Transforming Growth Factor-β in Cardiac Ontogeny and Adaptation

W. Robb MacLellan, Thomas Brand, Michael D. Schneider

The transforming growth factor-β (TGF-β) superfamily comprises a set of regulatory peptides with multiple effects on cell growth and differentiation. The elaborate regulation of TGF-βs during embryonic development of the heart, the upregulation of TGF-β after hemodynamic stress, and the impact of TGF-β on cardiac gene expression together imply a prominent functional role for this family of growth factors in cardiac organogenesis and hypertrophy. Basal and TGF-β-induced expression of skeletal α-actin, one of several genes specifically associated with developing or hypertrophied myocardium, each are contingent on transcriptional activation by serum response factor. A truncated form of the type II TGF-β receptor, created by deletion of the cytoplasmic kinase domain, acts as a dominant suppressor of TGF-β signal transduction in cultured cardiac muscle cells and may provide a suitable means to establish the functions of TGF-β in vivo. (Circ Res. 1993;73:783-791.)

**KEY WORDS** • transforming growth factor-β • cardiac gene expression • hypertrophy • development

Little is known of the trophic signals that govern the formation of cardiac muscle early in embryogenesis, the subsequent proliferative growth of cardiac myocytes, or the enlargement of ventricular muscle cells after birth. Although there is significant understanding of the function of polypeptide growth factors in the establishment of the body plan and the recruitment of skeletal muscle precursor cells into the myogenic pathway,1 the regulatory circuits that govern cardiac organogenesis are less clear. Correspondingly, despite recognition of the cardinal role for growth factors acting on vascular smooth muscle during atherosclerosis,2 there has been until recently little follow-up for the long-standing hypothesis that hypertrophy of adult myocardium after hemodynamic stress might involve the accumulation of autocrine or paracrine factors.3 One complexity in attempting to account for cardiac hypertrophy produced by load is the remarkably intricate pattern of gene regulation provoked by mechanical stress—including upregulation of β-myosin heavy chain (β-MHC), skeletal and smooth muscle α-actin, and atrial natriuretic factor (ANF)—which largely recapitulates the program of gene expression in embryonic myocardium.

Among the polypeptide growth factors that most plausibly might serve to regulate cardiac growth and differentiation are members of the type-β transforming growth factor family (TGF-β), the focus of this review. (For a more inclusive examination of cardiac growth factors, see Reference 4.) Taken together with developmental studies that revealed a strict temporal and spatial plan for expression of TGF-β in the early heart,4,5 evidence that TGF-β-related proteins modulate the likelihood of forming beating heart muscle in vitro has given credence to the hypothesis that this family of growth factors might direct critical aspects of the cardiac phenotype (C.A. Eisenberg, D.M. Bader, personal communication).6,7,126 Similarly, in pressure-overload hypertrophy, both circumstantial evidence (pressure-overload upregulates TGF-β1 in adult hearts8) and indications from cell culture (TGF-β reproduces the fetal program associated with hypertrophy in vivo9) support the interpretation that TGF-β participates in the cascade triggered by mechanical stress. The present review will summarize current evidence for the multiple roles of TGF-β in cardiac organogenesis and hypertrophy, review the known mechanisms for TGF-β signal transduction, with emphasis on skeletal α-actin (SαA) as a representative of the “fetal” class of cardiac genes, and describe a dominant-negative mutation of the TGF-β receptor, which blocks the TGF-β-signaling pathway and may help obtain a better understanding of the actions of TGF-β in embryonic and adult heart.

**TGF-βs and Their Receptors:**

**Structure and Biology**

*The TGF-β Superfamily*

TGF-βs constitute a series of multifunctional peptides that regulate cell growth and differentiation, the prototypal family within a more ramified superfamily that includes activins and inhibins, bone morphogenic proteins, and Müllerian inhibitory substance (see Table). TGF-β was first isolated via its ability to provoke anchorage-independent growth of fibroblasts59 but acts as a potent growth inhibitor for many cell types, arresting proliferation in the mid to late G1 phase of the cell.

