Differential STAT3 signaling in the heart
Impact of concurrent signals and oxidative stress

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Multiple lines of evidence suggest that the transcription factor STAT3 is linked to a protective and reparative response in the heart. Thus, increasing duration or intensity of STAT3 activation ought to minimize damage and improve heart function under conditions of stress. Two recent studies using genetic mouse models, however, report findings that appear to refute this proposition. Unfortunately, studies often approach the question of the role of STAT3 in the heart from the perspective that all STAT3 signaling is equivalent, particularly when it comes to signaling by IL-6 type cytokines, which share the gp130 signaling protein. Moreover, STAT3 activation is typically equated with phosphorylation of a critical tyrosine residue. Yet, STAT3 transcriptional behavior is subject to modulation by serine phosphorylation, acetylation, and redox status of the cell. Unphosphorylated STAT3 is implicated in gene induction as well. Thus, how STAT3 is activated and also what other signaling events are occurring at the same time is likely to impact on the outcome ultimately linked to STAT3. Notably STAT3 may serve as a scaffold protein allowing it to interact with other signaling pathways. In this context, canonical gp130 cytokine signaling may function to integrate STAT3 signaling with a protective PI3K/AKT signaling network via mutual involvement of JAK tyrosine kinases. Differences in the extent of integration may occur between those cytokines that signal through gp130 homodimers and those through heterodimers of gp130 with a receptor α chain. Signal integration may have importance not only for deciding the particular gene profile linked to STAT3, but for the newly described mitochondrial stabilization role of STAT3 as well. In addition, disruption of integrated gp130-related STAT3 signaling may occur under conditions of oxidative stress, which negatively impacts on JAK catalytic activity. For these reasons, understanding the importance of STAT3 signaling to heart function requires a greater appreciation of the plasticity of this transcription factor in the context in which it is investigated.

Introduction

Evidence suggests that overall the actions of the transcription factor STAT3 in the heart are beneficial. Some key studies involving genetic mouse models supporting a role for STAT3 in ischemic protection and preventing heart failure are listed in Table 1. For a comprehensive overview of this rather complicated topic, the reader is referred to several recent articles that chronicle the significant contribution that STAT3 plays in cardiac development, protection and remodeling. Of particular significance, this transcription factor has been implicated in the protection of cardiac myocytes that is provided by ischemic and pharmacological pre- and postconditioning, delayed ischemic preconditioning and post-infarct remodeling. Not surprisingly, many of the beneficial actions of STAT3 in the heart are ascribed to its transcriptional activity. STAT3 activation in the heart has been implicated (often based on circumstantial evidence) in the upregulation of anti-apoptotic (Bcl-xL), anti-oxidant (MnSOD and metallothioneins), and pro-angiogenic (VEGF and VE-cadherin) genes, as well as production of protective paracrine factors by endothelial cells. Some studies have also attributed anti-fibrotic and anti-inflammatory actions to STAT3 signaling in the heart through suppression of gene expression. STAT3 might suppress gene expression by well characterized means such as by competing with other transcription factors or cofactors. Alternatively, STAT3 might be linked to inhibition or induction of miRNAs that in turn determine the mRNA expression profile of cardiac cells.

Involvement of STAT3 in both early preconditioning and postconditioning would not by design involve gene expression. In this regard, recent evidence suggests that STAT3 has direct non-transcriptional actions at the level of the mitochondrion that are protective of cardiac function by limiting excessive reactive oxygen species (ROS) generation. These mitochondrial actions of STAT3 are poorly understood, but may have significance not only for both preconditioning and postconditioning, but heart failure as well. The nontranscriptional role of STAT3 may be related to the separate observations that STAT3 can serve as a scaffold protein and is redox-sensitive. The latter attribute of STAT3 may manifest itself by formation of higher order complexes that conceivably could affect its association with other proteins and subcellular distribution.

