

## REVIEW

# Fibroblast growth factors: key players in regeneration and tissue repair

Luigi Maddaluno, Corinne Urwyler and Sabine Werner\*

## ABSTRACT

Tissue injury initiates a complex repair process, which in some organisms can lead to the complete regeneration of a tissue. In mammals, however, the repair of most organs is imperfect and results in scar formation. Both regeneration and repair are orchestrated by a highly coordinated interplay of different growth factors and cytokines. Among the key players are the fibroblast growth factors (FGFs), which control the migration, proliferation, differentiation and survival of different cell types. In addition, FGFs influence the expression of other factors involved in the regenerative response. Here, we summarize current knowledge on the roles of endogenous FGFs in regeneration and repair in different organisms and in different tissues and organs. Gaining a better understanding of these FGF activities is important for appropriate modulation of FGF signaling after injury to prevent impaired healing and to promote organ regeneration in humans.

**KEY WORDS:** FGF, Regeneration, Repair, Blastema, Injury, Wound

## Introduction

The ability to regenerate injured tissues and organs, and even amputated body parts, is a long-standing aspiration for humankind and a major and highly challenging goal for clinicians, researchers and engineers. Unfortunately, the regenerative capacity of mammals is very limited, and injury to most tissues results in a wound-healing process that ultimately leads to scar formation. In this process, cells from the tissue adjacent to the insult, as well as progenitor cells recruited from the bone marrow, migrate and proliferate at the wound site in order to rapidly restore the lost tissue (Gurtner et al., 2008). However, this is often accompanied by a strong and long-lasting inflammatory response. Although this is beneficial for the defense against invading pathogens, it can limit the healing response and also cause inappropriate activation of fibroblasts and their differentiation into myofibroblasts (see Glossary, Box 1). This results in tissue contraction and in the deposition of large amounts of extracellular matrix (ECM), which prevents further regenerative processes (Gurtner et al., 2008; Eming et al., 2014). This is particularly obvious in a skin wound, where all insults that affect the epidermal and underlying dermal layers result in the formation of a scar. Such scar tissue is characterized by reduced elasticity and tensile strength compared with the non-injured skin and by a lack of all skin appendages, such as hairs, nails, sebaceous glands and sweat glands, which cannot regenerate (reviewed by Gurtner et al., 2008; Eming et al., 2014).

In contrast to mammals, various other organisms have a remarkable regenerative capacity (Fig. 1). Extreme cases include cnidarians, such as the freshwater polyp *Hydra*, or planarians, a subset of flatworms. These organisms are capable of re-growing major structures such as the head or tail, or even entire organisms from very small fragments of the body (reviewed by Tanaka and Reddien, 2011). In *Hydra*, this is achieved by the action of ectodermal and endodermal epithelial cells, as well as interstitial stem cells (see Glossary, Box 1), which enable the continuous production of new tissue. Regeneration in planarians, by contrast, involves the formation of a tissue outgrowth at the wound site, called a blastema, in which the missing tissues are regenerated. A population of dividing cells, called neoblasts, which include pluripotent stem cells, is responsible for regeneration of these organisms (reviewed by Tanaka and Reddien, 2011). Some vertebrate species, such as salamanders, frogs and fish can also re-grow certain parts of their body (reviewed by Brockes and Kumar, 2008). Particularly well-documented is appendage regeneration in adult salamanders and fish, as well as in tadpoles (Fig. 1). This regenerative response also involves the formation of a blastema at the amputation site, which consists of mesenchymal blastema cells covered by a simple wound epithelium. De-differentiated cells or stem cells provide lineage-restricted progenitors that are responsible for this process as shown by transplantation studies or genetic lineage tracing (Kragl et al., 2009; Knopf et al., 2011; Sandoval-Guzmán et al., 2014). Thus, the blastema includes a combination of cells with unique restricted potential and tissue origin, which together orchestrate the regeneration process (reviewed by Tanaka, 2016).

Both repair and regeneration are controlled by a large variety of cytokines, growth and differentiation factors (reviewed by Werner and Grose, 2003; Tanaka, 2016). Among them are the fibroblast growth factors (FGFs), which are master regulators of both organogenesis and tissue homeostasis. Mutations in FGF- or FGF receptor (FGFR)-encoding genes cause developmental/genetic diseases that affect different tissues and organs (reviewed by Beenken and Mohammadi, 2009; Ornitz and Itoh, 2015). In addition, abnormal FGF activity due to ligand or receptor overexpression or somatic mutations in FGFR genes have been demonstrated in different types of cancer (reviewed by Tanner and Grose, 2016). Therefore, a role for FGFs in the repair of injured tissues seemed likely, and indeed, numerous recent studies have reported roles for the FGFs in regeneration and repair and highlight the interplay between FGFs and other key signaling molecules. Here, we summarize these recent insights into the roles of FGFs in repair and regeneration of different tissues and organs, ranging from planarians to mammals. We focus on data obtained in functional *in vivo* studies that address the roles of endogenous FGFs in repair/regeneration. Numerous studies of the therapeutic activities of exogenous FGFs are not covered in this Review as they have been summarized elsewhere (we refer readers to reviews by Zhang and Li, 2016; Yun et al., 2010; Nunes et al., 2016).

Institute of Molecular Health Sciences, Department of Biology, Swiss Federal Institute of Technology (ETH) Zurich, Otto-Stern-Weg 7, 8093 Zürich, Switzerland.

\*Author for correspondence (Sabine.werner@biol.ethz.ch)

 S.W., 0000-0001-7397-8710

**Box 1. Glossary**

**Calvarium.** The portion of a skull including the braincase and excluding the lower jaw or lower jaw and facial portion.

**Club cells.** Bronchiolar exocrine cells found in the small airways (bronchioles) of the lung. They are important for the protection of bronchiolar epithelial cells. Club cells were previously known as Clara cells.

**Critical-size defect.** A bone defect that will not heal without intervention.

**Endochondral bone.** Any bone that develops within and replaces cartilage.

**Epidermal  $\gamma\delta$  T cells.** Unconventional T cells that are defined by their expression of heterodimeric T-cell receptors composed of  $\gamma$  and  $\delta$  chains. They are abundant in mouse epidermis, but much less frequent in human epidermis, and play important roles in wound healing, UV response and skin tumorigenesis.

**Granulation tissue.** New connective tissue that develops in a wound. It includes fibroblasts, blood and lymphatic vessels, various types of immune cells as well as nerve cells. It takes its name from the large number of cell nuclei that gives the tissue a granular appearance.

**Hepatic stellate cells.** Cells residing between the hepatocytes and small blood vessels in the liver. Their activation after liver injury leads to deposition of collagen and formation of scar tissue, leading to fibrosis/cirrhosis.

**Interstitial stem cells.** Multipotent cells that give rise to differentiated progeny cells during the growth and budding of *Hydra* polyps.

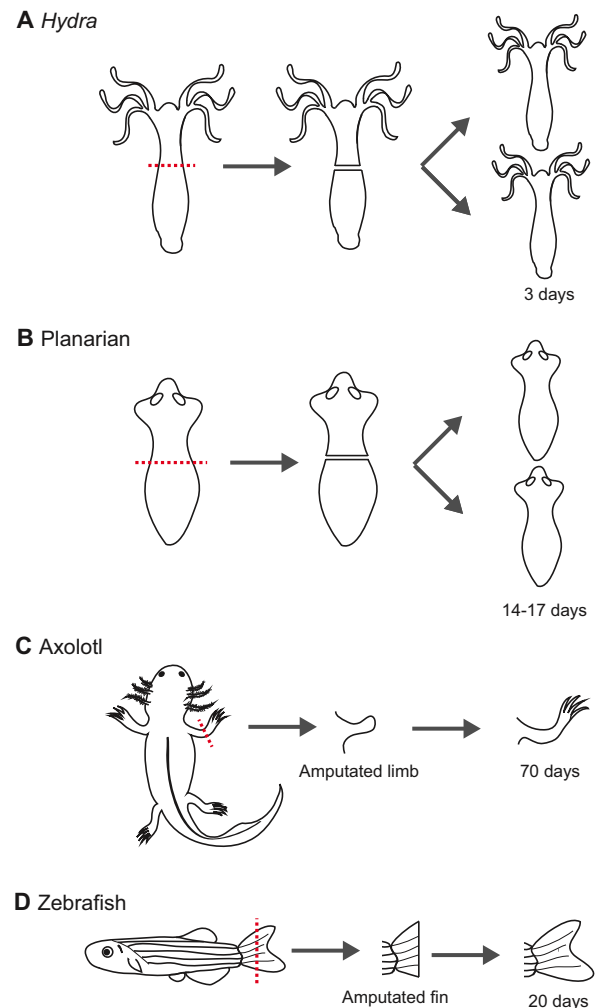
**Müller glial cells.** Most common type of glial cells in the vertebrate retina. They are named after Heinrich Müller, who first described them.

**Myofibroblasts.** Fibroblasts with contractile properties similar to smooth muscle cells. These cells are involved in tissue contraction and production of large amounts of ECM.

