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STAT transcription in the ischemic heart

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Abbreviations: AT, angiotensin; CHD, coronary heart disease; CM, cardiac myocytes; CT-1, cardiotrophin 1; EGCG, epigallocatechin 3 gallate; IL, interleukin; I/R, ischemia reperfusion; JAK, Janus kinase; LIF, leukemia inhibitory factor; PIAS, protein inhibitor of activated STATs; PKC, protein kinase C; RISK, reperfusion injury salvage kinase; SOCS, suppressor of cytokine signaling; STAT, signal transducers and activators of transcription; SAFE, survivor activating factor enhancement; TNF, tumor necrosis factor

All seven STAT proteins are expressed in the heart, and in this review we will focus on their contribution to cardiac physiology and to ischemic heart disease and its consequences. A substantial literature has focused on the roles of STAT1 and STAT3 in ischemic heart disease, where, at least in the acute phase, they appear to have a yin-yang relationship. STAT1 contributes to the loss of irreplaceable cardiac myocytes both by increasing apoptosis and by reducing cardioprotective autophagy. In contrast, STAT3 is cardioprotective, since STAT3-deficient mice have larger infarcts following ischemic injury, and a number of cardioprotective agents have been shown to act, at least partly, through STAT3 activation. STAT3 is also absolutely required for preconditioning—a process where periods of brief ischemia protect against a subsequent or previous prolonged ischemic episode. Prolonged activation of STAT3, however, is strongly implicated in the post-infarction remodeling of the heart which leads to heart failure, where, possibly together with STAT5, it augments activation of the renin-angiotensin system.

Introduction

The signal transducer and activator of transcription (STAT) family contains seven members, STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B and STAT6 (reviewed in ref. 1), all of which are expressed in the heart. All have a similar modular structure, including an N-terminal oligomerization domain, a coiled-coiled domain, a DNA binding domain, and linker, SH2 and C-terminal transactivation domains. However, despite this structural similarity, there is considerable functional diversity between these seven proteins. There are two key residues between the SH2 domain and the C-terminus (for example, tyr701 and ser727 in STAT1), whose phosphorylation is required for maximum transcriptional activity and for homo- and hetero-dimerization. All STATs can form homodimers or higher order structures, with the exception of STAT2, which can only heterodimerize with STAT1. All the individual STAT proteins have been genetically deleted in mice, although only STAT3 deletion is embryonically lethal, suggesting that it plays a major developmental role.

Binding of a number of extracellular ligands, such as cytokines, to their cognate receptors activates members of the Janus kinase (JAK) family, of which there are four members, JAK1, JAK2, JAK3 and TYK2 (only JAK1, JAK2 and TYK2 are expressed in the heart). In turn, JAKs recruit and activate specific STAT proteins, and JAK-activated STATs translocate to the nucleus where they transactivate target genes.

STATs are also inhibited by a number of mechanisms. Cytokines which activate STATs also induce expression of suppressors of cytokine signaling (SOCS), which form a classical negative feedback loop. The protein inhibitors of activated STATs (PIAS) also inhibit STAT activity, though these are not specific inhibitors of STAT proteins. In addition, STATs can be targeted for proteasomal degradation by a number of ubiquitin E3 ligases.

STATs in Cardiac Physiology

The heart is dependent, to a greater degree than many other organs, on a large supply of high energy phosphates generated by mitochondrial respiration. It has been calculated that, in the canine heart, basal ATP turnover is of the order of 5 μg/g/min[2] and this can increase by up to 10-fold under conditions of extreme exercise.[2] The mechanisms by which change in ATP consumption is coupled to change in ATP production remain only partially understood, although mitochondrial Ca clearly plays a role,[3] but STAT proteins, and in particular STAT3, may also contribute.

