REVIEW ARTICLE

Mouse Models for Investigating the Developmental Basis of Human Birth Defects

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ABSTRACT: Clinicians and basic scientists share an interest in discovering how genetic or environmental factors interact to perturb normal development and cause birth defects and human disease. Given the complexity of such interactions, it is not surprising that 4% of human infants are born with a congenital malformation, and cardiovascular defects occur in nearly 1%. Our research is based on the fundamental hypothesis that an understanding of normal and abnormal development will permit us to generate effective strategies for both prevention and treatment of human birth defects. Animal models are invaluable in these efforts because they allow one to interrogate the genetic, molecular and cellular events that distinguish normal from abnormal development. Several features of the mouse make it a particularly powerful experimental model: it is a mammalian system with similar embryology, anatomy and physiology to humans; genes, proteins and regulatory programs are largely conserved between human and mouse; and finally, gene targeting in murine embryonic stem cells has made the mouse genome amenable to sophisticated genetic manipulation currently unavailable in any other model organism. (*Pediatr Res* **59: 749–755, 2006**)

Gene targeting takes advantage of endogenous cellular machinery that directs recombination between homologous DNA sequences to precisely replace an endogenous sequence with one that has been altered in a defined manner. With this approach, the effects of disrupting a specific regulatory sequence, protein domain or the function of an entire locus can to be determined in the absence of confounding genetic or environmental variability (1). The impact of gene targeting on the conduct of contemporary biomedical science has been profound and ever increasing as strategies have evolved from relatively straightforward germline knock-outs of gene function, to complex multi-allele systems for conditional regulation of gene expression, analyses of cell lineage, and for controlling survival of discrete cell populations.

Fibroblast growth factors (*Fgfs*) comprise a large family of genes that encode polypeptide signaling proteins with many important functions during mammalian development. Fgf proteins activate Fgf receptors (FgfRs) which are trans-membrane receptor tyrosine kinases (RTKs) that regulate cell survival, growth, migration, differentiation and patterning during embryogenesis. Several observations have established the importance of Fgf signaling in human development and disease: Fgfs transform fibroblasts and are aberrantly expressed in human tumors; they prevent apoptosis and support tumor growth and angiogenesis; Fgfs are also expressed during normal wound healing; mutations in Fgf receptor genes cause common human skeletal malformation syndromes including Apert, Crouzon, and Pfeiffer syndromes and achondroplasia (2). An entire volume of "Cytokine and Growth Factor Reviews" was recently devoted to Fgf and FgfR biology (2a). We have developed a series of genetically-engineered mice to investigate the functions of Fibroblast Growth Factors during cardiovascular, pharyngeal and limb development.

Fgf8 is an important member of this family expressed in multiple regions of the early embryo (3). Complete ablation of *Fgf8* function results in embryonic lethality (4) due to defective gastrulation (5). To assess the roles of Fgf8 in later cardiovascular, pharyngeal, skeletal, CNS or craniofacial development, we and others have used a variety of genetic strategies to bypass embryonic lethality resulting from germline inactivation.

In this paper, I will describe how our investigations into the complex phenotypes resulting from embryonic Fgf8 deficiency in mice led to the discovery of a molecular pathway that likely contributes to the pathogenesis of human 22q11 deletion syndromes, such as the DiGeorge syndrome (6,7). I will also discuss how we used conditional mutagenesis in

Abbreviations: Fgfs, fibroblast growth factors; **FgfRs,** Fgf receptors; **RTKs,** receptor tyrosine kinases.

mice to dissect the unique and required functions of Fgf8 in different temporo-spatial expression domains and thereby determine how it contributes to normal cardiovascular development.

CREATING AN Fgf8-DEFICIENT MOUSE: THE *Fgf8* HYPOMORPH

One approach to circumventing embryonic lethality is to use a so-called hypomorphic allele: an allele with decreased function relative to the normal allele (Fig. 1A). The antibiotic resistance gene (neo^r) used for selection of recombinant embryonic stem cell clones can negatively impact expression of adjacent genes (8). By inserting neo^r in the 3′ untranslated region of *Fgf8*, we generated a hypomorphic allele ($Fgf8^H$; Fig. 1B) that produces less full-length *Fgf8* mRNA than a wild-type allele (6). In contrast to the early embryonic lethal phenotype resulting from complete loss of function in $Fgf8^{-/-}$ embryos, or the normal phenotype of *Fgf8*-null heterozygotes ($Fgf8^{+/-}$, Fig. 2A), animals whose only functional allele of *Fgf8* is hypomorphic ($Fgf8^{H/-}$, Fig. 2E) survive embryogenesis but are born with multiple congenital anomalies and die post-natally (6,9). The complex array of developmental defects exhibited by *Fgf8* hypomorphs revealed essential, non-redundant functions of *Fgf8* in multiple aspects of post-gastrulation morphogenesis (Fig. 2E–K).