**The FGF family: an overview**

FGFs and their receptors are highly conserved among the animal kingdom (Bertrand et al., 2014). In mammals, the FGF family includes 22 polypeptides that regulate migration, proliferation, differentiation, survival, metabolic activity and/or neural function in a wide variety of cells (reviewed by Ornitz and Itoh, 2015). Based on a phylogenetic analysis, FGFs can be arranged into seven subfamilies (Ornitz and Itoh, 2015) (Fig. 2A). However, other studies have proposed the existence of eight FGF families, with FGF3 forming a separate ‘family’ with only one member (Oulion et al., 2012).

With the exception of FGF11–14, which act intracellularly, FGFs bind to and activate four transmembrane tyrosine kinase receptors, designated FGFR1–4 (Ornitz and Itoh, 2015). Efficient receptor activation further requires the binding of FGFs to heparan sulfate proteoglycans, or – in the case of the endocrine-acting FGFs (FGF19 and its murine ortholog Fgf15, as well as FGF21 and FGF23) – to the co-receptor proteins klotho or  $\beta$ -klotho (Ornitz and Itoh, 2015). Further complexity among the FGFRs is achieved by alternative splicing. Of particular importance is alternative splicing of the RNA encoding the third immunoglobulin-like domain (Ig III) of FGFRs 1, 2 and 3, which generates the IIIb and IIIc variants, which differ in their ligand-binding specificities (Fig. 2B). The IIIb variants are mainly expressed in epithelial cells, whereas mesenchymal and other stromal cells express predominantly the IIIc variants (Ornitz and Itoh, 2015). Therefore, epithelial and stromal cells usually respond to a different set of ligands. Finally, a fifth member of the FGFR family, FGFR-like 1 (FGFRL1), has been described, which binds to at least some of the secreted FGFs. FGFRL1 lacks the intracellular tyrosine kinase domain present in other FGFRs and antagonizes some of the functions of FGFs (reviewed by Trueb, 2011).



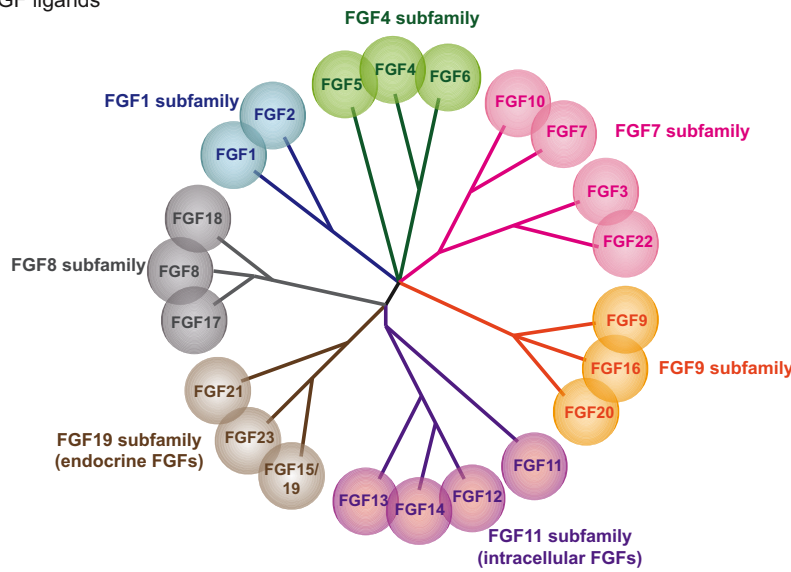
**Fig. 1. Regeneration in organisms with high regenerative capacity.**

Schematics of whole body regeneration in a *Hydra* (A), whole body regeneration in a planarian (B), limb regeneration in an axolotl (C) and fin regeneration in a zebrafish (D). The time shown beneath each regenerated structure indicates the time taken for regeneration to occur.

Upon binding to their high-affinity receptors, FGFs activate various signaling cascades, of which the Ras-Erk1/2 signaling pathway is most prominent. In addition, FGFs can activate the phosphatidylinositol 3-kinase/Akt pathway, as well as phospholipase C $\gamma$ , p38 and JNK kinases, and STAT1, STAT3 and STAT5 (Ornitz and Itoh, 2015). These pathways are activated in a receptor- and cell type-dependent manner. Their different usage might explain why FGFs stimulate the proliferation of some cells, but inhibit the proliferation and promote the differentiation of others.

FGFs act together with other signaling molecules, in particular the Wnt signaling pathway, to orchestrate important *in vivo* processes, including regenerative responses. In regenerating tissues, such as the mouse digit, *Xenopus* tail and zebrafish fin, FGFs frequently act downstream of Wnts (Lin and Slack, 2008; Takeo et al., 2013; Love et al., 2013; Wehner et al., 2014). Notch has been identified as a downstream regulator of Fgf8 during retinal regeneration in zebrafish (Wan and Goldman, 2017). Other factors acting in concert with FGFs are sonic hedgehog (Shh) and the bone morphogenetic protein (BMP) antagonist gremlin, which together with FGFs control the limb regeneration process in axolotls (Nacu

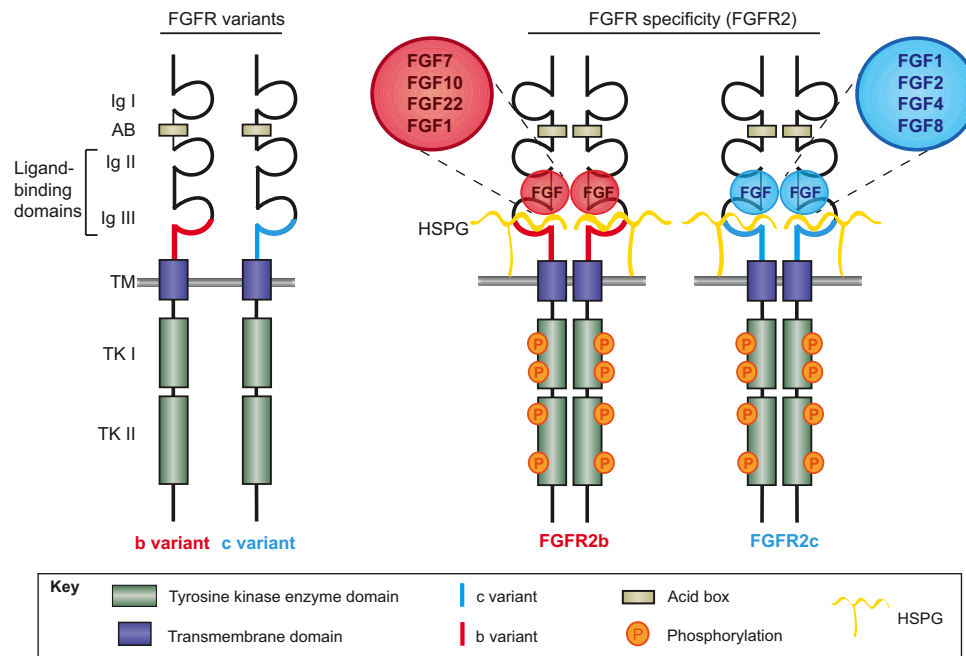
**A FGF ligands**



**Fig. 2. The FGF family and its receptors.**

(A) Schematic of the FGF family and its division into subfamilies in mice and humans based on phylogenetic analyses (Itoh and Ornitz, 2008). Note that mice have an Fgf15, whereas humans have the related FGF19, which does not exist in mice. (B) Left: Schematic of the structure of mammalian FGF receptors, including the difference between b and c variants. Right: Ligand-binding specificity of the FGFR2b and FGFR2c variants. The FGFs that bind to these receptor variants are indicated. HSPG, heparan sulfate proteoglycan; Ig, immunoglobulin-like domain; P, phosphorylated tyrosine residues; TK, tyrosine kinase domain; TM, transmembrane domain.

**B FGF receptors**



et al., 2016). This list is far from being complete, and the elucidation of factors that act synergistically or antagonistically with FGFs in different regeneration processes, and of their interaction with FGF signaling, is an important goal for the future. In the following sections, we summarize the role of FGFs in the regeneration of different tissues and organs and their interactions with other regulators of regeneration. A brief summary of the roles of FGFs in tissue repair and regeneration is provided in Table 1.