Small amounts (roughly 10% of the cytoplasmic levels) of STAT3 have been found in the mitochondria of several organs, including the heart.[4] STAT3 null cells show reduced oxygen consumption, which appears to predominantly affect Complexes I and II, and STAT3 binds to Complex I (and possibly Complex II), an interaction which requires Ser but not Tyr phosphorylation.[4,5] However, it has been disputed whether this occurs physiologically for stoichiometric reasons,[6] although previous studies have demonstrated an interaction between STAT3 and the Complex I component, GRIM-19, which does not apparently

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require the STAT3 C-terminus.[7,8] In addition, the deacetylase, SIRT-1, regulates STAT3 activity, and Sirt-1 null cells have increased phospho-STAT3 with a parallel increase in mitochondrial bioenergetics.[9] Clearly, more work is required to clarify some of these inconsistencies, and, in particular, to assess whether STAT3 null cardiac myocytes are as able to increase ATP production as wild-type cells. No other STAT has been implicated in potential interaction and regulation of electron transport chain Complexes, although in tumors STAT1 transcriptionally regulates expression of a number of metabolic genes, including those involved in glycolysis and the citric acid cycle as well as oxidative phosphorylation.[10] However, these long-term transcriptional effects cannot contribute to the rapid changes in ATP production in response to increased energy consumption.

In contrast to STAT3, relatively little is known about the contribution of other STATs to cardiac physiology. STAT1 has been detected in cardiac mitochondria,[11] and ROS production in response to TNF-α has been reported to be reduced in livers from STAT1 knockout mice,[12] although whether mitochondrial cardiac STAT1 contributes to changes in cardiac bioenergetics is unknown.

STATs in Cardiac Pathology

Ischemic heart disease. Over 180,000 people die each year in the UK from coronary heart disease (CHD), and this accounts for one third of all deaths per annum.[13] Coronary artery occlusion results in the death of irreplaceable cardiac myocytes (CM) by a variety of cell death mechanisms, including apoptosis, necrosis and autophagy[14] and many patients die from the acute effects of the loss of functional CM. While the relative contribution of the different death modalities to the overall size of the infarct remains debatable, considerable attention has recently been devoted to the apoptotic death of CM, since the molecular mechanisms of the apoptotic pathway are well understood and therefore allow the possibility of rational therapeutic intervention.

The role of STAT1 in ischemic heart disease. Over 10 y ago, it was demonstrated that ischemia/reperfusion (I/R) injury in the rat heart led to STAT1 activation together with an increase in its transcriptional activity, particularly on the promoter of caspase-1, an enzyme important in the execution of apoptosis, and on both Fas, a cell surface death receptor and its ligand FasL. Activated STAT1 was colocalized with apoptotic CM, and STAT1 knockdown reduced I/R-induced cell death.[15] These transcriptional effects required serine but not tyrosine phosphorylation,[16] and the C-terminal STAT1 domain was sufficient to enhance I/R-induced apoptosis in CM.[17] Since the C-terminus of STAT1 does not bind DNA and therefore cannot directly transactivate target genes, it must be acting as a coactivator. One possible interacting partner is the well-known pro-apoptotic protein, p53, which has been implicated in the apoptosis of CM following I/R,[18] and STAT1 has been shown to enhance the transcriptional activity of p53 on pro-apoptotic targets such as Bax.[19]

One mechanism by which STAT1 becomes activated following I/R is through free radicals, and it has been shown that the free radical scavenger, Tempol, a known cardioprotective agent, inhibits tyrosine phosphorylation of STAT1, an effect that is reversed by gamma interferon.[20] Similarly, another antioxidant, epigallocatechin-3-gallate (EGCG), a component of green tea, exerts cardioprotective effects in parallel with a reduction in STAT1 activation.[21] The free radical link cannot be the whole story, however, since other flavonoids that exert both anti-oxidant and STAT1 inhibitory actions are more potent cardioprotective agents than those that only have anti-oxidant activity.[22] Indeed, EGCG has been shown to induce expression of SOCS1, a known inhibitor of STAT1.[23]

The role of STAT1 in CM apoptosis can only be confined to its initiation, since STAT1 is one of the several hundred substrates cleaved by caspases, particularly caspase-3, during execution of the apoptotic program.[24]

In contrast with the relatively extensive literature on the role of STAT1 in apoptotic death, there is scant evidence implicating STAT1 in other death subroutines. It has been reported that autophagy enhances gamma interferon-mediated STAT1 tyrosine phosphorylation in macrophages,[25] although a recent report suggests that, perhaps paradoxically, hearts from STAT1 deficient mice are protected from I/R injury because of increased autophagy.[26] This would suggest that STAT1 activation in the heart challenged by I/R is harmful in two ways: it is directly implicated in apoptotic death and also inhibits potentially protective autophagy.

