PERGAMON Progress in Biophysics & Molecular Biology 78 (2002) 105–137

Progress in Biophysics & Molecular Biology

www.elsevier.com/locate/pbiomolbio

Review

Mechanical stress-induced apoptosis in the cardiovascular system

Florian Wernig, Qingbo Xu*

Department of Cardiological Sciences, St George's Hospital Medical School, Cranmer Terrace, Tooting, London SW17 0RE, UK

Abstract

All tissues in the body are subjected to physical forces originating either from tension, created by cells themselves, or from the environment. Particularly, the cardiovascular system is continuously subjected to haemodynamic forces created by blood flow and blood pressure. While biomechanical force at physiological levels is essential to develop and maintain organic structure and function, elevated mechanical stress may result in cell death leading to pathological conditions. In recent years, however, it has been widely recognized that cell death, namely apoptosis, is not just the response to an injury but a highly regulated and controlled process. Therefore, physical stimuli must be sensed by cells and transmitted through intracellular signal transduction pathways to the nucleus, resulting in cell apoptosis. Disturbances in the regulatory mechanisms of apoptosis often precede the development of a disease. Exploration of the molecular signalling mechanisms leading to mechanical stress-induced apoptosis in cardiovascular disorders revealed the crucial role of apoptosis in the pathogenesis of these diseases. For instance, heart failure, hypertension and atherosclerosis are believed to be related to sustained mechanical overloading or stress. In this review we summarize the recent data focusing on molecular mechanisms of mechanical stress-induced apoptosis and highlight the role of apoptosis in the development of cardiovascular disorders, which may lead to new therapeutic strategies for these diseases.

Keywords: Mechanical stress; Apoptosis; Signal transduction; Heart failure; Atherosclerosis

Contents

1. Introduction 106

2. Mechanical stress 107
   2.1. Stretch 107
   2.2. Shear 108

*Corresponding author. Tel.: +44-20-8725-2817; fax: +44-20-8725-2812.
E-mail address: qxu@sghms.ac.uk (Q. Xu).

0079-6107/02/$ - see front matter
PII: S0079-6107(02)00008-1

3. Apoptosis . . . 109
3.1. Molecular mechanisms of apoptosis . . . 109
3.2. Detection of apoptosis . . . 111

4. Pathophysiological mechanisms of apoptosis . . . 112
4.1. Heart failure . . . 113
4.1.1. Role of Bcl-2 protein family and p53 . . . 113
4.1.2. MAPK signalling . . . 115
4.1.3. G proteins . . . 116
4.1.4. Lack of survival signals . . . 116
4.2. Hypertension . . . 117
4.2.1. Remodelling . . . 118
4.2.2. Mechanotransduction . . . 118
4.3. Atherosclerosis . . . 120
4.3.1. Smooth muscle cell apoptosis . . . 121
4.3.2. Restenosis . . . 122
4.3.3. Vein graft arteriosclerosis . . . 123
4.3.4. Protein kinase C . . . 124
4.3.5. Checkpoint p53 . . . 125
4.4. Mechanical stress-induced heat shock protein induction . . . 125
4.4.1. Acute hypertension . . . 126
4.4.2. Chronic hypertension . . . 126
4.5. The dual role of the transcription factor NF-κB . . . 127

5. Perspectives . . . 128

Acknowledgements . . . 129

References . . . 130

1. Introduction

Organic tissues and organisms are subjected to a continuous turnover. It is a dynamic process of remodelling which leads to changes in shape and composition during embryo- and organogenesis, but also in the course of life as a result of an intrinsic program or in response to external stimuli. Accordingly, cells proliferate, divide, differentiate, degenerate, and are eventually renewed again. The concept of tissue remodelling is very old. In his work on morphology and metamorphosis of plants and animals, the German poet and scientist J. W. v. Goethe points out that whenever one wants to study living organisms, one has to consider that things in nature are constantly changing and that there are continuous processes of remodelling and tissue turnover going on (Goethe, 1817).

Remodelling processes are highly regulated and depend on the communication, or (in scientific terms) signal transduction, between the organic structure and its environment. The term 'signal transduction' refers to the relaying of a signal by conversion from one physical or chemical form to another. In this review, we will focus on the way by which mechanical stress, which is an extracellular physical signal, is converted into a cellular response leading to apoptosis. We will try to fit the destiny of single cells into an organic context which should lead us to a better

understanding of the complex molecular interplay occurring in mechanical stress-induced tissue remodelling.

The equilibrium between proliferation and degeneration crucial for the decision between health and disease. Only very recently, it has been recognized (at least at a molecular level) that cell death is not just an incidental part of life but rather a highly controlled and complex element of existence. Death is just as important as growth; one could not exist without the other. We will see later that controlled cell death is a physiological condition and that both lack and excess of cell death may cause disease. We will see furthermore that disturbances in the regulation of cell death often represent one of the first steps in the development of a disease, before other adaptive changes may take place.

We already mentioned briefly that this concept itself is not new. The new thing, however, is that we are getting more and more insights into the molecular mechanisms by which programmed cell death is carried out and controlled. During the last years, we have accumulated a lot of knowledge about cytokine-mediated cell death acting via specific cell death surface receptors; we know that check points within the cell can initiate signalling pathways leading to death when the cell's genomic integrity has been affected; we start to get information about the molecular regulation of cell death during development. Herein, we will focus on mechanical stress, which is able to induce both cell proliferation and cell death. Mechanical stress plays a crucial role in organ development, in functional maintenance of organic structures throughout life, but also in the pathogenesis of various diseases, and is one of the most important factors in the regulation of cell death. We will mainly consider the cardiovascular system, given the importance of mechanical stress-induced apoptosis in the development of cardiovascular disease.

2. Mechanical stress

In the cardiovascular system, blood vessels and organs are dynamically subjected to mechanical forces in the form of stretch and shear stress resulting from the pressure of the blood inside against the dynamic vascular wall (and adjacent structures). The major determinant of stretch stress is blood pressure. In comparison, shear stress results from blood flow, more precisely, from the friction of blood against the vessel wall. Whereas stretch stress exerts its effects in a radial and tangential manner affecting all cell types in the vessel wall, shear stress acts in parallel to the vessel wall and is therefore mainly sensed by endothelial cells that are located on the vessel surface. In the cardiovascular system, acute changes in the level of mechanical stress result in immediate changes in vessel diameter, mediated through release of vasoactive substances or change in myogenic tone. Longer lasting, chronically altered mechanical forces usually lead to adaptive alterations of the vessel or the organ wall, shape and tissue composition (Xu, 2000a).

2.1. Stretch

Embryonic heart cells are subjected to cyclic strain as the developing heart circulates blood to the embryo. It is supposed that cyclic strain may have an important regulatory role in the formation of the adult structure in organ development. In an experimental model, cyclic strain

induced proliferation of cultured embryonic heart cells without changing the viability of the cells (Miller et al., 2000).

In a model of cultured rabbit aorta, abnormally low intraluminal pressure (10 mm Hg) resulted in decreased contents of smooth muscle marker proteins h-caldesmon and filamin. This was not the case in aortic segments kept at physiological intraluminal pressure (80 mm Hg) (Birukov et al., 1998). Previous experiments showed that cyclic stretching of cultured vascular smooth muscle cells (SMC) not only increased the total expression of smooth muscle myosin heavy chains and myosin light chain kinases (Smith et al., 1995) but augmented also the smooth muscle specific myosin heavy chain SM-1 and SM-2 protein content and decreased the non-muscle myosin-A compared with SMC which were not subjected to stretch stress (Reusch et al., 1996). These experiments show that a certain level of mechanical stretch is necessary to develop and maintain a differentiated and contractile phenotype (Lehoux and Tedgui, 1998).

Overstretching, however, leads to hypertrophy, hyperplasia and cell migration which have been considered to be key events in the development of atherosclerosis, including angioplasty-induced restenosis, venous bypass graft arteriosclerosis, and spontaneous atherosclerosis (Li and Xu, 2000; Zou et al., 1998b). Mechanosensors include platelet-activated growth factor (PDGF) receptors, integrin receptors, ion channels, G protein-coupled receptors (such as receptors for angiotensin II or endothelin), or G proteins themselves (Cattaruzza et al., 2000; Hu et al., 1998, 1999; Leri et al., 1998; Shattil and Ginsberg, 1997). After transduction of the stretch signal, protein kinase C and mitogen activated protein kinases (MAPKs) are activated, leading to increased c-fos and c-jun gene expression and enhanced transcription factor AP-1 DNA-binding activity (Li and Xu, 2000). At the same time, overstretching induces signalling pathways leading to growth inhibition and apoptosis (Cheng et al., 1995; Leri et al., 1998; Mayr et al., 2000). These signalling pathways will be discussed in more detail later.

2.2. Shear

As outlined above, shear stress is mainly sensed by the superficial cell layer of a vessel, formed by endothelial cells. Fluid shear stress seems to represent an essential survival signal for endothelial cells and potently inhibits endothelial cell apoptosis via phosphorylation of the serine/threonine kinase Akt (Dimmeler et al., 1998), which subsequently mediates the activation of endothelial nitric oxide synthase (eNOS) leading to increased nitric oxide (NO) production (Dimmeler et al., 1999b). Another anti-apoptotic mechanism occurs via upregulation of integrin transcription and protein expression leading to enhanced endothelial cell adhesion. Shear stress time-dependently increased the mRNA expression and the protein levels of the fibronectin receptor subunits α_5 and β_1 in endothelial cells. Despite the fact that NO itself induces upregulation of integrins, inhibition of eNOS did not reduce the shear stress-mediated upregulation of integrins, which indicates that there has to be a second NO-independent pathway by means of which shear stress upregulates integrin expression (Urbich et al., 2000).

