Review Article

Growth factors and their roles in cardiac development and regeneration: a narrative review

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Background and Objective: A network of signaling events is required for the formation of a mature cardiovascular system. During embryogenesis, secretion, or suppression of several growth factors at critical time points and proximities drive this development, including epidermal growth factors (EGFs), fibroblast growth factors (FGFs) and insulin-like growth factors (IGFs) which will be discussed in this review.

Methods: PubMed was searched looking for literatures published between 1990 and March 2022 in English. A review of the literature was completed to find relevant research and terminology around the topics of cardiac development, growth factor signaling and recent advances in cardiovascular regeneration.

Key Content and Findings: Each family of growth factors has been extensively examined by investigators in pre- and postnatal contexts to uncover therapeutic potential against heart disease—a leading cause of death globally. Select ligands with predicted clinical value have been further assessed, such as neuregulin-1 (NRG1)—which binds to the family of EGF receptors (EGFRs) to dimerize and ultimately activate myocyte proliferation through a signal cascade, and IGF-1—which binds to IGF receptors (IGFRs) to regulate metabolism, proliferation, apoptosis, and autophagy.

Conclusions: Collectively, this review supports the need for further investigation on how these critical signaling pathways function during heart development and how the new knowledge might be leveraged to treat cardiovascular disease and promote heart regeneration.

Keywords: Cardiac development; growth factors; regeneration

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Introduction to cardiac development

During embryogenesis, the heart initially forms a linear heart tube structure, followed by cardiac looping and septation, and eventually grows into a four-chambered heart (1). Meanwhile, multiple components need to form, including the epicardium, myocardium, coronary vascular network, endocardial cushion and ventricular septum and trabeculae (2). This process can be divided into early, mid, and late gestational stages. In the early stages, the linear and looping heart contain two layers of cells. These are the outer layer, comprised of cardiomyocytes (CMs) and an inner layer, comprised of endothelial cells (ECs) (3). Later, the heart will be surrounded by the third layer, known as the epicardium, in the mid stage. The mid stage will also include development of the atrial-ventricular and outflow tract endocardial cushions (1). The process requires constant communications among many cell types such as cardiac progenitor cells, CMs, ECs and fibroblasts. If heart development goes awry, congenital heart disease (CHD)

Table 1 The search strategy summary

Items	Specification
Date of search	March, 2022
Databases and other sources searched	PubMed
Search terms used	Growth factor signaling, epidermal growth factors, fibroblast growth factors, insulin-like growth factors, cardiovascular development, and cardiovascular regeneration
Timeframe	1990s to March 2022
Inclusion	Inclusion: publications in the English language and growth factor actions on either the developmental stage or regeneration potential of the heart
Selection process	All authors identified pertinent material for the review

follows. CHD remains to be the number one birth defect in live births worldwide (4).

Importantly, normal cardiac development relies on the proper expression and suppression of growth factors and transcription factors at the right time and place (5). This manuscript will review some of the important signaling events required for proper cardiac development such as epidermal growth factors (EGFs), fibroblast growth factors (FGFs) and insulin-like growth factors (IGFs). We will briefly go over their molecular interactions and outline their noteworthy downstream components. Lastly, we will examine important models which have led to our understanding of the field in recent years and consider how therapeutic heart regeneration can be influenced by the current knowledge of growth factors and their downstream signals. We present this article in accordance with the Narrative Review reporting checklist (available at https://pm.amegroups.com/article/view/10.21037/pm-22-17/rc).

Methods

A review of the literature in PubMed was completed to find relevant research from the mid-1990s to present day—March 2022 (*Table 1*). The papers were focused more on recent advances and understandings, but we refer to older research to appreciate the progress which has been made over the past 30 years. The primary search terms included Growth Factor Signaling, EGFs, FGFs, IGFs—all as they relate to cardiovascular development. Finally, we compiled recent advances in the field of cardiovascular regeneration and focused on manuscripts relating to the previous growth factors mentioned.

