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FGF10 signaling in heart development, homeostasis, disease and repair

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Abstract

Essential muscular organ that provides the whole body with oxygen and nutrients, the heart is the first organ to function during embryonic development. Cardiovascular diseases, including acquired and congenital heart defects, are the leading cause of mortality in industrialized countries. Fibroblast Growth Factors (FGFs) are involved in a variety of cellular responses including proliferation, differentiation and migration. Among the 22 human/mouse FGFs, the secreted FGF10 ligand through the binding of its specific receptors (FGFR1b and FGFR2b) and subsequent activation of downstream signaling is known to play essential role in cardiac development, homeostasis and disease. FGF10 is one of the major marker of the early cardiac progenitor cells and a crucial regulator of differentiated cardiomyocyte proliferation in the developing embryo. Increasing evidence support the hypothesis that a detailed understanding of developmental processes is essential to identify targets for cardiac repair and regeneration. Indeed the activation of resident cardiomyocyte proliferation together with the injection of cardiac progenitors represent the most promising therapeutical strategies for cardiac regenerative medicine. The recent findings showing that FGF10 promotes adult cardiomyocyte cell cycle reentry and directs stem cell differentiation and cell reprogramming toward the cardiogenic lineage provide new insights into therapeutical strategies for cardiac regeneration and repair.

Keywords

FGF10, FGFR1/2, heart development, cardiomyocyte, cardiac regeneration

1 **Introduction**

2
3 The heart is an essential muscular organ that pumps blood and provides the whole body with
4 oxygen and nutrients. During embryonic development, the heart is the first organ to form and
5 cardiac morphogenesis is a tightly regulated process. Cardiovascular diseases including
6 congenital and acquired heart diseases are the leading cause of mortality in industrialized
7 countries (Writing Group *et al.*, 2016).

8
9 By mediating a variety of cellular response, Fibroblast Growth Factors (FGFs) are known to
10 play an essential role in cardiac development, homeostasis and disease. The human/mouse FGF
11 family comprises 22 members including secreted and intracellular FGFs. Secreted FGFs bind
12 and activate cell surface tyrosine kinase receptors (FGF receptors; FGFRs) encoded by four
13 genes (FGFR1-4). The alternate splicing of FGFR genes results in the generation of seven
14 different receptors, each of them displaying distinct ligand-binding properties (Zhang *et al.*,
15 2006). In contrast to secreted FGFs, intracellular FGFs serve as cofactors for voltage gated
16 sodium channels and other molecules (Ornitz and Itoh, 2015). Interaction between secreted
17 FGFs and their specific receptors is tightly regulated by extracellular binding proteins including
18 heparan sulfates and the Klotho family proteins that serve as cofactors and confer unique ligand-
19 receptor binding properties. Activated tyrosine kinase FGF receptors (FGFRs) mediate diverse
20 intracellular signaling cascades including the RAS-MAPK, PI3K-AKT, PLC γ , and STAT
21 signaling pathways (Ornitz and Itoh, 2015). Phylogenetic analysis suggest that secreted FGFs
22 can be grouped into 5 subfamilies of paracrine FGFs and one subfamily of endocrine FGFs.
23 The current consensus suggests that FGF10 belongs to a subfamily that comprises FGF3, FGF7,
24 FGF10 and FGF22. Receptor-ligand specificities are well described. Indeed, FGF3, 7, 10, and
25 22 have been shown to activate preferentially the IIIb splice variant of FGFR2. In addition
26 FGF3 and FGF10 also activate the IIIb splice variant of FGFR1 (Zhang *et al.*, 2006).
27 Nevertheless, ablation studies together with overlapping expression patterns strongly suggest
28 potential functional redundancy between FGF family members in the developing and adult
29 heart. Finally, the existence of heterodimer formation between FGFs and FGFRs may further
30 increase receptor-ligand interaction possibilities (Sun *et al.*, 2002) and thus the diversity of FGF
31 signaling.

32
33 Here we will review a detailed understanding of FGF signaling in cardiovascular development,
34 homeostasis, disease and repair, focusing on the particular role of the FGF10/FGFR1/FGFR2
35 pathway.