**FGF signaling in limb, tail and fin regeneration**

Limb amputation in mammals initiates a wound healing process but does not result in regeneration. However, the regenerative response is not completely abrogated, as digits can regenerate if the level of amputation is within the nail bed (Takeo et al., 2013). By comparison, other organisms, such as salamanders, can regenerate

a whole amputated limb; this is best documented for the axolotl (Fig. 1). The high regenerative capacity of the axolotl is likely to be related to the fact that it retains its larval features throughout its life, a condition called neoteny (Rosenkilde and Ussing, 1996). Both digit and limb regeneration in axolotls are highly dependent on innervation, and experimental denervation induces wound healing instead of regeneration (reviewed by Kumar and Brockes, 2012). Interestingly, regeneration in denervated salamanders can be rescued by the implantation of Fgf2-soaked beads during a nerve-dependent phase (Mullen et al., 1996). In this study, it was also shown that nerves produce an FGF family member during regeneration, which acts as a neurotrophic factor that is required for limb regeneration. Other studies have shown that nerve deviation to wounded skin or the application of exogenous BMPs combined with Fgf2 plus Fgf8 to wounds induces blastema formation in

**Table 1. An overview of FGF and FGF receptor functions in the repair and regeneration of different tissues and organs**

Organ/tissue	Protein	Functions	References
Limb (amphibians)	Fgf2, Fgf8	Promote limb regeneration	Mullen et al., 1996; Makanae et al., 2014
Limb ( <i>Xenopus</i> )	Fgf8+Fgf9+Fgf10	Promote limb regeneration	Nacu et al., 2016
	FGFR (type not defined)	Promotes limb regeneration	D'Jamoos et al., 1998
	Fgf10	Promotes limb regeneration	Yokoyama et al., 2001
Digit (mouse)	Fgf2	Promotes digit regeneration	Takeo et al., 2013
Tail (axolotl)	Fgf2+Fgf8	Promote tail regeneration	Makanae et al., 2016
Tail ( <i>Xenopus</i> )	FGFR (type not defined), Fgf20	Promote tadpole tail regeneration	Lin and Slack, 2008; Love et al., 2013
Fin (zebrafish)	FGFR (type not defined) Fgf20	Promotes fin regeneration Promotes fin regeneration via miR-133, laminin beta1a, Sdf1	Poss et al., 2000; Lee et al., 2005; Whitehead et al., 2005; Shibata et al., 2016; Yin et al., 2008; Chen et al., 2015; Bouzaffour et al., 2009; Shibata et al., 2016; Wehner et al., 2014
	Fgf3, Fgf10	Promote cell proliferation during fin regeneration	Del Rio-Tsonis et al., 1998; Hayashi et al., 2004
Lens (newt)	FGFR (type not defined)	Promotes lens regeneration	Fukui and Henry, 2011
Lens ( <i>Xenopus</i> )	FGFR (type not defined)	Promotes lens regeneration	Cebria et al., 2002
Neural tissue (planarians)	FGFRL-like molecule	Inhibits neural tissue regeneration	
Cerebellum (zebrafish)	FGFR (type not defined)	Promotes regeneration of the cerebellum	Koster and Fraser, 2006
Spinal cord (zebrafish)	FGFR (type not defined)	Promotes spinal cord regeneration	Goldshmit et al., 2012
Retina (chick)	FGFR (type not defined)	Promotes retinal regeneration in embryos	Spence et al., 2004; 2007
Retina (zebrafish)	FGFR (type not defined)	Promotes retinal regeneration	Qin et al., 2011; Hochmann et al., 2012
	Fgf8	Promotes retinal regeneration	Wan and Goldman, 2017
Sciatic nerve (mouse)	Fgf2	Promotes sciatic nerve regeneration, but reduces sensory recovery	Jungnickel et al., 2006, 2010
	Sprouty 2	Inhibits regeneration	Marvaldi et al., 2015
Spinal cord (mouse)	Sprouty 4	Enhances inflammation and astrocytic gliosis, reduces neuronal survival	Goldshmit et al., 2015
	Fgf2	Reduces inflammation and astrocytic gliosis, promotes neuronal survival	Goldshmit et al., 2014
Facial nerve (mouse)	Fgf2	Promotes facial nerve functional recovery	Seitz et al., 2011
Neocortex (mouse)	Fgfr1+Fgfr2+Fgfr3	Promote astrocyte activation, but also glial scar formation	Kang et al., 2014
Heart (zebrafish)	Fgf17b via Fgfr2, Fgfr4	Promote regeneration, EMT and neovascularization	Lepilina et al., 2006
Heart (mouse)	Fgf2 via Fgfr1+Fgfr2	Promote functional recovery after infarction, hypertrophic response and angiogenesis after ischemia reperfusion injury	Virag et al., 2007; House et al., 2015
	Fgf2	Promotes fibrosis after angiotensin II treatment	Matsumoto et al., 2013
	Fgf16	Attenuates inflammation and fibrosis after infarction or angiotensin II treatment	Hu et al., 2017; Matsumoto et al., 2013
	Fgf23	Promotes fibrosis after infarction or ischemia reperfusion injury	Hao et al., 2016
Skeletal muscle (mouse)	Fgf2, Fgf6, at least in part via Fgfr4	Promote regeneration	Lefaucheur and Sebillé, 1995; Floss et al., 1997; Armand et al., 2005; Zhao et al., 2006
Skeletal muscle (zebrafish)	FGFR (type not defined)	Promotes regeneration of extraocular muscle	Saera-Vila et al., 2016
Bone (mouse)	Fgf9+Fgf18	Promote calvarial healing	Behr et al., 2010a
	Fgf9	Promotes long bone repair	Behr et al., 2010b
	Fgf18	Promotes repair of the tibia	Behr et al., 2011
Skin (mouse)	Fgf7+Fgf10 via Fgfr1b+Fgfr2b	Promote wound re-epithelialization	Werner et al., 1994; Jameson et al., 2002; Meyer et al., 2012
	Fgf2 via Fgfr1+Fgfr2	Promote wound angiogenesis	Broadley et al., 1989; Ortega et al., 1998; Numata et al., 2006; Oladipupo et al., 2014
	Fgfbp1, sprouty 2	Inhibit wound angiogenesis	Tassi et al., 2011; Wietecha et al., 2011
Lung (mouse)	Fgf9	Promotes hair follicle neogenesis after wounding	Gay et al., 2013
	FGFR (type not defined)	Reduces susceptibility to hyperoxia and promotes lung recovery	Hokuto et al., 2004
	Fgfr2b	Promotes alveolar regeneration in a long-term injury model	Perl and Gale, 2009
	Fgf2	Promotes recovery after bleomycin-induced or naphthalene-induced injury	Guzy et al., 2015; Guzy et al., 2017; Ju et al., 2012

Continued

Table 1. Continued

Organ/tissue	Protein	Functions	References
Intestine (mouse)	Fgfr1+Fgfr2+Fgfr3	Promote bleomycin-induced fibrosis via fibroblasts	Volckaert et al., 2011
	Fgf10	Promotes repair after naphthalene-induced injury	
	Fgf7	Protects from injury, promotes repair upon DSS treatment	
Liver (mouse)	Fgf2	Promotes repair and reduces inflammation in colitis models	Song et al., 2015
	Fgfr1+Fgfr2	Promote liver regeneration after partial hepatectomy	
	Fgf15 via Fgfr4	Promotes hepatocyte survival and proliferation during regeneration	
Liver (zebrafish)	Fgf7	Promotes expansion of liver progenitor cells after toxin-induced injury	Takase et al., 2013
	FGFR (type not defined)	Promotes liver regeneration after partial hepatectomy	Kan et al., 2009

amphibians, which allows regeneration to occur instead of a normal wound healing process (Makanæ et al., 2014). Recently, endogenous Fgf8 was identified as a key player in axolotl limb regeneration (Nacu et al., 2016). This study showed that limb amputation induces the formation of anterior and posterior blastema cells and also their proliferation. Consequently, anterior cells express Fgf8 and gremlin, whereas posterior cells express Shh. Shh promotes the sustained expression of anterior Fgf8, as well as of Fgf9, Fgf10 and Fgf17, which are expressed in both the anterior and posterior compartments, and late expression of Shh requires Fgf8. The coordinated expression of Fgf8 and of Shh and their co-dependency were shown to be crucial for the proper regeneration of amputated limbs, and inhibition of FGFR signaling suppresses this process.

Interestingly, the role of FGFs seems to be highly conserved, as inhibitors of FGFR inhibit the normal limb outgrowth that occurs during pre-metamorphic hindlimb regeneration in *Xenopus laevis* (D'Jamoos et al., 1998). The responsible ligand is most likely Fgf10, which stimulates limb regeneration ability when introduced into non-regenerative *Xenopus* limb stumps (Yokoyama et al., 2001). Remarkably, the application of Fgf4 to amputated chicken limbs results in outgrowth of stump tissues and the development of a virtually complete cytoskeleton, demonstrating that the early stages of limb regeneration can also be induced by FGFs in chick (Kostakopoulou et al., 1996). Finally, a role for FGF signaling in mouse digit regeneration has been demonstrated (Takeo et al., 2013). This study found that *Fgf2* expression is upregulated in the nail epithelium after digit amputation during the phase of blastema growth, but that enhanced *Fgf2* expression is abrogated after denervation, a procedure that inhibits blastema growth and digit regeneration. The inhibition of Wnt signaling in epithelial cells has a similar deleterious effect on mouse digit regeneration; this correlates with the loss of *Fgf2* expression in the nail epithelium. In the regenerating digit, *Fgf2* expression correlates with the expression of *Fgfr1* and with Erk activation in mesenchymal blastema cells (Takeo et al., 2013). These results strongly suggest that Wnt activation in the epithelium promotes mesenchymal cell proliferation through induction of nerve-dependent Fgf2 expression. Taken together, these studies highlight that FGF signaling plays a key role in limb/digit regeneration across species.