The protective role of STAT3. In the heart, STAT1 and STAT3 have a similar inverse functional relationship as they do in cancer. Thus, in tumors, as in the heart, STAT1 is pro-apoptotic while STAT3 is a tumor promoter and a cardioprotective factor. Hearts from mice with a cardiac-specific knockout of STAT3 have larger infarcts after I/R, with increased numbers of apoptotic CM, and these mice show greatly increased mortality.[27] In addition, deletion of STAT3 in cardiomyocytes renders hearts more susceptible to inflammatory damage,[28] such as that which accompanies I/R injury, especially after necrotic myocyte death. STAT3 is also an essential component of the survivor activating factor enhancement (SAFE) pathway,[29] an intrinsic protective signaling pathway that is paradoxically activated by TNFα in the heart at reperfusion. The SAFE pathway may also be responsible for the observation that coronary effluents from a preconditioned heart confer protection and STAT3 activation in recipient hearts.[30] Several cardioprotective agents have been shown to activate STAT3, particularly on tyrosine, including cardiotrophin-1 and the urocortins,[31] opioids,[32] insulin,[33] leptin,[34] erythropoietin,[35] resveratrol and melatonin,[36] though whether STAT3 activation is essential for the protective properties of most of these agents is unknown. Indeed, there is some evidence that the protective effects of erythropoietin are mediated via STAT5 (as well as STAT3)[37,38]

The downstream protective targets activated by STAT3 have not been systematically investigated, although it is likely that at least some of the changes in gene expression associated with urocortin-mediated protection are mediated by STAT3.[39] In chick embryo hearts, STAT3 has also been implicated in the activation of components of the reperfusion injury salvage kinase (RISK)

pathway, including PI3K, Akt, GSK3β and ERK2.[40] Kinases of the RISK pathway have been shown to inhibit opening of the mitochondrial permeability transition pore (MPTP) during reperfusion[41] and activation of the RISK pathway is induced by several cardioprotective agents,[42] such as insulin, which is a known STAT3 activator.[33] Inhibition of MPTP opening has also been shown to involve an interaction between mitochondrial STAT3 and cyclophilin D.[11] Metallothionein, an endogenous cardioprotective agent that acts as a free radical scavenger and a stabilizer of biomembranes, is also induced by STAT3[43] and may also contribute to its protective effects.

Ischemic conditioning. The heart can be conditioned to better tolerate a severe I/R injury by pharmacological and mechanical means, as well as by short non-lethal periods of I/R before (preconditioning), during or after (postconditioning) a severe lethal I/R episode (reviewed in ref. 44). Ischemic conditioning does not even need to be applied to the heart itself, but is also cardioprotective when given to a distant organ or tissue such as the intestine or a limb. However, preconditioning is clearly academic in the clinical environment where patients can only receive medical intervention after myocardial infarction has occurred.

The crucial role of STAT3 in ischemic preconditioning is underlined by the fact that mice with a cardiac specific deletion of STAT3 cannot be preconditioned.[45] STAT3 activation is required for preconditioning in both normal[46] and hypertrophic myocardium,[47] and involves IL-6/gp130 signaling (since IL-6 deficient mice cannot be preconditioned), acting classically through JAK1/2 to produce tyrosine phosphorylation,[48] and PKCε and the Raf-1/p42:44 MAPK pathway to induce serine phosphorylation.[49] STAT1, STAT5 and 6 are also activated by ischemic preconditioning, but this would appear not to mediate the cardioprotective effects, since the increased phosphorylation of STAT5 and 6 in the STAT3-deficient heart compared with wild-type hearts does not result in cardioprotection.[50] However, remote preconditioning by arm ischemia in patients undergoing coronary bypass surgery has been shown to increase expression of activated STAT5 in the myocardium and to confer cardioprotection.[51] There is also some evidence that cardioprotective agents such as urocortin, which acts, at least in part, by activation of STAT3, can also precondition in the absence of brief ischemia.[52]