On the other hand, there is evidence that STAT3 activation in cardiac myocytes may be harmful in certain cases. STAT3 has been linked to pathological cardiac hypertrophy through both canonical (phosphorylation on Y705) and noncanonical (unphosphorylated STAT3 accumulation in the nucleus) means.
Table 1. Key genetic mouse models supporting a role for STAT3 in cardiac ischemia-reperfusion and heart failure

| Ischemia-reperfusion | Heart failure |
|----------------------|--------------|
| • Ischemic pre/post-conditioning lost in TNFα knockout,1 TNFα receptor knockout,2 IL-6 knockout3 or cardiac-myocyte STAT3 knockout mice4 | • Cardiac myocyte-specific gp130 knockout mice develop heart failure in response to pressure overload accompanied by increased cardiac myocyte apoptosis |
| • Cardiac myocyte STAT3-deficient mice show enhanced sensitivity to ischemia-reperfusion injury5 | • Mice with reduced STAT3 activity/levels have increased susceptibility to doxorubicin-induced heart failure and greater susceptibility to LP5-induced toxicity |
| • Cardiac myocyte-targeted STAT3 knockout mice show greater sensitivity to inflammation, cardiac fibrosis and heart failure with advanced age6 | • Mice with cardiac myocyte-targeted STAT3 overexpression develop cardiac hypertrophy but are resistant to doxorubicin-induced cardiomyopathy8 |

Although, as noted by others, convincing evidence linking STAT3 activation under normal levels of expression to actual physical hypertrophy of cardiac myocytes is scant.13 However, a recent study reported that uncontrolled STAT3 activation downstream of a mutant intractable gp130, the common receptor of interleukin-6 (IL-6) cytokines, is harmful to the heart in myocardial infarction by causing excessive inflammation (upregulation of IL-6 and complement-activating mannose-binding lectin C), ventricular rupture, and heart failure.30 A genetic reduction in cardiac myocyte STAT3 levels was able to rescue the mutant gp130 phenotype. In addition, hearts of mice with cardiac myocyte-targeted deletion of SOCS3, the STAT3-induced inhibitor of gp130, were characterized by development at about 25 weeks of age of cardiac contractile dysfunction, various ventricular arrhythmias and signs of heart failure that were preceded by abnormalities in Ca²⁺ handling and troponin I hypophosphorylation.31 Given previous reports linking STAT3 signaling to cardiac remodeling, it is notable that only “minimal histological abnormalities” were seen in SOCS3 cardiac knockout (KO) failing hearts, although cardiac myocyte hypertrophy was present. The SOCS3 KO phenotype was rescued by simultaneous cardiac-specific gp130 KO.

How can STAT3 activation be both beneficial and harmful to the heart? Unfortunately studies often approach the question of the role of STAT3 in the heart from the perspective that all STAT3 signaling is equivalent, particularly when it comes to signaling by the IL-6 type cytokines, which share the gp130 signaling protein. However, we propose that the context of how STAT3 is activated and also what other signaling events are occurring at the same time will impact on the outcome ultimately linked to STAT3 activation. As an extension of this proposition, a better understanding of the events that regulate STAT3 activation and its transcriptional and nontranscriptional (mitochondrial) behaviors is imperative. To complicate matters further, STAT3 “activation” for the purposes of transcription, and likely for its mitochondrial actions, may involve either tyrosine (Y705) or serine (S727) phosphorylation alone,32-36 both (more typically/canonically)37 or neither,38 and is now known to be impacted as well by acetylation and cellular redox status.13,39,40 In canonical signaling, S727 phosphorylation occurs in either the cytoplasm or nucleus on STAT3 that is likely already phosphorylated on Y705, as prior S727 phosphorylation seems to block Y705 phosphorylation.41 Interestingly S727 phosphorylation may play a critical role in the mitochondrial actions of STAT3.42 Thus, STAT3 S727 phosphorylation in the cytoplasm may function as a switch favoring the mitochondrial actions of STAT3 over its canonical nuclear actions, although this is conjecture and will need to be investigated.