As in limb regeneration, a combination of Fgf2/Fgf8/BMPs induces the early stages of tail regeneration in axolotls (Makanæ et al., 2016), indicating that an evolutionarily conserved mechanism plays a role in the neural tissue-governed regeneration of both body parts. However, additional, as-yet-unidentified factors are required for the formation of a patterned tail. Furthermore, the endogenous FGFs that control tail regeneration remain to be determined. A role

for endogenous FGFs has, however, been identified in *Xenopus laevis* tadpole tail regeneration: blocking FGFR signaling by an FGFR kinase inhibitor abrogates the regeneration process (Lin and Slack, 2008). This study further showed that FGFs act downstream of Wnts under these conditions, and overexpression of Fgf20, which is strongly upregulated after tail amputation (see below), rescues the regeneration defect that occurs upon Wnt inhibition (Lin and Slack, 2008). In a search for the early signals that activate the regeneration process, *Xenopus* tadpole tail amputation was found to induce the sustained production of reactive oxygen species (ROS), which are required for cell proliferation and tail regeneration. Mechanistically, this resulted from ROS-mediated activation of Wnt/ $\beta$ -catenin signaling, including the upregulation of the major Wnt target *Fgf20* (Love et al., 2013).

Efficient regeneration occurs also upon amputation of the zebrafish fin (Fig. 1D). This correlates with expression of *Fgfr1* in undifferentiated mesenchymal cells that underlie the wound epidermis during blastema formation and in blastemal tissue during regenerative outgrowth of the amputated fin (Poss et al., 2000). Concomitantly, a zebrafish FGF family member is expressed in the regenerating epidermis, suggesting a role for paracrine FGF signaling in regeneration. Indeed, an inhibitor of Fgfr1 blocks blastema formation without having an obvious effect on wound healing. At later stages, Fgfr1 inhibition prevents further outgrowth of the fin (Poss et al., 2000). A follow-up study revealed that the level of FGF signaling determines the proliferation of blastemal cells and the rate of regenerative outgrowth (Lee et al., 2005). Using an *in vivo* mutagenesis screen, Fgf20 was discovered as an essential regulator of the early stages of zebrafish fin regeneration. *Fgf20* expression is upregulated soon after fin amputation, and a missense mutation in the *Fgf20* gene abrogates blastema formation and regeneration (Whitehead et al., 2005). Early upregulation of *Fgf20* occurs in the wound epithelium, with Fgf20 acting in a paracrine manner on mesenchymal cells to induce blastema formation (Shibata et al., 2016). The important role of FGFs in fin regeneration appears to be partly mediated by the FGF-induced downregulation of microRNA-133 (miR-133), an inhibitor of fin regeneration (Yin et al., 2008). Furthermore, Fgf20 is required for the amputation-induced upregulation of laminin beta1a, a component of the ECM that is required for the formation of a signaling-competent regeneration epidermis (Chen et al., 2015). Another target of FGF signaling during blastema formation in the fin is stromal cell-derived factor-1 (Sdf1; also known as Cxcl12), which exerts negative feedback on the FGF pathway through downregulation of *Fgf20a*. This ensures transient rather than long-term expression of Fgf20a target genes at the onset of regeneration (Bouzaffour et al., 2009). Various other FGFs are also expressed in

the regenerating zebrafish fin, and it has been shown that *Fgf3* and *Fgf10* produced by blastemal cells are required for cell proliferation (Shibata et al., 2016). These results show that FGFs have crucial roles at different stages of fin regeneration, during which FGFs act downstream of Wnt. Thus, Wnt/ $\beta$ -catenin signaling in the fin blastema controls epidermal patterning via induction of *Fgf3* expression and the subsequent activation of the Fgfr-Ras signaling pathway (Wehner et al., 2014).

Taken together, these results have identified FGFs as crucial regulators of regeneration following amputation of large parts of the body. The regeneration of specific tissues and organs also requires FGFs, as we move on to discuss in the sections below.

### FGF signaling in lens regeneration

Certain vertebrates can completely regenerate a lens. In newts, for example, the new lens arises from the pigmented iris epithelium, via the de-differentiation and transdifferentiation of retinal epithelial cells, in a process called Wolffian lens regeneration (reviewed by Henry et al., 2013). In frogs, a new lens arises during larval stages from basal cells of the corneal epithelium, which are possibly uncommitted epithelial stem cells. In both cases, the essential regenerative signals are provided by the retina (Henry et al., 2013).

During Wolffian lens regeneration, several FGFs are upregulated and most likely act via *Fgfr1* on the de-differentiating pigment epithelial cells of the dorsal iris (Del Rio-Tsonis et al., 1998; Hayashi et al., 2004). These studies also showed that FGF signaling is functionally important, as the application of an FGFR tyrosine kinase inhibitor or of a soluble FGFR ligand trap inhibits lens regeneration in newts. Different FGFs and FGFRs are also expressed during cornea lens regeneration, with *Fgf1*, *Fgf8* and *Fgf9* being expressed by retinal cells (Fukui and Henry, 2011). These FGFs are therefore potential triggers of the regeneration process. The functional involvement of FGFR signaling was verified in a *Xenopus laevis* larvae eye culture system, in which lens regeneration was blocked by an FGFR tyrosine kinase inhibitor (Fukui and Henry, 2011). This finding demonstrates the functional importance of FGF expression and upregulation for regeneration after lens removal.

Remarkably, the lens can also regenerate in some mammals, such as in New Zealand albino rabbits, if the anterior and posterior capsules of the lens are left relatively intact. Although the molecular mechanisms underlying this process are not well-understood, it has been shown that FGFs are important for lens fiber differentiation during this process (Gwon, 2006).

### FGF signaling in the repair of neural tissue

#### FGF signaling in brain outgrowth/regeneration in planarians and zebrafish

The functional role of FGFs in neural tissue regeneration is highly conserved and has been reported in planarians (Cebrià et al., 2002). In this study, the expression of a gene encoding an *Fgfr1*-like molecule, *nou-darake*, was inhibited by RNA interference (RNAi) in the planarian *Dugesia japonica*. This resulted in induction of ectopic brain tissue throughout the body, which could be prevented by knockdown of *Fgfr1* and *Fgfr2*. However, the ligands responsible for this effect remain to be identified.

Regeneration of neural tissue is also possible in zebrafish and also involves FGFs. For example, the inhibition of FGF signaling shortly after ablation of the cerebellum in zebrafish embryos abrogates the regenerative capacity of cerebellar neuronal cells and the subsequent re-formation of cerebellar structures (Koster and Fraser, 2006). Regenerative capacity is maintained in adult zebrafish through the

action of neuroepithelial stem cells (Kaslin et al., 2017), although it is not yet clear whether this process also involves FGF signaling. It has been shown, however, that regeneration of the injured spinal cord in adult zebrafish involves Fgf-mediated shape changes of glial cells, which form a bridge between the two sides of the resected spinal cord and thereby direct the migration of regenerating axons (Goldshmit et al., 2012). Interestingly, activation of cultured primate astrocytes from the marmoset cerebral cortex by FGF2 results in the formation of a similar glial morphology as that seen in zebrafish (Goldshmit et al., 2012). This suggests that insufficient FGF activity might partly underlie the limited regenerative capacity of neurons in mammals.

### FGF signaling in retinal regeneration

Regeneration of the retina after its complete removal has been described in chick embryos (reviewed by Fischer, 2005). This process can occur via transdifferentiation, but also via stem/progenitor cells located in the anterior margin of the eye. The stem cell-dependent regeneration process can be inhibited in chick embryos by treatment with FGFR kinase inhibitors. In this context, FGF expression is upregulated by Shh, and both pathways work together to promote cell proliferation and survival during retinal regeneration (Spence et al., 2004, 2007).

In adult zebrafish, the ablation of photoreceptors by intense light treatment induces re-entry of Müller glial cells (see Glossary, Box 1) into the cell cycle and the production of new rod and cone photoreceptors. This is dependent on FGF signaling, probably due to the potent cytoprotective and pro-mitogenic activities of FGFs as shown using dominant-negative FGFR mutants (Qin et al., 2011; Hochmann et al., 2012). In addition, FGFs synergize with a set of other growth factors and cytokines in the reprogramming of Müller glia to generate multipotent progenitors (Wan et al., 2014), demonstrating that FGFs regulate retinal regeneration at multiple levels. After needle-poke or toxin-induced retinal injury in zebrafish, *Fgf8* expression rapidly declines, which is important for suppression of Müller glial cell proliferation. This resulted from a relief of *Fgf8*-mediated activation of Notch signaling, which is required to drive Müller glial cells to an activated state with a lower proliferative threshold to injury-related factors (Wan and Goldman, 2017).