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The Transforming Growth Factor-β Superfamily

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<td>TGF-β</td>
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<tr>
<td>TGF-β1</td>
<td>+</td>
<td>Upregulates SkA, SmA, β-MHC, and ANF*&lt;br&gt;Downregulates α-MHC and SRCA2&lt;br&gt;Stimulates cardiac muscle development in ES cells and axolotl&lt;br&gt;Maintains contractility; antagonizes IL-1; decreases NO&lt;br&gt;Decreases neutrophil adherence to endothelium and reduces reperfusion injury&lt;br&gt;Knockout causes generalized inflammatory disorder with prominent myocarditis</td>
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<tr>
<td>TGF-β2</td>
<td>+</td>
<td>Upregulates SkA; downregulates α-MHC</td>
<td>11</td>
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<td>TGF-β3</td>
<td>+</td>
<td>Upregulates SkA; downregulates α-MHC&lt;br&gt;Required for induction of valve precursor cells</td>
<td>11†&lt;br&gt;18</td>
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<td>TGF-βA (avian)</td>
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<td>Stimulates cardiac myogenesis in quail cardiac progenitors</td>
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<td>+</td>
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<td>Inhibin</td>
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<td>Activin A (βA)</td>
<td>+</td>
<td>Inhibits cardiac myogenesis in Xenopus and ES cells&lt;br&gt;Induces dorsal axis and mesoderm including heart in Xenopus</td>
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<td>DVR</td>
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<td>ND</td>
<td>Decreases neutrophil adherence to endothelium and reduces reperfusion injury</td>
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<td>BMP-2/BMP-2a</td>
<td>+</td>
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<td>ND</td>
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<td>+</td>
<td>ND</td>
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<tr>
<td>Nodal</td>
<td>ND</td>
<td>Essential for mesoderm formation</td>
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<td>MIS</td>
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<td>Müllerian inhibitory substance</td>
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TGF-β indicates transforming growth factor-β; DVR, decapentaplegic-Vg-related; MIS, müllerian inhibitory substance; BMP, bone morphogenetic protein; OP-1, osteoprotein-1; SkA, skeletal muscle α-actin; SmA, smooth muscle α-actin; β-MHC, β-myosin heavy chain; ANF, atrial natriuretic factor; α-MHC, α-myosin heavy chain; SRCA2, slow/cardiac sarcoplasmic reticulum Ca2+-ATPase; ES, embryonic stem; IL-1, interleukin-1; NO, nitric oxide; and ND, not determined.

*For the distribution of TGF-β-related transcripts and peptides in cardiac muscle, endocardial cushion, and other structures, see the text and references noted.

†C.A. Eisenberg, D.M. Bader, personal communication.

cycle. Recent work by Koff et al. has identified the mechanism for this characteristic effect as interference with the activation of cyclin-dependent protein kinases (CDKs), including a block to the assembly of the cyclin E–CDK2 complex. Inhibition of G1 CDKs, in turn, is expected to impair phosphorylation of the retinoblastoma gene product and delay or arrest G1 progression. In mammals, the TGF-β family is composed of TGF-β1, -β2, and -β3, with ~70% sequence similarity at the amino acid level. TGF-β1 is initially synthesized as a 390-amino-acid precursor protein, which is cleaved to generate an N-terminal remnant and a monomer of mature TGF-β and then is secreted as a latent complex: the mature TGF-β1 dimer (25 kD) is noncovalently the mechanism for this characteristic effect as interference with the activation of cyclin-dependent protein kinases (CDKs), including a block to the assembly of the cyclin E–CDK2 complex. Inhibition of G1 CDKs, in turn, is expected to impair phosphorylation of the retinoblastoma gene product and delay or arrest G1 progression. In mammals, the TGF-β family is composed of TGF-β1, -β2, and -β3, with ~70% sequence similarity at the amino acid level. TGF-β1 is initially synthesized as a 390-amino-acid precursor protein, which is cleaved to generate an N-terminal remnant and a monomer of mature TGF-β and then is secreted as a latent complex: the mature TGF-β1 dimer (25 kD) is noncovalently...
TGF-β and Signal-Transducing Receptors