Leukemia Inhibitory Factor (LIF) and LIF Receptor

A number of factors have been shown to induce STAT3 activation in the heart, including prolactin, granulocyte colony-stimulating factor (G-CSF), tumor necrosis factor-α (TNFα), erythropoietin (Epo), opioids, leptin, angiotensin II and insulin.13,43-47 Pre- and postconditioning-induced STAT3 activation is attributable to the release of paracrine factors, such as TNFα and IL-6.10,13,48-50 The increase in ROS that accompanies ischemia-reperfusion (IR) in the heart is also associated with marked STAT3 activation;51 whether ROS activates STAT3 via an effect on upstream JAK kinases, phosphatases or largely through the upregulation or release of paracrine factors is not known.52 Notably, cardiac IR is associated with the production of IL-6 cytokines, which are prominent activators of STAT3 signaling.10-13 The IL-6 family of cytokines signal through the common signaling subunit gp130.10-13 They can be further classified according to those that signal through gp130 homodimers (IL-6 and IL-11), gp130 heterodimers with the LIF receptor [LIF, cardiotoxiph-1 (CT-1), ciliary neurotrophic factor (CNTF), cardiotoxiph-like cytokine (CLC) and oncostatin M (OSM) in humans], or gp130 heterodimers with the OSM receptor (OSM). Additional ligand-binding proteins are needed for IL-6, IL-11, CNTF, CLC and probably CT-1. LIF likely binds first to the LIF receptor (LIFR/CD118), which then dimerizes with gp130.53 LIFR is ubiquitously expressed in the normal heart and unlike gp130 does not appear to be down-regulated (at least appreciably) in human heart failure,54-57 although cardiac levels of LIF are increased in heart failure.58,59

The IL-6 family cytokines are frequently described as characterized by functional redundancy, particularly with regard to signaling in the heart.12 Although different actions among them have been noted, the differences are commonly explained away as...
due to differences in expression levels of receptors or duration of STAT3 activation. However, marked qualitative differences have been noted among the gp130-related cytokines as far as gene induction and functional impact, in particular between IL-6 and LIF, which are not amenable to a straightforward explanation based on the strength of their respective signaling responses. At an early time, it was noted that IL-6 and LIF differ as far as the induction of acute-phase genes.\(^6\) More recently, LIF and IL-6 have been shown to counter-regulate development of the T lymphocyte lineages with IL-6 coupling moreover to the activation of a gene response that downregulates LIF signaling.\(^6\)

How IL-6 and LIF might exert dissimilar actions is not known. Based on conjecture, LIFR likely modifies the character of the gp130 signal, but how this occurs is not known. LIFR and gp130 are structurally very similar (Fig. 1).\(^6,6\) Both contain three box regions with the two membrane proximal ones being important for association with a JAK family kinase and the distal one playing a role in STAT3 and Src-family kinase Hck activation (and possibly reinforcing ERK activation at least for gp130).\(^6,6\) gp130 has four YXXQ motifs that upon tyrosine phosphorylation by a JAK family kinase serve as docking sites for STAT3 (and potentially STAT1); the cytoplasmic domain of LIFR is shorter and has three YXXQ motifs.

Both gp130 and LIFR contain a more membrane proximal YXXV motif that upon phosphorylation is important in signal termination by recruiting SHP2 or SOCS3.\(^6\) SHP2 terminates signaling through its tyrosine phosphatase activity, but also serves as a scaffold protein linking gp130 and LIFR to additional signaling pathways (Fig. 2).\(^6,6,6\) SOCS3 is induced by STAT3 and terminates LIF/IL-6 signaling by inhibiting JAK activity directly through the N-terminal kinase inhibitory region (KIR) or by facilitating ubiquitination and proteasomal degradation of the JAKs, other signaling components, or receptors.\(^3\) A significant difference between the YXXV site of LIFR and gp130 is that the former shows little affinity for SOCS3, while the latter binds both SOCS3 and SHP2.\(^6\) This might explain why LIF-induced STAT3 signaling is in general sustained (although at a reduced level from the initial increase), while IL-6 activation of STAT3 is brief (personal observation).

In addition to JAK-STAT3 signaling, LIFR and gp130 couple to the activation of 2 major signaling pathways: SHP2/MAPK and phosphatidylinositol 3-kinase (PI3K)/AKT.\(^6,10,12,6\) Whether LIFR and gp130 couple equally well to both of these pathways is not known. The MAPK that have been best studied are ERK1/2 and ERK5. Tyrosine phosphorylation of SHP2 leads to its interaction with Grb2 (growth factor receptor bound protein) and SOS (son of sevenless), which triggers the Ras/RAF/MEK/ERK cascade (Fig. 2). Tyrosine phosphorylated SHP2 was linked to ERK5 activation via Grb2-associated binder-1 (Gab1), a scaffolding/docking protein which likely contributes to protective signaling as well (Fig. 3).\(^6,6,7,6,7\) ERK5 activation is thought to be responsible for the unique hypertrophic phenotype of longitudinal elongation produced by the IL-6 type cytokines on cardiac myocytes. Details on how LIFR and gp130 couple to PI3K/AKT signaling is not known, although the PI3K regulatory subunit p85 likely associates with SHP2 via Gab1 as well (Fig. 3). PI3K in turn leads to activation of AKT and a diverse series of signaling pathways. STAT3 appears to serve as a scaffold protein that helps assemble an activation module with JAKs activating STAT3 and indirectly, PI3K and AKT, and in turn PI3K “activating” STAT3 by phosphorylating S727.\(^6,6,5,6,2,6\)