### FGFs in adult mammalian nervous system repair

Whereas peripheral nerves can regenerate to a certain extent, neurons within the central nervous system (CNS) of adult mammals exhibit limited regenerative capacity. This is a major problem in patients with injuries of the CNS. A regenerative response is usually initiated in the CNS but is rapidly blocked by the formation of a glial scar. However, studies have shown that exogenous FGFs can promote mammalian neuronal regeneration, mainly due to their neuroprotective activities. In particular, *Fgf2* is a key regulator of neuronal protection and repair after ischemic, metabolic or traumatic brain injury in mammals and promotes neurogenesis in the adult hippocampus after injury (reviewed by Alzheimer and Werner, 2002; Grothe et al., 2006). The positive effect of *Fgf2* on nerve regeneration was seen in a sciatic nerve regeneration model, in which transgenic mice that overexpress *Fgf2* show faster nerve regeneration as a result of enhanced Schwann cell proliferation, axonal regrowth and myelination (Jungnickel et al., 2006). Consistent with this beneficial activity of *Fgf2*, loss of the FGFR signaling antagonist sprouty 2 in mice promotes axon outgrowth and improves the long-distance regeneration of axons (Marvaldi et al., 2015). In response to spinal cord injury, mice lacking sprouty

4 show reduced inflammation and astrocytic gliosis combined with enhanced neuronal survival (Goldshmit et al., 2015). Although sprouty proteins also antagonize signaling by other receptor tyrosine kinases, FGF signaling activation through the loss of sprouty 4 might be particularly important in this situation, as the systemic administration of Fgf2 to wild-type mice with spinal cord injury has the same effect (Goldshmit et al., 2014). FGF signaling is also beneficial in response to facial nerve injury, as the already poor functional recovery of the facial nerve is further impaired in Fgf2-deficient mice (Seitz et al., 2011). However, negative effects of Fgf2 on nerve repair have also been reported; mice lacking this growth factor show faster sensory recovery after sciatic nerve crush, combined with increased axon and myelin size (Jungnickel et al., 2010). Thus, the combined effect of Fgf2 on neurons and Schwann cells does not always have a positive outcome after injury. Finally, FGFs also act on astrocytes: it has been reported that astrocyte activation is reduced after stab wounding of the neocortex in mice expressing a constitutively active Fgfr3 mutant in astrocytes compared with control mice. By contrast, strong astrocyte activation is seen in astrocyte-specific *Fgfr1 Fgfr2 Fgfr3* triple knockout mice in the same study (Kang et al., 2014). However, the glial scar is reduced in these triple mutant mice owing to loss of the pro-mitogenic effect of FGFs on astrocytes (Kang et al., 2014). Therefore, FGFs affect the repair process in the nervous system via different mechanisms and different target cells.

### FGF signaling in the repair of cardiac and skeletal muscle

#### FGF signaling in cardiac repair

The remarkable regenerative capacity of zebrafish is also obvious after heart injury. The heart regeneration process in these animals involves the formation of a blastema and of epicardial tissue that creates a new cover for the exposed myocardium. A subset of these epicardial cells undergoes epithelial-mesenchymal transition (EMT), enters the wound, and provides a new vasculature. It has been demonstrated that *Fgf17b* (now known as *Fgf17*) is upregulated in the myocardium after zebrafish heart injury, together with *Fgfr2* and *Fgfr4* in the adjacent cells that are derived from the epicardium. Furthermore, FGFR signaling blockade, using a dominant-negative receptor mutant, inhibits the EMT event and the subsequent neovascularization of the regenerating heart, causing premature arrest of the regeneration process (Lepilina et al., 2006).

In contrast to the zebrafish heart, the adult mammalian heart has a very poor regenerative capacity owing to the extremely low turnover rate of cardiomyocytes. This is a major problem after myocardial infarction, which results in rapid formation of non-functional scar tissue. Over time, this strongly increases the risk of heart failure (Tzahor and Poss, 2017). Therefore, it is of particular importance to identify factors that promote the cardiac repair process. One such factor appears to be Fgf2, as *Fgf2* knockout mice show poor functional recovery after infarction due to reduced cell proliferation, collagen deposition, endothelial cell proliferation and cardiomyocyte hypertrophy (Virag et al., 2007). In addition, the cardiac hypertrophic response to regional cardiac ischemia reperfusion injury is reduced in *Fgf2* knockout mice, combined with decreased vessel density and increased vessel diameter in the peri-infarct area (House et al., 2015). To determine whether this is a direct effect of FGF on endothelial cells, mice lacking Fgfr1 and Fgfr2 in this cell type were subjected to cardiac ischemia reperfusion injury. Indeed, it was shown that vascular remodeling and cardiac functional recovery are severely impaired in double mutant mice, whereas the hypertrophic response is unaffected

(House et al., 2016). A beneficial effect on the injured heart was also observed for Fgf16; treatment of mice with Fgf16 attenuates inflammation and interstitial fibrosis in response to myocardial infarction (Hu et al., 2017), and the cardiac hypertrophy and fibrosis that occur in response to angiotensin II treatment are enhanced in *Fgf16* knockout mice (Matsumoto et al., 2013). Surprisingly, the opposite effect was seen for Fgf2 in this study, suggesting that Fgf16 antagonizes a pro-fibrotic function of Fgf2. A pro-fibrotic effect in the heart was also demonstrated for Fgf23, which is upregulated in the heart and the bone after myocardial infarction and promotes myocardial fibrosis and exacerbates diastolic dysfunction in response to infarction or ischemia reperfusion injury (Hao et al., 2016). Taken together, it seems that the effect of FGFs on cardiac fibrosis depends on the type of FGF, the target cell affected, and potentially also the injury model.

#### FGF signaling in skeletal muscle repair

Similar to their roles in heart repair, FGFs are also key players in skeletal muscle regeneration in adult zebrafish. In an extraocular muscle injury model, in which regeneration occurs via a myocyte de-differentiation process and re-entry of the de-differentiated cells into the cell cycle, the blocking of FGFR signaling with a receptor kinase inhibitor or with a dominant-negative FGFR strongly impairs muscle regeneration (Saera-Vila et al., 2016).

Adult muscles in mammals also have a relatively good regenerative capacity, with repair occurring via stem cells, termed satellite cells, which are also required for physiological muscle growth. These cells are activated after muscle injury in mammals, re-enter the cell cycle and proliferate strongly for a limited period of time (Pawlikowski et al., 2017). Most of the satellite cells then differentiate and fuse, but a few return to quiescence to repopulate the stem cell pool (Pawlikowski et al., 2017). Initial evidence for a role of FGFs in skeletal muscle regeneration was obtained when it was shown that application of an Fgf2-neutralizing antibody impairs muscle regeneration following crush injury in mice (Lefaucheur and Sebillé, 1995). Another study reported that muscle repair is not affected in *Fgf2* knockout mice (Zhou et al., 1998). A severe regeneration defect with fibrosis and degeneration of myotubes has been reported for *Fgf6* knockout mice, indicating an important role for Fgf6 in muscle repair (Floss et al., 1997), although no muscle regeneration phenotype was seen in *Fgf6* knockout mice in a separate study (Fiore et al., 2000). These discrepancies might be due to different injury models or to different genetic backgrounds. Finally, a third study showed that Fgf6 promotes the proliferation and migration of myoblasts and muscle differentiation/hypertrophy after injury in mice (Armand et al., 2005). Consistent with an important role for FGFs in muscle regeneration, loss of *Fgfr4* was shown to cause a severe regeneration defect after toxin-induced muscle injury (Zhao et al., 2006). Therefore, FGFs may well have therapeutic efficacy. This could be particularly relevant in aged patients, in whom satellite cell self-renewal capability is severely decreased, resulting in age-induced muscle wasting (Sousa-Victor and Munoz-Canoves, 2016). Interestingly, aged satellite cells show impaired responsiveness to FGF, and ectopic activation of Fgfr1 results in a partial rescue of impaired muscle satellite cell renewal (Bernet et al., 2014). An important target of FGFR signaling in muscle progenitor cells is miR-29a, which suppresses the expression of different basement membrane proteins and thereby promotes Fgf2-induced proliferation of these cells. The upregulation of Fgf2 and its target miR-29 after cardiotoxin-induced muscle injury, combined with the finding that depletion of miR-29 inhibits muscle regeneration in this model, highlights the *in*

*in vivo* relevance of this interplay (Galimov et al., 2016). These findings raise hope that this key age-associated problem can be tackled by the activation of FGF signaling or the overexpression of FGF targets, such as miR-29.

### FGF signaling in bone repair

FGFs are key players in bone development, as reflected by the severe bone abnormalities in patients with different types of FGFR-activating mutations (reviewed by Ornitz and Marie, 2015; Ornitz and Legeai-Mallet, 2017). As important features involved in the development of endochondral bone (see Glossary, Box 1) are reactivated upon bone injury, a function for FGFs in fracture healing seemed likely, in particular because various FGFs and FGFRs are upregulated during this process (Ornitz and Marie, 2015). Indeed, the healing of defects in the calvarium (see Glossary, Box 1) is impaired in *Fgf9* and in *Fgf18* heterozygous knockout mice and can be rescued by local application of a collagen sponge soaked in the FGF that is reduced in the mutant mice (Behr et al., 2010a). Heterozygous *Fgf9* knockout mice also show defects in long bone repair due to impaired angiogenesis (Behr et al., 2010b). Furthermore, in a tibial defect model, heterozygous *Fgf18* knockout mice exhibit a reduced healing capacity (Behr et al., 2011). These results demonstrate that even a 50% reduction in the levels of certain FGFs is deleterious for bone repair, and highlight that these FGFs have non-redundant functions in this process.