Shear stress has been shown to modulate endothelial cell structure and function including the organization of F-actin microfilaments. Specific integrins and other focal adhesion-associated proteins such as vinculin and talin appear to play a major role in the modulation of these cytosceletal and morphological changes (Girard and Nerem, 1995), and as transducers of mechanical stress into chemical signals (Shyy and Chien, 1997). Focal adhesion kinase

protein-tyrosine kinase has been shown to be important in linking integrin receptors to intracellular signalling pathways. The association with different signalling proteins, such as Src-family protein-tyrosine kinases, $p130^{Cas}$, Shc, Grb2, phosphoinoside 3-OH kinase (PI3K), and paxillin, enables focal adhesion kinase to function within a network of integrin-stimulated signalling pathways leading to the transient activation of targets such as the extracellular signal regulated kinases (ERK) and c-Jun NH_2-terminal kinase (JNK)/mitogen-activated protein kinase pathways (Schlaepfer et al., 1999). Transcription and expression of a variety of genes encoding for growth factors (PDGF, transforming growth factor, vasodilatators (NO, prostacyclin), vasoconstrictors (endothelin), and adhesion molecules (intracellular adhesion molecule) appear to be regulated on shear stress stimulation (Lehoux and Tedgui, 1998). Interestingly, integrin receptors can initiate intracellular signals that synergize with those from growth factor receptor protein tyrosine kinases in modulating cell growth (Schlaepfer et al., 1999). Crosstalk between integrins and growth factor receptors seems to be a common and important phenomenon providing specificity during normal development and pathological processes in vascular biology (Eliceiri, 2001).

Taken together, shear stress seems to be essential to guarantee endothelial cell survival and function and to maintain tissue homeostasis. Alterations of haemodynamic forces, however, have significant impact on the development of cardiovascular disease such as atherosclerosis.

3. Apoptosis

This short overview of the effects of biomechanical force in the cardiovascular system shows that physical force is necessary and essential in order to develop organic shape and function and to maintain this functional phenotype throughout life. Stress initiates signalling pathways leading to proliferation or differentiation, or, to cell death. Since organs usually respond to mechanical stress in an adaptive way and strengthen their structure by increasing cell number, size and composition, the role of cell death does not immediately seem obvious. However, in a concerted remodelling process, it is the right balance between death and renewal that decides between health and disease. Programmed cell death or apoptosis is the *conditio sine qua non* for any change in tissue composition or renewal. In the following section, we will take a brief look at the role of apoptosis in organic development and throughout life, to see that alterations of signalling pathways leading to apoptosis often precede a developing disease. We will discuss the molecular principles of apoptosis, as known so far, since they provide the basis for a better understanding of the pathomechanisms and open possibilities for therapeutical concepts.

3.1. Molecular mechanisms of apoptosis

The first observations of cell death during animal development were made soon after the discovery that all living organisms are made of cells (Vogt, 1842). By eliminating unwanted cells, structures are sculpted, unneeded structures are deleted, cell homeostasis can be controlled, and abnormal, misplaced, non-functional, or harmful cells are eliminated (Jacobson et al., 1997). The ability to selectively induce cell death by activation of an intrinsic program seems to be uniformly present in metazoans.

The term *apoptosis* describes a morphologically distinct form of programmed cell death that plays a major role during development, homeostasis, and in many diseases including cancer, neurodegenerative disorders and, as it has now been recognized, in a number of common and threatening vascular diseases, including atherosclerosis (Kerr et al., 1972; Mallat and Tedgui, 2000; Steller, 1995). Apoptotic cells usually undergo a characteristic morphological change, in which the cell and the nucleus shrink and condense and frequently fragment into membrane-bound apoptotic bodies that are rapidly phagocytosed and digested by macrophages or by neighbouring cells. The expression of membrane-associated markers on the dying cell allows some phagocytes to recognize and selectively remove apoptotic cells. They are so rapidly phagocytosed that there is no leakage of cytosolic components and no inflammatory response. By contrast, cells that die accidentally—as a result of acute injury—usually swell and lyse thereby releasing cytoplasmic material which often triggers an inflammatory response—a process called *necrosis*.

The terminal apoptotic programme of most mammalian cells depends on the activation of a family of cysteine proteases known as caspases and their modification of protein substrates within the nucleus and cytoplasm. The caspases can broadly be divided into two groups: initiator caspases (such as caspase-8 and caspase-9) whose main function is to activate downstream caspases, and executor caspases (such as caspase-3, -6 and -7) responsible for the activation or inactivation of cellular target proteins. Several important caspase substrates have been identified in the last years, such as nucleases responsible for the formation of the nucleosomal ladder (Wyllie, 1980). As known so far, two distinct processes lie upstream of these effector events (Fig. 1): The first is the so called 'death receptor pathway', triggered by the members of the death receptor superfamily (such as Fas (CD95) and tumour necrosis factor receptor I, which activates ultimately caspase-8. The second originates from the mitochondria, which represent central targets for oxidative stress. Targeted mitochondria release a set of molecules such as cytochrome *c* and apoptosis-inducing factor. Cytochrome *c* binds to the adaptor Apaf-1 to form together with procaspase-9 a molecular cluster called apoptosome, which is then responsible for the activation of caspase-9. The mitochondrial pathway can be regulated by both pro-apoptotic and anti-apoptotic members of the Bcl-2 family which are in turn modified, in response to local survival factors, by PI3K and Akt (Green and Reed, 1998; Hengartner, 2000; Rich et al., 2000).

Apart from DNA damage, apoptosis-inducing stress signals resulting from outside the nucleus include MAPK cascades and protein kinase C (PKC). Important targets of activated MAPKs are transcription factors: ERK activation tends to favour survival whereas JNK and p-38 MAPK activation seems to contribute to growth arrest and cell death. Another transcription factor, the redox-sensitive transcription factor NF-κB, translocates to the nucleus after its activation by reactive oxygen species (ROS). NF-κB rather seems to protect cells from apoptosis. Several pathways seem to be able to stabilize p53, a well-investigated checkpoint in the decision between survival and apoptosis. Stabilized p53 might lead to an increase in expression levels of several membrane death receptors such as Fas (Bennett et al., 1998). Cell cycle regulators and other proteins are induced by p53, including p21, GADD45, and the proapoptotic bcl-2 family member Bax. Stabilized p53 increases the level of Bax while diminishing the concentration of Bcl-2. Another important p53-regulated protein is MDM2. Representing a negative feedback loop by targeting p53 for proteosomal degradation, MDM2 ensures that the p53 signal is transient and carefully controlled.

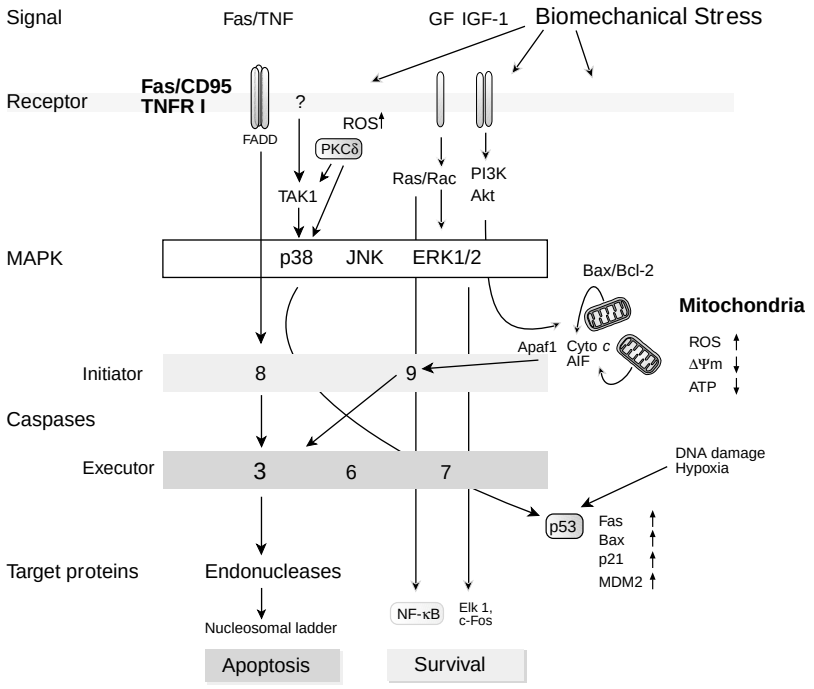

Fig. 1. Summary of the main pathways leading to apoptosis. The death receptor pathway is initiated by members of the death receptor superfamily, such as Fas/CD95 or tumour necrosis factor receptor I (TNFR I). Binding of the ligand to its receptor induces receptor clustering and the formation of a death-inducing signalling complex. This complex recruits via the adaptor molecule Fas-associated death domain protein (FADD) several procaspase-8 molecules resulting ultimately in the activation of caspase-8. Multiple signals such as Bax, oxidative stress, Ca^{2+} overload, or—indirectly—DNA damage, trigger mitochondria to release caspase-activating proteins, among which are cytochrome *c* and apoptosis inducing factor (AIF). Cytochrome *c* binds to the adaptor molecule Apaf-1 and stimulates its association with procaspase-9 to form a molecular complex called apoptosome. In turn, the apoptosome triggers an activating conformational change of caspase-9. The two pathways are regulated and linked by proapoptotic (e.g. Bax, Bid) and antiapoptotic (e.g. Bcl-X_L, Bcl-2) members of the Bcl-2 protein family. Both pathways converge at the level of caspase-3, which activates or inactivates cellular target proteins, such as endonucleases. The latter are responsible for the formation of the so-called nucleosomal ladder, which is one of the key features of apoptotic cell death. Apart from these two pathways, MAPK are able to mediate apoptosis, via the activation of several transcription factors or p53. p53 processes a variety of different signals, such as DNA damage, hypoxia, and probably mechanical stress. p53 is a transcription factor and induces multiple genes favouring growth arrest or apoptosis. It also induces the expression of its own inhibitor, MDM2. Multiple survival and protective signals interact with apoptosis pathways at different levels to modulate and regulate the cellular response.

