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Activated STAT-1 pathway in the myocardium as a novel therapeutic target in ischaemia/reperfusion injury



Abstract

The signal transducers and activators of transcription (STAT) factors function as modulators of cytokine signaling and sensors responding to UV and ischaemic stress. Recently, we have reported a number of transcriptional mechanisms that induce expression of pro-apoptotic genes following ischaemia/reperfusion (I/R) in cardiac myocytes. For example, we have shown that STAT-1 plays a role in enhancing apoptotic cell death in cardiac myocytes exposed to I/R. In contrast to STAT-1, STAT-3, which is activated by the IL-6 cytokine family, has

cardioprotective effects in cardiac myocytes exposed to I/R. The induction of apoptosis in cardiac myocytes by STAT-1 is dependent on STAT-1 phosphorylation on serine-727. Furthermore, we have demonstrated that the C-terminal transactivation domain of STAT-1 is required to mediate apoptotic cell death in cardiac myocytes exposed to I/R. Finally, cardiac myocytes isolated from mice expressing a truncated C-terminal STAT-1 were more sensitive to I/R-induced cell death. The isolated intact hearts from these animals exposed to I/R injury, had larger infarct size and a greater number of TUNEL-positive myocytes than control hearts. Hence, these studies demonstrate that activated STAT-1 is a pro-apoptotic factor in cardiac myocytes exposed to I/R, and is therefore a potential therapeutic target for the prevention of cell death following I/R injury.

Full text

Introduction

Signal transducers and activators of transcription (STAT) factors are a family of transcription factors that mediate intracellular signaling initiated at cytokine cell surface receptors and transmitted to the nucleus. STATs are activated by phosphorylation on conserved tyrosine and serine residues by the Janus kinases (JAKs) and MAP kinase families respectively, which allow the STATs to dimerise, translocate to the nucleus and thereby regulate gene expression. The C-terminal domains of STAT proteins all contain a transcriptional transactivation domain, plus the phosphorylation sites for JAKs and MAPK, which are essential for maximal STAT function. At present, seven different STAT family members have been characterized and have been shown to be encoded by distinct genes (STAT-1, STAT-2, STAT-3, STAT-4, STAT-5a, STAT-5b and STAT-6). Different STATs are activated by distinct groups of cytokines. For example, interferon- γ is a potent activator of STAT-1, whilst the interleukin-6 (IL-6) family members including IL-6, leukemia inhibitory factor (LIF) and cardiotrophin-1 (CT-1) primarily activate STAT-3 (for review see [1]).

Recently, the *in vivo* physiological role of individual STAT proteins has been assessed from STAT gene-knockout studies. These experiments demonstrated that STAT signaling is necessary for normal development, immune function and also regulation of cell growth and differentiation (for review see [2]). STATs have also recently been reported to modulate programmed cell death or apoptosis in

different cell types. In the present summary, we will discuss the role of STAT proteins in ischaemia/reperfusion-induced apoptosis in the heart.

Apoptosis in the heart

Ischaemia/reperfusion (I/R) injury in the heart, results in the death and loss of irreplaceable cardiac myocytes. Apoptosis has become increasingly recognized as one of the mechanisms of cell death during I/R injury in the heart (for review see [3]). In human studies, apoptosis has been observed in the infarct area of the heart following acute myocardial infarction [4]. A group of cysteine proteases (caspases) play a key role as effectors of the apoptotic cell death pathway initiated either by a death receptor pathway *via* caspase-8 or by agents leading to mitochondrial damage and leakage of cytochrome c leading to activation of caspase-9.

The Fas death receptor pathway has been reported to play an important role in the induction of apoptosis in cardiac myocytes. For example, hearts of mice, which lack functional Fas, show a considerable reduction in cell death following I/R as compared to control hearts [5]. Activated caspases have been detected in cardiac myocytes following simulated I/R injury [6]. Moreover, both a specific and a generalized caspase inhibitor abrogated apoptotic cell death in cultured cardiac myocytes, and also in the isolated, intact heart exposed to simulated I/R [7, 8]. We have recently reported that distinct upstream initiator caspases play a role in apoptotic cell death following ischaemia alone or I/R, in cardiac myocytes [7, 8]. We demonstrated, by using specific chemical or gene-based caspase inhibitors, that caspase-9 plays a key role in neonatal and adult cardiac myocyte apoptosis during simulated ischaemia, whilst caspase-8-induced death occurs during re-oxygenation. We have also substantiated our *in vitro* studies by demonstrating that the same caspases are activated, and induce apoptosis in the isolated, intact heart exposed to either ischaemia alone or I/R [8]. Thus, inhibiting the caspase pathway is a potential target for therapeutic intervention during I/R injury.

