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The Role of TGF - β Signaling in Cardiomyocyte Proliferation

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Abstract

Purpose of review—The loss of contractile function after heart injury remains one the major healthcare issues of our time. One strategy to deal with this problem would be to increase the number of cardiomyocytes to enhance cardiac function. In the last couple of years, reactivation of cardiomyocyte proliferation has repeatedly demonstrated to aid in functional recovery after cardiac injury.

Recent findings—The Tgf- β superfamily plays key roles during development of the heart and populating the embryonic heart with cardiomyocytes. In this review, we discuss the role of Tgf- β signaling in regulating cardiomyocyte proliferation during development and in the setting of cardiac regeneration.

Summary—Although various pathways to induce cardiomyocyte proliferation have been established, the extent to which cardiomyocyte proliferation requires or involves activation of the Tgf- β superfamily is not entirely clear. More research is needed to better understand cross-talk between pathways that regulate cardiomyocyte proliferation.

Keywords

TGF- β signaling; Proliferation; Cardiac Regeneration; Heart

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Introduction

Cardiovascular disease is the leading cause of death (1, 2). Treatment of this disease and its sequelae accounts for 17% of U.S healthcare expenditures due to significant mortality and morbidity(3). Although heart failure treatment has improved in the past decades, five year mortality rates remain around 50%(4). One of the major reasons for this poor outcome is the inability of the adult myocardium to regenerate after injury. The adult heart does not contain a progenitor cell population that can generate cardiomyocytes and repopulate the myocardium after injury (5–8). Moreover, adult cardiomyocytes do not proliferate frequently enough to meaningfully restore cardiac function(9).

In the past few decades, many strategies have been proposed to recover contractile function after cardiac injury. Since the most limiting factor in restoring normal cardiac function appears to be addition of new cardiomyocytes, one strategy to stimulate regeneration would be to enhance proliferation of endogenous cardiomyocytes (CMs). Near perfect heart regeneration has been observed in zebrafish, certain frogs, newts and neonatal mice(9–11). All of these animals regenerate their heart by repopulating their myocardium with new CMs, mostly through activation of endogenous CM proliferation. Zebrafish and certain urodele amphibians maintain this proliferative capacity throughout their entire life(10, 12). In contrast, mice lose the ability to stimulate CM proliferation in response to injury, and consequently their ability to regenerate from cardiac injury, soon after birth(12). Humans likely display a similar inability to stimulate CM proliferation after injury, since the overall proliferative capacity of CMs comes to a near-complete hold after 20 years of age (13). If adult humans could reactivate their proliferative potential, this would likely be therapeutically beneficial.

In recent years a number of publications have shown that it is possible to induce CM proliferation and promote functional regeneration after cardiac injury. Modulation of Neuregulin, Hippo and Cyclin/Cyclin Dependent Kinase signaling have shown promise in regulating endogenous CM proliferation and potential for functional recovery from cardiac injury (14–17). There are likely additional signaling pathways capable of regulating CM proliferation, including the Transforming Growth Factor β (Tgf- β) superfamily which has been implicated in cardiac cell cycle modulation. Some of the mechanisms known to influence CM proliferation, do so, at least partially, through manipulation of Tgf- β superfamily signaling or downstream signal transduction. The Tgf- β signaling pathway also appears to be important in the developmental transition in mammalian CMs where they lose their proliferative capacity with age. Alongside the Insulin Growth Factor (IGF) pathway, the Tgf- β pathway undergoes the most significant transcriptional changes as young mammalian CMs age from a proliferative to non-proliferative state (12, 17).

In this review we will summarize the basic structure of the Tgf- β signaling pathway, highlight portions of the Tgf- β pathway that have been implicated in the regulation of CM proliferation, and attempt to untangle the seemingly paradoxical ways in which components of the Tgf- β pathway alter CM proliferation.