Notably, PI3K/AKT signaling is essential for conferring cardioprotection in response to ischemic pre- and postconditioning stimuli, being involved in both the trigger and mediator phases.\(^7\) Its involvement in the latter forms part of the reperfusion injury salvage kinase (RISK) pathway that confers protection by attenuating opening of the mitochondrial permeability transition pore (MPTP) through as yet undefined means (Fig. 3). A second set of intracellular signaling events that operates independently of the RISK pathway as a trigger for protection and also confers protection during reperfusion by targeting MPTP opening was recently identified and involves STAT3 activation. It was named the survivor activating factor enhancement (SAFE) pathway.\(^6,6-8,6,6,8\) Details on how the SAFE pathway works at the level of the mitochondrion are not known. Thus, the IL-6 family of cytokines have in theory the potential of conferring protection to cardiac myocytes from IR injury by activating both the SAFE and RISK pathways (Fig. 3). Indeed, the LIFR ligand CT-1 was shown to protect isolated cardiac myocytes and the adult rat heart from injury when added either just prior to ischemia or at reoxygenation/reperfusion.\(^6,8,6,8,6,9,8\) This was not the case with human heart muscle preparations which required longer exposure to CT-1 to confer protection indicating that gene expression was involved.\(^6,8,6\)

A carboxyl-terminal acidic domain of gp130 was shown to couple to cellular proliferation and inhibition of stem cell differentiation through the binding and activation of Src family kinases, in particular Hck (Fig. 2).\(^6,6,6,6\) Hck represents an additional means by which gp130 may be linked to ERK activation. The relevance of this signaling pathway in the heart has not been explored. Neither is it known if Hck (or another Src family

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**Figure 1.** Schematic of the cytoplasmic regions of human gp130 and LIFR showing the relative locations of the three box motifs and the STAT3 YXXQ binding motifs. The YXXV domain is important for linking the receptors to PI3K/AKT and SHP2/MAPK signaling pathways and for termination of signaling by recruiting either SHP2 or SOCS3. SHP2 terminates signaling through its tyrosine phosphatase activity. SOCS3 terminates signaling by inhibiting JAK activity directly through the N-terminal kinase inhibitory region (KIR) or by facilitating ubiquitination and proteasomal degradation of signaling components.
member) modulates gp130 signaling; however, such a scenario might explain the increased tyrosine phosphorylation of gp130 in the face of reduced tyrosine phosphorylation of JAK2 in hearts of patients with end-stage dilated cardiomyopathy.\textsuperscript{57}

The focus of our research has been LIF, which has been shown to be produced by cardiac myocytes and to have protective effects on heart cells. Pretreatment of adult or neonatal cardiac myocytes with LIF protected against hypoxia-reoxygenation or doxorubicin-induced injury at a later (6–12 h) time point.\textsuperscript{15,16,82,83} LIF treatment was also shown to protect the heart from IR injury or myocardial infarction.\textsuperscript{84,85} These beneficial actions of LIF are attributed in part to the stimulation of angiogenesis and upregulation of MnSOD, Bcl-xl and VEGF.\textsuperscript{16,83,87} LIF was also shown to have effects on the growth, metabolism, contractility and Ca\textsuperscript{2+} handling of cardiac myocytes, which might overall be considered disadvantageous; however, because these studies mainly relied on cultured cells or isolated muscle the physiological significance of these effects is uncertain.\textsuperscript{74,75,88-95}