STAT signaling in the heart

The STAT-activated pathway was first demonstrated to be activated in rat cardiac myocytes following exposure to LIF, resulting in the activation of STAT-3 [9]. Subsequently, it was demonstrated in the intact heart that pressure

overload-induced hypertrophy leads to the activation of STAT-1 and STAT-3 [10]. Recently, it has also been reported that ischaemic preconditioning in the intact heart involves activation of the JAK-STAT pathway. Inhibition of STAT-1 and STAT-3 phosphorylation with the JAK inhibitor AG-490, blocked preconditioning cardioprotection [11]. Ourselves and others have also demonstrated activation of STAT-1 and STAT-3 in the isolated intact heart following I/R injury [12-14]. Pre-treatment with the JAK inhibitor reduced STAT-3 phosphorylation and enhanced apoptosis following I/R. Likewise, cultured cardiac myocytes treated with CT-1, which activates the STAT-3 pathway, enhanced cell survival following exposure to simulated I/R injury and reduced the level of apoptotic cell death [15]. Thus, these studies demonstrate that STAT-3 may be an anti-apoptotic signaling factor in the heart.

In contrast to STAT-3, STAT-1 plays a role in enhancing apoptotic cell death in cardiac myocytes following simulated I/R injury, by inducing the expression of the pro-apoptotic caspase-1, Fas and FasL genes leading to enhanced cardiac cell death [12, 13]. Moreover, inhibition of STAT-1 using an anti-sense approach, prevented the enhancement of caspase-1, Fas and FasL gene activity in cardiac myocytes exposed to simulated I/R, and protected cardiac cells from I/R-induced cell death [12, 13]. In addition, we have shown that STAT-1 also inhibits the promoters of genes encoding the genes for anti-apoptotic Bcl-2 and Bcl-x proteins [16]. Hence, STAT-1 activation appears to induce apoptosis in cardiac myocytes by activating pro-apoptotic genes as well as repressing anti-apoptotic genes. We previously examined the mechanism of STAT-1 activity in cardiac myocytes exposed to simulated I/R. We demonstrated that both the tyrosine 701 and serine 727 sites of STAT-1 are phosphorylated in cultured cardiac myocytes and also in the isolated intact heart exposed to I/R [12,13]. However, studies using STAT-1 mutant constructs, demonstrated that the induction of Fas and FasL, as well as enhanced apoptosis in cardiac myocytes exposed to simulated I/R, required the phosphorylation of STAT-1 on serine 727, but not on tyrosine 701 [13]. The phosphorylation of serine 727 of STAT-1 appears to be accomplished by p38 MAPK activated during I/R, since it can be blocked both by the chemical inhibitor SB203580 and by a dominant negative form of MKK6, the upstream activator of p38 MAPK [13].

Although phosphorylation of tyrosine 701 was originally thought to be essential for STAT-1 function, recent studies have shown that some genes can be induced by STAT-1 in a tyrosine 701-independent manner. In addition, serine

727 phosphorylation has also been reported to be critical for the activity of the C-terminal STAT-1 transactivation domain (TD) to bind to other co-activator molecules such as MCM5 and BRCA1.

Thus, our studies demonstrate that I/R-induced apoptosis requires serine 727 of STAT-1, but not tyrosine 701 in cardiac myocytes. This suggests that the activity of the C-terminal TD of STAT-1 - *via* phosphorylation on serine 727 - mediates the effects of cell death in cardiac myocytes exposed to I/R. Support for this comes from our finding that enhanced cell death is observed using a STAT-1 construct encoding only the C-terminal TD and lacking the DNA binding domain in cardiac myocytes exposed to I/R [17]. Moreover, exchanging serine 727 for a non-phosphorylatable alanine reduced the ability of the isolated C-terminal STAT-1 construct in promoting cell death in cardiac myocytes exposed to simulated I/R. Likewise, cardiac myocytes isolated from mice lacking the N-terminal domain of STAT-1 (amino acids 1-134), but expressing the C-terminal domain were more sensitive to I/R-induced cell death. The isolated intact hearts from these mice exposed to I/R injury had larger infarct size and a greater number of TUNEL positive myocytes than control hearts [17].

Interestingly, it has been shown that STAT-1 can be cleaved by caspases such as caspase-3 at position 694 [18]. We and others have demonstrated that caspases play an active role in apoptotic cell death in cardiac myocytes exposed to I/R [7, 8]. Cleavage of STAT-1 by caspase-3 at position 694 will ultimately release the C-terminal STAT-1 TAD. The N-terminal fragment containing the DNA-binding domain may behave as a dominant negative against intact STAT-1 protein, whilst the caspase-mediated generation of the pro-apoptotic C-terminal TAD fragment may be involved in amplifying and perpetuating the apoptotic loop in hearts exposed to ischaemia/reperfusion injury (Figure 1).