Fgf8 HYPOMORPHS PHENOCOPY HUMAN SYNDROMES RESULTING FROM DELETIONS ON CHROMOSOME 22q11

Fgf8 hypomorphs have a constellation of craniofacial, thymic and parathyroid, and cardiovascular defects (persistent Truncus Arteriosus, interrupted aortic arch) remarkably similar to that seen in human syndromes associated with deletions on chromosome 22q11, such as DiGeorge syndrome (Fig. 2) (6). This observation led us to consider the possibility that although *Fgf8* is not located within the typically deleted region of human chromosome 22q11, Fgf8 might function in molecular pathways affected by 22q11 genes and could thereby act as a modifier and contribute to phenotypic variability observed in patients with del22q11. To test this hypothesis in humans, we are using family studies and re-sequencing to examine variability at the *Fgf8* locus in controls and patients with cardiovascular disease related to del22q11. We are also investigating the possibility that *Fgf8* dysfunction
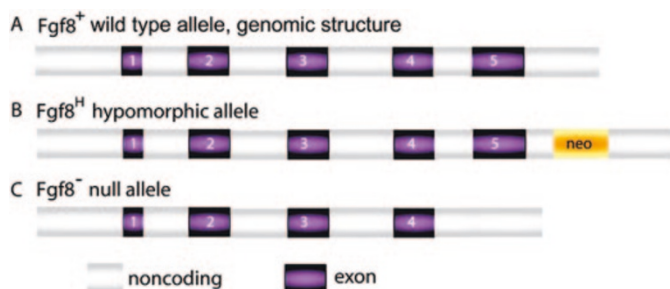

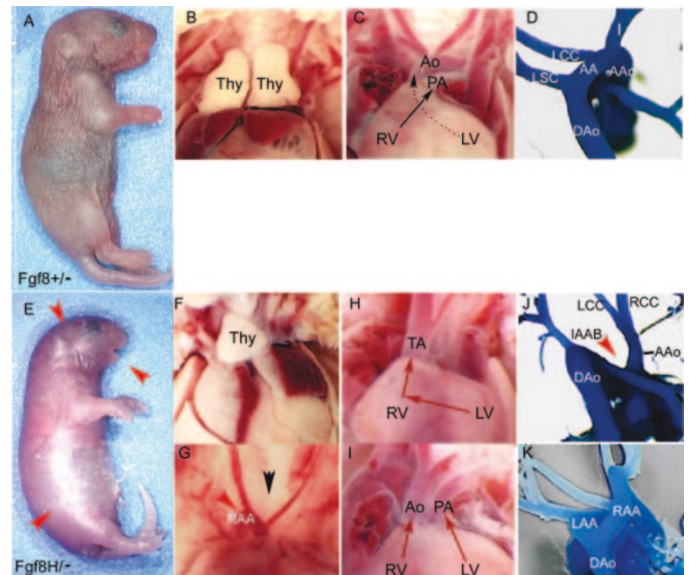**Figure 2.** Phenotypes of newborn Fgf8-null heterozygote (A, $Fgf8^{+/-}$) and an Fgf8 hypomorph (E, $Fgf8^{H/-}$). The normal thymus (B) is bi-lobed and sits at the base of the heart. Normal alignment of the aorta and pulmonary artery with respect to the ventricles is evident on dissection of the heart (C). A vascular cast viewed dorsally (D) shows a normal left transverse arch of the aorta which allows continuity between the ascending and descending aortae and gives rise to the head and neck vessels. Fgf8 deficiency in hypomorphs causes neonatal death associated with anasarca and obvious craniofacial defects (E, red arrowheads); thymic hypoplasia and ectopy (F) or thymic aplasia (G); lethal outflow tract defects in these mutants include persistent Truncus Arteriosus (H, a single outflow vessel from both ventricles) and transposition of the great arteries (I, aorta arises aberrantly from right ventricle and pulmonary artery from the left ventricle); aortic arch and vascular defects including right aortic arch (G, RAA) Interrupted aortic arch (J, IAAB) and double aortic arch (K). Thy, thymus; Ao, aorta; PA, pulmonary artery; RV, right ventricle; LV, left ventricle; I, innominate artery; LCC, left common carotid artery; RCC, right common carotid artery; LSC, left subclavian artery; AAo, ascending aorta; DAo, descending aorta; TA, persistent Truncus Arteriosus. Figure adapted from Frank *et al.*, Development 129:4591, copyright © 2002 The Company of Biologists Limited, with permission.

may independently contribute to heart malformations in humans.

In mice, we are investigating genetic and molecular interactions between *Fgf8*, its receptors, and genes within the murine genome homologous to those in *del22q11*.