Several recent observations support the conclusion that production of LIF by cardiac myocytes may have physiological importance under stress conditions in myocardial repair and regeneration beyond a protective action on the cells themselves. In the mouse, LIF was found to contribute to the homing of bone marrow-derived cardiac progenitors to the infarcted myocardium and the differentiation of resident cardiac stem cells into endothelial cells.\textsuperscript{96,97} Second, in a rat genetic model of heart failure, myocardium-produced LIF was shown to cause cholinergic transdifferentiation of cardiac sympathetic nerves, which might represent a means of protecting the heart from excessive sympathetic drive.\textsuperscript{59}

**STAT3—Too Much of a Good Thing?**

Two recent studies seem to provide evidence supporting the old adage that too much of a good thing is harmful in the case of STAT3 activation in the injured or aging myocardium. Or do they? In one study, sustained activation of STAT3 in cardiac myocytes following a stress stimulus (MI) was achieved by expressing a mutant gp130 protein (Y757F) that does not bind SOCS3 in mice that also have a heart targeted deletion of gp130.\textsuperscript{30} Thus, the “rescued” gp130 signaling in cardiac myocytes of these mice was refractory to inhibition by SOCS3. Of note, the

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**Figure 2.** Basic signaling similarities and differences of gp130 (right, blue) and LIFR (left, orange). Gp130 has 4 STAT3 binding sites and LIFR has 3. Both receptors couple to ERK1/2 activation through SHP2 functioning as a scaffold protein. The tyrosine phosphatase activity of SHP2 is thought to contribute to termination of receptor signaling. The SHP2 site on gp130 also binds SOCS3, which terminates signaling by inhibiting JAK activity. The tyrosine phosphatase SHP1 associates with JAK1 and contributes as well to termination of gp1320 and LIFR signaling. gp130 contains an acidic domain (light blue) comprising amino acids 771 to 811 that binds the Src-family kinase Hck and couples to ERK1/2 and Pyk2 (not shown) activation.\textsuperscript{64} Phosphorylation of LIFR on S1044 by ERK1/2 was shown to promote receptor degradation.\textsuperscript{66} For gp130, S782 phosphorylation may regulate cell surface expression.\textsuperscript{67} At least for gp130, other phosphorylation events have been reported. PKC\textgreek{d} that is associated with STAT3 may phosphorylate gp130 on T890, helping to stabilize STAT3-gp130 association.\textsuperscript{68}

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SOCS3 recruitment site is important for SHP2 binding and thus coupling of gp130 to MAPK, and possibly to PI3K/AKT, signaling would be expected to be impaired as well. Indeed, hearts harboring the mutant gp130 protein showed no increase over wild type mice in either ERK or AKT activation following MI, although STAT3 pY levels were markedly enhanced. The other study employed cardiac myocyte-targeted SOCS3 KO mice, which not only showed increased STAT3 pY levels, but increased AKT, ERK, and p38 MAPK activation. These signaling pathways were enhanced under basal conditions. The fact that STAT3 activation was enhanced under basal conditions in the SOCS3 KO model, but not the gp130 Y757F model, indicates that something was driving gp130 signaling (which could be characterized as complete) in the SOCS3 KO model in a feed-forward manner. Consequently, the SOCS3 KO mice developed cardiac hypertrophy and heart failure with age without additional stress. In these mice, imposition of stress by transverse aortic constriction further enhanced gp130 signaling and caused cardiac dysfunction. Together, both studies would seem to refute the oft-cited supposition that sustained STAT3 activation is beneficial and anti-inflammatory (as seen with IL-10), while brief STAT3 activation is harmful and pro-inflammatory (as seen with IL-6). 

STAT1 activation was not enhanced in either model. This is somewhat surprising as the genetic reprogramming of IL-6 signaling in SOCS3 KO macrophages was attributed to enhanced STAT1 activation, owing perhaps to enhanced recruitment of the two more membrane distal YXXQ STAT binding sites of gp130 that are capable of activating both STAT1 and STAT3. However, increased STAT1 activation was not found in the heart in either the SOCS3 KO (“preliminary observation”; personal communication, Dr Yajima) or the gp130 Y757F model. This would have neatly explained deleterious
consequences of enhanced gp130 signaling as several lines of evidence have shown that STAT1 has pro-apoptotic actions in cardiac myocytes, both as a transcription factor and as a signaling molecule.\textsuperscript{101}