3.2. Detection of apoptosis

Originally, apoptosis was described due to morphological changes detected by light and electron microscopy (Kerr et al., 1972). Since then, techniques to detect apoptosis have been extended to biochemistry, immunology and molecular biology in parallel to the expanding knowledge about the molecular mechanisms of apoptosis. These methods are mainly based on DNA fragmentation (TdT-mediated X-dUTP nick end labelling (TUNEL) or in situ nick end labelling), or cytoplasmic (caspase activity, calcium flux or mitochondrial dysfunction) and

membrane changes (membrane permeability or expression of lipid phosphatidylserine (van Heerde et al., 2000).

A variety of fluorometric and colorometric substrates are currently available to detect the activity of caspases (Gurtu et al., 1997). Other techniques to detect caspase activity include immunohistochemistry or detection of cleaved substates of caspases using specific antibodies. A very early but decisive event in apoptosis is the decrease in mitochondrial membrane potential ($\Delta\Psi m$). Collapse of the mitochondrial membrane potential can be detected by the diminished ability of various fluorescent dyes to accumulate in the mitochondria. The carbocyanine dye, JC-1, has been found to be a reliable and sensitive fluorescent probe to assess changes in the mitochondrial membrane potential (Di Lisa et al., 1995; Mathur et al., 2000; Reers et al., 1991). Furthermore, the release of several proapoptotic mitochondrial proteins, such as apoptosis inducing factor, cytochrome *c*, and procaspases 2, 3 and 9, from the intermembrane space into the cytosol can be measured using specific antibodies (van Heerde et al., 2000).

One of the key features of apoptosis is the presence of a nucleosomal DNA ladder, which has been used as a marker for apoptotic cell death. Caspase-activated endonucleases cut genomic DNA between nucleosomes, to generate fragments of approximately 180 base pairs, which appear as a DNA ladder on agarose gels (Itoh et al., 1995). Whereas detection of a DNA ladder does not pinpoint where apoptosis occurred, DNA strand break labelling techniques provide information on the localization of apoptosis in situ at a single-cell level, while preserving tissue architecture. The two commonly used techniques are the so-called TUNEL method and the in situ nick end labelling method. The TUNEL method uses the enzyme terminal deoxynucleotidyl transferase (TdT) to add biotinylated, BrdU or digoxygenin-labelled nucleotides to DNA strand breaks, whereas the in situ nick end labelling method employs a DNA polymerase (Gavrieli et al., 1992; Gorczyca et al., 1993). Variations in the DNA content can be assessed by flow cytometry and DNA probes like propidium iodide. Apoptotic cells have a decreased DNA content and show a characteristic sub-G1 peak. Due to the differences in the permeability of the cell membranes of live, dead and apoptotic cells, the combination of two DNA dyes—propidium iodide and Hoechst 3342—allows distinguishing between necrotic and apoptotic cells. Necrotic cells are permeable to both dyes, whereas apoptotic cells are able to exclude propidium iodide but not Hoechst (Darzynkiewicz et al., 1992).

Apoptotic cells expose the lipid phosphatidylserine, normally localized to the inner leaflet of the plasma membrane, on the outer-membrane leaflet. This selectively allows the uptake of apoptotic cells by some types of phagocytes (Fadok et al., 1992, 2000). By using FITC-labelled Annexin V, which binds to phosphatidylserine in a calcium-dependent manner, and flow cytometry, it is possible to detect apoptotic cells in the early execution phase of apoptosis (Andree et al., 1990; Vermes et al., 1995). By combining Annexin-FITC with propidium iodide, a similar profile to the combination of Hoechst and propidium iodide can be obtained. Living cells are negative for both dyes, necrotic cells are positive for both, while apoptotic cells are positive only for Annexin-FITC.

4. Pathophysiological mechanisms of apoptosis

A variety of signals are able to trigger apoptosis. Among the chemical ones are proteins from the tumour necrosis factor cytokine family (such as Fas ligand, TNFα), oxygen radicals (including

NO), calcium, angiotensin II, but also growth factor withdrawal or loss of extracellular matrix attachment. Physical signals that may induce apoptosis are for example radiation and biomechanical stress. We will focus on various pathological conditions of the cardiovascular system, in which mechanical stress-initiated apoptosis plays a pivotal pathogenetic role.

4.1. Heart failure

Apoptosis is usually associated with tissues where cells progress through the cell cycle. Therefore, the general belief was that in terminally differentiated adult cells, such as cardiac myocytes and neurons, no apoptosis could occur. Recent studies, however, have shown apoptosis in the human heart in various cardiac diseases, including myocardial infarction, cardiomyopathy, associated with heart failure, and arrhythmias (Feuerstein and Young, 2000; Mallat et al., 1996; Narula et al., 1996, 1999).

A variety of pathophysiological conditions, such as myocardial infarction, systemic hypertension, valvular diseases, viral myocarditis, dilated cardiomyopathy, and pulmonary embolism may lead to an increase in cardiac workload and thus in elevated mechanical stress on cardiomyocytes. Pressure overload induces compensatory hypertrophy of myocytes (increase in the size of myocytes and the expression of contractile and other proteins normally expressed only during foetal development), proliferation of non-muscle cells but also loss of cardiac muscle cells (Sadoshima and Izumo, 1997). As haemodynamic overload persists, the stressed heart may enter a critical transition from compensatory hypertrophy to decompensated heart failure. The pathogenetic mechanism for this transition leading to heart failure is not well understood. It has been suggested that apoptosis of myocytes may lead to progressive failure in myocardial function, culminating in chronic cardiomyopathy and end-stage heart failure (Bing, 1994).

4.1.1. Role of Bcl-2 protein family and p53

Very early in the development of cardiac failure, in a rat animal model of aortic stenosis, an increase in apoptotic cardiomyocytes could be observed during the first 7 days after operation with a peak at day 4. In contrast, cardiac growth continued for over 30 days (Teiger et al., 1996). However, comparing hearts with left ventricular hypertrophy and left ventricular dysfunction in a rat model of chronic pressure overload, apoptosis increased with the progression of cardiac disease probably due to higher levels of the pro-apoptotic protein Bax and a reduced ratio of the anti-apoptotic protein Bcl-2/Bax (Condorelli et al., 1999).

Significantly increased apoptosis was found in the myocardium of patients with end-stage dilated cardiomyopathy which occurred—in contrast to the data from the animal model—in spite of the enhanced expression of the antiapoptotic protein Bcl-2 (Narula et al., 1996; Olivetti et al., 1997). Another study demonstrated that mitochondrial cytochrome *c*-dependent activation of caspase-3, occurred only in patients with endstage cardiomyopathy but not in control hearts (Narula et al., 1999). This strengthened the hypothesis that cardiomyocyte loss is due to cell death by apoptosis and might play a crucial role in the development of cardial decompensation. Further confirmation came from a study focusing on the critical transition period by comparing hearts from failing and non-failing spontaneously hypertensive rats. TUNEL positive (apoptotic) cells were significantly increased in myocardial cross-sections of rats with heart failure compared to the non-failing hearts, which showed only low levels of apoptosis. Interestingly, treatment of the

failing hearts with the angiotensin-converting enzyme inhibitor captopril, which ameliorated heart failure in this model, reduced the number of apoptotic cells to the level of non-failure hearts (Li et al., 1997b). This is consistent with the observation that stretch-mediated release of Angiotensin (AT) II triggered myocyte apoptosis in vitro by activating p53 (Fig. 2). Apoptosis and p53