Effectors of ischemic preconditioning downstream of activated STAT3 include the kinase cascade of the RISK pathway.[42] In addition, STAT3-mediated preconditioning is associated with increased expression of known cardioprotective agents like COX-2 and HO-1, together with increases in anti-apoptotic proteins that protect against both the intrinsic mitochondrial apoptotic pathway (Mcl-1, BclxL) and the extrinsic death receptor pathway (c-FLIP).[53]

STAT3 has also been implicated in ischemic postconditioning. Thus, postconditioning is associated with its increased tyrosine phosphorylation, and STAT3 and JAK2 inhibition both abolish the postconditioning protection. Moreover, mice with a cardiac restricted STAT3 deletion cannot be postconditioned[54] and there is increased tyr705 phosphorylated STAT3 together with increased Complex I respiration in mitochondria isolated from postconditioned pig hearts.[55] Activated STAT3 activates the RISK pathway via PI3K, although, paradoxically, postconditioned cardiac specific STAT3 knockout hearts also show protective effects.[56] This suggests that activation of STAT3 may be one, but not the only pathway of RISK activation in postconditioning, and that other parallel initiators are also involved. Moreover, pharmacological inhibition of kinases involved in the RISK pathway did not inhibit the protective effects of postconditioning in pigs.[57] In contrast, another study has implicated the SAFE pathway and demonstrated that TNFα is required for postconditioning. Indeed, TNFα itself can result in postconditioned protection. Postconditioning by ischemia and TNFα both activate STAT3 and the postconditioning effects of TNFα are lost in STAT3-deficient hearts.[58] Perhaps relevant in the management of clinical myocardial infarction, postconditioning does not confer protection, nor result in STAT3 activation, in depressed rats.[59]

Post-infarction remodeling. After a myocardial infarct, the left ventricle undergoes progressive anatomical changes in both the infarcted and non-infarcted areas. In the infarcted area, thinning of the ventricular wall occurs by a sliding movement of the myocytes, termed "slippage," and which mainly results from degradation of collagen. Subsequently, cardiac fibroblasts deposit new collagen fibers, resulting in scar formation. In the non-infarcted area, there is compensatory hypertrophy; initially, this is beneficial since it counteracts the functional deficit of the infarcted area. However, over time, a hypertrophic myocardium becomes detrimental, since its increased oxygen demand cannot be fully supplied by the compromised ventricle. Chronic myocyte hypertrophy and death, together with interstitial fibrosis and an inflammatory response lead inexorably to heart failure (reviewed in ref. 60).

The renin-angiotensin-aldosterone system. The renin-angiotensin (AT)-aldosterone system is one of the main mechanisms implicated in heart failure following myocardial infarction. ATI, produced in the liver, is converted to ATII by AT-converting enzyme (ACE), and ATII, acting largely through the ATII type I receptor, induces myocyte apoptosis and hypertrophy and promotes myocardial fibrosis (reviewed in ref. 61). ATII induces the rapid phosphorylation of STATs 1 and 2 with delayed phosphorylation of STAT3,[62] the activation of STAT3 being mediated by a Rac-1-dependent mechanism.[63] STAT5A and B are also rapidly phosphorylated by ATII, though their phosphorylation is sustained for up to an hour.[64]

In the remote non-ischemic area of rat hearts exposed to I/R, there is rapid phosphorylation of STATs 1, 3, 5A and 6, together with JAK2 activation, and STATs 1, 3 and 5A remain activated for up to 7 d post I/R. These activated STATs, and particularly STAT3 and STAT5A, have been shown to bind to the STAT consensus sequence in the angiotensinogen promoter, and may thus contribute to the continued activation of the autocrine ATII loop.[65,66]