36 **Developmental role of the FGF10 signaling**

37
38
39 Heart development is an extremely complex process that can be divided in two major growth
40 phases distinguished by a shift in the major site of proliferation from an extracardiac progenitor
41 cell population to fetal cardiomyocytes. The early embryonic phase relies on the extensive
42 proliferation of cardiac progenitor cells termed the Second Heart Field (SHF) and their
43 progressive addition to the developing heart tube. Precise spatiotemporal control of second
44 heart field progenitor cell proliferation-differentiation balance is required for normal heart tube
45 elongation. Cardiac neural crest (CNC) cells, a second extracardiac cell population, play a
46 critical role in early heart development (Hutson and Kirby, 2007). Concomitant with SHF cell
47 addition to the outflow tract (OFT) of the heart, CNC migrate from the dorsal neural tube into
48 the OFT. Interactions between CNC cells and SHF progenitors are critical determinants for the
49 correct addition of SHF cells to the heart tube. In contrast to early heart tube development, fetal
50 heart growth is achieved through the proliferation of differentiated cardiomyocytes which tight

51 control is essential for the correct morphogenesis of the heart. Indeed, perturbations in the
52 regulation of fetal cardiomyocyte proliferation lead to congenital heart defects.

53

54 During the early embryonic phase of heart morphogenesis, proper communication between
55 cardiac progenitor cells is a prerequisite for correct heart tube elongation, looping and arterial
56 pole alignment. FGFs are among the critical signals required for proper early cardiac
57 morphogenesis (Kelly, 2012). By ensuring communication within and between developing
58 heart progenitors, FGF signaling leads to their tight regulation of proliferation and specification.
59 Indeed, transgenic mouse models with conditional inactivation of *Fgfr1/2*, conditional
60 overexpression of *Sprouty2* (*Spry2*, which encodes an FGF signaling antagonist) or conditional
61 ablation of *Frs2* (encoding a MAPK/ PI3K signaling adaptor protein) within the second heart
62 field progenitor cell population revealed that interrupting autocrine FGF signaling in SHF
63 mesoderm, by compromising SHF progenitor cell proliferation and by indirectly reducing
64 cardiac neural crest cell recruitment into the outflow tract cushions, causes outflow tract
65 misalignment and subsequently impaired cardiac morphogenesis (Park *et al.*, 2008; Zhang *et al.*,
66 2008). While FGFR-dependent regulation of second heart field proliferation seems to
67 depend on the PI3K/AKT pathway (Luo *et al.*, 2015), the Ras/Erk downstream signaling seems
68 to be required in the regulation of myocardial specification (Hutson *et al.*, 2010; Rochais *et al.*,
69 2009). All these studies thus strongly reveal iterative roles for FGF signaling in OFT
70 development.

71

72 Multiple FGF ligands have been described to be expressed in cardiac progenitors and
73 surrounding tissues (**Figure 1A-C**). FGF10 was identified as a specific endogenous marker of
74 the SHF (Kelly *et al.*, 2001). While *Fgf10* expression is restricted to SHF progenitors (Kelly *et al.*,
75 2001), *Fgf8* is also expressed in the adjacent pharyngeal ectoderm and endoderm (Ilagan *et al.*,
76 2006; Mesbah *et al.*, 2012). *Fgf15* expression has been detected in the pharyngeal endoderm
77 (Vincentz *et al.*, 2005) and *Fgf3* is expressed in the pharyngeal endoderm and ectoderm (Urness
78 *et al.*, 2011).

79 The Wnt/ β -catenin signaling pathway a key regulator of SHF development transcriptionally
80 controls *Fgf10* expression within SHF progenitors (Cohen *et al.*, 2007). Crucial transcription
81 factors of SHF cardiac progenitor cell deployment are also known to control *Fgf10* expression.
82 ISL1 and NKX2-5 control the expression of *Fgf10* in the SHF, through competitive binding to
83 common regulatory elements in an intronic cardiac enhancer, thus respectively activating
84 expression in progenitor cells and repressing transcription in differentiated myocytes
85 (Watanabe *et al.*, 2012). TBX1 also activates *Fgf10* through T-box binding sites in the same
86 enhancer element (Watanabe *et al.*, 2012).