Fgf8 GENETICALLY INTERACTS WITH THE DELETION 22Q11 GENE, *Crkl*

Crkl (*CRK-like*) is located within the typically deleted region of *del22q11* and disruption of its mouse orthologue, *Crkl*, causes cardiovascular, craniofacial and glandular features of *del22q11* syndrome (10). *Crkl* and its family members encode adaptor proteins that play important roles during intercellular signaling; they function in recruitment and activation of signaling complexes at focal adhesions and transduce signals downstream of several classes of RTKs (11,12). Since Fgf8 signaling *via* RTKs is required for cardiovascular and pharyngeal development and *Fgf8*-deficient mice phenocopy *del22q11* syndrome, we hypothesized that Fgf8 and Crkl function in a common molecular pathway that, when disrupted by haploinsufficiency of *Crkl*, contributes to the pathogenesis

Figure 1. Schematics of wild-type Fgf8 genomic (A), Fgf8 hypomorphic (B), and null alleles (C).

of human *del22q11* syndromes. Thus, we tested whether Crkl mediates Fgf8/FgfR signal transduction required for normal pharyngeal arch and cardiovascular development in mice (7). Our studies revealed that *Crkl* and *Fgf8* genetically interact during morphogenesis of structures affected in *del22q11* syndrome. For example, formation of the pharyngeal arch arteries occurs normally in *Fgf8*-null heterozygotes ($Fgf8^{+/-}$), but is disrupted at lower levels of Fgf8 that occur in *Fgf8* hypomorphs indicating that pharyngeal vasculogenesis is very sensitive to levels of Fgf8 signaling (6,9,13). Combined *Crkl/Fgf8* dysfunction profoundly perturbs pharyngeal vascular development in $Fgf8^{+/-};Crkl^{+/-}$ and $Fgf8^{+/-}; Crkl^{-/-}$ mutants and causes aortic arch and other vascular defects, similar to Fgf8 deficient hypomorphs (Fig. 3A–D). Similarly, outflow tract and intracardiac defects not observed in *Fgf8*-null heterozygotes occur in $Fgf8^{+/-};Crkl^{-/-}$ mutants. Fgf8 and Crkl also interact in craniofacial and appendicular skeletal development, and during formation of the pharyngeal glands (7). These phenotypes suggest that Crkl is required for normal responses to Fgf8 signaling in multiple morphogenetic pathways during embryogenesis.

DISRUPTED Fgf8 SIGNALING DUE TO Crkl DEFICIENCY MAY CONTRIBUTE TO THE PATHOGENESIS OF HUMAN *22q11* DELETION SYNDROMES

We questioned whether the interactions we observed between Fgf8 and Crkl reflect function of Crkl downstream of Fgf receptors, *versus* function in parallel molecular pathways that regulate these developmental events. To this end, we examined the effects of Crkl deficiency on behavior of Fgf8-dependent cell populations and expression of Fgf8 target genes and discovered that Crkl deficiency caused increased apoptosis of neural crest en route to the pharyngeal arches and outflow tract (Fig. 3E–N), and decreased the ability of cells to migrate in response to Fgf8. We further determined that Crkl deficiency disrupts MAP kinase activation and other aspects of intracellular biochemical signal transduction downstream of Fgf8-activated FgfRs *in vivo* (Fig. 4) (7). Modulation of Fgf/FgfR signal transduction by Crkl provides a mechanism for generating dosage-sensitive, context-dependent cellular responses (14–17). Our findings provide a molecular and biochemical basis for disrupted intercellular interactions resulting from 22q11 deletion, and provide new insight into the pathogenesis of the complex malformations seen in affected humans (7).

Fgf8 INTERACTS WITH MULTIPLE del22q11 GENES

Another critical *del22q11* gene is *TBX1*, a member the TBX family of transcription factors. *TBX1* mutations are associated with del22q11-like phenotypes in patients without detectable 22q11 deletions (18) and mice bearing deletions encompassing *Tbx1* or null mutations in *Tbx1* have cardiovascular and glandular features of the human syndrome (6,9,10,19–21). Recent studies by Guris and colleagues demonstrate that *Tbx1* and *Crkl* genetically interact and confirm that del22q11 phe-
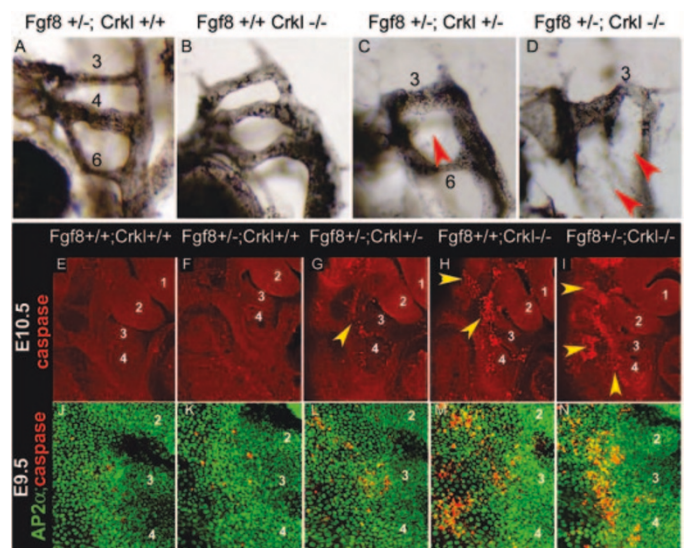

