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CELL CYCLE MOLECULES AND DISEASES OF THE CARDIOVASCULAR SYSTEM

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1. ABSTRACT

Injury to the cardiovascular system causes an elevated expression of endothelin-1 (ET-1) and activation of several important signaling pathways including the mitogen-activated kinase (MAPK) cascade. The activation of these pathways has been implicated in the pathogenesis of cardiovascular disease caused by hypoxia, infections, and ischemia /reperfusion injury, cardiomyopathy and restenosis after balloon angioplasty. Important downstream targets of the MAPK and ET-1 pathways are the cell cycle regulatory molecules (cyclins, cyclin-dependent kinases, and cyclin-dependent kinase inhibitors). Regulation of these molecules contributes to remodeling throughout the cardiovascular system. In addition, cell cycle molecules are important in the regulation of angiogenesis. These new data have led to the development of potential therapeutic modalities targeting these regulatory molecules in order to ameliorate various cardiovascular disease states.

2. INTRODUCTION

Coronary arteriosclerosis and subsequent ischemia and infarction are common causes of myocardial injury. Cardiomyopathy and congestive heart failure (CHF) may result from a variety of etiologies including infarction and infection. The molecular basis of the pathogenesis of cardiovascular diseases has become the focus of intense investigation in many laboratories. In that regard, dysregulation of cell cycle regulatory molecules (cyclins, cyclin-dependent kinases and cyclin-dependent kinase inhibitors) has been demonstrated to be associated with disorders of the cardiovascular system. The alterations in the expression of the cell cycle regulatory molecules result in remodeling of the myocardium and its vasculature. In the cardiovascular system, the cell cycle regulatory molecules are targets of interrelated pathways including the mitogen activated protein kinase (MAPK) cascade, endothelin-1 (ET-1) and angiotensin II.

The role of cell cycle regulatory molecules in arteriosclerosis, restenosis, angiogenesis, cardiac hypertrophy and myocarditis has been examined. The MAPK-ET-1-cyclin pathways are involved in modulating the cell cycle machinery in cardiac myocytes and the endothelium and may thus serve as potential targets for therapeutic modalities that include the repair of myocardial tissue following ischemia or infarction and reversal of restenosis following angioplasty.

3. ENDOTHELIN

Endothelial cells and cardiac myocytes are major sources of ET-1, a potent vasoconstrictor and mitogen (1). The synthesis of ET-1, the result of the action of endothelin converting enzyme (ECE), has several pharmacological actions in the cardiovascular system which are mediated by the ET-1 receptors (ET_A or ET_B) (2,3). The discovery of these receptors stimulated the development of receptor antagonists and significantly increased the research into the

molecular biology, physiology and pharmacology of ET-1 (4,5). The primary effects of ET-1 are mediated by the ET_A receptor including vasoconstriction and smooth muscle cell proliferation (6). ET-1 activates the MAPK cascade and the transcription factor activator protein-1 (AP-1), thus promoting smooth muscle cell proliferation (7). In addition, the presence of an AP-1/Jun-binding site in the 5'-flanking region of the ET-1 gene leads to the rapid induction of ET-1 mRNA (4).

Plasma ET-1 levels are elevated in humans and experimental animals with CHF, myocardial infarction, septic shock and infectious agents including murine viral and *Trypanosoma cruzi* -induced myocarditis (Chagas' disease) (4, 5, 8-13). Locally produced ET-1 acts on cardiac myocytes in both an autocrine and/or paracrine manner, increasing the contraction of smooth muscle cells and induces chronic myocardial hypertrophy and cardiac myocyte injury. ET-1 improves contraction in the failing heart and up-regulation of ET-1 provides short-term inotropic support for the failing myocardium despite vasoconstriction (4). Treatment with an ET_A receptor antagonist improves the survival of animals with CHF (4, 14,15) and is accompanied by improvement in left ventricle (LV) dysfunction and in ventricular remodeling. Recently, an endothelin receptor antagonist was demonstrated to ameliorate murine viral myocarditis (14). This suggests that upregulation of ET-1 may also be a potential target for therapeutic intervention. ET-1 production is increased in the myocardium of rats with CHF. The increased expression of myocardial ET-1 correlates positively with LV diastolic pressure. Plasma ET-1 levels also correlate with infarct size and severity of CHF in humans. In many pathological states elevated ET-1 levels reflect the degree as well as a mechanism of endothelial cell and cardiac myocyte damage.