Cells interact with TGF-β through a potentially perplexing number of cell surface proteins with distinct structural and functional properties. In addition to cell-specific TGF-β-binding proteins of uncertain significance, most cell types, including neonatal cardiac myocytes, express three characteristic TGF-β receptors identified by cross-linking -TGF-β, designated types I, II, and III. The type III receptor, betaglycan, is dispensable for signal generation, as illustrated by the intact TGF-β response despite lack of type III receptor in L6 myoblasts. Expression cloning of the type II receptor (TβRII) disclosed its identity as a 75-kD glycoprotein containing a presumptive serine/threonine kinase intracellular domain, distinct from the cytoplasmic regions of more familiar receptors for platelet-derived, epidermal, insulin-like, or fibroblast growth factors (FGFs), which share protein tyrosine kinase activity. Together, these constitute an altogether novel class of transmembrane signaling proteins. Although TβRII is competent by itself to bind TGF-β, cooperative interaction with type I receptor (a 53-kD protein of unknown structure) is necessary for signal transduction to occur.

TGF-β in Cardiac Morphogenesis

Establishment of the body plan and commitment of pluripotent cells to more specific fates in the early vertebrate embryo are thought to be directed by an ordered sequence of inductive events. In Xenopus laevis, diffusible signals from cells of the vegetal hemisphere (future endoderm) to cells of the animal hemisphere (future ectoderm) are responsible for the induction of dorsal mesoderm, the progenitors of skeletal muscle, in the marginal zone (equatorial region) of the embryo. Growth factors of the TGF-β, FGF, and Wnt families can substitute for vegetal cells, including dorsal mesoderm in isolated animal caps, and have been implicated as mediators of cell specification. TGF-β and TGF-β each possess mesoderm-inducing activity in these assays. TGF-β enhances induction by basic FGF; and the dorsal mesoderm-inducing factor in Xenopus XTC cells has been identified as the more distant TGF-β homologue activin A. Activins A and B each stimulate the formation of dorsal mesoderm yet are detected only later than endogenous mesoderm-inducing activity, and it is uncertain which factors best account for this function in vivo.

A fundamental integrative strategy to correct ambiguities inherent in the use of exogenous growth factors has relied on dominant-acting loss-of-function mutations. Mutations that delete the intracellular domain of receptor tyrosine kinases and some point mutations that extinguish kinase activity act as dominant-negative suppressors of growth factor signal transduction. A truncated FGF receptor constructed along these lines prevents dorsal mesoderm induction in Xenopus embryos, as does a type II activin receptor deleted in its serine/threonine kinase domain. Such results imply that members of the activin and FGF families are each essential for dorsal mesoderm induction in vivo. Insight into the corresponding signals that govern this process in mammalian embryos has lagged behind simpler systems for obvious technical reasons. To isolate genes important for embryonic organization in the mouse, one strategy—insertional mutagenesis—uses retroviruses that insert randomly into genomic DNA, and a small number of mutant animals produced by this approach will possess interesting phenotypes. A recessive embryonic-lethal mutation that prevents mesoderm formation in mice was traced to a gene that displays extensive homology to the decapentaplegic-Vg-related and inhibit subgroups of the TGF-β superfamily, designated nodal on the basis of its localization to the node at the anterior of the primitive streak, a structure analogous to Spemann’s organizer in Xenopus. This novel TGF-β homologue is thought to be essential for mesoderm induction and axis formation in early mammalian development.

