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FGF signalling controls expression of vomeronasal receptors during embryogenesis

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Abstract

Fibroblast growth factors (FGFs) have been shown to control formation and differentiation of multiple organ systems in the developing vertebrate embryo. The analysis of differential gene expression during embryogenesis is, therefore, a potent tool to identify novel target genes regulated by FGF signalling. Here, we have applied microarray analysis to identify differentially regulated genes in FGF mutant mouse embryos. Surprisingly, transcripts corresponding to vomeronasal receptors (VRs), which so far have been only detected in the vomeronasal organ (VNO), were found to be downregulated in FGF mutant embryos. VR expression was detected in the developing olfactory pit and the anlage of the VNO. Interestingly, several FGFs can be detected in the developing olfactory pit during mouse embryogenesis [Bachler, M., Neubuser, A. 2001. Expression of members of the Fgf family and their receptors during midfacial development. Mech. Dev. 100, 313–316]. FGF signalling may thus control expression of VRs in the olfactory pit and formation of the VNO. Moreover, VR expression was detected in unexpected locations within the developing embryo including retina, dorsal root ganglia and neural tube. The relevance of VR expression in these structures and for formation of the VNO is discussed.

Keywords: Fibroblast growth factor; Vomeronasal organ; V2R; Dorsal root ganglia; Inner ear

1. Introduction

Fibroblast growth factors (FGFs) make up a family of polypeptide growth factors found throughout the animal kingdom. During embryonic development, they regulate cell proliferation and differentiation, body plan formation and organogenesis (Ornitz and Itoh, 2001; Reuss and von Bohlen und Halbach, 2003). Several FGF members, among them FGF3, FGF8, FGF10 and FGF19, have been implicated in different stages of inner ear development (Barald and Kelley, 2004; Ladher et al., 2005). The stage of the otic vesicle (otocyst) is of high importance for inner ear development, because at this time point the inner ear undergoes subdivision into defined compartments, ultimately leading to the generation of the highly complex differentiated organ (Brigande et al., 2000). This process of regionalization and specification is very likely to be regulated by different genetic pathways. Indeed, previous studies, based on a cDNA subtraction screening technique, have identified numerous candidate genes potentially involved in controlling patterning of the inner ear at the otocyst stage (Powles et al., 2004). Recently, it was shown that FGF3 and FGF10 synergistically control formation of the otic vesicle in mice (Alvarez et al., 2003; Wright and Mansour, 2003). Since next to the inner ear, FGF3 and FGF10 have been shown to control the formation of other embryonic structures, including lung, limbs and tail (Mansour et al., 1992; Min et al., 1998; Sekine et al., 1999) during mouse development, mouse mutants for these FGF genes may serve as an ideal tool to identify novel downstream effectors of FGF signaling.

The vomeronasal organ (VNO) originates from the medial wall of the olfactory (nasal) pit where the VNO anlage is induced as a bud which will form the vomeronasal groove

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(Garrosa et al., 1998). In adult mammals, the mature VNO located in the nasal septum contains sensory neurons, which project their axons to the accessory olfactory bulb (AOB). In turn, neurons of the AOB project to discrete loci within the vomeronasal amygdala (Halpern, 1987). After direct contact with pheromone sources, these non-volatile chemicals enter the VNO and elicit complex behavioral responses. It is believed that the pheromone molecules are bound by vomeronasal receptors (VRs) from the multigene family of G-protein-coupled receptors, which trigger a phospholipase C (PLC)-dependent signal cascade (Holy et al., 2000). The mature VNO is organized into two distinct layers. Vomeronasal receptor neurons with cell bodies in the apical part of the sensory epithelium express the G-protein α-subunit $G\alpha_{i2}$ and project to the anterior part of the AOB, whereas basally located neurons of the VNO express the G-protein α-subunit $G_{o\alpha}$ and project to the posterior region of the AOB (Berghard and Buck, 1996; Jia et al., 1997; Shinohara et al., 1992). In addition, apical neurons express receptors of the V1R family, whereas basal sensory neurons express those of the V2R family. Each of these VR multigene families comprises more than a hundred genes (Dulac and Torello, 2003), thereby adding significant complexity to the analysis of individual VR genes. Recently, it was shown that small peptides which serve as ligands for major histocompatibility complex (MHC) class I molecules can stimulate V2R receptors (Leinders-Zufall et al., 2004).

In the present report we have used microarrays to screen for novel targets of FGF signaling by hybridising them with cDNAs derived from FGF3, FGF10 and homozygous compound mutants for these genes. As expected we found genes normally expressed in the otic vesicle to be down-regulated in FGF mutants. Unexpectedly however, we also found transcripts derived from the V2R gene family to be downregulated in these mutants. Expression of V2R transcripts was detected in the olfactory pit, retina, dorsal root ganglia (DRG) and neural tube of mouse embryos. FGFs thus appear to control early expression of V2Rs in the developing embryo. These results suggest potential roles of FGF signaling during formation of the VNO and other embryonic structures expressing V2R transcripts.