Studies on the putative role for ET-1 in the modulation of cardiovascular structure has focused on the role of ET-1 in the induction of smooth muscle cell proliferation. ET-1 stimulates smooth muscle cell hypertrophy, protein synthesis and the incorporation of ^{3}[H] thymidine. These effects of ET-1 are mediated, in part, by activation of the smooth muscle cell ET_A receptor. Activation of this receptor results in the synthesis of Types I and III collagen and reduction in collagenase activity (5,16).

Vasospasm and abnormal vascular smooth muscle proliferation are important complications of both arteriosclerosis and vascular wall trauma such as seen following balloon angioplasty. Arteriosclerosis and angioplasty are associated with enhanced ET-1/ECE immunoreactivity. Elevated levels of ET-1 promote acute vasospasm and chronic vascular remodelling. Following experimental balloon injury there is neointima formation (17,18) associated with an induction of ET-1, ECE and endothelin receptor mRNA and protein expression. Administration of endothelin receptor antagonists and ECE inhibitors results in a reduction in neointima formation suggesting a role for ET-1 in the pathogenesis of restenosis (19). The relationship between ET-1 and induction of cell cycle regulatory molecules has only recently been explored (20). It appears that extracellular signal-regulated kinase (ERK) and protein kinase C are involved in this process. Another vasoactive peptide, angiotensin II, activates cyclin D1 expression, DNA synthesis and cellular proliferation (21).

4. MITOGEN ACTIVATED PROTEIN KINASE (MAPK) CASCADE

Myocardial ischemia and hypoxia activate the MAPKs and the transcription factor, AP-1(85). AP-1 is an important factor in the activation of ET-1 and the Ras-MAPK signaling pathway is critical to the activation of AP-1(Figure 1). There are 3 major MAPKs in mammalian cells, ERK, SAPK/JNK and p38. However, only ERK and JNK, contribute to the activation of AP-1. Each MAPK affects AP-1 activity through specific phosphorylation of different substrates (TCF/ELK-1, c-Jun and ATF-2). The two MAPKs that affect AP-1 activity differ in their responses to extracellular stimuli. For example, growth factors and phorbol esters stimulate ERK, while (JNK) activity is induced by UV irradiation (22-24). Although both kinases are stimulated in response to Ras activation (25), JNK also responds to Ras-independent signals (26). Ras is a GDP/GTP-regulated binary switch that resides at the inner surface of the plasma membrane. It functions downstream of receptor tyrosine kinases and acts to relay extracellular signals to cytoplasmic signaling cascades. The major pathway leading from Ras to ERK are through the Raf cascades (Raf→MEK→ERK) providing a complete link between the cell surface and the nucleus (25). Ras and the other small GTP-binding proteins, Rac1, Rho and Cdc42 function upstream to activate JNK cascades (27). Smooth muscle cell proliferation is important aspects of vascular damage and repair. Activation of phosphorylated ERK (ERK1/2) contributes to smooth muscle cell proliferation and contraction. In that regard, PD98059, an inhibitor of MEK1, prevents the growth of smooth muscle cells in response to a variety of stimuli. Activation of ERK1/2 and medial smooth muscle cell proliferation occurs as a result of balloon injury to carotid and coronary artery in animal models that is markedly reduced by the administration of PD98059 (28).