The Tgf- β Signaling Pathway

The Tgf- β superfamily is one of the major ways in which cells communicate with each other. On the sending end, this includes small soluble growth factors, such as Tgf- β s, Bone Morphogenetic Proteins (BMPs), Activins and Nodal. While most cells express receptor complexes that allow receiving Tgf- β signaling, secretion of the ligands is a locally controlled process. For an effective signal after production of the growth factor, it first needs to be cleaved to proteolytically remove the pro-domain by proteins such as Furin (18, 19). The ligand is then secreted along with the associated Latency Associated Peptide (LAP) and Latent Tgf- β -Binding Protein 1–4 (LTBP1–4) complexes, which can sequester the ligand in the extracellular matrix until the ligand escapes the LAP and LTBP complex (18, 19). Only then is the ligand able to bind Tgf- β receptors on receiving cells (18, 19). Tgf- β receptors are categorized into Type I, II and III receptors, which are found in the plasma membrane of most cells. Activated Tgf- β is a dimer that binds to type II receptors, which then recruits type I receptors forming a heterotetrameric signaling complex (20). The recruitment of type I receptors exposes the cytoplasmic kinase domain on the type II receptor, which then phosphorylates and activates the type I receptor (20). The type I receptor can then act as a kinase and phosphorylate various cytoplasmic proteins (Figure) (20). Fine-tuning of activation of the Tgf- β receptor complex occurs through expression and activity of co-receptors, such as the Tgf- β Type III receptor or Endoglin. The consequences of these larger receptor complexes will modulate activity and directionality of signaling downstream of the receptor complex (21).

Downstream of the Tgf- β receptor, signaling is typically mediated by phosphorylation of cytoplasmic targets, and the Tgf- β signaling pathway is typically segregated into two separate categories: canonical and non-canonical (Figure). In the canonical pathway, the type I receptor will phosphorylate a receptor-regulated SMAD (R-SMAD) protein. In the case of Tgf- β or Activin signaling, this is usually SMADs 2/3, while downstream of BMP signaling, it is usually SMADs 1/5/8. Upon phosphorylation, R-SMADs will bind to a co-SMAD, SMAD4. These R-SMAD/co-SMAD complexes then translocate to the nucleus where the SMAD complex forms the basis for a larger transcriptional complex to regulate gene transcription (21). The SMAD transcriptional complexes regulate numerous genes, and SMAD signaling has been implicated in regulation of cell cycle progression and proliferation in various cell types (22). SMAD4 can bind to over two thousand separate loci, and a number of these are differentially expressed in response to Tgf- β signaling (23). Alternatively, there are also inhibitory SMADs (i-SMADs) which can interfere with SMAD signaling. SMAD6 can sequester R-SMADs away from SMAD4, while SMAD7 can disrupt R-SMAD activation (24, 25).

The non-canonical arm of the Tgf- β pathway results in signaling downstream of the receptor ligand complex, independent of SMAD phosphorylation. In the non-canonical arm, the type I receptor will phosphorylate and activate proteins that are not SMADs, resulting in either cytoplasmic regulation of cell function, or modulation of gene transcription independent of SMAD complexes. Non-canonical signaling can result in either activation or suppression of the targeted protein (26). Some major non-canonical Tgf- β regulated proteins that have been linked to CM proliferation include ERK, p38 and JNK, all of which are also targets of the

Mitogen-Activated Protein Kinase (MAPK) signaling pathway (27, 28). Other non-canonical Tgf- β targets can also be phosphorylated through additional signaling pathways. Thus, Tgf- β signaling may be sufficient but not necessary to induce activation of these non-canonical pathways. Below, we will discuss the various components of the Tgf- β signaling pathway and their potential functions in regulation of CM proliferation.

Ligands of the Tgf- β Superfamily

The archetypal Tgf- β pathway is activated by Tgf- β 1, Tgf- β 2 or Tgf- β 3 ligands. Activation of Tgf- β signaling initiates with secretion of a ligand. The ligands are expressed in many cell types in the body and are not specific to the myocardium. They are usually secreted in an inactive form and require proteolytic cleavage, before they can functionally bind to their target receptors (29). Expression of these ligands is sufficient to induce proliferation in some specific cell types such as fibroblasts and smooth muscle, although they can also be inhibitors of proliferation as occurs in endothelial cells, and the effect could be dependent on the effective concentration of Tgf- β growth factor (30–33). While these three ligands are expressed in many cell types and are not specific to the myocardium, they exhibit dramatic changes in expression after cardiac injury (34). Although ligand modulation is essential for wound healing processes such as scar formation, secretion of Tgf- β ligands does not appear to be sufficient to induce proliferation of cardiomyocytes in any detectable way. Knockout of any of the three Tgf- β ligands does not alter proliferation of CMs; genetic deletion of either Tgf- β 1 or Tgf- β 3 does not result in heart defects(35–37), suggesting that Tgf- β 1/3 do not play a critical role in myocardial development. In contrast, Tgf- β 2 null mice display a plethora of cardiac defects, but ventricular hypoplasia is not among them (38). Thus, Tgf- β 2 also does not appear essential for regulating CM proliferation. These findings indicate that loss of the core ligands is either unimportant in CM cell cycle or that there is sufficient redundancy to activate Tgf- β signaling in absence of one of these ligands.