87

88 *Fgf10*-null embryos, which die at birth due to lung aplasia, display altered heart morphology.
89 In addition to the absence of pulmonary arteries and veins, *Fgf10* knockout embryos display an
90 abnormal positioning of the ventricular apex in the thoracic cavity (Marguerie *et al.*, 2006;
91 Rochais *et al.*, 2014). Nevertheless, early SHF deployment and subsequent heart tube
92 elongation are not affected by *Fgf10* deletion. In contrast, deletion of the main FGF10 receptor,
93 *Fgfr2b*, leads to major congenital heart defects including ventricular septal defects, OFT
94 alignment defects, and thin and poorly trabeculated ventricles (Marguerie *et al.*, 2006) strongly
95 suggesting the existence of functional redundancy between FGF10 and other FGFR2b ligands
96 during the early steps of heart development. FGF8 appears to be the major ligand regulating
97 cardiac progenitor cell deployment. A series of conditional loss of function experiments has
98 revealed that *Fgf8*, through a cell-autonomous mechanism, is required for SHF expansion and
99 thus OFT elongation, septation and subsequent ventriculoarterial alignment (Ilagan *et al.*, 2006;
100 Park *et al.*, 2008). Interestingly, the fact that heterozygous deletion of *Fgf10* in combination

101 with homozygous loss of mesodermal *Fgf8* expression results in more severely altered anterior
102 heart development (Watanabe *et al.*, 2010) strongly supports mesodermal FGF8 and FGF10
103 functional redundancy. In addition, FGF3 and FGF10 have been also shown to play redundant
104 and dosage sensitive requirement during heart tube elongation (Urness *et al.*, 2011). All these
105 studies highlight that critical FGF dosage, including FGF10, is crucial for SHF proliferation
106 and deployment and thus for normal cardiac morphogenesis.

107
108 During the second phase of heart development (after embryonic day E10.5), subsequent growth
109 and remodeling of the myocardium occur without significant further addition of cardiac
110 progenitor cells to the heart. Instead, regulated proliferation of cardiac myocytes drives growth
111 of the atrial and ventricular chambers. Tight spatio-temporal regulation of fetal cardiomyocyte
112 proliferation thus appears to be required for proper heart formation and impairment of
113 cardiomyocyte proliferation during fetal stages also results in congenital heart defects (Ahuja
114 *et al.*, 2007). FGF signals, through cell-autonomous or paracrine mechanisms, have been
115 described as crucial regulators of fetal cardiomyocyte proliferation (**Figure 1D-E**) (Smith and
116 Bader, 2007). FGF ligands originating from the endocardium and the epicardium, including
117 FGF9, FGF16 and FGF20, have been shown to regulate cardiomyocyte proliferation (Hotta *et al.*
118 *et al.*, 2008; Lavine *et al.*, 2005; Lu *et al.*, 2008). Recent reports revealed the implication of FGF10
119 in the regulation of fetal cardiomyocyte proliferation. *Fgf10* mutant heart analysis demonstrates
120 that FGF10 signaling, through a cell-type autonomous mechanism, specifically controls fetal
121 right ventricular cardiomyocyte proliferation. In fact, at fetal stages, FGF10/FGFR2b signaling
122 promotes cardiomyocyte proliferation through the phosphorylation of the FOXO3 transcription
123 factor and subsequent downregulation of the cyclin dependent kinase inhibitor p27^{kip1}
124 expression (Rochais *et al.*, 2014). In addition, myocardial FGF10 signaling, through the
125 paracrine activation of FGFR1 and FGFR2 in the epicardium, has been suggested to promote
126 epicardial-derived cell migration into the compact myocardial layer (Vega-Hernandez *et al.*,
127 2011). In this study, the impairment in cardiac fibroblast numbers observed in *Fgf10*-mutant
128 hearts, results indirectly in reduced fetal cardiomyocyte proliferation.

129
130 Despite cardiomyocyte proliferation, FGF signals, through redundant function of FGFR1 and
131 FGFR2, originating from the epicardium and the endocardium, play pivotal role in coronary
132 vasculature development (**Figure 1D**). In fact, in embryonic mouse heart, myocardial FGFR1/2
133 signaling by triggering Hedgehog signaling activation, *Vegf* and *Angiopoietin-2* expression,
134 indirectly participate to the coronary vascular plexus formation and thus coronary vessel
135 deployment (Lavine *et al.*, 2006). Here the precise requirement of the FGF10 ligand has not
136 been explored.