5. CELL-CYCLE REGULATORY MOLECULES

5.1. Cardiac cell cycle

Cyclins are proteins that are synthesized and destroyed during each mammalian cell cycle (29). Eight cyclins have been described: A, B1,2 3, C, D1,2,3 E, F, G, H. They all share a 150 amino acid region of homology, the "cyclin box." They bind to the N-terminal end of specific cyclin-dependent kinases (CDKs) (30). G_1 cyclins (D1-3, E and A) are short-lived proteins that function during the G_1 phase and the G_1-S transition before their destruction via the ubiquitin pathway. Cyclins A and B remain stable during interphase and are rapidly destroyed by proteolysis during mitosis. Different cyclins specifically bind to different CDKs to form complexes at specific phases of the cell cycle thereby driving cells from one stage to another. The CDKs are protein kinases, which bind to and are

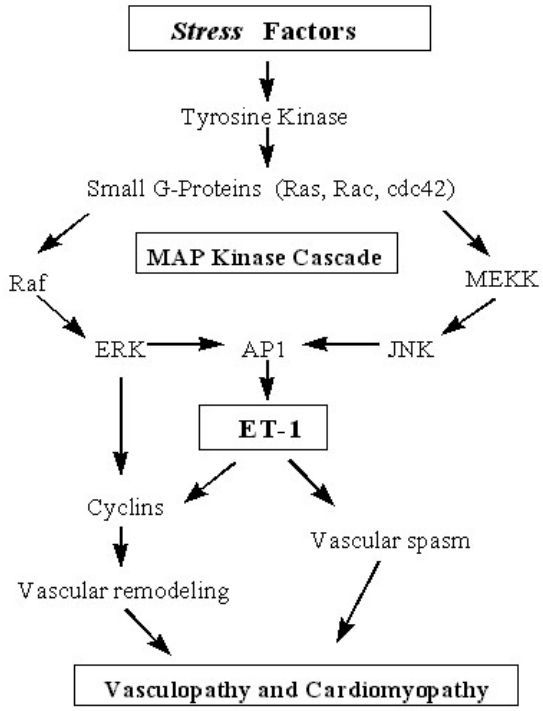

Figure 1. The relationship between the MAPK cascade, ET-1 and the cyclins. Note also that that ET-1 may activate ERK and that angiotensin II may activate the MAPK cascade, ET-1 and the cyclins.

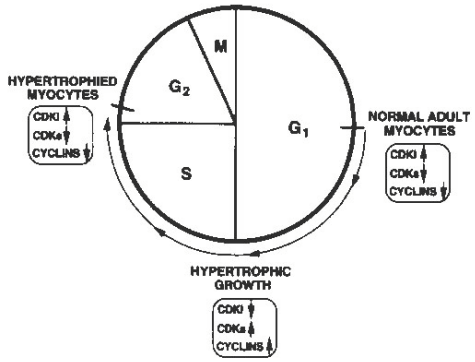

Figure 2. Hypothesis of Brooks *et al* (32) for the involvement of cell cycle regulatory molecules in the development of hypertrophic growth of cardiac myocytes. They have hypothesized that normal adult cardiac myocytes are arrested in the G_0/G_1 phases of the cell cycle. This is associated with an increase in the expression of CDKIs and a decrease in cyclins and CDKs. Hypertrophic growth is associated with the cells proceeding through the G_1, S and arresting in the G_2 phase. This is associated with an increase in the expression of cyclins and CDKs with an accompanying decrease in CDKI expression. The hypertrophied cardiac myocyte is unable to undergo mitosis. (Reprinted from Cardiovascular Research, 39, Brooks G, Poolman RA, Li J-M, Arresting developments in the cardiac myocyte cell cycle: Role of cyclin-dependent kinase inhibitors, 301-311 (1998), with permission from Elsevier Science).