Although core Tgf- β ligands themselves are not implicated in regulation of CM proliferation, there are several Tgf- β superfamily ligands, which have been directly shown to modulate CM proliferation. The best studied of these ligands, *Inhbaa* and *Mstnb*, signal through the type 2 activin receptors in zebrafish. Similar to the Tgf- β receptor, the activin receptor also acts to phosphorylate R- SMADs such as SMAD2/3 (39). Myostatin, a ligand for signaling through the type 2 activin receptor, has been demonstrated to inhibit cultured rat CMs from entering S-phase of the cell cycle (40). Myostatin treatment also results in an increase in SMAD2 phosphorylation (40). A similar phenomenon can be observed in zebrafish heart regeneration with *Mstnb*: the zebrafish homolog to Myostatin. After cryoinjury, expression of the *Mstnb* ligand is inhibited, while expression of *Inhbaa* is stimulated to promote CM proliferation. This zebrafish response is restricted to trabecular myocardium and is not significant in the compact myocardium (39). In adult mammals, Myostatin expression is directly correlated with skeletal muscle size, but not cardiac myocyte size (41, 42).

The Receptors

The various ligands of the Tgf- β superfamily bind to type II receptors, which can then recruit the type I receptor. There are many combinations of type I and type II receptors that can induce SMAD signaling. However, only a few combinations of receptors have currently been shown to modulate CM proliferation. The best studied of these receptors is TGFBR1, also known as Alk5. Myocardial specific TGFBR1 knockout mice have no detectable cardiac defects during embryonic development (43). However, two different groups have noted the important role of TGFBR1 in adult zebrafish heart regeneration. Zebrafish were treated with SB-431542, which blocks the kinase activity of TGFBR1, preventing it from phosphorylating SMAD3 (44). SB-431542 treatment inhibits CM proliferation both under baseline conditions and after various injuries (45). Thus, TGFBR1 activity is required for CM proliferation in zebrafish. Furthermore, SB-431542 treatment also prevents activation of a promoter fragment that is specifically activated in heart and fin regeneration in zebrafish, called the *careg* regulatory sequence, which plays a key role in demarcating the peri-injury zone after cardiac injury(46). To what extent TGFBR1 activity limits CM proliferation is not entirely clear.

In adult mammals, the most potent stimulation of CM proliferation is accomplished through modulation of core cell cycle regulators, via 2 different strategies: 1) overexpression of 4 core cell cycle activators, CDK1, CDK4, Cyclin B and Cyclin D or 2) overexpression of CDK1 and CDK4, in conjunction with treatment with Wee1 inhibitor Mk1775 and TGFBR1 inhibitor SB-431542. This cocktail of cell cycle regulators enables substantial repopulation of the myocardium after a myocardial infarction in adult mice. It is worth noting that this combination of cell cycle regulators can accomplish complete cell division in adult murine CMs, as opposed to S phase progression or nuclear division unaccompanied by cytokinesis (17). The above studies highlight the importance of the canonical Tgf- β pathway in regulation of cardiac cell cycle completion, even in adults. TGFBR1 can complex with various type 2 receptors, requiring additional research to know exactly what receptor combination is inhibited to accomplish cardiomyocyte proliferation.

The other core Tgf- β receptor, TGFBR2, appears to limit heart size. Although proliferation has not been explicitly measured, there are dramatic changes in cardiac morphology in TGFBR2 null mice. These mice display an enlarged myocardium, particularly the right ventricle (38). This enlargement results in loss of trabeculae and a host of other congenital defects. Although heterozygous deletion of TGFBR2 does not result in any apparent malformation (38), it is likely that TGFBR2 signaling has some inhibitory role in CM proliferation. Additionally, TGFBR2, but not TGFBR1, activity is required for Periostin expression. Periostin is a protein that cardiac fibroblasts secrete following myocardial infarction (47). It is thought that Periostin injection promotes full murine CM cell cycle progression and post injury regeneration in a manner that is independent of the non-canonical Tgf- β superfamily member p38 (48). Tgf- β superfamily signaling may still be involved in Periostin signaling, but not through the p38/MAPK pathway. However, there are some conflicting results regarding the pro-proliferative phenotype of Periostin (49). A more thorough investigation of the precise manner of Periostin-mediated cardiomyocyte signaling

seems appropriate to be able to assess whether Periostin indeed stimulates cardiomyocyte proliferation.