137 Several members of the FGF family are expressed in the vascular network (Beenken and
138 Mohammadi, 2009; Presta *et al.*, 2005). While the most studied FGF member, FGF2, is a potent
139 inducer of angiogenesis, other FGFs (FGF 1, 2, 5, 7, 8, 16 and 18), but not FGF10, are expressed
140 in endothelial and vascular smooth muscle cells (Antoine *et al.*, 2005). Despite predominant
141 FGFR1 and FGFR2 expression endothelial cells (Presta *et al.*, 2005), mouse specific deletion
142 of *Fgfr1* and *Fgfr2* in both endothelial and hematopoietic cell lineages has no impact on normal
143 vascular development (House *et al.*, 2016; Oladipupo *et al.*, 2014). In contrast, in zebrafish,
144 global FGFR signaling inhibition using allosteric inhibitor or a dominant negative form of the
145 receptor revealed the critical requirement for FGF signaling in the maintenance vascular
146 function and integrity (De Smet *et al.*, 2014; Murakami *et al.*, 2008). This strongly suggests, in
147 mammals, the existence of functional redundancy between FGFR1, FGFR2 and FGFR3 that
148 also expressed in endothelial cells.

149
150

151 **FGF10 signaling in cardiac homeostasis**

152

153 Soon after birth, the ability of cardiomyocytes to proliferate is strongly reduced, and cardiac
154 growth transitions from hyperplastic to hypertrophic (Pasumarthi and Field, 2002). For nearly
155 a century, the adult heart has been considered to be a post-mitotic organ; however, recent studies
156 have highlighted the importance of the homeostasis of the adult heart in physiological
157 conditions. Indeed extensive studies on adult mammalian hearts including the human heart have
158 led to a consensus that new cardiomyocytes are indeed generated throughout life (Bergmann *et*
159 *al.*, 2009; Bergmann *et al.*, 2015; Soonpaa and Field, 1998). In the healthy adult murine and
160 human heart, cardiomyocyte renewal is currently estimated at 0.5% to 2% per year
161 (Eschenhagen *et al.*, 2017).

162

163 Diverse FGFs and downstream signals, including FGF1, FGF2, FGF10 and p38 MAP kinase
164 have been shown to be involved in the regulation of adult cardiomyocyte renewal (**Figure 2**).
165 *In vitro* studies initially described FGF2 as a potent positive regulator of cardiomyocyte
166 proliferation (Pasumarthi and Field, 2002). *In vitro* and *in vivo* experiments indicate that p38
167 MAP kinase inhibition alone (Jopling *et al.*, 2012b) or in combination with FGF1 treatment
168 (Engel *et al.*, 2006; Engel *et al.*, 2005), leads to partial cardiomyocyte dedifferentiation and
169 cell-cycle progression. Furthermore, in the adult zebrafish, epicardial cells addition to the
170 ventricle has been shown to support cardiac homeostasis in an FGF-dependent fashion (Wills
171 *et al.*, 2008). Finally, in the adult mouse heart, FGF10 has been described to be a potent
172 regulator of cardiomyocyte proliferation. Indeed, temporal *Fgf10* overexpression rapidly
173 enhanced adult cardiomyocyte cell cycle re-entry leading to increased ventricular wall
174 thickness. While FGF10 regulation of fetal cardiomyocyte proliferation seems to occur through
175 the FGFR2b, FGF10 may activate predominantly the FGFR1b to promote adult cardiomyocyte
176 proliferation (Rochais *et al.*, 2014).

177

178 **Implication of FGF10 signaling in cardiovascular diseases and repair**

179

180 Cardiovascular diseases are the leading cause of mortality in industrialized countries (Writing
181 Group *et al.*, 2016). Characterized by any molecular, cellular and physiological change in the
182 myocardium, coronary vessels or valves, cardiac diseases result in cardiomyocyte loss and
183 impaired cardiac function that ultimately lead to congestive heart failure. Multiple FGFs
184 including FGF10 signaling have been described to play pathophysiological roles in the
185 cardiovascular system (Itoh *et al.*, 2016).

186

187 Diverse studies highlighted a role for the FGFR1/2 signaling in the neovascularization after
188 injury (**Figure 2**). In the zebrafish injured heart, epicardial *Fgfr2* expression is upregulated and
189 FGFR signaling blockade leads to a failure in coronary neovascularization, resulting in severely
190 impaired cardiac regeneration (Lepilina *et al.*, 2006). In addition, neovascularization and
191 vascular remodeling in response to injury is severely impaired in endothelial specific FGFR1/2
192 deficient mice (House *et al.*, 2016; Oladipupo *et al.*, 2014). Finally, endothelium-targeted
193 overexpression of constitutively active FGFR2 post-myocardial infarction results in anti-
194 apoptotic action with enhanced angiogenesis (Matsunaga *et al.*, 2009).