The precise role of the individual STATs activated by ATII in cardiac remodeling has not been fully elucidated. However, cardiac specific deletion of STAT3 is associated with the spontaneous development of heart failure in later life, and

STAT3 would appear to be particularly important in protecting the heart from doxorubicin-induced cardiomyopathy.[67] Moreover, ATII also induces suppressor of cytokine signaling (SOCS)-3, which associates with JAK-2 and inhibits activation of the JAK2-STAT1 pathway, although the effects of SOCS-3 induction on STAT3 signaling were not investigated in this model.[68] In contrast, transgenic mice overexpressing STAT3 in the myocardium have enlarged left ventricles with increased expression of hypertrophic genes such as β myosin heavy chain as early as 12 weeks of age.[69]

An alternative mechanism has been suggested for the development of postpartum cardiomyopathy in female mice with a cardiac myocyte-specific deletion of STAT3.[70] Here, the activity and expression of cathepsin D is increased, resulting in the generation of an angiogenic and proapoptotic form of prolactin. Correspondingly, inhibition of prolactin with bromocriptine prevents the development of the cardiomyopathy.

Gp130 signaling. The pro-hypertrophic effects of STAT3 are reinforced by studies on the effects of the interleukin (IL)-6 family of cytokines and their receptors. Receptors for the IL-6 family consist of a specific ligand binding subunit and a shared non-ligand binding transducer, gp130, which undergoes dimerization upon binding of an IL-6 family ligand and signals through JAK-STAT and ERK1/2 pathways. Mice overexpressing both IL-6 and its specific receptor, and in which there is therefore constitutive activation of gp130, show sustained elevation of activated STAT3 and ventricular hypertrophy.[71] There is also evidence that at least some of the hypertrophic effects of ATII may be mediated through the IL-6 family, since ATII has been reported to increase expression of IL-6 itself, and of the family members leukemia inhibitory factor (LIF) and cardiotrophin-1 (CT-1) in cardiac fibroblasts.[72] There is also increased expression of both IL-6 and LIF in the hypertrophied ventricles of rats with a hyperactive renin-angiotensin system as well as in the ventricles of spontaneously hypertensive rats.[73] However, the increased expression of CT-1 in failing hearts is accompanied by reduced gp130 expression,[74] suggesting that there may be a compensatory mechanism inhibiting overactivation of gp130 signaling. In cultured cardiomyocytes, both LIF and CT-1 induce a hypertrophic response, which in both cases is mediated by activated STAT3.[31,75] Deletion of Shp2, an SH2 domain containing tyrosine phosphatase that is known to dephosphorylate Tyr705 of STAT3,[76] also leads to enhanced STAT3 activation and cardiomyopathy.[77]