Finally, of potential interest is the role of the Tgf- β co-receptor Endoglin, a type III Tgf- β receptor, in regulating cardiomyocyte proliferation. Endoglin can bind to and modulate activity of many type I and type II Tgf- β receptors (50). Endoglin null embryos display abnormally enlarged ventricles, suggesting an important role for Endoglin in cardiac development, and potentially in inhibition of CM proliferation (51). However, the effect of Endoglin on Tgf- β stimulation varies substantially by cell type. Although Endoglin lacks a cytoplasmic kinase domain, it can bind to other Tgf- β receptors and modulate downstream signaling. Endoglin induction of Tgf- β signaling can cause cell division in some cell types, while abolishing cell division in others (50). More investigation is needed to clarify the mechanism by which Endoglin modulates proliferation and explore whether CMs even express Endoglin.

SMADs

The canonical SMADs 2 and 3 appear to play key roles in proliferation, as exemplified by the dependency of zebrafish CM proliferation on SMAD signaling, as discussed above. Through activity of the Acvr1b/TGFBR1 receptor complex, Mstnb promotes SMAD2 activation while suppressing SMAD3 activity. Inhbaa does the inverse, activating SMAD3 and inhibiting SMAD2 activation (52). These studies might suggest an antagonistic relationship where SMAD2 activation inhibits zebrafish CM proliferation while SMAD3 might activate proliferation. To what extent this antagonistic relationship holds true in mammalian cells is not clear.

Overall, phosphorylation of SMADs 1/5/8 appears to stimulate proliferation, while phosphorylation of SMAD2 appears to inhibit proliferation. The exact mechanisms through which these divergent phenotypes are accomplished, are not entirely clear. SMAD4 might be a downstream facilitator of these different phenotypes, as both BMP-dependent SMADs 1/5/8 and Tgf- β -dependent SMADs 2/3 require SMAD4 binding to act as transcription factors. Accordingly, SMAD4 has been identified as an essential regulator of proliferation in both zebrafish and murine hearts. Morpholino-induced SMAD4 knockdown during zebrafish development leads to underdevelopment of the heart. However, this phenotype appears to be mediated mostly by apoptosis with negligible changes in proliferation (53). SMAD4 appears to more directly mediate proliferation in mice. Embryonic SMAD4-deficient mouse CMs have a decreased proliferative capacity and produce an uncommonly thin compact myocardium (54). Intriguingly, deletion of SMAD4 leads to a reduction of SMAD2 phosphorylation and an increase in SMAD1/5/8 phosphorylation (54). These findings suggest the possibility of a negative BMP-dependent and a positive Tgf- β -dependent feedback loop.

As the major transcriptional effectors of Tgf- β , more investigation is needed on the role of SMAD signaling in CM proliferation. Much of the diversity of SMAD signaling is determined by co-transcription factors utilized and the specific DNA targets of SMADs. However, this is not well investigated in the context of cardiomyocytes. A better

understanding of SMAD activity within the nucleus would be valuable to our understanding of how SMADs regulate CM proliferation.

Bone Morphogenetic Protein (BMP) Signaling

The BMP signaling pathway appears to be of utmost importance to regulation of CM cell cycle activation. Zebrafish heart regeneration is characterized by an increase in CM proliferation especially at the border zone of an injury (55). One of the major transcriptional changes in the border zone is an increase in BMP signaling and activation of its downstream targets(55). The BMP2 ligand appears to play a notable role, being expressed in proliferative CMs which also display increased phosphorylation of the BMP-dependent R-SMADs(1/5/8) (55). Without the BMP receptor *bmpr1aa*, zebrafish display reduced CM proliferative capacity after injury. Furthermore, overexpression of BMP2 or the BMP receptor *bmp2b*, increased the proliferative capacity after injury (55). Interestingly, induction of BMP2 or BMP2b does not result in enhanced proliferation during development (55).

