

THE FUNCTION OF FGF SIGNALLING DURING EARLY CRANIOFACIAL DEVELOPMENT

A Leibbrandt², M Bachler², J Partanen³ & A Neubüser^{1,2}

¹ *Institute of Biology I Department of Developmental Biology University of Freiburg Germany.*

² *Institute of Molecular Pathology Vienna Austria,* ³ *Institute of Biotechnology Viikki Biocenter University of Helsinki Finland*

INTRODUCTION: The FGF family of signaling molecules has a central role in the regulation of many aspects of vertebrate embryogenesis and many processes in the adult. In the developing face *Fgf3*, 8, 9, 10, 15, 17, and 18 are expressed in partially overlapping domains in the ectoderm of the early facial region, and *Fgfr1* and *Fgfr2* are both expressed in the underlying neural crest mesenchyme and continue to be expressed in the mesenchyme of the developing palatal shelves [1]. To study the function of *Fgf8* and *Fgfr1* during early craniofacial development we have analyzed the phenotype of mice with a conditional inactivation of these two genes in the facial region.

METHODS: Mice homozygous for a conditional allele of *Fgf8* (*Fgf8^{lox/flox}*) were bred to mice carrying a null allele of *Fgf8* and a *cre*-cDNA under control of the *Foxg1* promoter (*Fgf8^{+/+}; Foxg1cre⁺*) as described in [2]. In *Fgf8^{lox/flox}; Foxg1cre⁺* embryos (in the following referred to as *Fgf8* mutant embryos) Cre-mediated recombination leads to a loss of functional *Fgf8* message in the prospective midfacial ectoderm and forebrain by the 12-somite stage. To inactivate *Fgfr1* in neural crest cells mice homozygous for a conditional allele of *Fgfr1* (*Fgfr1^{lox/flox}*) were bred to mice carrying the conditional allele and *Wnt1::Cre* (*Fgfr1^{lox/+}; Wnt1::Cre*). In *Fgfr1^{lox/flox}; Wnt1::Cre* embryos Cre-activity leads to loss of functional *Fgfr1* protein in migrating neural crest cells [3]. Embryos were isolated from pregnant females between 8 and 18 days post coitum, fixed and processed for analysis using histological stainings, TUNEL and cell proliferation assays, and in situ hybridization with panel of markers of facial development.

RESULTS: *Fgf8* mutant embryos are born with severe brain and midfacial defects and die within an hour after birth. In the facial region, defects first become apparent around E9.5 as a reduction in the amount of mesenchyme. Nile blue sulfate staining and TUNEL assays revealed a dramatic increase in the amount of cell death in the facial mesenchyme at E9.5, and BrdU incorporation assays suggest that cell proliferation is also slightly reduced. In contrast, immigration of neural crest cells into the facial area is not affected.

We also found that in the remaining nasal tissue *Erm*, *Pea3*, and *Tbx2*, genes that we had previously identified as FGF regulated genes [4], fail to be expressed close to the facial midline at E9.5. At E10.5, their expression surrounding the nasal pits is largely unaffected. Further analysis revealed that expression of *Fgf9* and *Fgf10*, which are expressed in an overlapping region with *Fgf8* at E10.5 but not E9.5, is maintained in *Fgf8* mutant embryos. Experiments *in vitro* confirmed that *Fgf9* can substitute for *Fgf8* in inducing and maintaining expression of these transcription factors when tested in facial explants. Therefore *Fgf9* seems to partially compensate for the lack of *Fgf8* after E10. In addition to its functions in the facial mesenchyme *Fgf8* is also essential for normal development of the olfactory epithelium.

Facial defects in *Wnt1::Cre; Fgfr1^{lox/flox}* embryos are much milder than in *Fgf8* mutants. Embryos develop midfacial clefts and cleft palate with incomplete penetrance. The midfacial cleft first becomes apparent at E11 as an increased distance of the media nasal processes, which subsequently fail to fuse. Analysis of *Alx3*, *Alx4*, *Msx1*, *Tbx2*, *Fgf8* and *Shh* expression revealed no differences between mutant and wildtype embryos at E10.5 and E11.5. Analysis of secondary palate development revealed normal outgrowth of mutant palatal shelves lateral to the tongue between E12.5 and E14. In contrast, elevation of the palatal shelves in the majority of the mutants was severely disturbed.

DISCUSSION & CONCLUSIONS: Our results demonstrate that *Fgf8* is essential for survival and normal development of the neural crest derived facial mesenchyme and suggest that other Fgf receptors in addition to *Fgfr1* are involved in the reception of the *Fgf8* signal.

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ACKNOWLEDGEMENTS: This work was supported by the DFG (SFB592-A12).