Although there is less complete understanding of the molecular signals that control the induction of lateral plate mesoderm and subsequent cardiac muscle formation, three classes of results implicate the TGF-β superfamily in cardiac organogenesis. First, expression of TGF-βs during ontogeny displays spatial and temporal characteristics suggestive of a paracrine role in cardiac muscle induction, as well as in septation and formation of the cardiac valves (reviewed in References 4 and 56). Second, TGF-β has been shown to promote cardiac myogenesis in a variety of in vitro models. TGF-β2 promotes cardiac differentiation by presumptive cardiac mesoderm explanted from the axolotl. In QCE-6 cells, derived from splanchic mesoderm of the Japanese quail, TGF-β2 and -β3 induce an array of sarcomeric proteins including myosin heavy chain, titin, α-actinin, and cardiac troponin I (C.A. Eisenberg, D.M. Bader, personal communication). In murine ES-5 embryonic stem cells, TGF-β and -β both increased cardiac muscle formation. Cardiac muscle formation by P19 embryonal carcinoma cells, likewise, is stimulated by TGF-β2 and, interestingly, is repressed by activin A. However, limits to interpreting the effects imposed by exogenous growth factors were noted above. Third, antibodies directed against TGF-β and antisense oligonucleotides that, more specifically, interfere with expression of TGF-β1 have both been shown to disrupt the induction of valve- forming mesenchyme in the endocardial cushion. Inhibitors of TGF-β expression or function have not yet been applied to the corresponding issue of cardiac myogenesis. However, development of the cardiovascular system (like that of the embryo more generally) is evidently normal in mice deficient for TGF-β1, created by homologous recombination in em-
bryonic stem cells. By contrast, these mice altogether lacking TGF-β develop a diffuse inflammatory disorder in the first weeks of life that culminates in an inflammatory cardiomyopathy and early death. To be discussed subsequently, the absence of a discernible impact on embryogenesis could result from overlapping or redundant function among the isoforms of TGF-β. Similar constraints apply to verifying the in vivo function of TGF-β in the setting of myocardial hypertrophy, illustrated below.

**TGF-β and Mechanical Load**

The postulate that a myocardial growth factor might mediate the response of cardiac muscle cells to hemodynamic load (hypertrophic growth, the characteristic fetal program of cardiac-specific gene expression, or both) would gain strength from corroboration that (1) appropriate upregulation of TGF-β occurs in stressed myocardium, (2) a plausible mechanism can be invoked to link mechanical stress to the TGF-β-signaling pathway, (3) cardiac myocytes indeed are targets for the actions of TGF-β, and (4) TGF-β can evoke effects associated with mechanical load.

The first of these prerequisites has been established clearly. Induction of TGF-β in the adult ventricle, along with other growth factors, is observed during hypertrophy imposed by aortic banding, as well as the compensatory hypertrophy of myocardium surviving infarction. Marked elevation of TGF-β mRNA also is found during genetically determined hypertrophy in Syrian hamsters. How mechanical load might be coupled to the generation of intracellular signals for gene regulation has begun to receive systematic study in cultured cardiac myocytes subjected to passive stretch. This mechanical stress is sufficient to activate a myriad of second-messenger pathways, including protein kinase C, tyrosine kinases, Ras, and mitogen-activated protein (MAP) kinase, to upregulate immediate-early genes such as the transcription factors fos and jun, to induce RNA and protein synthesis, and to activate the fetal program (SkA, β-MHC, and ANF) in isolated cardiac myocytes (reviewed in Reference 68). At least a portion of this cascade can be activated by load in the isolated heart as well. Sadoshima and Izumo have shown recently that stretched cardiac myocytes release one or more “hypertrophic factors,” which activate MAP kinase and induce c-fos. The initial factor released under these conditions may be angiotensin II (S. Izumo, personal communication), which is known to induce TGF-β at least in the vascular wall. Although the sensor for mechanical load and the mechanisms of factor release remain to be proven, these studies establish a rational basis for investigations of autocrine/paracrine pathways for induction and maintenance of the hypertrophic phenotype.