Although both models were associated with increased STAT3 activation (tyrosine phosphorylation) there are marked differences in the phenotypes of the two models that are illuminating and indicate that prolonged STAT3 activation was not functionally equivalent in both (Table 2). Of note, the gp130 Y757F model was associated with enhanced cardiac inflammation, while the SOCS3 KO model was not (although inflammation in the gp130 Y757F model was assessed in hearts subjected to MI, whereas in the SOCS3 KO model inflammation was assessed under basal conditions). Since inflammation was observed in the instance where there was no concomitantly enhanced PI3K/AKT signaling, enhanced STAT3 signaling may not be inflammatory perhaps as long as it is balanced (both in magnitude and temporally) by enhanced PI3K signaling. Consistent with this possibility is the finding that cardiac myocyte-targeted STAT3 overexpression was associated with cardiac hypertrophy, but was protective against doxorubicin-induced heart failure.\textsuperscript{9}

While STAT3 signaling is important for the anti-inflammatory actions of IL-10, the basis for why IL-10-induced STAT3 signaling is anti-inflammatory is still unresolved; however, evidence was recently presented that a subset of STAT3-induced anti-inflammatory genes in macrophages in response to IL-10 treatment is dependent upon PI3K-mediated signaling.\textsuperscript{102} In this scenario, cytokine-induced activation of inflammatory genes by PI3K/AKT-mediated activation of NF\textsuperscript{k}B (or by STAT3) may be offset by the actions of PI3K/AKT on STAT3 signaling (as long as the latter is sustained). Moreover, NF\textsuperscript{k}B p65 and p50 are known to physically interact with functional consequences regardless of whether STAT3 is tyrosine and/or serine phosphorylated.\textsuperscript{103} Conceivably, different phosphorylation (or acetylation) profiles of STAT3 might be associated with different gene expression profiles linked to STAT-NF\textsuperscript{k}B association.

PI3K signaling and JAK-STAT signaling could converge at multiple sites in both the cytoplasm and nucleus to affect expression of a particular gene, but of note the PI3K/AKT pathway also induces acetylation of lysine residues on STAT3 that affect dimerization and transcriptional behavior.\textsuperscript{39,40,104-106} In this regard, the gp130 Y757F model was associated with increased levels of cardiac STAT3, which may have been due to STAT3-induced STAT3 expression.\textsuperscript{38} Accumulating evidence shows that accumulation of unphosphorylated STAT3 in the nucleus can drive expression of a set of pro-inflammatory genes, including IL-6, which was in fact increased in the gp130 Y757F model.\textsuperscript{30,38,107} Thus, under certain conditions STAT3 activation sets into play a series of events that lead to a sustained inflammatory response.

Redox Sensitivity

Coupling of LIF and the other IL-6 family cytokines to a balanced PI3K-STAT3 response requires JAK activation. The increased STAT3 “activation” (i.e., increased tyrosine phosphorylation) that is observed with IR need not reflect a JAK-mediated event as protein tyrosine phosphatases are known to be inactivated by oxidative stress.\textsuperscript{108} In fact, we found that oxidative stress inhibits LIF-induced JAK1 and JAK2 activity in cardiac myocytes.\textsuperscript{109} Since then site-directed mutagenesis experiments were performed to demonstrate that two nearby cysteine residues in the amino-terminus region of JAK2’s catalytic domain act together as a redox-sensitive switch.\textsuperscript{110} The presence of this switch would thus permit the catalytic activity of JAK2 to be directly regulated by the redox state of the cell. Of note, these cysteine residues are highly conserved in both JAK1 and JAK2 among mammalian and most lower-order species.

Table 2. Comparison of two genetic mouse models of sustained cardiac STAT3 activation

| Baseline | Stress (MI) | Baseline | Stress (TAC) |
|---------|------------|----------|-------------|
| pY STAT3 | ≡ | † (/> > WT)** | † | † (/> > WT) |
| pS STAT3 | nr | nr | nr | nr |
| STAT3 | ≡ | † (later times) | ≡ | ≡ |
| pYSTAT1 | ≡ | ≡ | ≡ (initial screening) | nr |
| STAT1 | ≡ | ≡ | ≡ | nr |
| PI3K/AKT | ≡ | ≡ (\(\)) | † (AKT)’ | † (AKT) (> WT)? |
| SHP2/MAPK | ≡ | ≡ (modest †) | † (ERK, p38)’ | † (ERK) (> WT7) |
| Phenotype | Normal cardiac function and morphology | † mortality | Cardiac hypertrophy | Cardiac hypertrophy |
| | ↑ LV rupture | ↑ cardiac inflammation | Heart failure | Heart failure |
| | Heart failure | (No cm disarray, necrosis, apoptosis, inflammation or interstitial fibrosis) | Cardiac dysfunction | Cardiac dysfunction |