EGF signaling

EGF signaling regulates important processes throughout the body, including the development of a fully functional cardiovascular system. ErbB receptors are well-studied receptor tyrosine kinases (RTKs) which transmit signals from the extracellular environment into the cell via the EGF family of ligands (6). Several ligands have been identified as stimulators of ErbB receptor dimerization, including EGF, neuregulins (NRGs), transforming growth factor-α, amphiregulin, heparin-binding EGF-like growth factor (HB-EGF), betacellulin, epiregulin and epigen (7). These ligands first bind to a monomer to initiate a conformational change in the extracellular domain—for members 1, 3, and 4—which expose a dimerization arm to facilitate pairing (8).

As EGF signaling ligands, NRGs are secreted by endocardial cells and can be tethered to the surface of the cells to assist in the regulation by a transactivating signal discussed later (9). There are 4 NRGs: NRG1–4, with many isoforms for each (10). The NRG-ErbB pathway is mostly involved in ventricular trabeculation and the development of the valves between chambers (11). These steps are valuable in shaping the myocardium and are required for the proper development. Notably, these pathways are involved in differentiation or proliferation, but not simultaneously (12,13).

The first identified EGF receptor (EGFR) is also known as ErbB1 or HER1 (*Figure 1*) (14). The four members of the receptor family include ErbB1–4, also known as HER1–4 (6). Due to their implications in many diseases—notably with HER2 and breast cancer—they are very well studied and characterized (12). These receptors reside in the plasma membrane in a mostly inactive conformation