**TGF-β and Cardiac Gene Expression**

To investigate the hypothesis that cardiac muscle cells might be targets for the action of TGF-β and to ascertain whether TGF-β control of myocardial genes resembles the global suppression of tissue-specific genes produced by TGF-β in skeletal myoblasts, or, instead, the “fetal” phenotype associated with cardiac hypertrophy, neonatal rat ventricular muscle cells stimulated with TGF-β were analyzed for expression of seven representative cardiac genes. Four of these, in small mammals, are expressed at higher abundance in embryonic ventricle than in adult ventricle and are induced by aortic banding: β-MHC, ANF, SkA, and smooth muscle α-actin, the first α-actin to be expressed during cardiac ontogeny. Two, α-MHC and the slow/cardiac Ca2+-ATPase of sarcoplasmic reticulum (SR), are more abundantly expressed in adult myocardium than in the embryo and are downregulated by load. The seventh gene, cardiac α-actin, is relatively unaffected by mechanical interventions causing hypertrophy. TGF-β, produced a continuum of inhibitory and stimulatory responses: partial suppression of genes for α-MHC and the SR Ca2+-ATPase, no effect on cardiac α-actin, and selective upregulation of the array of fetal cardiac genes that are characteristic of myocardial hypertrophy.

Defining the molecular basis for TGF-β control of gene expression and how these signals relate to those produced by mechanical load is paramount to understanding the potential hierarchical mechanisms that govern so complex an array of cardiac-specific genes. Like other growth factors, TGF-β regulates gene expression through a cascade that involves activation of Ras and immediate-early genes such as c-fos and c-jun. Similarities in transduction of mechanical signals were noted earlier. Upregulation of Fos/Jun transcription factors by both mechanical load and TGF-β suggests that these complementary signaling pathways might converge on similar or identical response elements. Presently, three canonical genes associated with the fetal or hypertrophic program of gene expression in the heart are known to be upregulated by cotransfected Fos and Jun: ANF, SkA, and β-MHC. A functional Fos/Jun binding site mediates autoinduction of the promoter for TGF-β1 and provides a plausible mechanism for upregulation of TGF-β by mechanical stretch and a positive feedback loop.

Two generic models can be envisioned for TGF-β-regulated expression of cardiac genes: (1) the existence of TGF-β response factors distinct from the DNA-binding proteins that mediate basal expression or (2) interaction of TGF-β-dependent signals with the same factors that drive cardiac-specific transcription. Proteins of the former class, which have been reported to confer TGF-β-regulated transcription, include NF-182 and Fos/Jun81 among others. An especially informative example of the latter mechanism is repression of skeletal muscle genes by TGF-β, which involves a block to transcriptional activation by myogenin distal to DNA binding but is thought to be mediated at least in part by Fos or Jun. To distinguish between these contrasting possibilities and identify molecular mechanisms underlying TGF-β control of the cardiac phenotype, our laboratory has studied the cis-acting sequences that mediate tissue-restricted and TGF-β-dependent transcription of the SkA gene.

Deletion analysis revealed that the proximal 202 bp of the skeletal actin promoter encoded all the elements necessary for full basal and TGF-β–induced activity. This portion of the SkA promoter contains consensus recognition sites for factors critical in transcriptional regulation of other muscle-specific genes: serum response factor (SRF), the SV40 enhancer binding factor TEF-1, Sp1, and helix-loop-helix proteins. Functionally
important SRF binding sites have been identified for cardiac and skeletal α-actin, dystrophin, α-MHC, and muscle creatine kinase. An alternate function for SRF is shown by its involvement in the activation of immediate-early genes following trophic signals, including cytoskeletal β-actin, Egr-1, and the c-fos proto-oncogene. The canonical serum response element (SRE) in the c-fos promoter is activated by serum itself, peptide growth factors including FGF, and postulated components of the growth factor signal transduction cascade, such as Ras, protein kinase C, Raf-1 kinase, and casein kinase II. In cardiac muscle cells, this element also is thought to mediate induction of Fos by mechanical stress. SRF, in turn, is one member of a complex set of “MADS box” transcription factors, which includes the SRF-related muscle enhancer factor-2 (MEF-2) family of proteins. A factor crucial for expression of the cardiac troponin T gene has recently been shown to be indistinguishable from TEF-1; consensus TEF-1 sites are present in the regulatory regions of many other muscle-specific genes like dystrophin and β-MHC. Multiple forms of TEF-1, whose detailed tissue distribution and functional dissimilarities are presently unknown, are thought to exist.