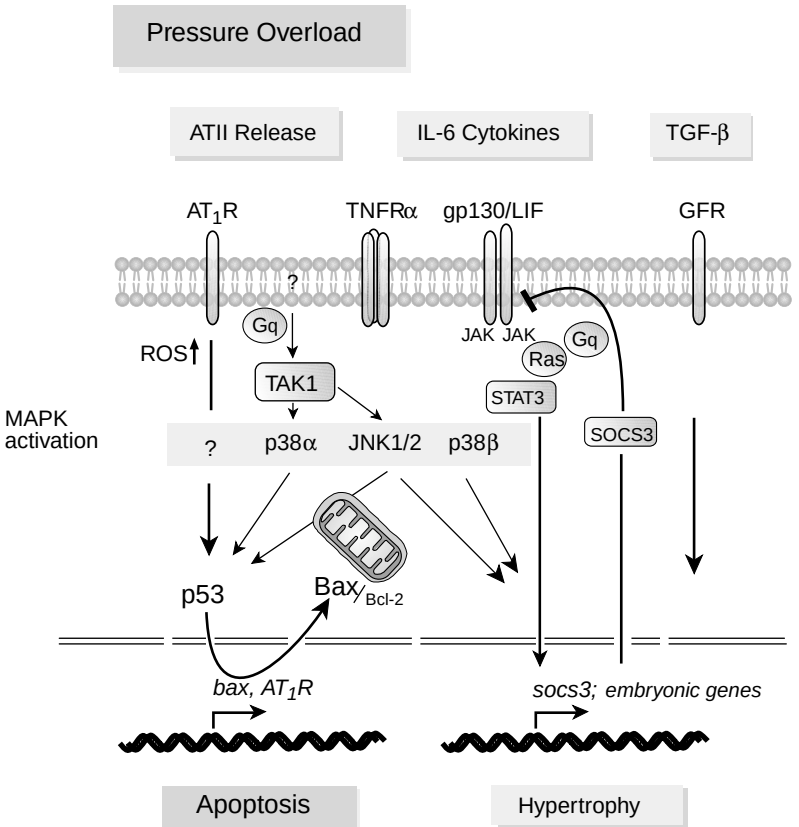

Fig. 2. Molecular signalling pathways leading to mechanical stress-induced apoptosis of myocytes in heart failure. Pressure overload of the heart induces Angiotensin II (ATII) release, which stabilizes and activates p53 via Angiotensin receptor 1 (AT_1R). Activated p53 translocates to the nucleus and stimulates the expression of multiple genes, such as *bax*, *angiotensinogen*, and AT_1R, ultimately leading to apoptosis. Additionally, activated AT_1R induce the generation of ROS, which contribute to mechanical stress-induced cardiac remodelling and apoptosis. Two different isoforms of the MAPK p38 show different functions in response to mechanical stress. p38α induces apoptosis via the activation of p53, whereas p38β results in hypertrophic changes. JNK seems to be able to signal in either direction depending on the other factors involved. Co-activation of JNK and p38 triggers apoptosis, whereas activation of JNK alone leads to hypertrophic changes. The MAPK kinase TAK1 is an important upstream regulator of p38. G proteins transduce signals from a variety of transmembrane receptors. The subunit Gqα was found to mediate mechanical stress-induced apoptosis via the activation of p38 and JNK. Another apoptosis pathway in heart failure employs tumour necrosis factor receptor-α (TNFα pathway not shown). High levels of TNFα are present in conditions of heart failure. The absence of survival signals also favours apoptosis in heart failure. Ligands of the gp130 receptor, the IL-6 cytokines cardiotrophin and LIF, are induced upon elevated mechanical stress in the heart. Via JAK and STAT3, gp130 mediates the transcription of several genes favouring cell survival as well as transcription of its own inhibitor, SOCS3. Overexpression of SOCS3 might contribute to the pathogenesis of decompensated heart failure.

activation could be abolished by the AT_1 receptor antagonist, Losartan (Leri et al., 1998). ATII stimulation enhanced p53 binding to the promoters of Angiotensinogen, AT_1 receptor and bax genes. The subsequent decrease of the Bcl-2-to-Bax protein ratio might be responsible for the increased susceptibility of stretched myocytes to undergo apoptosis (Leri et al., 1998). Introduction of a dominant-negative p53 mutant in adult ventricular myocytes interfered with both stretch induced ATII formation and apoptosis, indicating that p53 is a major modulator of myocyte renin-angiotensin system and cell survival after mechanical stretching (Leri et al., 2000a).

4.1.2. MAPK signalling

At least in part, activated Angiotensin receptors trigger the production of ROS in stretched myocytes. The increase in myocardial oxidative stress in animal models of haemodynamic overload may contribute to myocardial remodelling (Dhalla et al., 1996; Dhalla and Singal, 1994). In vitro, tonic mechanical stretch of rat papillary muscle increased the production of ROS, which seem to be involved in the induction of myocyte apoptosis in this model (Cheng et al., 1995). Interestingly, a small increase in myocyte oxidative stress caused by partial inhibition of CuZn-superoxide dismutase (SOD) resulted in myocyte hypertrophy, whereas a higher level of oxidative stress due to more complete inhibition of SOD caused apoptosis (Siwik et al., 1999). According to that, increasing levels of cyclic strain caused an amplitude-dependent increase in ROS in cardiac myocytes in vitro together with an amplitude-dependent phenotype shift, from hypertrophy at low levels of stretch, to apoptosis at high levels of mechanical stretch. In this model, mechanical stress activated in a ROS-dependent manner ERK1/2 at both low and high amplitude, whereas JNK was activated only at high-amplitude stretch (Pimentel et al., 2001).

It seems to be the case that myocytes undergoing pressure-induced hypertrophic changes become more susceptible to apoptosis. Biomechanical stress activates multiple parallel and converging signals for hypertrophy and apoptosis. Pressure overload in mouse hearts increased significantly p38 MAPK activities, in coincidence with the onset of ventricular hypertrophy. Co-infection of cardiomyocytes with a p38 upstream activator (MKK3b) and two different isoforms of p38 MAPK (β and α, respectively) revealed divergent functions for different members of the p38 MAPK family (Fig. 2): Forced induction of the p38β activity resulted in characteristic features of hypertrophy, whereas the induction of p38α activity led to myocyte apoptosis suggesting a crucial role of the p38 MAPK pathway in the onset of pressure-induced cardial hypertrophy and the transition to heart failure (Wang et al., 1998b). Furthermore co-activation of both, JNK and p38 MAPK, in cardiomyocytes provoked cytopathic responses and suppression of hypertrophic changes. In contrast, specific activation of the JNK pathway induced characteristic features of hypertrophy (Wang et al., 1998c). In a very interesting study, Zhang et al. identified an upstream regulator of p38 MAPK activity, the transforming-growth-factor-β-activated kinase (TAK1) (Zhang et al., 2000). TAK1 kinase activity was upregulated 7 days after aortic banding in adult mouse myocardium. The mechanical load was sufficient to induce hypertrophy and expression of transforming growth factor β in the model. An activating mutation of TAK1 expressed in the myocardium of transgenic mice led to p38 MAPK phosphorylation, cardiac hypertrophy, interstitial fibrosis, severe myocardial dysfunction, induction of 'foetal' genes, apoptosis and early lethality indicating a crucial role of TAK1 in the delayed response to mechanical stress and the development of fulminant heart failure.

4.1.3. G proteins

Another group of proteins involved in the signal transduction of mechanical stress in heart failure are G proteins of the Gq family. They transduce signals from a variety of membrane receptors to generate diverse, tissue-specific effects, such as contraction, secretion or regulation of cell proliferation (Post and Brown, 1996). Overexpression of the α subunit of Gq in cultured cardiac myocytes resulted in hypertrophic growth. Interestingly, expression of a constitutively activated mutant of Gαq, which further increased Gq signalling, led initially to hypertrophy, which rapidly progressed to cardiomyocyte death by apoptosis. This was consistent with the sequence of Gαq stimulated hypertrophy, apoptosis, and heart failure in Gαq overexpressing mice in which the transition from hypertrophy to apoptosis correlated with the level of Gαq expression. Progression from hypertrophy to apoptosis in vitro and in vivo was coincident with activation of p38 and Jun kinases (Adams et al., 1998).

Other reports demonstrated the hypertrophic (Yokoyama et al., 1997) and proapoptotic effect of tumour necrosis factor-α, high levels of which are present in advanced heart failure in rodent cardiomyocytes (Bozkurt et al., 1998; Kubota et al., 1997). High levels of tumour necrosis factor-α are also present in patients with advanced heart failure.

4.1.4. Lack of survival signals

Cytokines seem to play an important role as modulators in this critical transition between cardiac hypertrophy and heart failure in response to biomechanical stress. In particular, the common receptor of the IL-6 family of cytokines, gp130, has been shown to play an important role in the pathogenesis of cardiac failure (Hirota et al., 1999). The gp130 cytokines, such as cardiotrophin-1 and leukaemia inhibitory factor, may induce cardiomyocyte hypertrophy and also serve as myocyte survival factors. Both factors are induced by biomechanical stress in the heart (Sheng et al., 1997; Wollert et al., 1996). Additionally, mice that harbour a ventricular-restricted knockout of gp130 show a rapid-onset dilated cardiomyopathy and massive myocyte apoptosis in response to biomechanical stress associated with transverse aortic constriction (Hirota et al., 1999). Although the function of gp130 in the physiological response of the heart is not known, gp130 pathways are rapidly activated in response to the mechanical stress of pressure overload and seem to mediate a myocyte survival pathway that acts to block the onset of myocyte apoptosis during pressure overload (Fig. 2).