activated by specific cyclins. Thus the cell cycle progression is regulated by CDKs which are positively regulated by association with cyclins and negatively regulated by CDK inhibitors (CDKIs). Cdk 4, cdk5 and cdk6 complex mainly with the cyclin D family and function during the G_0/G_1 phases. Cdk2 can also bind to members of the cyclin D family but more commonly associates with cyclins A and E and function during G_1 and G_1-S transition. CDKIs comprise two major families, Ink4 and Cip/Kip. The Cip/Kip family is composed of $p21^{Cip1}$, $p27^{Kip1}$ and $p57^{Kip2}$ and inhibits many CDKs including Cdk2, Cdk4 and Cdk6. Ink4 includes p14, p15, p16, p18 and p19 and inhibits mainly G_1 cyclins (29).

During early cardiac development cardiac myocytes differentiate from mesoderm into striated muscle. They contract and despite this, retain their capability of dividing. During fetal development the increase in cardiac mass is the result of increased cardiac cell proliferation. Cardiac myocyte division ceases asynchronously over fetal life, so that by early neonatal life any subsequent increase in cardiac mass is the result of an increase in myocyte size (31,32). The reasons for the irreversible withdrawal of the cardiac myocyte from the cell cycle are not entirely understood (33). However, it appears that the adult heart retains the ability to undergo DNA synthesis following hemodynamic overload or injury. Cardiac myocytes are capable of re-entering the cell cycle although there is little evidence that these cells undergo mitosis. A hypothesis to explain the involvement of cell cycle regulatory molecules and the development of cardiac myocyte hypertrophic growth is illustrated in Figure 2 and reviewed by Brooks *et al* (32).

The inability of mature cardiac myocytes to undergo cell division leads to major consequences following severe injury such as myocardial infarction, since the heart is unable to regenerate new cardiac myocytes to replace damaged tissue. Therefore, understanding the mechanisms by which cell cycle molecules participate in cardiac myocyte proliferation and hypertrophy may lead to new therapeutic strategies targeted at the regeneration of new cardiac myocytes from healthy cells that surround infarcted areas. Controlling the growth of cardiac myocytes in the myocardium may result in the re-initiating DNA synthesis and cell division thus controlling the repair of damaged myocardium.

5.2. Myocardial infarction

Injury to the myocardium results in alterations in cardiac cell cycle molecules. Several studies have examined DNA synthesis of adult cardiac myocytes under normal and pathological conditions. It has been reported that in injured or hypertrophied rat cardiac myocytes, there are mitotic cells most of which are in the S and G_2/M phases (34-36) (Figure 2). However, other studies have failed to confirm this increase in DNA synthesis (37,38). It has been suggested that these differences may be due to differences in animal species as well as methodology. Anversa and his colleagues (39,40) have suggested that cardiac myocytes undergo mitosis but these conclusions have been questioned (41).

Myocardial infarction is associated with an upregulation in the expression of cyclins E, A and B, Cdk2 and Cdc2 in the remaining viable LV cardiac myocytes (42). It is of interest that cyclins A, B, E and Cdc2 are also detected in neonatal cardiac myocytes. There is a minority opinion that cardiac myocytes may not be terminally differentiated and that their regeneration significantly contributes to the pathophysiology of the failing heart. The transition from hyperplastic to hypertrophic growth in heart involves the key regulators of the cell cycle including CDKIs (Figure 2). In acute and end-stage heart failure there is a significant change in CDKI expression. Levels of $p21^{Cip1}$ and $p27^{Kip1}$ are reduced while the $p57^{Kip2}$ levels, expressed in fetal cardiac myocytes, are increased. These observations are consistent with the notion that injury to the myocardium may result in the reversion to a fetal pattern of expression. Cardiac myocytes stimulated to divide may be driven towards apoptosis. Evidence for this is derived from studies in which DNA synthesis induced myocytes transfected with E1A gene triggered apoptosis rather than division (43). However, the link between altered CDKI expression and apoptosis requires further investigation. Observations in animal models of myocardial infarction suggest that apoptosis may contribute substantially to cell death in the central infarct area with 5% to 33% of cardiac myocytes staining positive for DNA fragmentation. In addition, myocytes in the periinfarct region are shown to activate the apoptotic regulatory proteins bax and bcl-2. However, at present, the relative importance of apoptotic and nonapoptotic cell death in both acute and chronic phases of myocardial infarction is not known(86).