Figure 3. Fgf8 and Crkl genetically interact in vascular and pharyngeal development. A–D) Ink injections into the pharyngeal arch arteries of embryonic day (e) 10.5 controls and mutants reveals that decreasing dosage of Fgf8 and Crkl disrupts formation of the pharyngeal arch arteries (red arrowheads). Genotypes are listed above the panels and the pharyngeal arch arteries are numbered. Note loss of artery 4 (which forms the arch of the aorta) in the double heterozygote (C) and loss of both artery 4 and 6 in the $Fgf8^{+/-}; Crkl^{-/-}$ mutant. E–I) Whole mount anti-caspase immunohistochemistry reveals increasing apoptosis in the pharyngeal arches of embryos (bright red signal, yellow arrowheads) with decreasing *Fgf8* and *Crkl* gene dosage. J–N) Whole mount immunohistochemistry for anti-caspase (red) and anti-Ap2α (green labels ectoderm and neural crest cells) reveals abnormal apoptosis of neural crest cells (double labeled, yellow signal) en route to the outflow tract and pharyngeal arches of *Fgf8;Crkl* mutants.

notypes are caused by haploinsufficiency of at least two genes in the deletion and del22q11 is therefore a contiguous gene syndrome (22).

Mounting evidence from our lab and others indicates that Fgf8 operates in critical shared developmental and molecular pathways with Tbx1 during development. As with *Crkl*, *Fgf8* genetically interacts with *Tbx1* to increase penetrance of aortic arch defects (23), possibly through effects on *Gbx2* (24). *Fgf8* and *Tbx1* are both expressed in the pharyngeal epithelia and mesoderm, and Fgf receptors 1 and 2 are also widely expressed in all pharyngeal tissues (Moon, unpublished) so Fgf and Tbx1 pathways may overlap in some tissues (Fig. 4). There is an apparent cell-type-specific relationship between Tbx1 transcriptional activity and *Fgf8* expression: *Fgf8* is expressed in the pharyngeal ectoderm, but absent in endoderm, of *Tbx1*-null mutants (23) and a Tbx1-responsive enhancer regulates expression of an *Fgf8*-transgene in the developing outflow tract (25).

In summary, *Fgf8* interacts with at least two genes that are deleted in human *del22q11* that regulate cardiovascular, pharyngeal and craniofacial morphogenesis (Fig. 4). Additional studies are underway to determine whether Crkl dysfunction disrupts transduction of other Fgf signals in the pharynx and to determine whether Fgf8 effectors and Tbx1 interact at the transcriptional level.
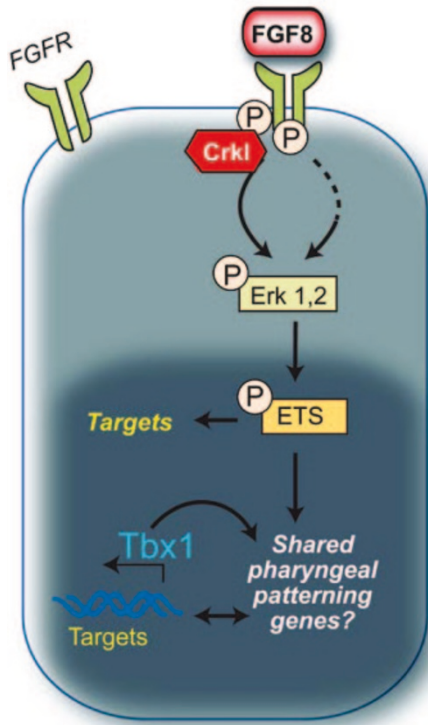
Figure 4. Schematic of a cell showing Crkl-mediated signal transduction in a cell responding to Fgf8 ligand. Note activation of MAP kinases (Erk1,2) and ETS proteins resulting in expression of Fgf8 target genes. Tbx1 is expressed in many Fgf8 target cells and may share activation of common target genes with the Fgf/Crkl pathway.