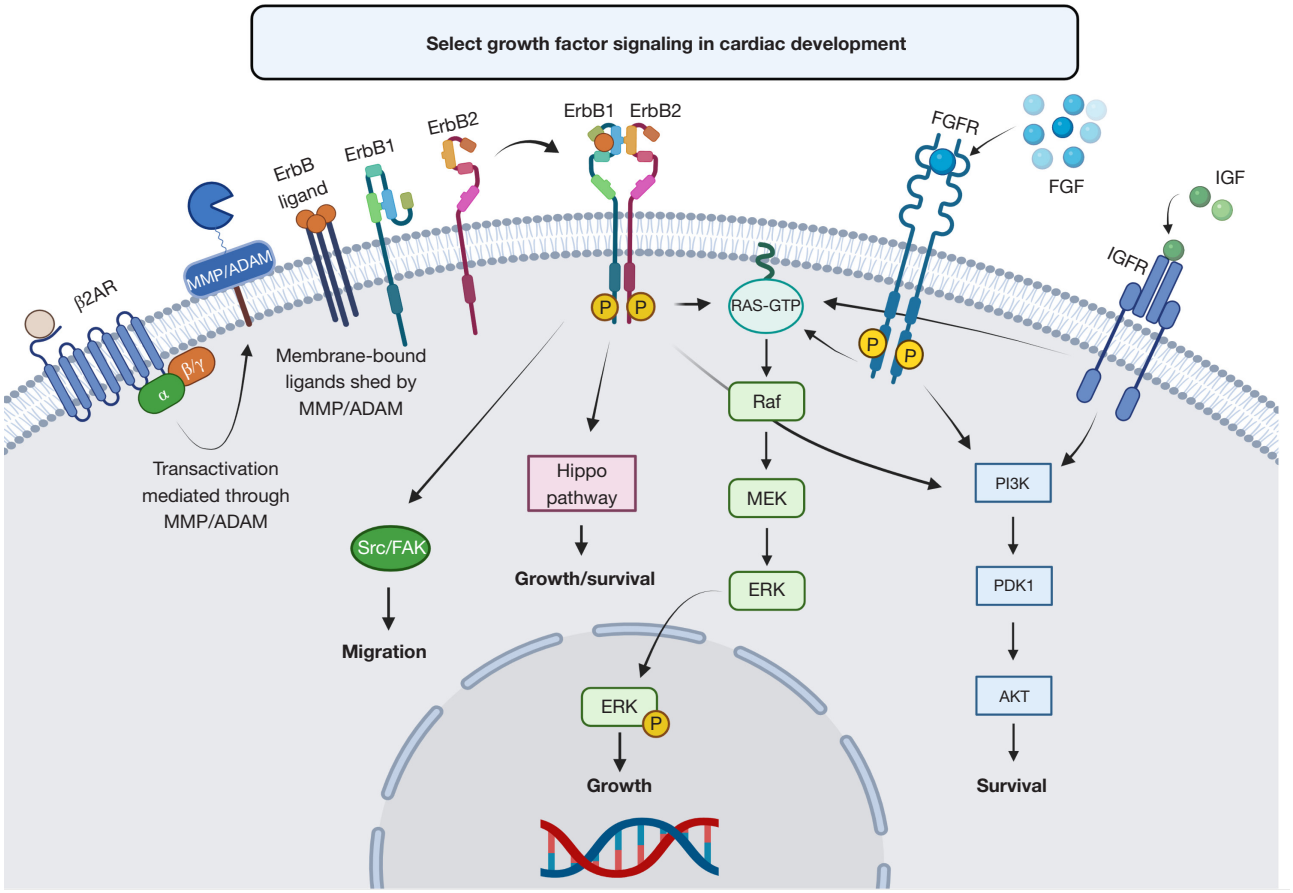

Figure 1 The interconnected pathways discussed in the review: EGFs, FGFs, and IGFs and their downstream consequences in the cardiovascular system. β2AR, outlining the current understanding of GPCR transactivation with a typical example. Created with BioRender.com. β2AR, beta-2 adrenergic receptor; MMP, matrix metalloproteinase; ADAM, A disintegrin and metalloproteinase; ErbB, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; FGF, fibroblast growth factor; IGF, insulin-like growth factor; IGFR, insulin-like growth factor receptor; Src, proto-oncogene tyrosine-protein kinase; FAK, focal adhesion kinase; Ras-GTP, rat sarcoma guanosine triphosphate hydrolase oncogene; Raf, serine/threonine-specific protein kinase oncogene; MEK (MAPKK), mitogen-activated protein kinase kinase; ERK, extracellular-signal regulated kinase; PI3K, phosphoinositide 3-kinase; PDK1, phosphoinositide-dependent kinase 1; AKT, protein kinase B; EGF, epidermal growth factor; GPCR, G protein-coupled receptor.

until activated by their respective ligands. This interaction leads to homo- or heterodimerization with another member of the ErbB family and initiates a downstream signal (15,16). As with other RTKs, the dimerization is followed by transphosphorylation in the cytosolic domains, creating docking sites for proteins to bind and propagate a signal (17). ErbB2 is unique in that it does not have a known ligand. Due to this phenomenon, it typically maintains an active conformation, always revealing a dimerization arm for heterodimerization to take place. It is classified by scientists as a "co-receptor" and tends to be the favorite binding partner (18,19).

ErbB receptors often begin the mitogen-activated protein kinase (MAPK) pathway, by signaling Ras-GTPase and ultimately leading to extracellular-signal regulated kinase (ERK) translocation into the nucleus (*Figure 1*); however, more work needs to be done to determine the comprehensive role in cardiac development (20). ErbB signaling is also linked to the Hippo pathway, with the nuclear translocation of Yap/Taz regulating NRG1 production (9).

ErbB activity can also be mediated by transactivation

via G protein-coupled receptors (GPCRs). The ErbB system is known to have many of the pro-ligands tethered to the surface of cell membranes (16). After enzymatic cleavage of these ligands, the paracrine signal can be initiated immediately. This transactivation system links GPCRs and ErbBs through various proteinases such as matrix metalloproteinase (MMP) or A disintegrin and metalloproteinase (ADAM) (14). When one of these metalloproteinases is activated, this can lead to an autocrine or paracrine response, which helps to coordinate the signal to nearby cells. This is an important feature of a multicellular system as these GPCR ligands potentially stimulate the receptor of the resident cell followed by activation of a nearby EC, CM, or fibroblast. This creates an opportunity for these GPCRs to directly impact the crosstalk between cell types (21). Within the cardiovascular system, the most common ligand which is shed by metalloproteinase activity is HB-EGF which directly binds to either ErbB1 or ErbB3 (22). A particular example is ADAM17, which is critical for the shedding of NRG1 (23,24). Adding to the intricacy of the system, many relay molecules can also modulate metalloproteinases, including intracellular calcium, protein kinase C, Src kinase and reactive oxygen species (14,25,26).

Early genetic knockout studies have revealed the importance of various ErbB receptors, such as ErbB2, 3, and 4—the most abundant receptors in heart development (19,27-29). Knockout of *ErbB2*, *3*, or *4* led to an embryonic lethal condition between day 10.5 and 13.5 in mice (30,31). This death is attributed to defects in heart morphology, such as hypotrabeculation, thin myocardium and defective cardiac cushion formation (32,33). Knockout of *Nrg1* in mice resulted in similar embryonic lethality and cardiac phenotype (34). Taken together, these studies suggest that EGF signaling is critical for early heart morphogenesis, and its continued investigation might be impactful for adult heart regeneration.