FGF function in the context of bone repair has been linked to the DJ-1 protein (also known as Park7), which is a promoter of bone repair and enhances healing of a critical-size defect (see Glossary, Box 1) of the skull in rats by promoting angiogenesis and osteogenesis. This activity is abrogated in the presence of an FGFR kinase inhibitor (Kim et al., 2012). Overall, these roles for FGFs and FGFRs in bone repair are likely to be of therapeutic relevance. Indeed, it has been reported that the healing of a closed fracture of the tibiae is accelerated in transgenic mice expressing an 18-kD isoform of Fgf2 (Hurley et al., 2016), and that FGFs, in particular Fgf2, can promote bone repair in different pre-clinical models (reviewed by Ornitz and Marie, 2015).

### FGF signaling in skin wound healing

The expression of several FGFs, in particular Fgf7, is strongly induced upon skin wounding (reviewed by Werner and Grose, 2003). Fgf7 is mainly produced by fibroblasts in granulation tissue (see Glossary, Box 1) and in the dermis adjacent to skin wounds, as well as by epidermal  $\gamma\delta$  T cells (see Glossary, Box 1), and it acts in a paracrine manner on keratinocytes via activation of Fgfr2b (Werner and Grose, 2003) (Fig. 3A,B). This expression pattern suggested that Fgf7 functions in wound re-epithelialization. Surprisingly, however, the repair of incisional wounds was not affected in *Fgf7*-deficient mice (Guo et al., 1996), suggesting functional redundancy among different FGFs (Fig. 3A,B,D), in particular as other Fgfr2b ligands (Fgf10 and Fgf22) are also expressed in normal skin and *Fgf22* is upregulated after skin wounding (Werner and Grose, 2003). Indeed, mice lacking Fgf7- and Fgf10-producing  $\gamma\delta$  T cells, as well as transgenic mice that express a dominant-negative Fgfr2b mutant protein in keratinocytes, show a delay in wound re-epithelialization (Jameson et al., 2002; Werner et al., 1994). To identify the responsible receptor(s), mice lacking Fgfr1 and/or Fgfr2 in keratinocytes were subjected to full-thickness excisional wounding. This approach showed that wound contraction and re-epithelialization are severely impaired in the double knockout mice (Meyer et al., 2012); these mutants exhibit impaired keratinocyte

migration at the wound edge owing to reduced expression of major focal adhesion components, but proliferation is not affected.

In contrast to mice, the rapid re-epithelialization in zebrafish skin wounds does not require FGF signaling. However, the mitogenic effect of FGFs is important for restoring normal epidermal thickness after wound closure (Richardson et al., 2016). These results reveal fundamental mechanistic differences between skin wound re-epithelialization in zebrafish versus mammals.

FGFs also function as crucial regulators of wound angiogenesis. For example, the application of Fgf2 neutralizing antibodies to rat wounds, and wound-healing studies in FGF2-deficient mice, have revealed a role for Fgf2 in the early phase of wound angiogenesis and in granulation tissue formation in general (Broadley et al., 1989; Ortega et al., 1998). Furthermore, the wound healing-promoting effect of histamine in mice occurs through Fgf2 upregulation, stimulating macrophage accumulation and angiogenesis. This is abrogated in the presence of an FGFR kinase inhibitor (Numata et al., 2006). The effect of Fgf2 and potentially other FGFs on wound angiogenesis is mediated via Fgfr1 and Fgfr2 (Fig. 3C), as deletion of these receptors in endothelial cells and in hematopoietic cells affects neovascularization after skin wounding in mice, which is associated with delayed wound repair (Oladipupo et al., 2014). By contrast, the overexpression of FGF binding protein 1, a secreted protein that binds different FGF family members and enhances their activities by facilitating their release from the ECM, promotes wound angiogenesis and accelerates the repair process (Tassi et al., 2011). Consistent with an important role for FGFR signaling in wound angiogenesis, mice lacking the FGFR signaling inhibitor sprouty 2 show enhanced vascularization of healing skin wounds (Wietecha et al., 2011).

In contrast to wounds in humans and to small wounds in mice, in which hair follicles cannot regenerate, hair follicle regeneration can occur in large excisional wounds in mice and requires Fgf9. Fgf9 is initially produced by dermal  $\gamma\delta$  T cells and subsequently by wound fibroblasts, and its overexpression promotes hair follicle neogenesis at the wound site via induction of Wnt expression (Gay et al., 2013) (Fig. 3).

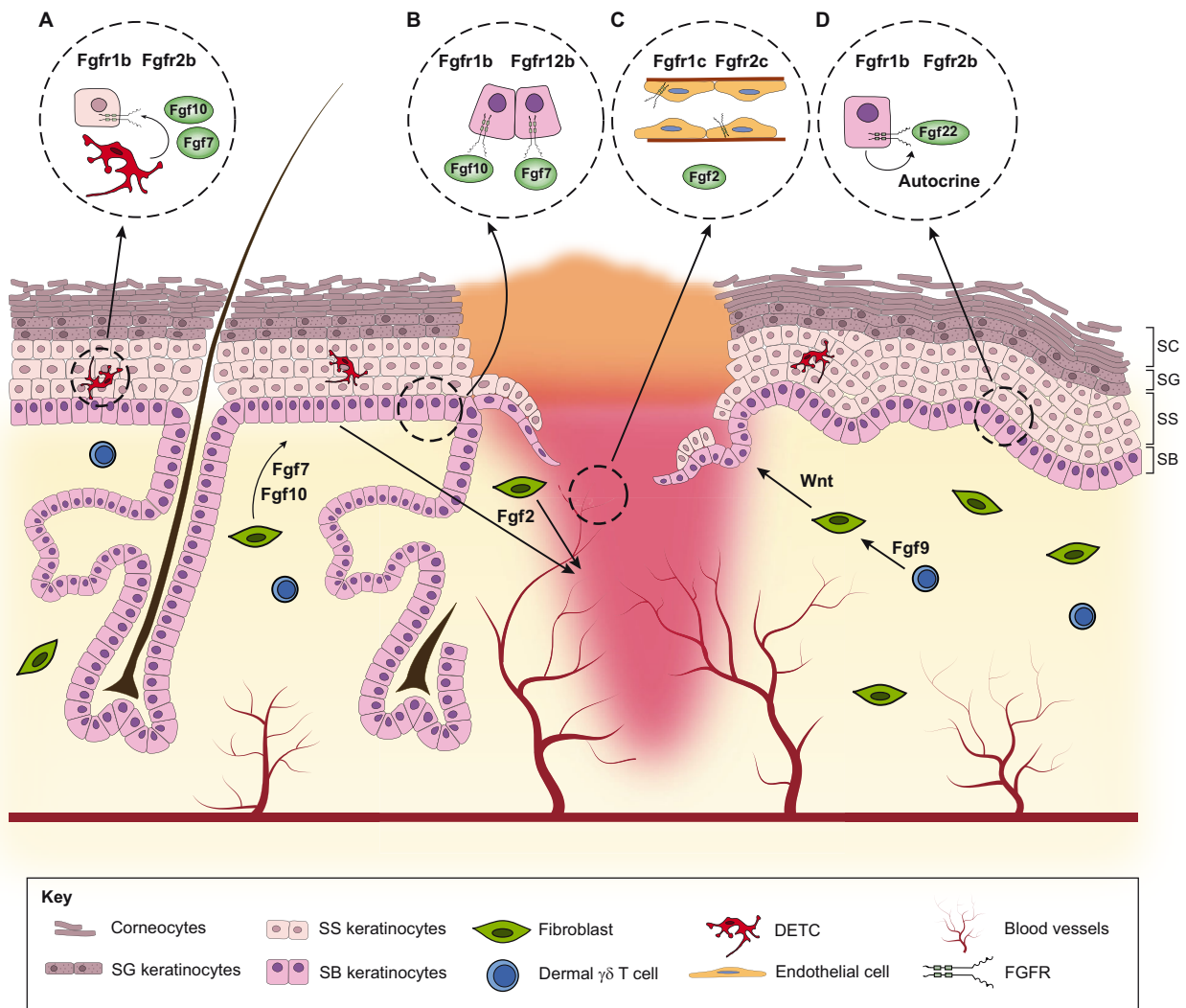
Taken together, these results identify FGFs as crucial regulators of different phases of the wound healing process in mammals, although their upregulation after wounding does not induce a regenerative response as seen in various lower organisms.