GENERATING A CONDITIONAL MUTAGENESIS SYSTEM

The complex phenotypes of *Fgf8* hypomorphs suggest that different *Fgf8* expression domains have discrete roles in diverse processes during embryonic organogenesis. However, the hypomorphic system does not allow us to dissect the functions of individual *Fgf8* expression domains or determine the cellular targets of Fgf8 signaling. Therefore, we developed a conditional mutagenesis system that permits us to ablate *Fgf8* function in specific temporo-spatial expression domains. With this approach, it is possible not only to bypass embryonic lethality, but to examine the effects of *Fgf8* loss-of-function in a particular tissue without the confounding effects of other developmental abnormalities.

The basic components of our conditional mutagenesis system are: 1) a conditional allele that functions as well as a wild-type allele until it is inactivated by a developmentally regulated molecular switch; and, 2) a molecular switch consisting of the Cre-recombinase/loxP system adapted from bacteriophage P1 (26). LoxP sites are 34 base pair DNA sequences recognized by the site-specific Cre recombinase enzyme. When two LoxP sites are correctly oriented with respect to one another, Cre catalyzes the deletion of DNA located between them (Fig. 5A–C). LoxP sites can be inserted anywhere in the genome using standard gene targeting. Thus Cre-mediated recombination/deletion can be used to change the structure of a gene; by controlling the timing and location of Cre activity, we can switch a gene off (or on) at a particular time and location (27).

To generate *Fgf8*-conditional alleles, we flanked exons critical for Fgf8 protein function with loxP sites, but positioned the sites in non-coding sequences to avoid disrupting *Fgf8* function before Cre recombination (Fig. 5A) (13,28,29). To avoid the hypomorphic effects associated with retention of neo^r in the targeted locus, we also used a different recombinase system (Flp/frt) to remove the neo^r gene (Fig. 5A,B) (30).

Mice with the conditional allele are bred to mice bearing the chosen Cre switch. In progeny containing the requisite conditional and Cre-expressing alleles, Cre-mediated recombination conditionally ablates *Fgf8* function (Fig. 5D) in the
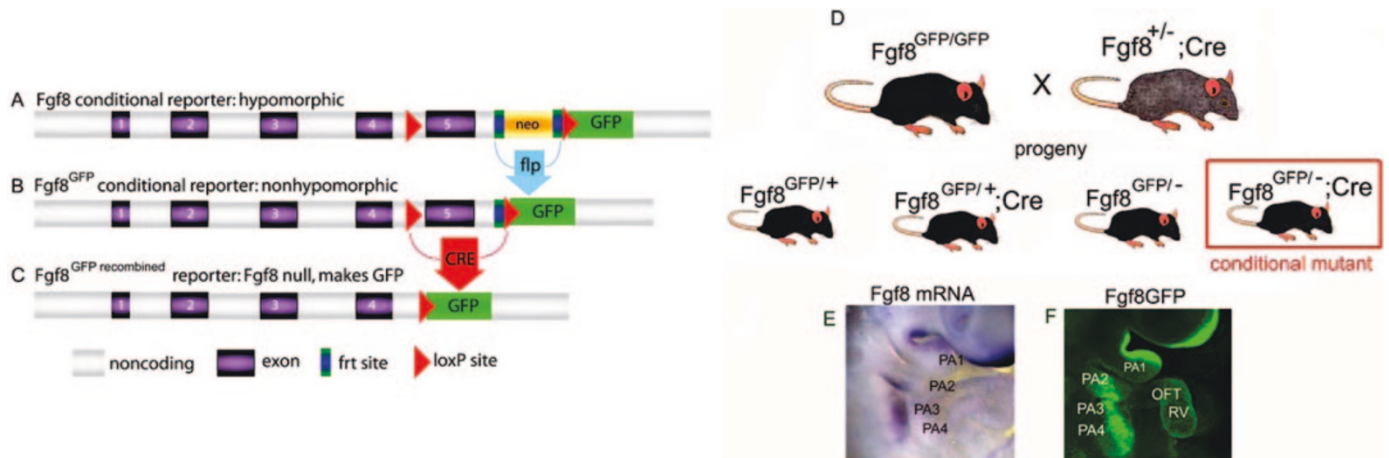

Figure 5. The Fgf8-conditional mutagenesis system. A–C) Schematic showing the targeted conditional reporter before removal of neo^r (A); note that loxP sites are positioned in non-critical region of the Fgf8 locus which permits normal allele function after Flp-mediated removal of neo^r (B); this is the non-hypomorphic conditional reporter. The Cre-recombined, GFP-expressing reporter allele (C); recombination of the conditional allele by Cre deletes exon 5, making this a null allele for producing Fgf8 protein, but brings a GFP reporter into frame to be produced under control of Fgf8 regulatory sequences in cells that would normally produce Fgf8. D) Breeding scheme used to generate conditional mutants for analysis. E) *In situ* hybridization reveals Fgf8 expression in the pharyngeal arches at e9.5 (blue signal, right lateral view, arches are numbered). F) Function of the GFP reporter; Fgf8^{GFP} (bright green fluorescence) is expressed in all the normal pharyngeal *Fgf8* expression domains after Cre recombines the locus.

desired temporo-spatial domain so that the resulting anatomic, cellular and molecular phenotypes can be assessed.