The role of BMP signaling in regulating CM proliferation in mammals is less clear compared with zebrafish. BMP2 can induce CM proliferation in mammals and overexpression of BMP2 results in enhanced CM proliferation within embryoid bodies (56). The same proliferative phenotype was also observed *in vivo*, where mice were engineered with increased expression of BMP2 in a cardiac specific manner (56). Cardiac overexpression of BMP2 induces SMAD1/5/8 phosphorylation without phosphorylation of SMAD2 (57, 58). However, ectopic treatment with BMP2 failed to promote proliferation in cultured rat CMs. Additionally, BMP10 appears to be required for CM proliferation during early cardiac development. BMP10 null mice have hypoplastic ventricular walls as early as embryonic day 9, likely due to an inability to progress to the S phase of the cell cycle. This cell cycle arrest can be prevented by culturing the hearts in Bmp10 supplemented media (59). Clearly, BMP signaling is an essential regulator of CM proliferation during development, but more research is required to see if it remains a potent modulator in adult mammals.

P38 Signaling

P38 MAP-Kinase is a powerful cell cycle regulator that is required for proliferation in many cells. P38 is activated by a diverse set of extracellular mitogenic signals and can activate many potent transcription factors such as Myc, MEF2 and Pax6 (60–62). It can also respond directly to Tgf- β signaling. Tgf- β stimulation, via Tgf- β activated Kinase 1 (TAK1) signaling is initiated by the type I receptor Alk-1 and the co-receptor Endoglin can activate MKK3/6 (26). MKK3/6 in turn can phosphorylate p38 allowing it to translocate to the nucleus and act as a transcription factor. This signaling cascade represents a direct link between Tgf- β signaling and MAPK signaling, whereby Tgf- β or BMP ligand binding can initiate p38 signaling. This link between Tgf- β signaling and p38 activation does appear to be important after murine myocardial infarction (63). P38 has been shown to be a potent proliferative inhibitor in murine CMs. Activation of p38 via its upstream kinase MKK3bE, reduces the rate at which fetal CMs progress through S-phase of the cell cycle. Inversely, inhibition of p38 promotes CM cell cycle progression in embryonic stem cell derived human

CMs (64). P38 null murine CMs display enhanced S- and M-phase progression. Interestingly, this phenotype is not entirely recapitulated in neonatal rat CMs *in vitro*, where p38 inhibition alone does not promote cell cycle progression. However, when paired with Neuregulin or Fibroblast Growth Factor 1 (Fgf1) treatment, P38 inhibition promotes full cell cycle progression and cytokinesis (65). P38 inhibition paired with Fgf1 also appears to be therapeutic to infarcted adult mice (27). From these findings, it is clear that p38 plays a notable role in regulating CM cell cycle progression. Moreover, Tgf- β signaling is a potent activator of p38, but it remains unclear to what extent Tgf- β signaling controls p38 activity. P38 is clearly a potent cell cycle inhibitor in CMs, but more research is needed to understand why p38 inhibition stops being sufficient to inhibit proliferation later in development.

Erk Signaling

ERK signaling is one of the most important MAP-Kinase pathways to accomplish cell cycle activation and proliferation in non-CMs (66). Upstream Ras signaling relays growth factor signaling downstream to ERK to activate cell cycle progression. To what extent Ras signaling and ERK activation are important to regulate CM proliferation is not entirely clear. Another potential regulator of ERK activation is Tgf- β signaling, which is thought to be important for CM cell division during development (67, 68). Mice with increased ERK1/2 phosphorylation resulting from genetic Dusp6 deletion show enhanced CM proliferation during embryonic and early postnatal development (28). While ERK signaling is important in regulating CM proliferation during development, there is evidence that this may not be the case in adult cardiomyocytes (69, 70). How and why CMs change their response to ERK activation during the early postnatal period is not well understood, but has been suggested to be in part due to localization of activated ERK signaling. With the advent of sensitive probes that allow subcellular quantification of ERK activity, it might be possible to assess localized ERK activity within CMs to understand why ERK activation induces proliferation before birth, but leads to CM hypertrophy after birth (71, 72).