*At 3 mo; **Both level and duration; † At 15 weeks, but not 8 weeks. Symbols/abbreviations: ≡, no difference compared with wild type; cm, cardiac myocyte; nr, not reported; WT, wild type; †, increase; †, decrease.
STAT3 transcriptional response may also be directly affected by oxidative stress due to the presence of redox-sensitive cysteines.\textsuperscript{25,111} Oxidative stress undoubtedly impacts on STAT3 transcriptional signaling indirectly in multiple ways as well. We recently presented evidence that oxidative stress may attenuate LIF-induced gene expression in human microvascular endothelial cells by causing serine phosphorylation and degradation of STAT3’s binding partner and transcriptional cofactor p300/CBP.\textsuperscript{112}

Mitochondrial STAT3

Recent studies demonstrate STAT3 localizes to mitochondria of different cell types and regulates respiration.\textsuperscript{23,32,113–116} STAT3 deletion reduced respiration of cardiac myocyte mitochondria due to 50% decrease in activities of complexes I and II.\textsuperscript{115} Loss of mitochondrial STAT3 decreased ATP production and enhanced ROS generation.\textsuperscript{115} These findings are consistent with reports of increased ROS formation by complex I with decreased activity, as seen in the heart with ischemia or age.\textsuperscript{116,117} Besides regulating the electron transport chain, STAT3 associates with matrix-localized cyclophilin D, the target of mitochondrial permeability transition pore (MPTP) inhibitor cyclosporine.\textsuperscript{115} In fact, mitochondria from STAT3\textsuperscript{114}/ STAT3\textsuperscript{115} hearts undergo MPTP opening at lower calcium levels, which has significance for ischemia-reperfusion injury.\textsuperscript{118} Recently, cardiac myocyte-specific over-expression of mitochondria-targeted STAT3 was found to partially block complexes I and II with no increased basal ROS production; however, there was no ischemia-induced ROS release from complex I and less reduction in complex I activity with ischemia.\textsuperscript{118} How STAT3 regulates complexes I/II is unknown, but some intermediary process is likely, since the ratio of complexes I and II to mitochondrial STAT3 is ~10.\textsuperscript{119,120} S727 phosphorylation would seem to be important in STAT3’s mitochondrial actions. Impaired activity of complexes I and II of mitochondria from STAT3\textsuperscript{114}/ STAT3\textsuperscript{115} pro-B cells could be restored by a mimic of constitutively serine phosphorylated STAT3 (STAT3 Y705S/F727D), while STAT3 Y705S/F727A was ineffective.\textsuperscript{121} Also, a number of studies report that STAT3 pS727 is enriched in mitochondria compared with the cytoplasm;\textsuperscript{119–122} however, this may not be the case for all species.\textsuperscript{123} The question of whether ischemic pre- or post-conditioning dynamically regulate mitochondrial STAT3 levels or phosphorylation profile has not been determined.\textsuperscript{124} Conceivably, translocation of STAT3 to mitochondria could function as part of a feedback loop to control mitochondrial generation of ROS.

Conclusions and Future Directions

The transcription factor STAT3 has been implicated in a protective and reparative response in the heart. Thus, increasing duration or intensity of STAT3 activation ought to minimize damage and improve heart function under conditions of stress. However, given the many ways that STAT3 is post-translationally modified and its interaction with other signaling networks, the assumption that enhanced STAT3 activity is solely a reflection of increased tyrosine phosphorylation must be construed as a gross over-simplification. Understanding the beneficial importance of STAT3 signaling to heart function will require a greater appreciation of the context in which it is activated. In this regard, defining the differences in the impact on the heart between gp130 homodimer and heterodimer STAT3 activation will be illuminating.

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