The power of this approach was evident when we used it to test several long-standing models of tissue interactions and Fgf function during limb outgrowth and patterning by specifically ablating *Fgf8* and *Fgf4* (a related Fgf) in the ectoderm of early mouse limbs (28,29,31). The results of our experiments, and similar work by Martin and colleagues (32–34), revealed that aspects of earlier models were incorrect, and contributed to the formation of new hypotheses of how signaling centers expressing these growth factors regulate limb outgrowth and patterning.

By generating additional Cre lines and refining our conditional alleles, we have adapted our conditional mutagenesis system to allow increasingly more precise manipulation of gene expression and better methods of assaying the conditional mutagenesis events. We have developed inducible Cre systems that provide additional levels of temporal regulation of Cre activity. One of the most informative adaptations we made was to design conditional alleles so that the recombination event not only disrupts endogenous gene function, but concurrently generates a unique allele that produces a reporter molecule instead of the regular gene product (such as Green Fluorescent protein, Fig. 5B,C). This type of conditional reporter allele allows us to detect functionally relevant inactivation of *Fgf8* in the cells that express it (Fig. 5E,F) (13,28,29). This feature is enormously helpful in characterizing the activity of the various Cre-expressing switches relative to expression of *Fgf8*, (Fig. 6A,B; Fig. 7D,H,I) which in turn contributes to more accurate interpretation of the effects of a particular conditional ablation event.

DISSECTING THE ROLES OF *Fgf8* IN DIFFERENT EXPRESSION DOMAINS USING CONDITIONAL MUTAGENESIS

We have used conditional mutagenesis to determine how *Fgf8* dysfunction causes the various components of the *Fgf8* hypomorphic phenotype, with an emphasis on cardiovascular development. Cardiovascular morphogenesis requires specification, differentiation, proliferation and migration of cells from diverse locations in the embryo. Inductive interactions between cell populations and regionally-specific transcription factor hierarchies regulate distinct morphogenetic events during heart development; disruption of these events by mutation or environmental insult usually results in malformations that affect discrete cardiac substructures and leave the rest of the organ structurally and functionally intact (35,36). The observation of complex cardiovascular defects affecting the heart itself (ventricular septal defects, valve abnormalities), the outflow tract (rotation and septation defects, Fig. 2H,I) and the vasculature (aortic arch and subclavian artery defects, Fig. 2J,K) in *Fgf8* hypomorphs led us to investigate the required functions of Fgf8 in discrete aspects of cardiac and vascular development.

The embryonic pharyngeal arch arteries are remodeled to form the arch of the aorta and the great vessels of the head and neck; these vessels develop adjacent to *Fgf8*-expressing pha-
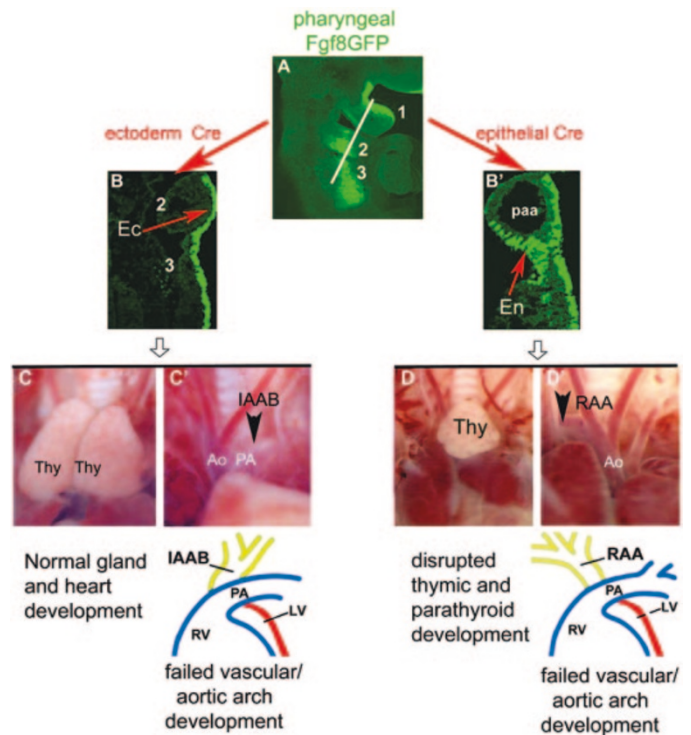