Hippo Signaling

One of the strongest CM proliferative mediators, which may be related to Tgf- β signaling is HIPPO signaling. Hippo pathway activation can induce startling degrees of cardiomyocyte proliferation and cardiomegaly (15, 73, 74). In non-cardiac cell types SMAD1 is one of the major inhibitors of Hippo activation (75). Additionally, HIPPO signaling may also converge with the core Tgf- β signaling pathway. In mouse embryogenesis, YAP/TAZ activation is one of the key proteins that permits active SMAD2/3 complexes to enter the nucleus and modulate transcription (76). Once in the nucleus, YAP/TAZ can also complex with SMADs, affecting the DNA binding affinity (77). The integration between Tgf- β and HIPPO signaling pathways is clearly complex and vital in non-CM cell types. Unfortunately, little is known about how these signals are integrated in the heart and to what extent the cross talk observed in other cell types is conserved in cardiac signaling. They may represent fully independent pathways, which can be activated synergistically. Another possibility is that they overlap in downstream signaling, and that these shared downstream members may serve as ideal targets for proliferative induction. Importantly, Hippo and Tgf- β are some of the strongest known

regulators of adult CM cell cycle. More investigation into the interaction of these two pathways in CMs is clearly warranted.

Conclusion

Many successful attempts to reactivate cardiomyocyte proliferation incorporate Tgf- β superfamily signaling, clearly highlighting the importance of Tgf- β signaling in regulating CM proliferation. Unfortunately, the core members of the Tgf- β pathway do not represent ideal therapeutic targets. This is because the Tgf- β superfamily signaling is extremely pleiotropic—it's impacts extend well beyond proliferation and the Tgf- β superfamily plays key roles in many aspects of cardiac biology and infarct recovery (78). In a therapeutic context, the ideal regulator will not solely induce the highest levels of cell division, but also improve other aspects of infarct repair. The upstream members of the Tgf- β superfamily are poorly suited for therapeutic development due to their incredible degree of pleiotropy. Instead more effort needs to be focused on downstream pathway members to achieve a more specific and discrete proliferative phenotype. A better understanding of the intracellular signaling pathways, and cross-talk between Tgf- β signaling and pro-proliferative stimuli is needed to identify therapeutic strategies that could be developed.

Much of the current research into Tgf- β superfamily signaling and CM proliferation has been performed in either zebrafish or embryonic mice. These are systems in which cardiomyocytes still maintain their proliferative capacity. The precise reasons why CMs withdraw from cell cycle, and why reactivation of CM proliferation appears to be such a challenging task is not entirely clear. It is likely that many of the known proliferative modulators lose efficacy later in murine development. A better understanding of this transition is of particular importance, since adult patients without proliferative capacity of their CMs are the largest patient population that could benefit from this research. To address this shortfall, more research into both the mechanisms via which the regenerative potential is lost, and which known regulators of proliferation are conserved after the transition is needed.

Repopulation of lost CMs represents one of the most promising therapeutic strategies for treating post-infarct patients. Currently there are only few known modulators of adult CM proliferation, which includes Hippo signaling, specific miRNAs and overexpression of core cell cycle regulators. The precise role of Tgf- β signaling in regulating CM proliferation is still unclear, although this review pointed out many modes of regulation of CM proliferation by Tgf- β signaling, including crosstalk with known modulators of CM proliferation. It is possible that many of the known modulators of proliferation represent a network of regulatory signals. More research is needed to identify potential synergy between known signaling pathways that can coerce cardiomyocyte into division.

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Components of the Tgf- β signaling pathway that have been implicated in cardiomyocyte proliferation.

Binding of the Tgf- β ligand leads to assembly and activation of the Tgf- β receptor complex. This complex phosphorylates SMADs2/3 (canonical signaling) and TAK1 (non-canonical signaling). Mstbn promotes this process in SMAD2 while inhibiting it in SMAD3. Inhbaa does the inverse: promoting phosphorylation of SMAD3 while inhibiting it in SMAD2. SMAD7 can inhibit phosphorylation of both SMAD2/3. The phosphorylated SMAD2/3 can then complex with SMAD4. This complexing can be inhibited by SMAD6. The SMAD2/3/4 complex can translocate to the nucleus and act as a transcription factor. In the non-canonical signaling pathway, phosphorylated TAK1 can activate MKK3 which in turn phosphorylates p38. Activated p38 can translocate to the nucleus and act as a transcription factor. Bmp2/10 ligands can activate the BMP receptor complex, which will in turn phosphorylate SMADs1/5/8. These phosphorylated SMADs can then bind to SMAD4, translocate to the nucleus and act as a transcription factor (20, 24, 26, 39, 55–59). **Note:** The Tgf- β signaling pathway is much more complex than depicted here, and includes crosstalk with other signaling pathways that can regulate cardiomyocyte proliferation, such as cell cycle progression regulation and Hippo signaling.