Mutational analysis of the SkA promoter revealed a requirement for Sp1 and TEF-1, acting in concert with SRF for efficient transcription in cardiac cells (W.R. MacLellan, T-C. Lee, R.J. Schwartz, M.D. Schneider, unpublished results). Due to the proximity in muscle-specific genes of binding sites for TEF-1 and MADS box factors, it has been postulated that synergy might exist between these two transcription factor families. Analogously, collaboration of SRF with Sp1 and a third factor is required for tissue-restricted transcription of the cardiac α-actin promoter in either skeletal or cardiac muscle cells—MyoD or unidentified helix-loop-helix proteins, respectively.

The kinase-defective transforming growth factor-β (TGFβ) receptor, ΔKβR, is a dominant-negative suppressor of TGFβ signal transduction in cardiac muscle cells. A, Schematic representation is shown of the wild-type and truncated type II TGFβ receptor (TβRII) illustrating the predicted structural domains. Arrowheads indicate the positions of polymerase chain reaction amplification primers. E and H indicate EcoRI and HindIII, respectively. B, ΔKβRII blocks induction of the skeletal α-actin (SkA) promoter by all mammalian isoforms of TGFβ. Neonatal rat ventricular muscle cells, transfected with SkA-luciferase and constitutive β-galactosidase reporter genes, were cultured in the absence or presence of TGFβ for 36 hours. Luciferase expression, corrected for transfection efficiency, is shown relative to the SkA promoter in control (vehicle-treated, vector-transfected) cells. Error bars denote standard error of the mean. Open bar indicates the control vector, lacking insert; solid bar, ΔKβRII, the kinase-defective TβRII; and hatched bar, ΔkAcRII, an analogous truncation of the type II activin receptor. (Adapted with permission from J Biol Chem. 1993;268:11500-11503.)

**TGF-β in Ischemic Heart Disease**

Apart from their suggested role in pressure-overload hypertrophy, TGF-βs have been implicated in the development of other forms of cardiovascular pathology. TGF-β is upregulated during progressive coronary artery occlusion in experimental animals and coronary artery restenosis in humans. (In cell culture, TGF-β acts both as a stimulator and inhibitor of vascular smooth muscle growth, and its role in vivo remains to be shown. Methods that might be applied to this issue are discussed below.) The distantly related factor BMP-2A is likewise induced in restenotic lesions, where it is thought to promote calcification. The reported ability of TGF-β to reduce myocardial infarct size and its induction in myocardium surrounding infarction have raised speculation that TGF-β may act as a cardioprotectant, perhaps by modulating the local inflammatory response to reperfusion. Indeed, both TGF-
\(\beta_1\) and BMP-7 (osteoprotein-1) decrease neutrophil adherence to endothelium. The fact that TGF-\(\beta\) can protect cultured cardiac muscle cells from the depressant effects of endogenous interleukin-1 on beating, by preventing increased nitric oxide formation, suggests a role for TGF-\(\beta\) in augmenting contractile function under pathological conditions in which cytokine levels are high. Conversely, TGF-\(\beta\) is known to elevate the expression of multiple components of the extracellular matrix and to cause the accumulation of extracellular matrix in certain pathological states, potentially including myocardial fibrosis.