Biomechanical stress induces induction of the gp130 ligands, such as LIF, which bind to the receptor and trigger receptor dimerization and activation of the *janus* kinase (JAK). In turn, JAK rapidly phosphorylates tyrosine residues of these receptors and subsequently recruits various signalling molecules to the receptor complex such as Ras, PI3K or signal transducer and activator of transcription 3 (STAT3). Activated STAT3 dimers translocate to the nucleus to induce transcriptional activation of downstream target genes, including the intrinsic inhibitor of the JAK-mediated gp130 cascade, suppressor of cytokine signalling 3 (SOCS3). Adenovirus-mediated gene transfer of SOCS3 to ventricular cardiomyocytes completely suppressed both LIF-induced hypertrophy and antiapoptotic phenotypes by blocking three independent signalling survival pathways which are co-induced by LIF and which lie downstream of JAK: (1) STAT3, (2) MEK1-ERK1/2, and (3) Akt (Yasukawa et al., 2001). SOCS3 function initially is to control and limit the compensatory hypertrophic changes in response to increased loading in the heart. Once

compensation is no longer possible, overexpressed SOCS3 might play an important role in the transition leading to decompensated heart failure.

4.2. Hypertension

We have discussed previously that a certain amount of mechanical stress is essential to develop and to maintain differentiated and functional organic structures.

Due to the haemodynamic changes at birth, closure and regression of the human umbilical vessels and the ductus arteriosus take place. Lack of arterial blood flow as well as contraction and constriction of SMC due to the exposure to oxygen and endothelin-1 provoke the morphological changes during which apoptosis of SMC plays a crucial role. Here, lack of strain induces apoptosis by upregulation of proapoptotic proteins such as Bax and Bcl-xS (Kim et al., 2000; Slomp et al., 1997).

In contrast, remodelling processes of large and small arteries due to pathologically elevated blood pressure contribute to the development and complications of hypertensive disease.

Hypertension is the single most important risk factor in both coronary heart disease and cerebrovascular accidents; it may also lead directly to congestive heart failure, renal failure and aortic dissection. In most cases, hypertension is primary (idiopathic). If secondary, hypertension is mostly related to renal disease or (less often) to renal artery stenosis (renovascular), endocrine abnormalities, vascular malformations, or neurogenic disorders. Blood pressure is determined by the complex interaction of multiple factors, including genetic, environmental and psychogenic factors that regulate the relationship between cardiac output and total arteriolar resistance. The major site of generation of vascular resistance are so called 'resistance arteries', which are vessels with lumen diameters measuring less than 400 μm when relaxed, including small arteries and arterioles (Intengan and Schiffrin, 2000). The fundamental cause of increased peripheral resistance is a decrease in lumen diameter: According to Hagen–Poiseuille's law, resistance varies inversely with the fourth power of the radius, thus a small decrease in the lumen markedly increases resistance (Box 1). The major haemodynamic abnormality underlying elevated blood pressure is an increase in vascular resistance. Disappearance of microvessels (microvascular rarefaction) during hypertension constitutes a process that exacerbates the hypertensive condition (Gobe et al., 1997; Price and Skalak, 1994). However, it is not known whether abnormalities in microvessels are a cause or consequence of high blood pressure (Noon et al., 1997).

Box 1
Hagen–Poiseuille law

The laminar flow of a fluid or a gas through a pipe is described by the *Hagen–Poiseuille law*, stating that the flow rate (V) flowing per unit time (t) is proportional to the pressure difference ($p_1 - p_2$) between the ends of the pipe (l) and the *fourth* power of its radius (r):

$$V = \frac{\pi \times r^4}{8 \times \eta \times l} \times (p_1 - p_2) \times t$$

V, volume of the fluid; t, time; r, radius of the pipe; p, pressure; η, coefficient of viscosity; l, length of the pipe.

4.2.1. Remodelling

Two kinds of structural changes in resistance arteries can be distinguished: Inward eutrophic remodelling, in which the outer and lumen diameters are reduced, and the media cross-sectional area remains unaltered; and hypertrophic remodelling, in which the media thickens to encroach on the lumen, resulting in increased media cross-sectional area and media/lumen ratio. Eutrophic remodelling is associated with conditions of mild hypertension or animal models in which the renin-angiotensin system may play a pathogenetic role (spontaneously hypertensive rats), whereas hypertrophic changes predominate in severe hypertension of animal models in which the endothelin system is activated. The common outcome is luminal narrowing of small arteries and arterioles, thereby increasing vascular resistance. Possibly, initial eutrophic remodelling may evolve towards hypertrophic remodelling, as hypertension progresses (Intengan and Schiffrin, 2000, 2001). The underlying mechanisms of these vascular structural changes are not well understood. However, mechanical stress seems to be an important signal stimulating pathways usually used by growth factors. In vitro, pulsatile stress stimulated growth factor receptor activation (PDGF receptor α) and the induction of multiple genes, including genes encoding for growth factors, in cultured vascular endothelial cells and SMC (Chien et al., 1998; Hu et al., 1998). Another important signalling molecule seems to be NO, since fluid shear stress elevated endothelial NOS in cultured endothelial cells (Ranjan et al., 1995). Apart from its role in controlling the vessel diameter (vasodilatation), NO inhibits SMC proliferation and migration, but also apoptosis probably by inhibition of ICE-like and cysteine protease protein (CPP)-32-like cysteine proteases (Dimmeler et al., 1997a,b, 1999c), and seems to have an overall protective role (Fig. 3).

In parallel with hypertrophic/hyperplastic changes (growth, inflammation, and fibrosis), increased apoptosis has been found in the heart, kidney, and brain of spontaneously hypertensive rats and mice as well as in cultured vascular SMC (Hamet et al., 1995). Apoptosis appears to start with a certain delay, but at the very beginning of the development of hypertensive disease. Apoptosis was significantly increased in mesenteric small resistance arteries of spontaneously hypertensive rats after 8 and 12 weeks of age, in parallel with the rise in systolic blood pressure and the increase in media/lumen ratio. At 4 weeks of age, no apoptosis was detectable, whereas the media/lumen ratio was already significantly greater in spontaneously hypertensive rats (Rizzoni et al., 2000).

4.2.2. Mechanotransduction

Among various candidates that modulate mechanical stress-induced apoptosis are ROS, NO, AT II, and the endothelin system (Intengan and Schiffrin, 2001). Oxygen radicals are involved in the pathogenesis of hypertensive disease and are able to induce apoptosis in SMC via a PKC-dependent mechanism (Li et al., 1997a, 1999b). The role of AT_1 and AT_2 receptor subtypes in the induction of apoptosis seems to vary in different cell types. In contrast to the findings of Leri et al. in postinfarcted hypertrophied myocytes (Leri et al., 1998, 2000b), ATII was shown to induce apoptosis via the AT_2 receptor in rat pheochromocytoma cell line and a mouse fibroblast cell line (which do not express AT_1 receptors) possibly through dephosphorylation of the survival factor MAPK (Fig. 3). MAPK phosphatase-1 (MKP-1) dephosphorylated Bcl-2 inhibiting its antiapoptotic affect (Horiuchi et al., 1997; Yamada et al., 1996). These observations are consistent with those made in an in vivo model: ATII infusion in normotensive rats raised blood

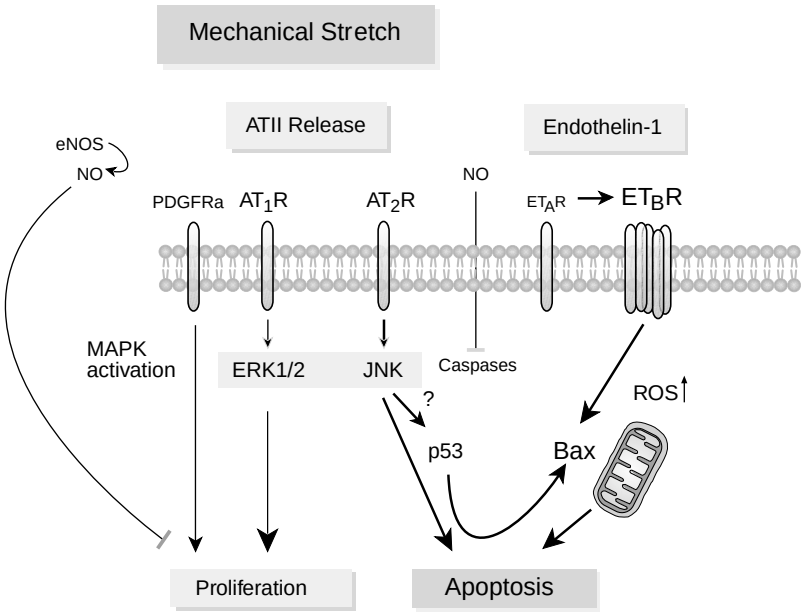

Fig. 3. Signal transduction pathways mediating mechanical stretch-induced apoptosis of vascular SMC in hypertension. Stimulation of growth factor receptors, such as PDGF receptor-α, triggers cell proliferation. Shear stress stimulates the endothelial production of NO, which inhibits SMC proliferation and migration, but also apoptosis probably by inhibition of caspases. Mechanical stretch induces the release of AngiotensinII (ATII). ATII receptor subtypes display divergent functions upon stimulation by ATII. AT_1 receptor favours proliferation via activation of ERK, while AT_2 receptors trigger apoptosis via JNK and probably p53. Mechanical stretch stimulates the synthesis of endothelin-1 (ET-1). Usually, the ET_A receptor subtype mediates SMC proliferation, whereas ET_B induces apoptosis. Upon elevated mechanical stress, an ET receptor subtype shift, from ET_A to ET_B, takes place resulting in increased apoptosis.

pressure and increased apoptotic rate in thoracic aortae by activation of both AT_1 and AT_2 receptor subtypes (Diep et al., 1999). AT_1 receptor blockade in spontaneously hypertensive rats lowered systolic blood pressure and increased SMC apoptosis, an effect, which could be attenuated by coadministration of an AT_2 receptor antagonist. However, AT_2 receptor antagonist given alone did not affect growth or apoptosis, which might be explained by the possibility that AT_2 receptors do not induce proapoptotic effects in SMC under basal conditions in spontaneously hypertensive rats. Furthermore, AT_2 receptors inhibited AT_1 receptor antagonist-triggered apoptosis but did not affect the antihypertensive effect of AT_1 antagonists, which shows that the causal relation between blood pressure and SMC apoptosis is complex and modulated by endocrine factors (Tea et al., 2000).