CONCLUSION

Myocardial ischaemia/reperfusion injury plays a major role in cell death and the loss of irreplaceable cardiac myocytes, which have been suggested to contribute to heart failure. It is clear that STAT-1 plays a critical role in inducing pro-apoptotic genes and apoptosis in cardiac myocytes exposed to I/R. Thus, the STAT-1-activated pathway is a potential therapeutic target in the prevention of ischaemic heart disease. Agents that are likely to inhibit STAT-1 and not STAT-

3 activity may lead the way for developing strategies for therapies that may ultimately lead the way for the prevention of heart failure.

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Figures

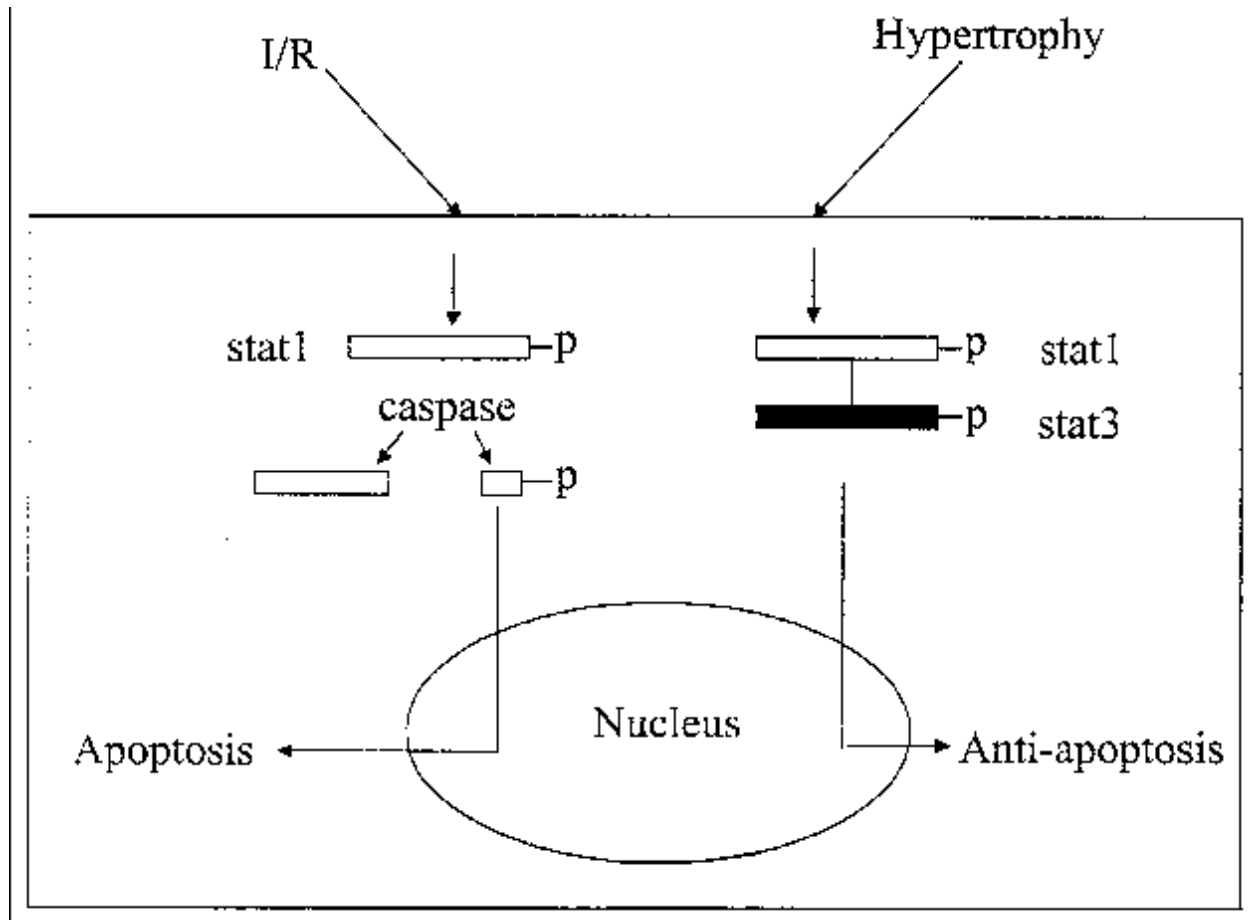


Figure : 1 STAT activation in hypertrophy.

A hypothetical pathway demonstrating activation of STAT-1 or STAT-3, or both, following exposure of cardiac myocytes to either ischaemia/reperfusion (I/R) injury or events leading to hypertrophy. I/R leads to phosphorylation of STAT-1 (p), followed by caspase cleavage of the C-terminal domain of STAT-1, which activates apoptotic genes leading to cell death. In contrast, a hypertrophic signal leads to both STAT-1 and STAT-3 activation, which signals to activate anti-apoptotic genes and cell survival.

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