195

196 While zebrafish adult heart fully regenerates after injury (Poss *et al.*, 2002), damaged adult
197 mammalian myocardium is replaced by fibrotic scar tissue. The MAPK pathway plays a crucial
198 role in adult zebrafish heart regeneration (**Figure 2**). Indeed, the induction of p38-MAPK
199 activity prevents cardiomyocyte proliferation and subsequent heart regeneration (Jopling *et al.*,
200 2012a). In the adult mouse heart, p38 inhibitor injection, after acute myocardial injury,

201 enhances cardiomyocyte and endothelial cell proliferation and preserves cardiac remodeling
202 and function (Engel *et al.*, 2006) strongly revealing that downstream FGF signaling may be
203 beneficial to improve the limited innate regenerative capacities of the adult mammalian heart.
204 In contrast to the adult heart, neonatal mammalian heart, including mouse, pig and human,
205 possesses extensive regenerative capacities (Haubner *et al.*, 2016; Porrello *et al.*, 2011; Zhu *et*
206 *al.*, 2018). Nevertheless, the rapid and dramatic decrease in cardiomyocyte proliferation rate
207 during the first week of postnatal life (Pasumarthi and Field, 2002) results in severely limited
208 regenerative capacities in adult, strongly supporting the hypothesis that a detailed
209 understanding of the regulation of fetal cardiomyocyte proliferation is essential to identify
210 targets for cardiac regeneration. As described above, FGF10 has been identified as a crucial
211 regulator of fetal cardiomyocyte proliferation (Rochais *et al.*, 2014). The fact that decreased
212 myocardial *Fgf10* expression has been observed in mouse postnatal heart during the time
213 window where cardiomyocytes exit from the cell cycle (Rochais *et al.*, 2014), coinciding with
214 the loss of regenerative capacities, suggests that FGF10 signaling may play a role in cardiac
215 regeneration. However *Fgf10* overexpression in the neonatal mouse heart does not promote
216 beneficial effects on post-natal cardiac regeneration (Rubin *et al.*, 2013). Nevertheless, the
217 ability for FGF10 to specifically induce adult cardiomyocyte cell-cycle reentry in physiological
218 conditions suggests that FGF10 might be able to promote cardiomyocyte renewal in the adult
219 injured heart (Rochais *et al.*, 2014).

220

221 Together with the stimulation of existing cardiomyocyte renewal, cell therapy using the
222 injection or tissue-based implantation of cardiac progenitor cells and direct reprogramming
223 represent relevant therapeutic options for cardiac regenerative medicine (Tzahor and Poss,
224 2017). Several studies revealed the requirement of FGF10 signaling during stem cell
225 specification into the cardiogenic lineage (**Figure 2**). Indeed, FGF10 signaling has been shown
226 to play an important role in promoting cardiomyocyte differentiation in both embryonic and
227 induced pluripotent stem cells (Chan *et al.*, 2010). Furthermore, in addition to improve the
228 quality of cardiac reprogramming in mouse fibroblasts, and in combination with FGF2 and the
229 vascular endothelial growth factor (VEGF), FGF10, through the RAS-MAPK and PI3K/AKT
230 pathways, is able to convert partially reprogrammed cells into functional cardiomyocyte-like
231 cells (Yamakawa *et al.*, 2015).

232

233 **Conclusions**

234

235 All the studies described in this review highlighted the crucial role for the FGF10 ligand and
236 the related FGFR1/2 signaling in heart development, homeostasis and disease. The recent
237 findings revealing a crucial role for FGF10 in controlling both adult cardiomyocyte cell cycle
238 reentry and stem cell differentiation and cell reprogramming toward the cardiogenic lineage
239 provide potential therapeutic strategies for cardiovascular diseases.