Circumferential stretch represents one of the most potent stimuli for the synthesis of endothelin-1 (ET-1), which is predominantly synthesized in endothelial cells and which is the most potent vasoconstrictor peptide known so far. Additionally, ET-1 is an important mitogen for SMC; an effect, which is mediated by the ET_A receptor subtype (Cattaruzza et al., 2001). Very interesting is the observation of a shift in the ET receptor subtype upon mechanical stress

exposure (Fig. 3). In rat aortic SMC, cyclic stretch increased the expression of ET_B receptor mRNA whereas ET_A receptor mRNA was reduced. Exogenous ET-1 induced apoptosis via ET_B receptor as apoptosis was completely suppressed by a ET_B receptor antagonist but not by a ET_A receptor antagonist (Cattaruzza et al., 2000).

4.3. Atherosclerosis

The hallmark of atherosclerotic disease are intimal fibrofatty plaques, the atherosclerotic lesions, which develop principally in large and medium-sized muscular and elastic arteries and are characterized by SMC proliferation, extracellular matrix formation and deposition, and lipid accumulation (Ross, 1993). Since lesions occur mainly in bifurcations and curved regions of the arteries where blood flow is disturbed, the impact of haemodynamic disturbances caused by blood flow are evident (Box 2). Interestingly, low shear stress levels were found in the locations of atherosclerotic plaques in the carotid artery (Ku et al., 1985). A recently presented computer model of 'How blood flows through human arteries' confirms that some curved or branching stretches of an artery are simultaneously exposed to low shear and high mechanical strain. And that is precisely where atherosclerotic lesions usually develop (Xu, 2000b).

These areas of unsteady and turbulent flow are characterized by higher endothelial cell turnover rates (Davies et al., 1986), suggesting increased endothelial cell apoptosis. In fact, it has been shown that laminar shear stress at physiological levels protects endothelial cells from apoptosis, whereas endothelial cells cultured under static conditions or after blood flow reduction in vivo undergo apoptosis (Cho et al., 1997; Dimmeler et al., 1996; Kaiser et al., 1997). As outlined above, the apoptosis-suppressive effect of shear stress occurs via activation of survival pathways, such as activation of Akt (Dimmeler et al., 1998), upregulation of Cu/Zn SOD and NOS (Dimmeler et al., 1999b), or upregulation of integrin expression (Urbich et al., 2000). In contrast, endothelial cell death in plaque regions may contribute not only to plaque formation but also to plaque disruption and thrombosis. It has been shown that apoptotic endothelail cells markedly increase their procoagulant activity and become proadhesive for non-stimulated platelets (Bombeli et al., 1997, 1999). A very interesting study examined carotid atherosclerotic plaques from patients undergoing en bloc carotid endarterectomy in the longitudinal axial direction, upstream and downstream from the stenosis. Endothelial cell apoptosis was significantly increased in the

Box 2
Laminar and turbulent flow

For sufficiently small velocities the flow of a fluid or gas will be *laminar* i.e. layered, without layer separation. As the velocity increases past a critical value, depending upon the viscosity and density of the fluid, vorticity appears and the flow becomes *turbulent*. The *Reynold's number*, a dimensionless parameter expresses the ratio of inertia forces to viscous forces in a flow field (A high Reynold's number implies that the flow is turbulent, while a low Reynold's number implies that the flow is laminar). Atherosclerotic lesions develop in areas of unsteady and turbulent flow:

$$R = \frac{\rho \times v \times r}{\eta}$$

R, Reynold's number; ρ, density of the fluid; v, velocity; r, radius; η, viscosity of the fluid.

downstream part of the plaque, where blood flow is low and disturbed. In contrary to the findings in luminal endothelial cells, apoptosis in the inside of the plaque was higher in the upstream part than in the downstream part, which could possibly be attributed to the high stretch stress in this area (Tricot et al., 2000).

4.3.1. Smooth muscle cell apoptosis

The endothelial injury or rather dysfunction resulting from multiple stresses (including biomechanical stress) induces compensatory responses that alter the normal homeostatic properties of the endothelium, which leads to increased endothelial adhesiveness and permeability with respect to leukocytes or platelets. Furthermore, the atherogenic stresses induce the endothelium to have procoagulant instead of anticoagulant properties and to produce vasoactive molecules, cytokines, and growth factors, which in turn stimulate the migration and proliferation of vascular SMC intermixed with an area of inflammation to form a so called intermediate lesion. Thickening of the arterial wall can initially be compensated by gradual dilatation (up to a point the lumen remains unaltered). As the process continues, further accumulation of mononuclear cells, migration and proliferation of SMC, and formation of fibrous tissue result in further enlargement and restructuring of the lesion, which becomes covered by a fibrous cap that overlies a core of lipid and necrotic tissue, the so termed advanced lesion. When the artery is no longer able to compensate by dilatation, the lesion may intrude into the lumen and alter the blood flow (Lusis, 2000; Ross, 1993, 1999).

Intimal SMC proliferation seems to play an important role in the development of atherosclerosis and has been subject to intensive research in the last decades (Schwartz et al., 1995). The mitogenic effect of mechanical strain on cultured SMC is well documented (Hu et al., 1998; Li et al., 1999a; Morawietz et al., 1999; Sudhir et al., 1993; Wilson et al., 1993; Xu et al., 1997a). In recent years, however, apoptosis of SMC has been implicated in both development and outcome of the disease (Geng and Libby, 1995; Han et al., 1995; Isner et al., 1995; Kockx, 1998; Newby and George, 1996). In human specimens from atherosclerotic lesions of coronary and carotid arteries, widespread apoptosis was found. Apoptotic cells were confined to the intima but were not detectable in the media. Both SMC and macrophages undergo apoptosis. Whereas SMC were found mainly in the fibrotic portion of the atheroma, macrophages clustered near or within the lipid-rich core of the lesion (Geng and Libby, 1995; Isner et al., 1995). Moss and Benitt (and later others) showed that SMC cultured from lesions had a shortened life span relative to normal medial cells (Dartsch et al., 1990; Moss and Benditt, 1975; Ross et al., 1984). Recently, it has been demonstrated that cultured SMC from human atherosclerotic plaques have higher susceptibility for apoptosis compared with SMC from normal vessels (Bennett et al., 1995). It is likely that specific alterations within the SMC itself elicit sensitivity to a particular stimulus that is associated with vascular disease (McCarthy and Bennett, 2000). In vivo, it is the lack of survival factors (such as cell-cell and cell-matrix interactions and growth factors) and the presence of proapoptotic factors (like oxidized low density lipoprotein, vasoactive substances, mediators of inflammation, and altered expression of apoptosis regulating genes), which ultimately leads to SMC death (Mayr and Xu, 2001). Increased JNK activation and p53 expression might be involved in the mediation of cell death in lesions (Metzler et al., 2000) (Fig. 4).

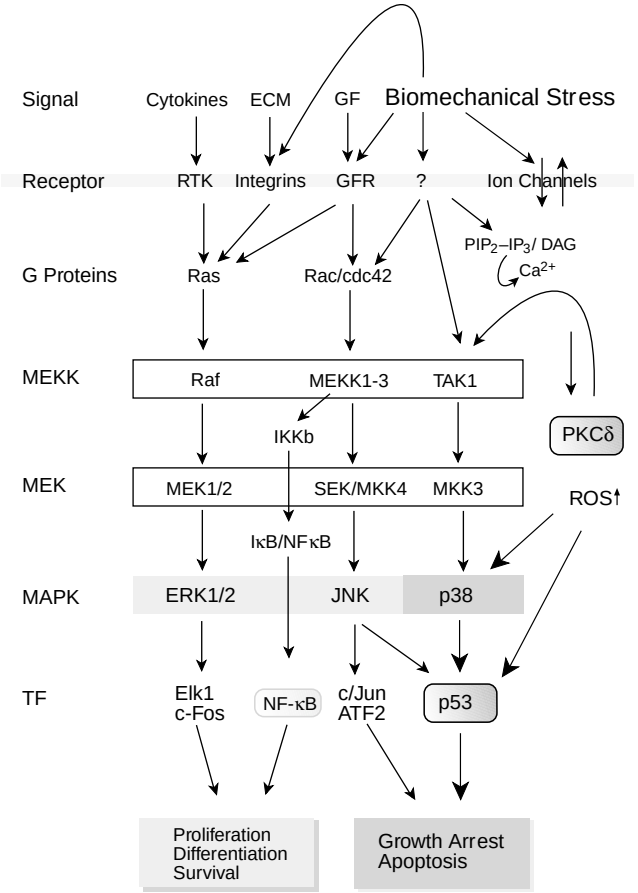

Fig. 4. Mechanical stress-induced molecular signal transduction in the pathogenesis of atherosclerosis. A variety of external signals, such as cytokines, extracellular matrix (ECM) composition, growth factors (GF), and biomechanical stress activate directly or indirectly MAPK. MAPK comprise a family of tyrosine/threonine kinases. Receptor activation initiates a cascade of phosphorylation events involving sequential activation of G proteins, MAPK kinase (MEKK), MAPK kinase (MEK) and finally MAPK. Activated MAPK, in turn, are responsible for the phosphorylation and activation of various other regulatory proteins and transcription factors, which induce the expression of genes involved in the regulation of cell proliferation and apoptosis. ERK kinases mediate cell survival and proliferation, whereas JNK and p38 tend to induce growth arrest and apoptosis. MAPK activation in response to mechanical stress plays a pivotal role in the development of atherosclerosis. In particular, activation of p38 and the presence of p53 and PKCδ appear to be essential in mechanical stress-mediated apoptosis in atherosclerosis. Furthermore, the NF-κB pathway, important for both prevention and pathology of atherosclerotic disease, is shown.