Figure 6. Conditional ablation of *Fgf8* in the pharyngeal ectoderm *versus* endoderm reveals domains-specific functions of Fgf8 signaling in the pharynx. *A)* Right lateral view of intact, embryonic day (e) 9.5 $Fgf8^{GFP\ recombined/+}$ mouse embryo stained with anti-GFP antibody (fluorescent green) shows all *Fgf8* expression domains at this stage. White line indicates plane of sections in panels *B* and *B'*. Pharyngeal arches are numbered. *B)* Coronal cross-section of e9.5 $Fgf8^{GFP/+}$; ectodermal Cre embryo stained with anti-GFP antibody has GFP staining (indicating recombination of the locus and inactivation of *Fgf8*) only in the ectodermal layer (Ec, red arrow). Pharyngeal arches 2 and 3 are numbered. HT, heart. *B')* Coronal cross-section of e9.5 $Fgf8^{GFP/+}$; epithelial Cre embryo stained with anti-GFP antibody has GFP staining (indicating *Fgf8* inactivation in the ectoderm and endoderm, En, red arrow). The third pharyngeal arch artery is labeled paa. *C, C')* Chest dissections of newborn *Fgf8* ectodermal conditional mutants. Specific ablation of *Fgf8* from the pharyngeal ectoderm permits normal thymic development (*C*) but causes aortic and vascular defects including interrupted aortic arch (*C'*, IAAB, black arrowhead). A schematic of the outflow and great vessels is shown below panel *C'*. D, D') Chest dissections of newborn *Fgf8* epithelial conditional mutants. Loss of *Fgf8* function in the endoderm disrupts thymus (thy) formation (*D*). Because *Fgf8* is also ablated in the ectoderm in these epithelial mutants, they have aortic arch defects, in this case, a right aortic arch (*D'*, RAA, black arrowhead). A schematic of RAA is shown below. Figure adapted from Macatee *et al.*, Development 130:6361, copyright © 2003 The Company of Biologists Limited, with permission.

ryngeal epithelia (Fig. 6*A,B*) and *Fgf8* hypomorphs have lethal aortic arch defects (Fig. 2*J,K*). The thymus and parathyroid glands are derived from the pharyngeal endoderm. We considered the fact that *Fgf8* is expressed in both the pharyngeal ectoderm and endoderm, and hypothesized that Fgf8 emanating from these different tissues has separable functions during pharyngeal vascular *versus* thymic and parathyroid gland development. To test this hypothesis, we generated novel Cre-expressing lines and used the $Fgf8^{GFP}$ conditional reporter allele to confirm that we could independently ablate *Fgf8* in ectoderm and endoderm (Fig. 6*B–D*). Remarkably, complete ablation of *Fgf8* in the pharyngeal ectoderm recapitulated the vascular defects seen in *Fgf8* hypomorphs but
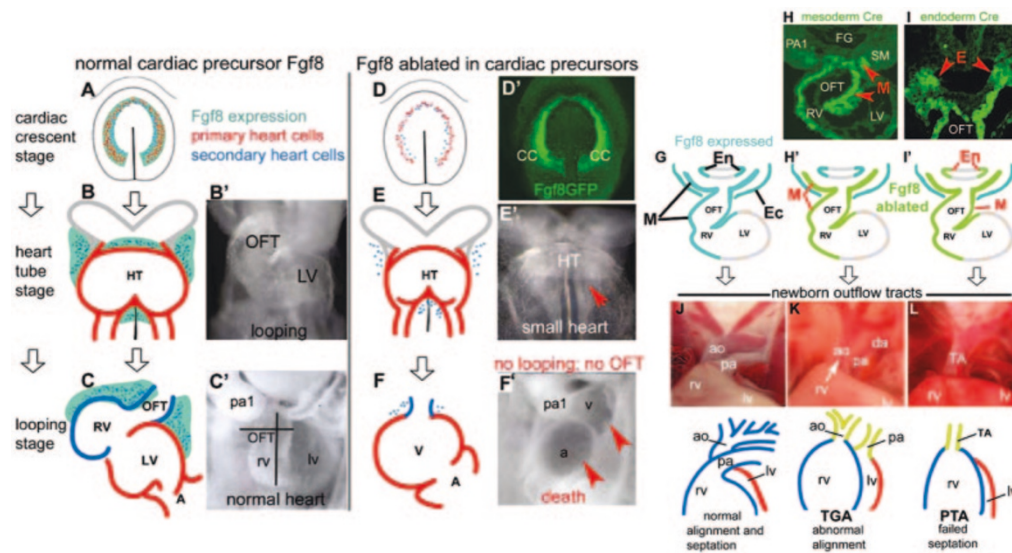
Figure 7. Evaluating the roles of Fgf8 in cardiac and outflow tract development. *A*) Schematic anterior view of a pre-somite mouse embryo showing Fgf8 expression in the cardiac precursor cells in the cardiac crescent. The Fgf8 expression domain is light blue, primary heart tube precursors are shown as red dots and secondary heart precursors that will contribute to the outflow tract are shown as blue dots. *B*) Schematic ventral view of the primitive heart tube before looping. *B')* Wild-type embryo heart at the early looping stage with early outflow tract formation. *C*) Schematic of normal heart at stage shown in B'. *C')* Normal heart of an e9.5 embryo. *D*) Schematic showing Fgf8 ablation from the cardiac crescent/heart precursors at the pre-somite stage. *D')* GFP-stained pre-somite embryo showing Fgf8 ablation throughout the cardiac crescent (cc). *E*) Schematic of small primary heart tube resulting from ablation of Fgf8 in cardiac precursors. *E')* Mutant embryo at the same somite stage as control shown in B'. Note small heart tube and no evidence of outflow tract formation. *F*) Schematic of failed addition of right ventricle and outflow tract, and absent looping resulting in ventricular dilatation and death in an Fgf8 mutant. *F')* Mutant embryo at the same somite stage as control shown in C'. Note single ventricle, marked cardiac dilatation and absent outflow tract; the embryo had a heart beat but no circulation. *G*) Schematic of transverse section of e8.5 embryo (looping stage) at the level of the second pharyngeal arch and heart. As noted above, *Fgf8* is expressed (blue color) in multiple domains including mesoderm of the right ventricle and outflow tract. *H*) Transverse section of e8.5 *Fgf8^{GFP/+}* embryo at the level of the second pharyngeal arch and heart after Cre-mediated ablation of Fgf8 in the cardiac and pharyngeal mesoderm (M). A schematic of this ablation is shown in H'. *I*) Transverse section of e8.5 *Fgf8^{GFP/+}* embryo at the level of the second pharyngeal arch and heart after Cre-mediated ablation of Fgf8 in the early pouch endoderm (E) and cardiac and pharyngeal mesoderm. A schematic of this ablation is shown in I'. *J*) Normal heart and outflow tract in a wild-type newborn mouse. *K*) Ablation of Fgf8 in the mesoderm disrupts alignment and rotation of the outflow tract relative to the ventricles causing transposition of the great arteries (TGA). A schematic is shown below the photo. *L*) Ablation of Fgf8 in the early pharyngeal endoderm prevents septation of the outflow tract, resulting in persistent Truncus Arteriosus (PTA). A schematic is shown below the photo.