References

- Ahuja, P., Sdek, P., MacLellan, W.R. (2007). Cardiac myocyte cell cycle control in development, disease, and regeneration. *Physiol Rev.* 87, 521-544.
- Antoine, M., *et al.* (2005). Expression pattern of fibroblast growth factors (FGFs), their receptors and antagonists in primary endothelial cells and vascular smooth muscle cells. *Growth Factors.* 23, 87-95.
- Beenken, A., Mohammadi, M. (2009). The FGF family: biology, pathophysiology and therapy. *Nat Rev Drug Discov.* 8, 235-253.
- Bergmann, O., *et al.* (2009). Evidence for cardiomyocyte renewal in humans. *Science.* 324, 98-102.
- Bergmann, O., *et al.* (2015). Dynamics of Cell Generation and Turnover in the Human Heart. *Cell.* 161, 1566-1575.
- Chan, S.S., *et al.* (2010). Fibroblast growth factor-10 promotes cardiomyocyte differentiation from embryonic and induced pluripotent stem cells. *PLoS One.* 5, e14414.
- Cohen, E.D., *et al.* (2007). Wnt/beta-catenin signaling promotes expansion of Isl-1-positive cardiac progenitor cells through regulation of FGF signaling. *J Clin Invest.* 117, 1794-1804.
- De Smet, F., *et al.* (2014). Fibroblast growth factor signaling affects vascular outgrowth and is required for the maintenance of blood vessel integrity. *Chem Biol.* 21, 1310-1317.
- Engel, F.B., Hsieh, P.C., Lee, R.T., Keating, M.T. (2006). FGF1/p38 MAP kinase inhibitor therapy induces cardiomyocyte mitosis, reduces scarring, and rescues function after myocardial infarction. *Proc Natl Acad Sci U S A.* 103, 15546-15551.
- Engel, F.B., *et al.* (2005). p38 MAP kinase inhibition enables proliferation of adult mammalian cardiomyocytes. *Genes Dev.* 19, 1175-1187.
- Eschenhagen, T., *et al.* (2017). Cardiomyocyte Regeneration: A Consensus Statement. *Circulation.* 136, 680-686.
- Haubner, B.J., *et al.* (2016). Functional Recovery of a Human Neonatal Heart After Severe Myocardial Infarction. *Circ Res.* 118, 216-221.
- Hotta, Y., *et al.* (2008). Fgf16 is required for cardiomyocyte proliferation in the mouse embryonic heart. *Dev Dyn.* 237, 2947-2954.
- House, S.L., *et al.* (2016). Endothelial fibroblast growth factor receptor signaling is required for vascular remodeling following cardiac ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol.* 310, H559-571.
- Hutson, M.R., Kirby, M.L. (2007). Model systems for the study of heart development and disease. Cardiac neural crest and conotruncal malformations. *Semin Cell Dev Biol.* 18, 101-110.
- Hutson, M.R., *et al.* (2010). Arterial pole progenitors interpret opposing FGF/BMP signals to proliferate or differentiate. *Development.* 137, 3001-3011.
- Ilagan, R., *et al.* (2006). Fgf8 is required for anterior heart field development. *Development.* 133, 2435-2445.
- Itoh, N., Ohta, H., Nakayama, Y., Konishi, M. (2016). Roles of FGF Signals in Heart Development, Health, and Disease. *Front Cell Dev Biol.* 4, 110.
- Jopling, C., Sune, G., Faucherre, A., Fabregat, C., Izpisua Belmonte, J.C. (2012a). Hypoxia induces myocardial regeneration in zebrafish. *Circulation.* 126, 3017-3027.
- Jopling, C., Sune, G., Morera, C., Izpisua Belmonte, J.C. (2012b). p38alpha MAPK regulates myocardial regeneration in zebrafish. *Cell Cycle.* 11, 1195-1201.
- Kelly, R.G. (2012). The second heart field. *Curr Top Dev Biol.* 100, 33-65.
- Kelly, R.G., Brown, N.A., Buckingham, M.E. (2001). The arterial pole of the mouse heart forms from Fgf10-expressing cells in pharyngeal mesoderm. *Dev Cell.* 1, 435-440.
- Lavine, K.J., *et al.* (2006). Fibroblast growth factor signals regulate a wave of Hedgehog activation that is essential for coronary vascular development. *Genes Dev.* 20, 1651-1666.
- Lavine, K.J., *et al.* (2005). Endocardial and epicardial derived FGF signals regulate myocardial proliferation and differentiation in vivo. *Dev Cell.* 8, 85-95.
- Lepilina, A., *et al.* (2006). A dynamic epicardial injury response supports progenitor cell activity during zebrafish heart regeneration. *Cell.* 127, 607-619.

Lu, S.Y., *et al.* (2008). FGF-16 is required for embryonic heart development. *Biochem Biophys Res Commun.* 373, 270-274.

Luo, W., *et al.* (2015). Akt1 signaling coordinates BMP signaling and beta-catenin activity to regulate second heart field progenitor development. *Development.* 142, 732-742.

Marguerie, A., *et al.* (2006). Congenital heart defects in Fgfr2-IIIb and Fgf10 mutant mice. *Cardiovasc Res.* 71, 50-60.