Despite the controversy regarding the cardiac cell cycle and injury, it is clear that the reactivation of the cell cycle is important during the acute phase following myocardial infarction and ischemia. Cardiac hypertrophy is the physiological response to myocardial infarction, but becomes pathological when it exceeds normal limits, resulting in loss of contractile performance and in heart failure. In recent years, strategies have been attempted to improve cardiac function, in the heart following infarction. One such approach utilized the transplantation of myocytes that have proliferative capacity into infarcted myocardium. The results from the initial experiments were promising and suggested that cell transplantation had the potential to be used as an alternative therapy for regenerating damaged myocardial tissue. Another approach was to increase the number of viable cardiac myocytes by manipulating the expression of cell cycle regulatory molecules by gene transfer strategies that include oligonucleotide therapy, adenoviral and adeno-associated viral vectors and hemagglutinating virus of Japan-liposome-mediated transfer. Although these approaches are still in their preliminary stages of development preliminary data are encouraging (30,44). More recently $p27^{Kip1}$ knockout mice have been studied. These mice have multi-organ enlargement that includes the heart where there is prolonged proliferation of cardiac myocytes and a perturbation of cardiac myocyte hypertrophy (45). These data again underscore the possibility that specific targeting of certain cell regulatory molecules will enable the initiation of cell division in healthy cardiac myocyte that surround an injured area.

5.3. Cardiac hypertrophy and congestive heart failure

Adult cardiac myocytes respond to stimuli such as pressure overload by hypertrophy. This is a process associated with protein synthesis and expression of early genes (c-*fos*, c-*jun*, and hsp70) as well as those genes normally associated with cardiac development. Cardiac hypertrophy is a normal physiological response that becomes pathological when normal limits are exceeded. This results in decreased myocardial contractility and heart failure (30). The cardiac myocyte responds to pressure overload with a hypertrophic growth response (46) and if the response persists it may lead to CHF. In rats, pressure overload-induced LV hypertrophy is associated with upregulation in the expression and elevated activities of cyclin D2, cyclin D3, Cdk2, Cdk4 and Cdk6 whereas $p21^{Cip1}$ and $p27^{Kip1}$ mRNA and protein levels are downregulated (Figure 2). (30). Therefore, it appears that certain cell cycle regulatory molecules are associated with the development of cardiac myocyte hypertrophy. Recent data indicate that the Cip/Kip family of inhibitors is altered in heart failure. In human end-stage CHF, Burton *et al* (47) observed a reduction in the levels of $p21^{Cip1}$ and $p27^{Kip1}$ and an increase in $p57^{Kip2}$. This is of interest since $p57^{Kip2}$ is found only in fetal cardiac myocytes.

6. THE ENDOTHELIUM

6.1. Role of Cell cycle regulatory molecules in endothelial cell (EC) biology and angiogenesis.

Endothelial cells form a monolayer, which line the vascular network and are responsible for maintenance of laminar flow and hemostasis. They are quiescent with a doubling time of several years (48). This quiescent state is maintained through the contact inhibition of endothelial cell growth. The levels of cyclin A mRNA decreased in confluent bovine aortic endothelin cells (49). This was due to transcriptional regulation of the activating transcription factor (ATF)-1 protein that was required for cyclin A promoter activity. Contact inhibition also results in decreased p42/p44 MAPK activation with consequent inhibition of c-*fos* and cyclin D1 (50). Quiescent endothelial cells also express low levels of Cdk2 and cyclin E compared to proliferating cells.