4.3.2. Restenosis

One of the main therapeutic approaches for patients who have coronary artery obstruction with myocardial ischemia is balloon angioplasty, which often leads to a temporary relief, followed by restenosis of the vessel, thus limiting the primary success. In several studies, clinical restenosis has been found to occur in approximately 36–40% of patients within 6 months (Levine et al., 1995). MAPK activation in response to mechanical injury seems to play a crucial role in mediating SMC proliferation and neointima formation. In a rat model, carotid balloon angioplasty rapidly

activated MAPK (ERK2 and JNK1), followed by increase in c-fos and c-jun gene expression and enhanced AP-1 DNA-binding activity (Hu et al., 1997).

Interestingly, several studies have shown in animal models that apoptosis of medial SMC is an initial event in response to vascular injury induced by mechanical angioplasty (Malik et al., 1998; Perlman et al., 1997). In rat carotid arteries, 30 min after mechanical injury, 70% of medial SMC were found to be apoptotic together with a marked decrease in Bcl-X expression in the most luminal layers of the media (Perlman et al., 1997). A study in a porcine model revealed that the profile of apoptotic cells after angioplastic treatment shows regional differences and is cell specific. After balloon injury, apoptosis within the media, adventitia, and neointima peaked at 18 h, 6 h, and 7 days, respectively. In contrast, proliferation peaked at 3 days and profiles of apoptosis and cell proliferation were discordant in all layers of the artery except the neointima (Malik et al., 1998). Sata et al. showed that mechanical stress-induced apoptosis appears to occur independently of the Fas pathway. In a vascular injury model, there was no difference in the amount of apoptotic cells among wild-type, *Fas ligand* –/–, and *Fas* –/– mice (Sata et al., 2001).

4.3.3. Vein graft arteriosclerosis

In coronary bypass surgery, a section of a vein (usually the saphenous) is used to form a connection between the aorta and the coronary artery distal to the obstructive lesion. Veins usually do not develop spontaneous arteriosclerosis. Accelerated arteriosclerosis, however, develops rapidly in venous bypass grafts (Dietrich et al., 2000), obviously due to the increase in biomechanical load that the vein has to bear when transferred to an arterial system. Alternatively, anastomosis of one of both of the internal mammary arteries to the coronary artery distal to the obstructive lesion may be employed and is now preferred whenever possible (Lytle and Loop, 2001). The main complication with vein grafts is atherosclerotic obstruction occurring on a foundation of neointimal hyperplasia. During the first postoperative year up to 15% of venous grafts occlude, between 1 and 6 years the graft attrition rate is 1–2% per year, and between 6 and 10 years it is 4% per year. By 10 years after surgery only 60% of vein grafts are patent and only 50% of patent vein grafts are free of significant stenosis. In addition, native coronary artery disease progresses in 5% of patients annually (Motwani and Topol, 1998). Long-term patency rates are considerably higher for internal mammary artery implantations.

A recently developed mouse model of venous bypass graft arteriosclerosis provides new insights in the pathogenesis of venous graft arteriosclerosis. In this model, autologous or isogeneic vessels of the external jugular or vena cava veins were end-to-end grafted into carotid arteries. Vessel wall thickening was observed as early as 1 week after surgery and progressed continuously up to 18-fold of the original thickness in grafted veins in the course of 16 weeks. Neointimal hyperplasia caused significant narrowing of the lumen. Interestingly, marked loss of SMC in vein segments 1 and 2 weeks after grafting, massive infiltration of mononuclear cells in the vessel wall between 2 and 4 weeks, and a significant proliferation of vascular SMC between 4 and 16 weeks were found, indicating that SMC apoptosis is a very early and critical event in the development of graft arteriosclerosis (Zou et al., 1998a). Another study confirmed these results: 1, 4, and 8 weeks after bypass operation, 13%, 29%, and 21%, respectively, of TUNEL positive cells were found in vessel walls of mouse vein grafts. No apoptosis was detectable in control vein-to-vein grafts (Mayr et al., 2000). Additionally, in cultured mouse, rat, and human arterial SMC, cyclic strain stress induced apoptosis in a time- and strength-dependent manner (Mayr et al., 2000) and resulted in

rapid and transient p38 MAPK activation (Li et al., 2000). Activation of p38 MAPK and mechanical stress-induced apoptosis were abolished in SMC lines stably expressing dominant-negative ras or rac, or MAPK phosphatase-1 (a negative regulator of MAPK signalling pathways). These findings suggest that p38-MAPK activation is, at least in part, responsible for the signal transduction leading to apoptosis (Li et al., 1999a) (Fig. 4).

Interestingly, hyperlipidemia amplifies the mechanical stress-induced development of vein graft atheroma, which appears to be related to increased apoptosis (Fig. 5). In a mouse model of hypercholesterolemia (apoE deficient mice), biomechanical stress-induced atherosclerosis was significantly enhanced compared to wild-type mice. Concomitantly, apoptosis was markedly increased in lesions of hypercholesteremic mice. In addition, cultured SMC from hypercholesteremic mice showed a higher rate of spontaneous apoptosis and a higher sensitivity to a variety of apoptosis-inducing agents (Dietrich et al., 2000). Taken together, hyperlipidemia seems to render SMC more susceptible to various apoptotic stimuli, biomechanical stress in particular, and therefore worsens the pathologic condition of vein graft atheroma.

4.3.4. Protein kinase C

We already discussed previously that PKC isoenzymes play an important role in cellular signalling stimulated by mechanical stress (Li and Xu, 2000). The PKCδ isoform, ubiquitously expressed in most mammalian cells, was reported to inhibit growth, induce differentiation, and promote apoptosis in SMC and other types of cells (Fukumoto et al., 1997). Recently, PKCδ

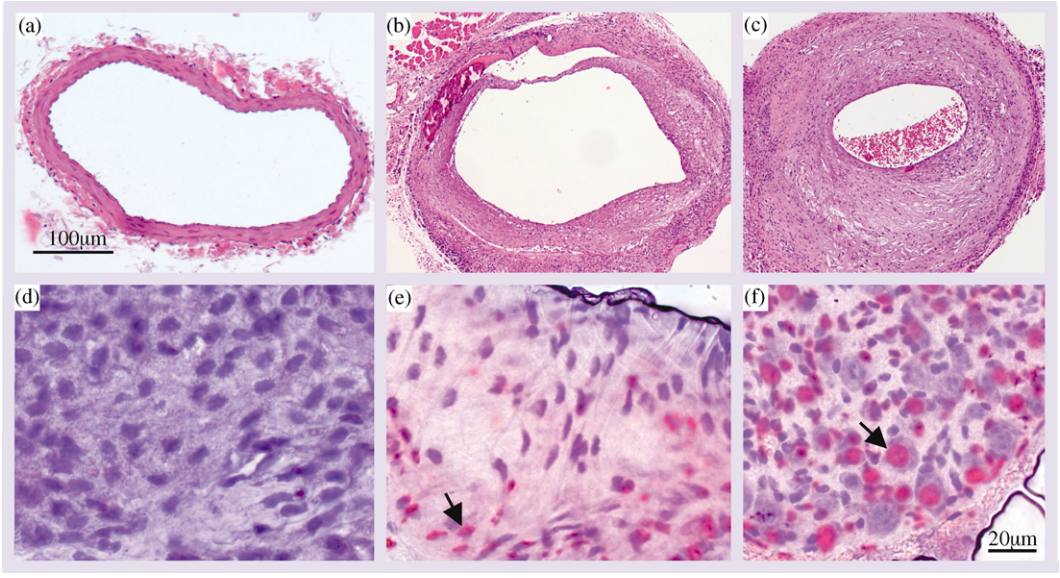

Fig. 5. Vein graft atherosclerosis and TUNEL-positive cells. Mouse control artery (a) and vein grafts harvested 4 (b and e) and 8 (c, d and f) weeks after surgery. Mice underwent anaesthesia and vena cava isografts into the common carotid artery of apoE-deficient mice. Grafted tissue fragments were harvested, fixed in 4% phosphate-buffered (pH 7.2) formaldehyde, embedded in paraffin, sectioned and stained with either HE (a–c) or TUNEL (e and f). Panel d is a negative control. Arrows indicate examples of TUNEL$^+$ cells in atherosclerotic lesions.

knockout mice were generated and vein bypass grafts were performed on theses animals as described previously. Interestingly, $PKC\delta^{-}/^{-}$ mice developed more severe vein graft arteriosclerosis with higher numbers of SMC and decreased SMC death in lesions compared to $PKC\delta^{+}/^{+}$ mice. Furthermore, SMC derived from $PKC\delta^{-}/^{-}$ mice were resistant to cell death, but responded similar to wild-type SMC with respect to mitogen proliferation in vitro. Treatment with several proapoptotic stimuli markedly diminished caspase-3 activation, poly (ADP-ribose) polymerase cleavage, and cytochrome *c* release in $PKC\delta^{-}/^{-}$ SMC relative to wild-type SMC, suggesting that loss of free radical generation and mitochondrial dysfunction in response to stress stimuli might lead to apoptotic resistance in $PKC\delta^{-}/^{-}$ mice (Leitges et al., 2001).