Matsunaga, S., *et al.* (2009). Endothelium-targeted overexpression of constitutively active FGF receptor induces cardioprotection in mice myocardial infarction. *J Mol Cell Cardiol.* 46, 663-673.

Mesbah, K., *et al.* (2012). Identification of a Tbx1/Tbx2/Tbx3 genetic pathway governing pharyngeal and arterial pole morphogenesis. *Hum Mol Genet.* 21, 1217-1229.

Murakami, M., *et al.* (2008). The FGF system has a key role in regulating vascular integrity. *J Clin Invest.* 118, 3355-3366.

Oladipupo, S.S., *et al.* (2014). Endothelial cell FGF signaling is required for injury response but not for vascular homeostasis. *Proc Natl Acad Sci U S A.* 111, 13379-13384.

Ornitz, D.M., Itoh, N. (2015). *The Fibroblast Growth Factor signaling pathway.* Wiley Interdiscip Rev Dev Biol. 4, 215-266.

Park, E.J., *et al.* (2008). An FGF autocrine loop initiated in second heart field mesoderm regulates morphogenesis at the arterial pole of the heart. *Development.* 135, 3599-3610.

Pasumarthi, K.B., Field, L.J. (2002). Cardiomyocyte cell cycle regulation. *Circ Res.* 90, 1044-1054.

Porrello, E.R., *et al.* (2011). Transient regenerative potential of the neonatal mouse heart. *Science.* 331, 1078-1080.

Poss, K.D., Wilson, L.G., Keating, M.T. (2002). Heart regeneration in zebrafish. *Science.* 298, 2188-2190.

Presta, M., *et al.* (2005). Fibroblast growth factor/fibroblast growth factor receptor system in angiogenesis. *Cytokine Growth Factor Rev.* 16, 159-178.

Rochais, F., Mesbah, K., Kelly, R.G. (2009). Signaling pathways controlling second heart field development. *Circ Res.* 104, 933-942.

Rochais, F., *et al.* (2014). FGF10 promotes regional foetal cardiomyocyte proliferation and adult cardiomyocyte cell-cycle re-entry. *Cardiovasc Res.* 104, 432-442.

Rubin, N., Darehzereshki, A., Bellusci, S., Kaartinen, V., Ling Lien, C. (2013). FGF10 Signaling Enhances Epicardial Cell Expansion during Neonatal Mouse Heart Repair. *J Cardiovasc Dis Diagn.* 1.

Smith, T.K., Bader, D.M. (2007). Signals from both sides: Control of cardiac development by the endocardium and epicardium. *Semin Cell Dev Biol.* 18, 84-89.

Soonpaa, M.H., Field, L.J. (1998). Survey of studies examining mammalian cardiomyocyte DNA synthesis. *Circ Res.* 83, 15-26.

Sun, S., *et al.* (2002). Expression, purification, and kinetic characterization of full-length human fibroblast activation protein. *Protein Expr Purif.* 24, 274-281.

Tzahor, E., Poss, K.D. (2017). Cardiac regeneration strategies: Staying young at heart. *Science.* 356, 1035-1039.

Urness, L.D., Bleyl, S.B., Wright, T.J., Moon, A.M., Mansour, S.L. (2011). Redundant and dosage sensitive requirements for Fgf3 and Fgf10 in cardiovascular development. *Dev Biol.* 356, 383-397.

Vega-Hernandez, M., Kovacs, A., De Langhe, S., Ornitz, D.M. (2011). FGF10/FGFR2b signaling is essential for cardiac fibroblast development and growth of the myocardium. *Development.* 138, 3331-3340.

Vincentz, J.W., McWhirter, J.R., Murre, C., Baldini, A., Furuta, Y. (2005). Fgf15 is required for proper morphogenesis of the mouse cardiac outflow tract. *Genesis.* 41, 192-201.

Watanabe, Y., *et al.* (2010). Role of mesodermal FGF8 and FGF10 overlaps in the development of the arterial pole of the heart and pharyngeal arch arteries. *Circ Res.* 106, 495-503.

Watanabe, Y., *et al.* (2012). Fibroblast growth factor 10 gene regulation in the second heart field by Tbx1, Nkx2-5, and Islet1 reveals a genetic switch for down-regulation in the myocardium. *Proc Natl Acad Sci U S A.* 109, 18273-18280.

Wills, A.A., Holdway, J.E., Major, R.J., Poss, K.D. (2008). Regulated addition of new myocardial and epicardial cells fosters homeostatic cardiac growth and maintenance in adult zebrafish. *Development*. 135, 183-192.