permitted normal glandular and cardiac development (Fig. 6 C,C'), whereas ablation of *Fgf8* in the pharyngeal endoderm disrupted formation of the thymus and parathyroid glands (Fig. 6D,D').

By completely ablating Fgf8 in cardiac precursor cells before formation of the early heart tube (Fig. 7A–D), we discovered that Fgf8 has a critical role in the survival and proliferation of these precursors: loss of Fgf8 in this domain results in small heart tubes and outflow tracts (Fig. 7E,F). *Fgf8* mesodermal ablation mutants (Fig. 7H,H') have defects of outflow tract alignment such as transposition of the great arteries (Fig. 7K) and double outlet right ventricle. In contrast, if we ablate *Fgf8* function in the early pharyngeal pouch endoderm (Fig. 7I,I'I), animals fail to septate the outflow tract and are born with persistent Truncus Arteriosus (Fig. 7L). These results explain why *Fgf8* hypomorphs can exhibit an entire range of developmentally distinct outflow tract defects (Fig. 2H,I) and demonstrate that the requirements for Fgf8 in the processes of outflow tract septation and rotation/alignment are not only temporally distinct from those for heart tube and outflow tract formation, but are spatially separate from one another (37).

Our studies have also begun to address the cellular targets and required Fgf receptors that respond to Fgf8. Although earlier *in vitro* assays suggested particular Fgf ligand/receptor

preferences exist and supported a model of primarily paracrine signaling (38,39), it has been unclear how accurately preferences exhibited by cultured cells reflect ligand/receptor interactions that occur in the highly plastic, tissue-specific environments of developing embryos. Our work reveals that Fgf8 (and other Fgfs) have both paracrine and autocrine activities and can signal through non-optimal ligand-receptor interactions depending on cellular context (7) (Francis and Moon, unpublished results).

Thus far, our work has focused on defining the required regions of Fgf signaling in cardiovascular and pharyngeal development and generating the genetic tools needed to predictably regulate gene functions in specific expression domains. We are now pursuing the molecular and cellular events that result from perturbed growth factor signaling and how they lead to altered cardiac form; we are also evaluating functional redundancy of different Fgfs and their receptors during pharyngeal and cardiovascular morphogenesis. Since the primitive cardiovascular system must support the entire organism while critical aspects of its own development are still underway, our ability to reliably create specific, homogeneous cardiovascular defects in mouse embryos will allow us to ultimately investigate how structural, hemodynamic and environmental inputs interact with, and modify, the genetic

and molecular programs controlling cardiac morphogenesis in normal and pathologic conditions.

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