### FGF signaling in lung, intestine and liver repair

#### FGF signaling in lung repair

A role for FGFs in lung repair has been suggested based on the strong induction of *Fgf7* and/or *Fgf10* in response to different types of lung injury (reviewed by Finch et al., 2013). Indeed, expression of a soluble FGFR mutant in respiratory epithelial cells enhances the susceptibility of mice to hyperoxia and inhibits their subsequent recovery (Hokuto et al., 2004). In a long-term lung injury mouse model, retinoic acid was shown to promote regeneration of the damaged pulmonary alveoli, and this could be inhibited by the expression of a dominant-negative *Fgfr2b* mutant (Perl and Gale, 2009). Inhibition of FGF signaling has also been proposed to block myofibroblast differentiation, although it is not clear whether FGFs directly or indirectly affect this process. In a recent study, *Fgf2* knockout mice showed poor recovery of epithelial integrity in response to bleomycin-induced lung injury, as well as impaired repair of naphthalene-induced damage of the bronchial epithelium (Guzy et al., 2015). Although this study showed a positive effect of Fgf2 on lung repair, it has also been reported that activation of FGFR signaling in pulmonary fibroblasts promotes fibrosis, as





**Fig. 3. FGF activities during cutaneous wound healing.** Schematic of a mouse wound, highlighting the structures found in skin wounds and the FGFs/FGFRs expressed in normal and wounded skin. The red shaded area represents the lower part of the wound, which is filled by granulation tissue, and the orange area depicts the part that becomes re-epithelialized. Both Fgf7 and Fgf22 are upregulated after skin injury in mice. Fibroblasts also produce Fgf2 and Fgf10. Fgf9 is produced by dermal  $\gamma\delta$  T cells in skin wounds and induces the expression of Wnt, which then promotes hair follicle neogenesis in large mouse wounds. (A,B) Epidermal  $\gamma\delta$  T cells (dendritic epidermal T cells, DETCs) (A) and fibroblasts (shown in the main panel) produce Fgf7 and Fgf10. These FGFs stimulate the migration and proliferation of keratinocytes in the basal layer via Fgfr1b and Fgfr2b, thereby promoting wound re-epithelialization. (C) Fgf2 stimulates wound angiogenesis in a paracrine manner via Fgfr1c and Fgfr2c on endothelial cells. (D) Keratinocyte-derived Fgf22 activates keratinocytes in an autocrine manner via Fgfr1 and Fgfr2. SB, stratum basale; SC, stratum corneum; SG, stratum granulosum; SS, stratum spinosum.

demonstrated by the reduced bleomycin-induced fibrosis in mice with combined inducible knockout of Fgfr1, Fgfr2 and Fgfr3 in fibroblasts (Guzy et al., 2017). A similar effect has been observed upon treatment of wild-type mice with a soluble Fgfr2c ligand trap, which inhibits signaling by the FGFs that activate stromal cells (Ju et al., 2012). Therefore, the repair-promoting effects of FGFs are unfortunately associated with induction of fibrosis through the action of FGFs on fibroblasts. By contrast, activation of FGF signaling in lung epithelial cells is generally beneficial. For instance, naphthalene-induced lung injury induces the expression and secretion of Fgf10 around the bronchi, and this results in the activation of club cells (see Glossary, Box 1), which then undergo a transient EMT to initiate the repair process (Volckaert et al., 2011). The repair-promoting activities of FGF10 are likely to be relevant for humans, as *FGF10* haploinsufficiency is associated with chronic obstructive pulmonary disease (Klar et al., 2011). Together with results from preclinical studies, which show that Fgf10 and Fgf7

promote repair and prevent fibrosis in different lung injury/disease animal models, these findings indicate that some FGFs might have therapeutic potential in patients with acute or chronic lung injury/lung disease (reviewed by Finch et al., 2013).

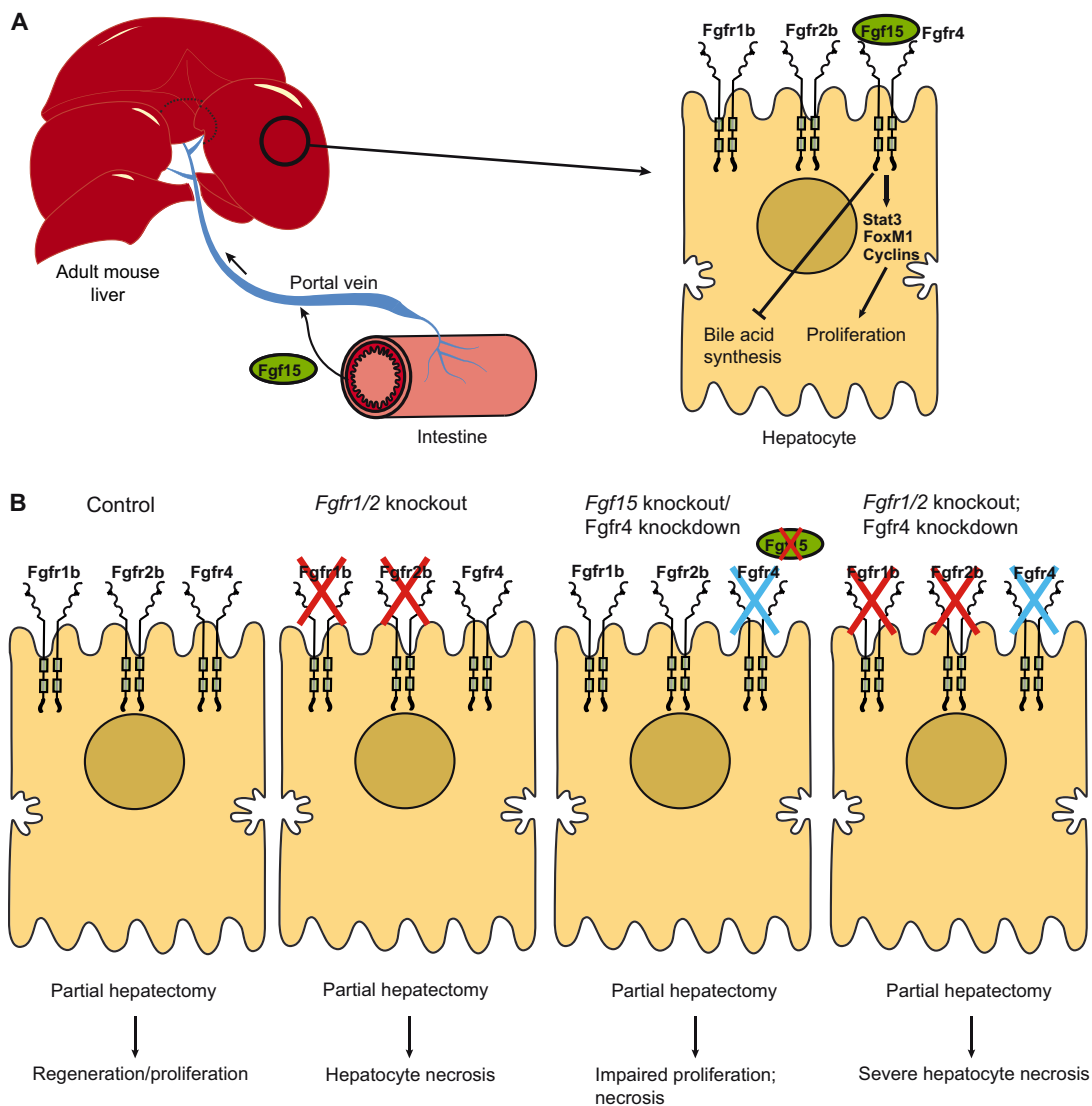
#### FGF signaling in intestinal repair

A role for FGFs in intestinal repair has been suggested based on the strong overexpression of FGF7 in human inflammatory bowel disease, which is characterized by severe tissue damage in the intestine (reviewed by Danopoulos et al., 2017). The increased FGF7 levels most likely prevent more severe injury and promote intestinal epithelial repair, as mice lacking either *Fgf7* or Fgf7-producing intestinal  $\gamma\delta$  intraepithelial T lymphocytes are more susceptible to dextran sodium sulfate (DSS)-induced colitis than are wild-type controls, and they exhibit delayed repair of intestinal tissue after termination of the treatment (Chen et al., 2002). Fgf2 might have a similar protective function, as *Fgf2* knockout mice

show impaired intestinal epithelial cell proliferation, increased outgrowth of pro-inflammatory microbiota, and, subsequently, a worse pathology in two different mouse colitis models (Song et al., 2015). In the injured gut of wild-type mice, dysregulated microbiota cause upregulated TGFβ1 expression, which in turn promotes *Fgf2* expression through regulatory T cells. *Fgf2* then cooperates with interleukin 17 (IL-17) to induce the expression of a panel of repair-associated genes in intestinal epithelial cells. These findings identified a novel *Fgf2*-IL-17 cross-talk that is important for intestinal injury repair (Song et al., 2015). This could be therapeutically relevant, given that *Fgf1*, -2, -7, -10 and -20 promote intestinal repair and reduce the inflammatory response in rodent models of colitis (reviewed by Danopoulos et al., 2017).