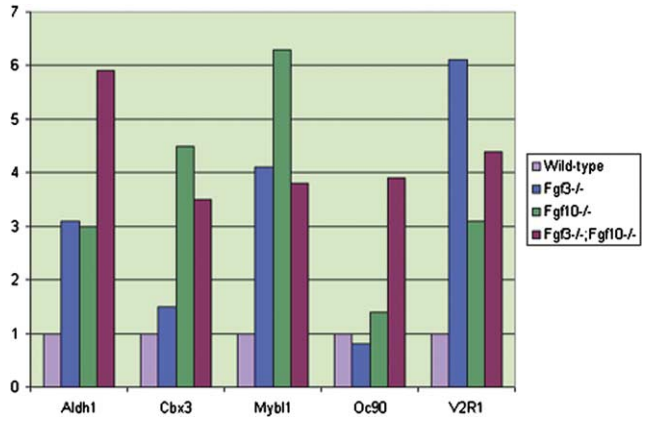

Fig. 1. Selected genes, downregulated in $Fgf3^{-/-}$, $Fgf10^{-/-}$ and compound $Fgf3^{-/-}/Fgf10^{-/-}$ mouse mutant embryos compared to wild-type embryos at E10. Signal fold changes showing fold-down-regulation between wild-type and mutant samples are indicated.

2. Results

2.1. A microarray-based screen for FGF-regulated genes

FGF3 and FGF10 have been shown to control formation of the otic vesicle in mice (Alvarez et al., 2003; Wright and Mansour, 2003). Double mutant homozygous $Fgf3^{-/-}/Fgf10^{-/-}$ embryos lack otic vesicles or only form microvesicles at the otic vesicle stage from embryonic day 9 (E9) to E10. To reveal the genes regulated by FGF signaling during embryogenesis we employed gene chips covering the expression of 36.000 known mouse genes and expressed sequence tags (U74ABC V.2 set from Affymetrix; see Section 4). Due to the very limited amount of otic tissue in $Fgf3^{-/-}/Fgf10^{-/-}$ mutants we opted to isolate RNA from embryonic tissue at E10 corresponding to the area of the otic vesicle and neighbouring tissue, including periotic mesenchyme and the neural tube (see Section 2). Both of the latter tissues have been shown to be essential for formation of the inner ear and influence the expression of otic genes (Baker and Bronner-Fraser, 2001). In our analysis, we compared three groups of embryos against their wild-type littermates: $Fgf3^{-/-}$, $Fgf10^{-/-}$, and double $Fgf3^{-/-}/Fgf10^{-/-}$ mutants (see Section 4). As a result, a list of genes, which showed altered expression levels in the absence of FGFs, was generated. We discovered genes already reported to have expression in the otic vesicle, as well as genes not previously implicated during inner ear development (see below). We further focused our analysis on a set of selected genes downregulated in the FGF-deficient embryos, as presented in Fig. 1. These genes corresponded to *retinaldehyde-dehydrogenase 1* (*Aldh1 (Raldh1)*; Romand et al., 2004), *chromobox homolog 3* (*Cbx3*; Jones et al., 2001), *myeloblastosis oncogene-like 1* (*Mybl1*; Trauth et al., 1994), *otoconin 90* (*Oc90 (Oc95)*; Wang et al., 1998; Verpy et al., 1999) and vomeronasal receptors (V2Rs; Herrada and Dulac, 1997; Matsunami and Buck, 1997; Ryba and Tirindelli, 1997). A complete list of the microarray results obtained from the hybridizations of the U74A V.2 chips can be found in the Supplementary information.

To analyse more globally in the developing embryo the differential expression of the selected genes with an independent technique, we next employed semi-quantitative reverse-transcriptase polymerase chain reaction (RT-PCR). We extracted total RNA from wild-type, $Fgf3^{-/-}$, $Fgf10^{-/-}$ and $Fgf3^{-/-}/Fgf10^{-/-}$ embryos at E10, which then served as a template in a one-step RT-PCR with gene-specific primers (see Section 4). RNA amounts in samples were normalized by using two independent housekeeping genes, *beta-actin* and *glyceraldehyde 3-phosphate dehydrogenase* (*gapdh*). Among the genes we analyzed by RT-PCR, we found *Aldh1* and *V2Rs* to be downregulated in single and compound FGF mutants (Fig. 2 A,C), while *Oc90* was only downregulated in $Fgf3^{-/-}/Fgf10^{-/-}$ double mutants (Fig. 2 B), consistent with the results from the gene chips (Fig. 1). In contrast, *Cbx3* and *Mybl1* failed to show a significant change in mRNA levels