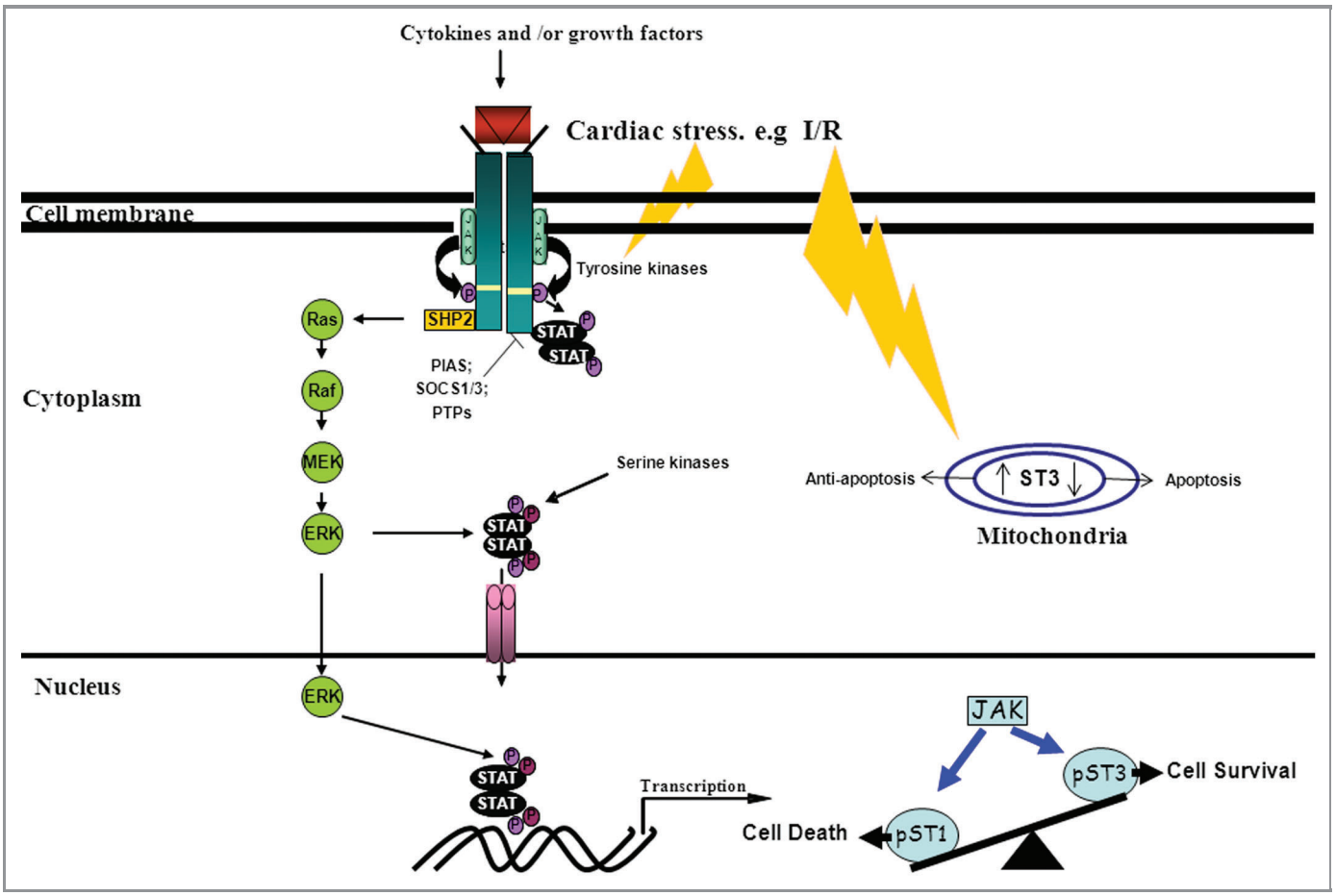

Figure 1. STAT1 (ST1) and STAT3 (ST3) regulate transcriptional processes and have opposing roles in ischemic pathology, with ST1 activation contributing to cell death signals, while ST3 activity largely mediates cytoprotective signals.

Concluding Remarks

Although all seven STATs have been described in the mouse heart,[78] studies on the role of STATs in cardiac physiology and pathology have largely been restricted to STAT1 and STAT3. In particular, strong evidence suggests that these two STATs play essentially antagonistic roles in ischemic pathology, with STAT1 activation contributing to death signals, and STAT3 largely being protective (**Fig. 1**). Prolonged STAT3 activation, however, is strongly implicated in the post-infarction changes leading to ventricular hypertrophy and heart failure. STAT1 and STAT3 can form heterodimers, and whether this occurs in the cardiac setting, and how heterodimerization modulates the activities of STAT1 and STAT3 homodimers remains unclear. There are also suggestions that other STATs may also contribute to the ischemic phenotype, and their precise role needs to be more carefully investigated.

Another area that has received only limited attention is whether both Tyr and Ser phosphorylation are required for STAT activity in the heart, although there is evidence that Ser phosphorylation alone is sufficient to allow death signaling by STAT1.[16] Moreover, transcription factors generally do not act in isolation, but their activity is modulated by other coactivators and corepressors, another area that remains to be systematically investigated in the heart.

Nevertheless, sufficient evidence has accumulated to emphasize the important role of the JAK-STAT pathway in ischemic heart disease. However, further insights into the complexities of the system are clearly required before we can begin to make translational predictions that are other than simplistic.

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