Angiogenesis is the process by which new vessels are derived from the pre-existing mature vasculature in the adult. This complex process requires dissolution of the surrounding matrix, migration of cells along a chemotactic gradient and re-differentiation into new vessels (48). As a part of this process endothelial cell must leave their normal quiescent state and re-enter the cell cycle. During angiogenesis, proliferative endothelial cells become apoptotic in response to antagonists of integrin $\alpha v\beta 3$ leading to the regression of angiogenic blood vessels (51). The ligation of vascular cell integrin $\alpha v\beta 3$ promotes a critical and specific adhesion-dependent cell survival signal during angiogenesis leading to inhibition of p53 activity, decreased expression of $p21^{WAF1/CIP1}$, and suppression of cell death (52). Interestingly, the cyclins also play a role in the onset of endothelial cell death, vessel regression and

dissolution. Apoptosis of human endothelial cells after growth factor deprivation is associated with rapid and dramatic up-regulation of cyclin A-associated Cdk2 activity (53). In apoptotic cells, the C termini of the CDKIs $p21^{Cip1/Waf1}$ and $p27^{Kip1}$ are truncated by specific cleavage. The enzyme involved in this cleavage is caspase-3 and/or a caspase-3-like caspase. After cleavage, $p21^{Cip1/Waf1}$ loses its nuclear localization sequence and exits the nucleus. Cleavage of $p21^{Cip1/Waf1}$ and $p27^{Kip1}$ results in a substantial reduction in their association with nuclear cyclin-Cdk2 complexes, leading to a significant induction of Cdk2 activity. These data suggest that the activation of Cdk2 through caspase-mediated cleavage of CDKIs may be critical in the execution of apoptosis following caspase activation. Thus, multiple stages of the angiogenic process appear to converge on the proliferative capacity of the endothelium, especially the cyclins, as a determinant of angiogenic potential. Further, many anti-angiogenic agents have mechanisms of action, which inhibit endothelial cell proliferation. One well-documented example is the fumagillin analogue AGM-1470, a potent inhibitor of angiogenesis *in vivo* and a powerful antitumor agent. AGM-1470 acts by altering the endothelial cell cycle and inhibiting growth factor induced proliferation (54). AGM-1470 inhibits phosphorylation of the Rb protein, and limits growth factor-induced mRNA expression of Cdc2 and cyclins A, E and D1 (54,55). In addition, AGM1470 also acts directly on the tumor cell cycle. These observations are of interest since gene therapy using cDNAs encoding cell cycle regulatory proteins, which directly manipulate the cell cycle of a tumor, can also inhibit the angiogenic potential of the surrounding vasculature. For example, restoration of functional p16 in human gliomas led suppression of tumor vascularization through down-regulation of vascular endothelial growth factor (VEGF) expression (56). Thus, regulating the ability of endothelial cells to proliferate is an important and readily manipulated component of the angiogenic process.