FGFs

Another secreted growth factor important in the developing heart is the FGF (*Figure 1*). This family of proteins has 22 known members divided into 7 subfamilies, which are also needed in the development of many organ systems (35,36). Signaling is typically accomplished through a ligand-receptor interaction, initiated in a paracrine or endocrine manner with a few members performing intracrine functions (37). Paracrine and endocrine ligands are secreted and function along with the cofactor heparan sulfate. This complex will facilitate the dimerization of FGF receptors (FGFRs), leading to the same RTK phenomenon as ErbB signaling (35).

There are 4 receptors in this family, classified as FGFR1–4, with multiple splice variants for each (38). The FGFRs are typically involved in the MAPK and Akt pathways or initiate Jak/STAT signaling and are required for both differentiation and proliferation at different stages and different ligand/receptor interactions (20,39,40). They are also involved in homeostatic events like cell survival, apoptosis or migration (41) as well as angiogenesis—helping to build the coronary network but could also improve the blood flow after an ischemic incident (42). These points highlight the importance of FGF in physiological and pathophysiological contexts.

There are many intricate details involved in the different FGF ligands and receptors, which are still being investigated. In this review, we will discuss only a handful which are involved in heart development. Of the growth factors discussed, this family is the most lacking in available evidence with some controversial discoveries. Multiple FGFs help to regulate CM proliferation via an epicardial- and endocardial-derived signal. These populations of ECs in close contact with the myocytes mediate growth through FGF and other mitogens through paracrine signaling (43).

Several FGFs have been implicated in cardiac development, however there are some disagreements about which ones are involved. There is now a consensus that FGF9, and likely FGF16 and 20, are all essential for myocyte proliferation and could also be necessary for coronary vascular development (1,44). The FGFR1/2 and FGF9-null mice have decreased myocyte proliferation with significant ventricular hypoplasia—an underdeveloped heart (45). Early research suggests that the only relevant FGFs are FGF9, 16 and 20, discounting the importance of others in a developing heart. Later, investigators highlighted the importance of several more ligands, including FGF8, 10, 15, 19 and several others (35). Early work had identified FGF2 as one member which is necessary (46), however this has since been disregarded with later knockout models showing no significant phenotype in mice during embryogenesis.

Elegant studies using embryonic stem cells observed the need for FGFR1 in the differentiation into functional CMs (1,20,47), using both genetic and pharmacological strategies to probe different parts of the signaling pathway. FGFR2 is also an essential component of a developing heart

but is lacking in the adult heart (48). Knockout models of *FGFRs 1* and *2* in the myocardium led to ventricular wall hypoplasia, giving us a good understanding of how FGF signaling leads to the maturation of the ventricles (33). These findings could help researchers uncover unique strategies in treating cardiovascular disease later in adulthood. There is some conflicting evidence when cells or systems are treated with inhibitors of downstream FGF signaling, such as ERK1/2 or mitogen-activated protein kinase kinase (MEK), with some investigators not able to replicate any findings (49,50). This could be variability in the methods used but could also highlight the importance of time and dose dependence of these signaling events.

IGFs

The last important element we will discuss in the coordinated effort of cardiac development is IGF signaling (*Figure 1*) (51). IGF-1 and IGF-2 are small growth hormones which perform endocrine, paracrine and autocrine signaling within the system (52,53). A majority of IGF-1 is made and secreted by the liver and expressed postnatally (52), while IGF-2 is predominantly expressed within a developing embryo (54). They are both, however, in many ways critical in embryogenesis and must be well regulated—requiring IGF-binding proteins to stabilize them in their soluble form (55,56). There are two IGF receptors (IGFRs), IGFR-1 and IGFR-2. When a ligand binds, these receptors will autophosphorylate and initiate an appropriate downstream cascade (54).