**FGF signaling in liver regeneration**

In contrast to other mammalian organs, the liver has the remarkable capacity to regenerate fully after acute injury. Thus, removal of up to two-thirds of the liver mass in rodents induces re-entry of the major liver cell types into the cell cycle and their proliferation until the initial liver mass is restored (reviewed by Böhm et al., 2010a). Several FGFs are expressed and upregulated after liver injury, either in the liver itself or in the spleen from where they reach the liver via the portal vein (Steiling et al., 2003). This is functionally important, as mice expressing a dominant-negative *Fgfr2b* mutant in hepatocytes are characterized by impaired hepatocyte proliferation after two-third (partial) hepatectomy (PH) (Steiling et al., 2003). This finding is consistent with the impaired liver regeneration



**Fig. 4. The role of FGF signaling in liver regeneration after partial hepatectomy.** (A) Schematic of an adult mouse liver, the hepatic portal vein and a segment of intestine. A detailed schematic of a hepatocyte is shown on the right. In response to feeding, bile acids that reach the small intestine induce production of the endocrine-acting *Fgf15*, which reaches the liver via the portal vein. Via activation of *Fgfr4* on hepatocytes, *Fgf15* activates a Stat3-FoxM1-cyclin pathway to promote hepatocyte proliferation. Furthermore, it inhibits the production of bile acids in the liver, thereby preventing toxicity caused by high levels of these molecules. In this way, loss of *Fgfr4* or of *Fgf15* impairs liver regeneration as a result of reduced hepatocyte proliferation and bile acid toxicity. (B) Schematic illustrating the effects of FGFR knockdown/knockout on hepatocytes. A control hepatocyte is shown on the left. The loss of *Fgfr1* and *Fgfr2* in hepatocytes causes liver necrosis after partial hepatectomy due to impaired detoxification of endogenous and exogenous compounds. The knockout of *Fgf15* or *Fgfr4* knockdown results in impaired hepatocyte proliferation and in necrosis. The loss of *Fgfr1* and *Fgfr2* combined with *Fgfr4* knockdown causes liver failure after partial hepatectomy due to severe necrosis, demonstrating that FGFR signaling is essential for liver regeneration. FoxM1, forkhead box protein M1; Stat3, signal transducer and activator of transcription 3.

observed in zebrafish that express a dominant-negative FGFR mutant in an inducible manner (Kan et al., 2009). However, because the dominant-negative Fgfr2b inhibits signaling through all FGFRs in response to common ligands, the type of receptor involved in liver regeneration could not be identified in this study. It has been shown that loss of *Fgf4* in mice can aggravate carbon tetrachloride-induced liver injury and fibrosis, but does not affect regeneration after PH (Yu et al., 2000). By contrast, liver regeneration after PH is severely impaired in mice with siRNA-mediated knockdown of Fgfr4 in hepatocytes, owing to enhanced liver cell death that is caused by strongly elevated levels of intrahepatic toxic bile acids. In addition, hepatocyte proliferation in this context is reduced, owing to the failure to activate an Fgf15-Fgfr4-Stat3 signaling pathway involved in normal liver regeneration. The discrepancy between the results obtained with *Fgf4* knockout versus Fgfr4 knockdown mice could be explained by the acquisition of compensatory mechanisms during embryonic and postnatal development of the knockout mice. The results obtained with the Fgfr4 knockdown mice also suggest that Fgf15, which is the major ligand of Fgfr4 and is produced in response to feeding by cells in the small intestine, reaches the liver through the portal vein and promotes hepatocyte proliferation via Fgfr4 (Padrissa-Altés et al., 2015) (Fig. 4). Consistently, mice deficient for Fgf15 show a similar and even more severe regeneration defect after PH compared with Fgfr4 knockdown mice (Uriarte et al., 2013; Kong et al., 2014).

When mice lacking Fgfr1 and/or Fgfr2 in hepatocytes, which do not have a phenotype in the non-challenged liver, are subjected to PH, severe hepatocyte necrosis occurs in the double, but not in the single, knockout mice. This is most likely caused by impaired expression of transcription factors that control the expression of P450 enzymes involved in compound detoxification. Accordingly, the liver tissue that remains after PH in the double knockout mice fails to metabolize endogenous compounds and the drugs applied for anesthesia or analgesia (Böhm et al., 2010b) (Fig. 4B). An important ligand of Fgfr2b on hepatocytes is Fgf7, which is upregulated in mice and in patients with severe liver injury (Takase et al., 2013; Steiling et al., 2004). This is functionally important, as in *Fgf7* knockout mice the expansion of liver progenitor cells is severely reduced and the mice show a higher mortality rate after toxin-induced liver injury compared with wild-type mice. By contrast, transgenic mice overexpressing Fgf7 in the liver show stronger expansion of these progenitor cells and have less severe hepatic dysfunction compared with wild-type mice (Takase et al., 2013).

Finally, it has been reported that *Fgf9* expression is induced in hepatic stellate cells (see Glossary, Box 1) in liver slice cultures after exposure to carbon tetrachloride, and that Fgf9 promotes <sup>3</sup>H-thymidine incorporation by hepatocytes *in vitro* (Antoine et al., 2007). These findings suggest that Fgf9 upregulation after toxin-induced liver injury promotes hepatocyte proliferation. Therefore, it appears that several FGFs and FGFRs are required for efficient liver regeneration, and that there is at least some redundancy among these receptors. Indeed, when Fgfr4 is knocked down in the hepatocytes of mice lacking both Fgfr1 and Fgfr2 in these cells, liver failure occurs within 2–3 days after PH as a result of massive liver necrosis (Padrissa-Altés et al., 2015) (Fig. 4B), confirming an essential role for FGFR signaling in liver regeneration.

### Perspectives

In recent years, FGFs have been identified as key regulators of repair and regeneration in numerous tissues and organs, and their roles in the regeneration of many other tissues remains to be discovered. In

most cases, FGFs promote cell proliferation, although it should be noted that they can negatively affect proliferation but promote differentiation in some tissues (see Box 2).

Most of the repair-promoting functions of FGFs are mediated by paracrine-acting FGFs, which do not diffuse far away from their site of origin. However, important roles for endocrine-acting FGFs in tissue repair are becoming increasingly recognized. A key example is Fgf15, which is produced by intestinal epithelial cells and stimulates repair of the injured liver in an endocrine manner (Uriarte et al., 2013; Kong et al., 2014; Padrissa-Altés et al., 2015) (Fig. 4). It seems likely that additional repair-promoting functions of Fgf15, of its human ortholog FGF19, and of other endocrine-acting FGFs such as Fgf21 and Fgf23 will be discovered in the future. This hypothesis is supported by the finding that epigenetic silencing of *klotho*, the essential co-receptor for Fgf21 and Fgf23, occurs in a mouse model of Duchenne muscular dystrophy, and that expression of a *klotho* transgene reduces muscle wasting and increases the pool of muscle-resident stem cells required for regeneration (Wehling-Henricks et al., 2016). However, it is as yet unclear whether this effect of *klotho* results from enhanced activity of an endocrine-acting FGF or from FGF-independent functions of *klotho*.

Another interesting question concerns the targets of FGF signaling involved in tissue repair. Interesting examples are matrix molecules, such as laminin beta1a, which is regulated by Fgf20 to form a signaling-competent regenerating epidermis during zebrafish fin regeneration (Chen et al., 2015). Fgf20 also negatively regulates miR-133, and this is important for fin regeneration. The relevant substrate of miR-133 is most likely Mps1 kinase (Ttk), a positive regulator of blastemal proliferation. Therefore, Fgf20-mediated downregulation of miR-133 prevents the loss of this important kinase (Yin et al., 2008). Targets of FGF signaling in mammalian repair processes are also emerging. These include focal adhesion proteins, which are positively regulated by FGFs in keratinocytes and thereby promote efficient skin wound re-epithelialization (Meyer et al., 2012). Fgf2, together with IL-17, also induces the expression of chemokines and matrix metalloproteinases in intestinal epithelial cells of the injured mouse gut (Song et al., 2015). Finally, the Fgf2-induced expression of miR-29a has been identified as an important mechanism for muscle regeneration (Galimov et al., 2016). Identification of further FGF targets in regenerating tissues, for example by means of large-scale transcriptomic and proteomic approaches, will shed further light on the mechanisms of FGF action during the repair of different organs. These FGF-regulated proteins

### Box 2. FGFs control cell differentiation during hair cell regeneration

In humans, the loss of mechanosensory hair cells in the inner ear, owing to their failed regeneration, results in deafness. By contrast, hair cells can regenerate in the zebrafish lateral line (which is a sensory system found on the surface of the fish), as well as in the chick cochlea. In the chick cochlea, the expression of *Fgf20* and *Fgf3* rapidly declines when supporting cells proliferate strongly. Gain-of-function studies suggest that FGF signaling inhibits hair cell proliferation during regeneration; however, FGF signaling blockade alone does not enhance hair cell proliferation (Ku et al., 2014). In the zebrafish lateral line hair cell regeneration model, inhibition of FGF signaling suppresses the regeneration of neuromasts (which are clusters of sensory cells), most likely as a result of a blockade of the differentiation process (Lee et al., 2016). Thus, in contrast to most other regenerative situations, FGFs do not act as mitogens during hair cell regeneration, but rather promote differentiation.

and non-coding RNAs might also provide promising targets for the treatment of impaired tissue repair, and their activation might enable a more specific therapeutic approach compared with the direct application of soluble FGFs, which can cause local and systemic side effects and may also have pro-tumorigenic activities. Finally, furthering our understanding of how FGFs act mechanistically in both tissue repair and regeneration might help to uncover ways to drive regenerative rather than repair processes in humans.

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#### Competing interests

The authors declare no competing or financial interests.

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