Writing Group, M., *et al.* (2016). Executive Summary: Heart Disease and Stroke Statistics--2016 Update: A Report From the American Heart Association. *Circulation*. 133, 447-454.

Yamakawa, H., *et al.* (2015). Fibroblast Growth Factors and Vascular Endothelial Growth Factor Promote Cardiac Reprogramming under Defined Conditions. *Stem Cell Reports*. 5, 1128-1142.

Zhang, J., *et al.* (2008). *Frs2alpha*-deficiency in cardiac progenitors disrupts a subset of FGF signals required for outflow tract morphogenesis. *Development*. 135, 3611-3622.

Zhang, X., *et al.* (2006). Receptor specificity of the fibroblast growth factor family. The complete mammalian FGF family. *J Biol Chem*. 281, 15694-15700.

Zhu, W., *et al.* (2018). Regenerative Potential of Neonatal Porcine Hearts. *Circulation*.

Figure 1: FGF10 signaling in the developing heart. (A) Lateral whole-mount view and (B) transverse section of an embryo carrying an *Fgf10-LacZ* transgene (Kelly *et al.*, 2001) at embryonic day E9.5. *Fgf10* transgene expression is observed in second heart field (SHF) progenitor cells, which are located in pharyngeal mesoderm adjacent to pharyngeal endoderm, and in the outflow tract (OFT). (B) Immunofluorescence on transverse section of an E9.5 embryo carrying an *Fgf10-LacZ* transgene, at the level of the dotted line in (A). The anti-AP-2 α (pink) antibody was used to detect cardiac neural crest (CNC) cells and ectodermal cells and the anti- β galactosidase (green) antibody to visualize SHF cells. (C) Table showing the overlapping expression patterns of key FGF ligands and receptors at E9.5 in the SHF and surrounding tissues. (D) FGF signaling role in fetal heart development. (E) Table showing the overlapping expression patterns of key FGF ligands and receptors in the fetal heart.

FIGURE 1

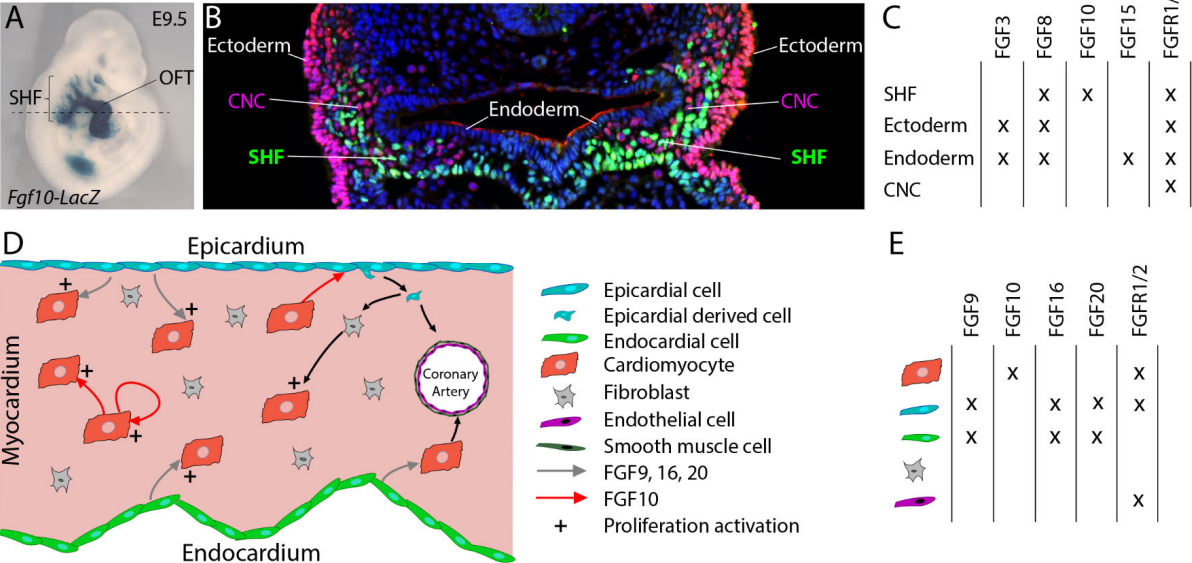


Figure 2: FGF10 signaling in cardiac homeostasis, disease and repair.