IGF-1 activates MEF2C, a key transcription factor in cardiac development. This has been confirmed using Luciferase reporter assays showing the promoter enhancement of MEF2C by IGF-1 signaling stimulation (57). IGF-1-induced p38-MAPK activation mediates enhanced phosphorylation of MEF2C. Conversely, inhibitors of phosphoinositide 3-kinase (PI3K) abolish the IGF-1 stimulated MEF2C transcriptional activation (57). IGF-1 also activates other signaling pathways including ERK1/2, PI3K, PKC, PKB, Jak/STAT and PLC (58-60).

IGF-1 and IGF-2 are important for heart development, metabolism, apoptosis, autophagy, and aging (61). An interesting study showed that clinical treatment with IGF-1 could strengthen the heart in late gestation and beyond (62). Downregulation of IGF-1 receptors generate a dilated cardiomyopathy phenotype, while upregulation has led to hypertrophy (51). This phenotype has been observed in a PI3K/Akt dependent manner (63). IGF-1 signaling may not contribute to the maturation of cardiac cells per se, but increases the total number of cells during the developmental phase which may be critical in CVD incidences into adulthood (64). Additionally, reduced amount of IGF-1 in the blood has been associated with a greater risk of acquiring cardiovascular abnormalities (53). IGF-1 could also be anti-fibrotic, an important mechanism which prevents permanent remodeling and scarring—revealing its cardioprotective role (61,65).

Expanding on the key regulatory components of IGF-1, an important consideration for disease risk is now known to be linked to the environment *in utero* (66). It is evident that issues in gestation are associated with energy balance and regulation throughout life, increasing risk for CVD (67). Many groups have identified a link between fetal growth restriction (FGR) and cardiovascular disease in rats, mice, chickens and sheep (68-70). Long-term consequences of FGR, which were identified over 30 years ago, include decreased cardiac stroke volume, cardiac output, early morbidity and mortality, metabolic impairments leading to obesity, and early CVD-related events as adults (71). IGF-1 was previously speculated as a main driver for this syndrome (72). Authors from the 90s have used sheep as a model of *in utero* development, particularly using IGF-1 as a treatment for restricted growth. In this sheep model (*Figure 2*), circulation to the fetus was restricted by partially occluding the umbilical artery and subsequently reintroduce recombinant IGF-1 to evaluate the overall health of the fetus (62). They found that IGF-1 treatment effectively increases the overall heart mass by increasing the proliferation of these ovine cardiac cells (62). These researchers have also taken these experiments a step further and to understand postnatal metabolic control in a myocardial infarction model. These models have shown that administration of various levels of the two IGFs may lead to enhanced healing (54). In a more recent study, scientists treated obese mice with IGF-1 and revealed an improvement in cardiac metabolism, reduction in fibrosis and a decrease in apoptosis; all suggesting a cardioprotective effect of the treatment. These results were accompanied by cardiac progenitor cell survival, proliferation and differentiation which the authors confirm is due to an increase in Akt phosphorylation (73).

Learning from these preclinical studies, an important issue to first understand is the right concentration of IGF to be administered to these FGR patients, as levels over and under the correct value are detrimental to development. This was evident after a series of clinical trials for delivering