The proliferative response of endothelial cells can be modulated by multiple factors. One common pathway which plays a dual role in endothelial cell proliferation is the protein kinase C (PKC) pathway. PKC activation is a necessary step for the proliferative effects of many pro-angiogenic factors, including fibroblast growth factor (FGF-2) and VEGF (57-60). Additionally, over-expression studies have shown that some individual isoforms of PKC are necessary for cell cycle progression in endothelial cells. Rosales *et al* (61) observed that PKCβ1 over-expression promoted bovine aortic endothelial cell growth and shortened bovine aortic endothelial cell doubling time. PKCθ activity is required for transition through the G_2/M stage of the cell cycle in microvascular endothelial cells (62). However, direct manipulation of the PKC pathway via multiple mechanisms, can also lead to endothelial cell cycle arrest and therefore decreased angiogenic potential. For instance, stimulation of large vessel endothelium with either PMA or diacylglycerol, two agents that activate PKC, leads to G_2 arrest through decreased activity of cdc2 kinase transition due to inhibition of tyrosine dephosphorylation (63). PMA also decreases cyclin B and cdc25B expression, which is thought to contribute to decreased cdc2 kinase activity. In venous, endothelial cells PKC stimulation during the early G_1 phase potentiates growth factor-stimulated DNA synthesis, the activation of cdc2 and cdk2 cyclin-dependent kinases, and the mRNA expression of cdc2, cyclins A, D1 and E. Conversely, PKC stimulation in the late G_1 phase inhibits DNA synthesis, activation of CDKs and prevents the transcriptional up-regulation of E2F1, cyclin A and cyclin E (64,65). While these studies suggest a strong relationship between PKC activation and regulation of endothelial cell proliferation, they do not assess the role of individual PKC isoforms. Rosales *et al* (61) demonstrated that overexpression of PKCα reduced proliferation of endothelial cells by increasing the accumulation of cells in the G_2/M phase of the cell cycle. One isoform that appears to regulate endothelial cell proliferation is PKCδ. Decreased PKCδ activity is a specific requirement of VEGF induced endothelial cell proliferation and migration (66). Over-expression of PKCδ in microvascular endothelial cells leads to an accumulation of cells in S-phase of the cells cycle associated with decreased cyclin A, E, and D1 activity and a failure of cyclin D1 to translocate to the nucleus (67,68). The sole causal factor was an up-regulation of the $p27^{kip1}$ gene. PKCδ activity is also specifically responsible for the up-regulation of $p21^{cip1}$ inhibition of endothelial cell cycle associated with phorbol ester treatment (69). Collectively, these data suggest that the regulation of different PKC isoforms may be an attractive therapeutic option to modulate angiogenesis and endothelial cell proliferation.

In atherosclerosis, one event implicated as the initial step in plaque formation is induction of endothelial dysfunction or endothelial denudation from the vessel wall. Endothelial cells perturbed by shear or cytokine exposure (such as that from activated macrophages) decrease the soluble factors and intercellular communication that maintain the quiescent, "contractile" smooth muscle cell phenotype (70). Although untested in restoring intimal integrity after angioplasty, intervention using antisense technologies has been successful in preventing multiple facets of graft failure in animal models. In rabbits, autologous vein grafts treated with anti-sense oligonucleotides to either proliferating cell nuclear antigen or cdc2 kinase have better endothelial function than untreated or control-transfected grafts (71). Treated grafts elaborated more nitric oxide, exhibited greater vasorelaxation, reduced superoxide radical generation, VCAM-1 expression and monocyte binding activity. Thus, arrest of vascular cell cycle progression preserves the normal endothelial phenotype and imparts resistance to atherosclerosis and restenosis.

6.2. Restenosis and neointima formation

Coronary angioplasty has become a commonly performed technique to establish myocardial revascularization. However, in 30-60% of cases restenosis occurs which may or may not be accompanied by recurrent angina (72). This injury to the vessel causes smooth muscle cell proliferation, migration and the production of extracellular matrix. This results in thickening/hyperplasia of the neointima (72). This is associated with an activation

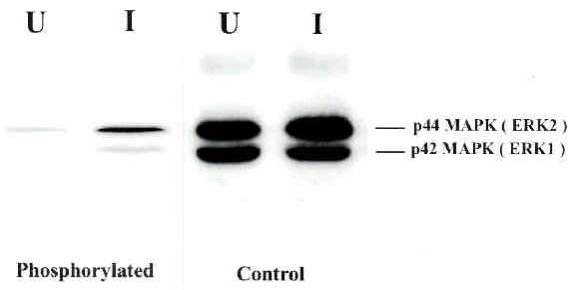

Figure 3. Western blot analysis of protein lysate obtained from *Trypanosoma cruzi*-infected (I) and uninfected (U) myocardial tissue. Note the increase in phosphorylated ERK (ERK1/2) in infected hearts.

