by transcription, reorganization of the cytoskeletal architecture, and cell adhesion. Many of these cellular processes are controlled by cell-to-cell communication mediated by secreted molecules such as polypeptide growth factors. Fibroblast growth factor (FGF) refers to a group of structurally related growth factors that were initially characterized based on the ability of these molecules to promote the proliferation and survival of a variety of cells; FGF has also been implicated in the regulation of cell morphology and migration (Szebenyi and Fallon, 1999). In

isolated animal cap (AC) explants of early *Xenopus laevis* embryos, which normally become ectoderm, basic FGF can induce the ventral mesodermal fate, mimicking the ventrovegetal mesoderm-inducing signal (Kimelman and Kirschner, 1987; Slack et al., 1987). Earlier, embryonic FGF (eFGF) was found to induce a gene for the T-box transcription factor, *Xenopus* brachyury (Xbra), which in turn activates the eFGF gene, thereby constituting a closed circuit of gene activations to amplify the signal for mesoderm induction (Conlon et al., 1996; Issac, 1997). Although FGF is known as a mesoderm-inducing factor, recent studies have revealed that it can regulate multiple cellular events, including cell shape change and migration (Burdine et al., 1997; Hacohen et al., 1998; Ribeiro et al., 2002; Schumacher et al., 2004; Sutherland et al., 1996; Yang et al., 2002). In *Xenopus*, FGF2 can induce cell motility, lamellipodia formation, and cellular polarization in gastrula stage cells (Wacker et al., 1998) and a functional

analysis of an FGF antagonist, sprouty2 (Nutt et al., 2001), showed that FGF plays an important role in the regulation of gastrulation cell movements. These observations suggest that FGF has a diverse array of biological functions during the course of early development. To dissect the temporally distinct roles of FGF in early *Xenopus* development, we attempted to screen for FGF target genes by treating embryos with SU5402, a chemical inhibitor of FGFR (Mohammadi et al., 1997), at different times in development, and examined the gene expression by microarray analysis. We successfully isolated transcriptionally regulated FGF target genes that are involved in MAPK-dependent mesodermal induction as well as in gastrulation cell movements (Chung et al., 2004).

Among the target genes that are affected by treating embryos with SU5402 from stage 10.5 to 11.5, we identified a gene that encodes a protein similar to mammalian p75NTR. p75NTR was originally identified as a receptor for neurotrophins, in addition to the well-characterized Trk receptor tyrosine kinases. Thus, neurotrophins are thought to exert their cellular activities through two different receptors, the Trks and p75NTR. In *Xenopus*, the neurotrophin receptor homologs (*NRHs*) encode two alleles, *NRH1a* and *NRH1b*, which are structurally related to p75NTR and are also called *NRH* (Genbank accession no. AY553184, Chung et al., 2004) and fullback (AF131890, Bromley et al., 2004), respectively. Recently, Sasai et al. (2004) reported the biochemical mechanism of NRH function in gastrulation cell movement via regulation of small GTPase proteins and JNK activation but it still remains unknown how NRH regulates cytoskeletal machinery to achieve dynamic cell shape changes and cell movements during convergent extension. Here, we report a newly identified function of NRH in vertebrate gastrulation and propose a possible mechanism for FGF's regulation of gastrulation cell movements through the activity of NRH. Since neurotrophins regulate neuronal and non-neuronal cell survival and determine cell numbers during development, this finding is somewhat unexpected. Nonetheless, it sheds light on this novel function of neurotrophins and their receptor during embryogenesis, and suggests an interaction between the FGF and neurotrophin pathways in neural development.

Materials and methods

Plasmid construction, RNA synthesis, and antisense morpholino oligonucleotides

We previously identified a cDNA fragment XL019m15, in our microarray screen, and subsequently found the full-length (FL) cDNA clone, XL103g21 in our *Xenopus* EST database (NIBB XDB, http://www.Xenopus.nibb.ac.jp). Using PCR, we constructed plasmids for microinjection and subcellular localization studies. A FL construct without the 5′UTR, a FL construct that included the 5′ UTR, and cleavage mutants of *NRH* were subcloned in-frame into the *Eco*R[2] and *Eco*RV sites of pCS2+ and pCS2− Venus to create a C-terminal Venus tag. The PCR primer pairs were as follows: 5′ CGGAATTCATGGAAATGAGGGGCCCACG-T 3′ and 5′ CCGCTCGAGTTACACCACAGAGCTGGCAT 3′ for NRH FL; 5′ CGGAATTCATGGAAATGAGGGG-CCCACGT 3′ and 5′ CCGCTCGAGCACCACAGAGC-TGGCAT 3′ for Venus tag tethered FL construct; 5′ CGGAATTCGGGCAGTTTTCATACAGGAGAA 3′ and 5′ CCGCTCGAGCACCACAGAGCTGGCAT 3′ for the 5′ UTR plus FL cDNA; 5′ CGGAATTCATGGAAATGAG-GGGCCCACGT 3′ and 5′ CCGCTCGAGTTAGCAGGTA-GTATAGCACTT 3′ for NRH ΔC; 5′ CGGAATTCATGTT-TAAGTGCTATACTACCT 3′ and 5′ CCGCTCGAGTTA-CACCACAGAGCTGGCAT 3′ for NRH C. Constitutively active constructs (QL) of RhoA and Rac (GenBank accession no. AF174644) were prepared by PCR using neurula cDNA. A plasmid bearing the gene for constitutively active MKK7 (MKK7 DED) was a kind gift from Dr. E. Nishida (Yamanaka et al., 2002). The plasmids bearing the gene for GAL4 (DBD)-tagged c-Jun and the dominant negative form of Xwnt11 were kind gifts from Dr. M. Tada. Xwnt11 (Tada and Smith, 2000), dominant negative Xwnt11 and pSP64T-Cdc42G12V (Djiane et al., 2000) were linearized with *Bam*HI. Plasmids to be used for microinjection were linearized with *Not*I, if there was no additional description. Capped mRNAs were synthesized with an mMESSAGE mMACHINE kit (Ambion, Austin, TX) and purified on a NICK column (Pharmacia, Uppsala, Sweden). All plasmids were transcribed with SP6 polymerase. Antisense Morpholino oligonucleotides (Mo) were obtained from Gene Tools. Mo sequences were as follows: NRH Mo 5′-GGATCTGGCCTCGTTCCTGTTCAGG-3′; fullback Mo 5′-GCCCCTCTTATCCATAGTTGGGATC-3′; control Mo 5′-CCTCTTACCTCAGTTACAATTTATA-3′.

Embryo manipulation

Xenopus eggs were collected as described (Yamamoto et al., 2001), and the embryos were staged according to Nieuwkoop and Faber (1967). ACs were dissected at the blastula stage, and the dorsal marginal zone (DMZ) or ventral marginal zone (VMZ) was isolated at stage 10.5. Dissected tissues were placed in 1× Steinberg's solution supplemented with 0.1% bovine serum albumin (BSA) until the stages of interest were reached or the tissue was processed further for other assays.

RNA isolation and RT-PCR assays

Total RNA was isolated from 10 explants using Trizol® reagent (Life Technologies), according to the manufacturer's instructions. Isolated RNAs from the explants were used for cDNA synthesis in the presence of 2 mM dNTP, 0.1 M DTT, 5× first-strand buffer, RNase inhibitor (Takara,