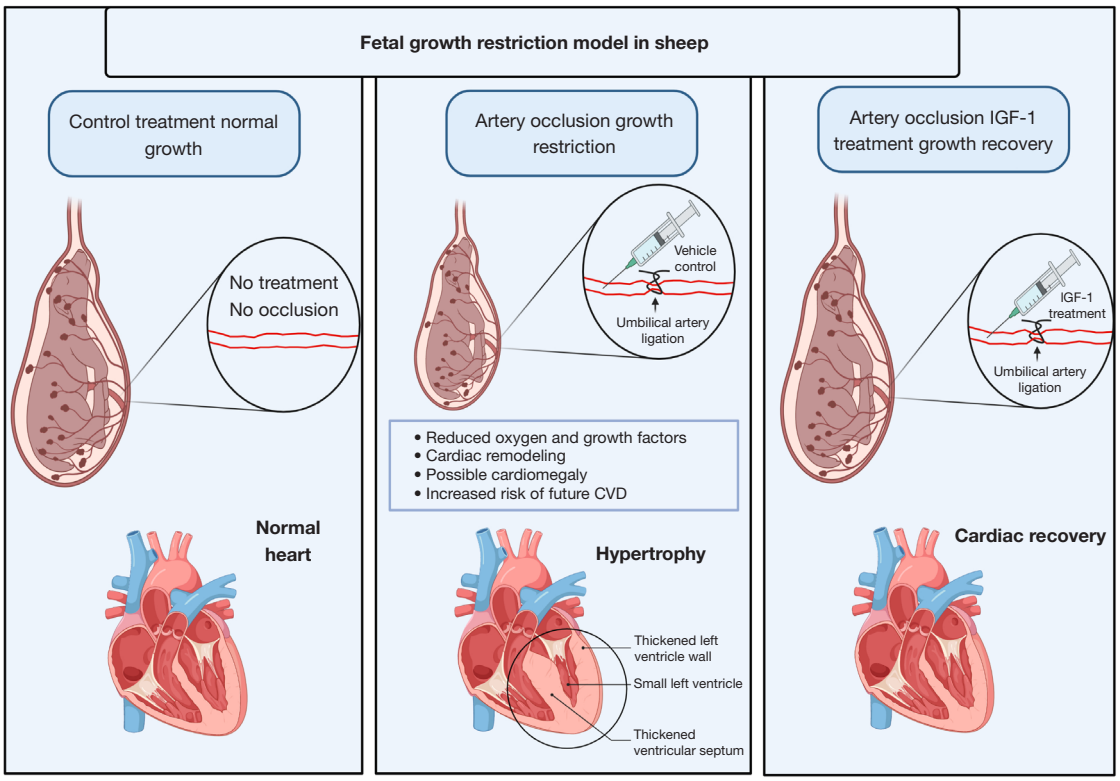

Figure 2 FGR ovine model. With the umbilical artery ligation, blood flow is restricted from the embryo leading to many growth abnormalities; however, with the addition of IGF, the embryonic hearts return to a normal phenotype. IGF-1 as a therapeutic option is being explored due to the long-term consequences of the syndrome. Created with BioRender.com. CVD, cardiovascular disease; IGF-1, insulin-like growth factor 1; FGR, fetal growth restriction; IGF, insulin-like growth factor.

the right dose of IGF-1 for FGR patients. These clinical trials reveal the complications associated with variable pharmacokinetic properties on fetal and preterm patients, which needed to be treated intravenously (74). In a mouse model of FGR, Alsaied *et al.* [2017] observe a higher weight gain postnatally compared to control mice, with a higher number of overweight adults (61). This was accompanied by systolic dysfunction at 3 months with no sign of recovery at the end of the experimental timeline (32 weeks).

Cardiac regeneration

Promoting cardiac repair and regeneration has been a huge challenge to the field for several decades. Treatment using stem cells, tissue patches, and trans-differentiation strategies have been tested preclinically and clinically (75). Some have also hypothesized that calling upon endogenous pathways could be the best way to stimulate CM self-renewal (76). Most recently, studies suggest the use of extracellular vesicles could have regenerative potential (77-79). Apparently, a better understanding of these growth signaling pathways such as what are being discussed herein during heart development may give us clearer pictures on when and how we can utilize these pathways to induce cardiac regeneration in adult heart.

Exogenous administration of recombinant NRG-1 into adult mice was shown to stimulate CM proliferation and heart regeneration to various degrees (78,80-82). However, this treatment option may only be effective during earlier periods of life (83). This narrow time window is consistent with an observation of the drastic reduction in the expression of ErbB2 after birth (32). This EGF signaling could be an important element in treating heart failure patients. IGF signaling may have similar effects on heart regeneration (84). Newborn mice can fully regenerate their hearts after injury, although they lose this ability 1 week after birth (56). Interestingly, ablation of IGF-2 in the developing endocardium abolished CM