**Dominant-Negative TGF-\(\beta\) Receptor: A Genetic Approach to the Actions of TGF-\(\beta\) In Vivo**

Although its pattern of expression and provocative in vitro models implicate the TGF-\(\beta\) superfamily in the hypertrophy of cardiac muscle, a regulator of growth, function, or both, the various roles postulated for TGF-\(\beta\) have not been tested mechanistically. The importance of this issue can be illustrated by dichotomous effects of TGF-\(\beta\) on skeletal muscle differentiation and by equally dichotomous effects as a agonist or antagonist for growth. Thus, as indicated earlier, the actions of TGF-\(\beta\) are contingent on the precise stage of differentiation, cell lineage, and coexisting growth factors, confounding any easy attempt to extrapolate from simpler preparations to the actions of TGF-\(\beta\) in vivo. Genetic analysis using loss-of-function mutants provides a powerful tool to move beyond descriptive studies and ascertain the role played by putative regulators of cardiac hypertrophy. One current strategy for engineering loss-of-function mutants in vivo (gene “knockouts”) by homologous recombination has proven to be inconsistent and unpredictable in the setting of complex multicogene families in which protein redundancy exists. A key example of this potential shortcoming is the apparently normal phenotype at birth in mice homozygous for the absence of TGF-\(\beta_1\), as cited earlier. The ability of such mice to develop normally to the perinatal stage may result from the ability of TGF-\(\beta\) isoforms to substitute for one another in at least a subset of functions. For example, all three are equivalent for induction of the SKA promoter in cardiac muscle cells. Other examples of unforeseeably normal mice produced by homologous recombination include knockout mutations of the myogenic determination factors MyoD and myf-5. Although this limitation could be overcome by independently disrupting each TGF-\(\beta\) gene locus and crossbreeding the single mutations in combinatorial fashion, there is a need for an alternative method that would more uniformly and efficiently suppress the action of this class of proteins, i.e., a dominant inhibitor for the action of all three mammalian TGF-\(\beta\)s.

Trans-dominant negative mutations have been implemented in various cultured cell systems to ascertain the functional role of signaling proteins in growth and differentiation and have also been applied successfully to developing Xenopus embryos. We have recently shown that a truncated kinase-defective type II TGF-\(\beta\) receptor (\(\Delta\)T\(\beta\)RII) introduced into cardiac muscle cells suppresses the transcriptional effects of all three mammalian TGF-\(\beta\) isoforms. \(\Delta\)T\(\beta\)RII impaired activation of the SKA promoter by TGF-\(\beta_1\), -\(\beta_2\), and -\(\beta_3\) and, conversely, impaired TGF-\(\beta\) inhibition of \(\alpha\)-MHC. Regulation of \(\alpha\)-MHC by thyroid hormone, in contrast, was unaffected. Thus, \(\Delta\)T\(\beta\)RII disrupted TGF-\(\beta\)-dependent signals for both negative and positive control of cardiac gene transcription, without affecting a TGF-\(\beta\)-independent pathway. It should be emphasized that mutation of the type II receptor was sufficient to repress signal transduction without mutation of other proteins that constitute the heteromeric TGF-\(\beta\)-binding complex.

One plausible mechanism for this dominant-negative effect, which has been suggested for the analogous deletion mutants of receptors with tyrosine kinase domains, is a block to the intermolecular autophosphorylation that follows ligand-induced dimerization. However, it is unproven whether an equivalent molecular mechanism accounts for ligand activation of the TGF-\(\beta\) receptor and, conversely, for the ability of kinase-defective mutations to suppress signaling by the wild-type receptor protein. Alternative explanations that require study include competition for TGF-\(\beta\) by the nonfunctional receptors, impaired expression of normal type II receptor, and competition for the type I receptor (required in concert with T\(\beta\)RII for signal generation). Expression of this and other dominant-inhibitory mutants in transgenic mice potentially provides a generic strategy for the construction and analysis of loss-of-function mutations in vivo. In addition, such methods may prove expedient for application to larger mammals in which conventional transgenic technology can be applied but suitable embryonic stem cell lines do not exist. We also have shown that recombinant adeno-virus mediates highly efficient gene transfer to adult ventricular myocytes in culture, providing a complementary approach for genetically modifying the adult cardiac muscle cell.

In summary, several complementary lines of evidence advance the hypothesis that TGF-\(\beta\) and members of the TGF-\(\beta\) superfamily play a significant role in the induction, differentiation, and adaptation of cardiac muscle cells, as well as other aspects of cardiac organogenesis. The importance of genetic proof is highlighted by the inherently inconclusive nature of circumstantial evidence, by the myriad of roles postulated for TGF-\(\beta\) (and other growth factors) from purely descriptive studies, and, in the case of TGF-\(\beta\) itself, by uncertainty as to whether its net effect in any context is fibrosis, growth, or growth arrest. A definitive understanding of the actions of TGF-\(\beta\) in the cardiovascular system is likely to be gained through the use of dominant inhibitors that specifically disrupt TGF-\(\beta\) signal transduction in vivo or prevent the interaction of TGF-\(\beta\) with the signal-transducing receptors.

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