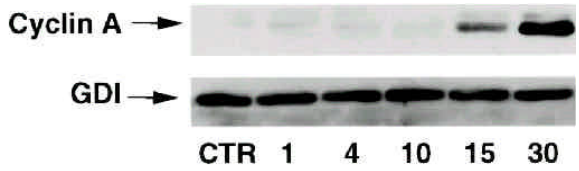

Figure 4. Western bot of infected myocardial tissue demonstrating induction of cyclin A over time (days post infection). Ctr (control, uninfected myocardial tissue).

of the MAPK-AP-1 pathways (73) and ET-1. Cell cycle regulatory molecules, downstream targets both of MAPK and ET-1, are associated with smooth muscle cell and fibroblast proliferation and hence are responsible for remodeling in the cardiovascular system. As stated previously, MAPK and ET-1 blockade has been reported to ameliorate restenosis. There is a marked induction of cyclins and CDKs shortly following angioplasty and is sustained for many days following injury. This is consistent with the period when neointimal growth is most rapid. Recently, studies have targeted the cell cycle regulatory molecules to reduce neoinitma formation following angioplasty. For example, the principal animal model used for gene therapy studies is the balloon-injured rat carotid artery model. There are reports that a single dose of antisense oligodexoynucleotides directed against Cdk2 and/or Cdc2, and proliferating cell nuclear antigen can inhibit the proliferation of vascular smooth muscle cells for an extended period of time following balloon injury (30,44,74,75). Chang *et al* (76) performed localized arterial injection of a nonphosphorylatable form of dominant negative retinoblastoma protein gene product (Rb) at the time of angioplasty, which blocked cellular proliferation. This indicates that phosphorylation of Rb is required for smooth muscle cell proliferation during neointimal remodeling after angioplasty. Moreover, Sylvester *et al* (75) extended these studies and investigated the mechanisms that regulate cyclin A gene expression following angioplasty. These authors demonstrated that a Ras-dependent mitogenic signaling pathway is essential for normal stimulation of cyclin A promoter activity and DNA synthesis in rat smooth muscle cells.

7. INFECTIONS OF THE CARDIOVASCULAR SYSTEM

7.1. *Trypanosoma cruzi* infection of the cardiovascular system

The role of the MAPK-ET-1-cyclin pathways in the pathogenesis of infections of the cardiovascular system has not been examined in detail. *Trypanosoma cruzi*, a hemoflagellate protozoan parasite, is the etiologic agent of Chagas' disease, an important cause of acute myocarditis and chronic cardiomyopathy in endemic areas of Latin America (77). This disease is accompanied by arrhythmias and CHF. Infection of the cardiovascular endothelium with this parasite increases the synthesis of ET-1 causing vascular spasm and myocardial ischemia (78,79,80,81). Therefore, we focused on the *T. cruzi* infection-associated activation of upstream pathways leading to activation of ET-1 (i.e., MAPK- AP-1 pathway) (13,82-86) and the downstream targets of MAPK and ET-1, the cell cycle regulatory molecules. These factors are important in the process of cardiovascular remodeling. We found that in the myocardium of *T. cruzi*-infected mice there was an upregulation of myocardial expression of ERK1/2 (Figure 3), AP-1 and ET-1 (13). In addition, Western bot analysis revealed an increased expression of cyclin D1 and cyclin A (Figure 4). Immunohistochemistry demonstrated localization of ET-1 and cyclin A and D1 to the vascular endothelium, the endothelium of the endocardium and fibroblasts. These observations underscore the critical importance of the endothelium in the pathogenesis of chagasic cardiomyopathy and in the role of cell cycle regulatory molecules in the process of remodeling following this infection.

8. CONCLUSIONS

The importance of the cell cycle regulatory proteins in the pathogenesis of a variety of cardiovascular disease states is now emerging. Whether knowledge of their regulation will lead to new therapeutic strategies awaits further investigations**.**

9. ACKNOWLEDGEMENTS

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