Table 2 Studies involving growth factors promoting heart regeneration and repair

Activated growth signaling	Organism	Intervention/transgenic model	References
NRG1	Zebrafish	Treatment of AG1478 (a small molecule inhibitor of ErbB receptors)	Polizzotti *et al.*, 2015 (83)
NRG1	Zebrafish	Homeobox-containing transcription factor prrx1b knockout heart	De Bakker *et al.*, 2021 (88)
NRG1/ErbB4	Rat, mouse	Injection of recombinant NRG1	Bersell *et al.*, 2009 (80)
ErbB2	Mouse	caErbB2 CM	D'Uva *et al.*, 2015 (32), Aharonov *et al.*, 2020 (81)
FGF	Zebrafish	hsp70:dn-fgfr1; fli1:EGFP transgenic	Lepilina *et al.*, 2006 (89)
FGF1	Rat	Treatment with FGF1 and p38 inhibitor SB203580	Engel *et al.*, 2006 (90)
FGF2 VEGFA	Mouse	Reactivation of the fetal epicardial program after MI	Zhou *et al.*, 2011 (91)
IGF-2	Zebrafish	hsp70:dn-igf1ra-GFP transgenic	Huang *et al.*, 2013 (92)
IGF-2	Zebrafish	Igf agonist NBI-31772	Choi *et al.*, 2013 (93)
IGFR-1	Mouse	Injection of IGF-1 modRNA-injected heart	Zangi *et al.*, 2017 (94)
Igf2bp3	Mouse	Pre-injection of AAV9-Igfbp3	Wang *et al.*, 2019 (95)

NRG1, neuregulin-1; ErbB, epidermal growth factor receptor; FGF, fibroblast growth factor; VEGFA, vascular endothelial growth factor A; IGF, insulin-like growth factor; IGFR, insulin-like growth factor receptor; Igf2bp, insulin-like growth factor 2 binding protein; PRRX1b, paired-related homeobox 1 transcription factor; hsp70:dn-fgfr1, mouse model dominant negative for the gene fibroblast growth factor receptor 1; caErbB2 CM, cardiomyocyte-specific overexpression of constitutively active ErbB2; fli1:EGFP, mouse model with enhanced green fluorescence protein on friend leukemia integration transcription factor 1 proto-oncogene; MI, myocardial infarction; AAV9, adeno-associated virus serotype 9.

proliferation capacity induced by injury on postnatal day 1 (85). This study illustrated that IGF-2 is an important paracrine factor involved in the early heart regeneration. However, the effects of IGF-1 on adult cardiac repair and regeneration are mixed. For instance, in an MI rat model, administration of IGF-1-adipocyte-derived mesenchymal stem cells in a myocardial infarction rat model showed improved left ventricle ejection fraction (LVEF) but did not decrease infarct scar size when compared to the control group (86). On the contrary, the application of microencapsulated IGF-1 in an ischemia-reperfusion porcine model was reported to improve cardiac function and significantly reduce fibrosis in treated animals compared to controls (79). In a recent study, the overexpression of IGF-1 in cardiac progenitor cells in a pig model of sub-acute myocardial infarction did not achieve significant cardiac functional improvement (87). The overall effects of these exogenous paracrine growth signals on injured hearts might be dependent on the dose, the time and the route of application, as well as the injury models being used. *Table 2* outlines some *in vivo* studies done using these approaches, highlighting which model was used and the therapeutic strategy involved.

Conclusions and future directions

This review has outlined some of the important growth factor cascades in mediating development of the cardiovascular system. These features are only a fraction of the full interconnected pathway which needs to take place for formation of a fully functioning pump system. EGF, FGF, and IGF families are major contributors to cardiac development and provide an immense number of potential targets for pharmacological or genetic manipulation. However, a more detailed analysis on each of the signaling component will gain a better understanding of these as targets. Growth factor signaling is a promising avenue, and some have even begun clinical treatments with them, such as treating FGR with IGF-1 (74). Similar work is desired to evaluate the therapeutic potential for these growth signals, but these approaches are mostly unresolved *in vivo* and must be approached with skepticism (82). An important consideration is that these pathways do intertwine at various points. For instance, the MAPK and PI3K pathways are common downstream signaling components for all three growth factor families discussed (*Figure 1*). Future work is needed to dissect the potential cross talks among these

signaling pathways during heart development.

As we have all recognized, developmental events often replay during regeneration, as also illustrated in this review. Hopefully, the knowledge we will gain by studying these signaling events will educate us and then apply them in stimulating heart regeneration.

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Footnote

Reporting Checklist: The authors have completed the Narrative Review reporting checklist. Available at https://pm.amegroups.com/article/view/10.21037/pm-22-17/rc

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://pm.amegroups.com/article/view/10.21037/pm-22-17/coif). All authors report that this work was supported by the National Heart, Lung, and Blood Institute R01 grant (No. HL153406). The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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