4.3.5. Checkpoint p53

Further insights in the signalling mechanisms leading to biomechanical stress-induced neointimal lesions in vein bypass grafts came from a p53 knockout model. Four weeks after grafting, neointimal hyperplasia of vein grafts in $p53^{-}/^{-}$mice was 2-fold increased compared to wild-type controls and consisted mainly of α-actin positive SMC. Most importantly, SMC apoptosis was significantly reduced in $p53^{-}/^{-}$vein grafts as determined by TUNEL method. At 4 weeks after operation, TUNEL positive cells in wild-type vein grafts were markedly increased in wild-type vein grafts, but remained virtually unchanged in $p53^{-}/^{-}$grafts. Furthermore, immunofluorescence analysis revealed increased p53 expression in neointimal SMC of wild-type mice, but not $p53^{-}/^{-}$mice, which coincided with oxidative DNA damage in vein grafts (Mayr et al., 2002). In cultured SMC of $p53^{-}/^{-}$mice, apoptosis was increased in response to the death receptor ligand TNFα, but decreased in response to the NO donor sodium nitroprusside suggesting that different signalling pathways are involved in TNFα– and NO-induced apoptosis, respectively (Mayr et al., 2002). Recent observations showed that mechanical stress-induced apoptosis in SMC is p53-dependent and associated with the transcription of p53-regulated and apoptosis-regulating genes, such as Bax (Mayr and Xu, unpublished data).

In summary, biomechanical stress-induced apoptosis in vein grafts seems to be dependent on a functional p53 checkpoint, involves the PKCδ signalling pathway and activation of p38 MAPK (Fig. 4).

4.4. Mechanical stress-induced heat shock protein induction

The arterial wall is continuously subjected to various stressors, including biomechanical stress. To maintain the homeostasis of the vessel wall, vascular cells produce high levels of stress proteins, also known as heat shock proteins (hsp). Hsp function as molecular chaperons during assembly and transport of newly synthesized proteins within the endoplasmatic reticulum. When the folding or assembly of proteins is not completed successfully (for example, if the protein structure is altered by a mutation or stress), the misfolded proteins, bound to the chaperones, are retained in the endoplasmic reticulum, then transported back to the cytoplasm, where they are finally degraded (Kuznetsov and Nigam, 1998). Hsp appear to be important in preventing damage and in cellular repair processes following injury and are highly expressed in cardiovascular tissues in response to stress stimuli (Benjamin and McMillan, 1998; Xu and Wick, 1996). Hsp can be subdivided into multimember families based on the molecular weights of the proteins encoded, i.e. hsp27, hsp60, hsp70, and hsp90. Hsp production is primarily regulated by heat shock

transcription factors (HSF) that interact with a specific regulatory element, heat shock element (HSE), which is present in the promoters of *hsp* genes (Morimoto, 1993). Two distinct HSF, HSF1 and HSF2, respectively, have been shown to exist in mammalian species and appear to be activated by distinct signals (Sorger, 1991).

4.4.1. Acute hypertension

Restraint (immobilization) stress resulted in the selective induction of *hsp70* mRNA and hsp protein in rat aortas, which was mainly confined to the SMC layer (Udelsman et al., 1993; Xu et al., 1995). Additionally, various agents that elevate blood pressure, such as phenylephrine, dopamine, cocaine, and air-jet stress, induced *hsp70* expression in rat aortas. *Hsp70* expression could be abolished by sodium nitroprusside, which lowers blood pressure non-specifically, suggesting that *hsp70* expression occur secondary to acute hypertension as a physiological response to the elevated haemodynamic stress. Further confirmation came from the findings that the pressure-elevating agents alone did not induce *hsp70* expression in primary cells in vitro (Xu et al., 1995). Consistent with that were previous observations that stretching of the myocardium induced *hsp70* expression in isolated perfused rabbit hearts, and that elevated load was sufficient to elicit *hsp70* induction in the heart (Izumo et al., 1988; Knowlton et al., 1991). Following studies showed that induction of the hsp70 gene in the arterial wall is mediated through the activation of HSF1 in response to high blood pressure (Xu et al., 1996). MAPK inhibition did not influence HSF1 activation by cyclic strain stress in cultured aortic SMC. Interestingly, stable transfection of vascular SMC lines dominant-negative rac abolished hsp-protein production and HSF1 activation induced by cyclic strain stress, whereas hsp70 expression was significantly reduced in SMC stably expressing dominant-negative ras (Xu et al., 2000). In addition, mechanical stress induced ROS generation including NO, which may contribute to HSF activation (Xu et al., 1997b).

In summary, the rapid induction of *hsp70* in response acute haemodynamic stress suggests an important role in protecting the vessel from injury and injury-induced apoptosis and maintaining cellular homeostasis. The stress-induced induction of *hsp70* is mediated by HSF1 activation and regulated by rac and ras G-proteins.

4.4.2. Chronic hypertension

In contrast to acute elevation in blood pressure, *hsp70* expression seems not to be elevated in conditions of chronic hypertension, at least in rats. Unstressed spontaneously hypertensive rats had no elevated *hsp70* expression even though their basal blood pressure (150 mm Hg) would be sufficient to induce *hsp70* gene expression in normotensive animals. However, further acute elevation in blood pressure in SHR induced *hsp70* expression (Xu et al., 1995). This was consistent with previous findings that SHR showed a greater increase in heat-induced *hsp70* expression than normotensive controls due to enhanced activation of HSF (Hamet et al., 1992; Hashimoto et al., 1991). Thus, *hsp70* expression seems to be dependent on acute haemodynamic changes regardless of the baseline blood pressure level in the absence of stress. Part of the adaptive response to chronic hypertension is the resetting of the threshold for *hsp70* induction in the arterial wall.

4.5. The dual role of the transcription factor NF-κB

Using immunofluorescence and immunohistochemical techniques, activated nuclear factor (NF)-κB could be identified in situ in human atherosclerotic plaques, whereas little or no activated NF-κB was detected in healthy vessels. Activated NF-κB was present in the intima and media of atheromatous regions of atherosclerotic lesions in SMC, macrophages, and EC (Brand et al., 1996). NF-κB is expressed in arterial SMC after balloon injury and induces in turn the expression of several genes, including leukocyte adhesion molecules, such as ICAM-1, VCAM-1, as well as chemokines (chemoattractant cytokines) macrophage chemoattractant protein (MCP-1) and IL-8, which help recruit circulating mononuclear leukodytes to the arterial intima (Landry et al., 1997).

NF-κB/Rel transcription factors are important in the inducible regulation of a variety of genes involved in inflammatory and proliferative responses of cells. NF-κB is rapidly activated in response to a variety of inflammatory and other stimuli, including atherosclerosis-related ones, such as tumour necrosis factor α, interleukin 1, platelet-activating factor, oxidized lipids, oxidatative stress, shear stress and cyclic stretch stress (Hishikawa and Luscher, 1997; Pahl, 1999). The activation of NF-κB is regulated by a family of inhibitors, IκB, that bind to NF-κB dimers and mask the nuclear localization sequence, which causes the retention of the complexes in the cytoplasm (Karin, 1999). One of the pathways leading to the release of NF-κB from its inhibitor is mediated by phosphorylation and activation of the IκB kinase (IKK) complex, consisting of IKK-α, IKK-β, and a structural component, IKK-γ. The activated IKK complex specifically phosphorylates IκB, which results in their rapid polyubiquitination and degradation by the proteosome. After being released from the inhibitor, NF-κB translocates to the nucleus, where it binds to target genes and stimulates transcription.

As we mentioned previously, activated NF-κB protects cells from apoptosis by inducing the expression of genes that promote resistance to apoptosis. One way to protect cells from apoptosis consists in the suppression of caspase activation by increasing the expression of the inhibitors of apoptosis (IAP) proteins, c-IAP1, c-IAP2, and XIAP, as well as the TNF receptor-associated factors (TRAF), TRAF1 and TRAF2 (Wang et al., 1998a). The other way may consist in the suppression of mitochondrial cytochrome *c* release through the induction of the Bcl-2 family member A1/Bfl-1 (Wang et al., 1999). Other products of target genes, including cyclin D1, may contribute to cell cycle progression and stimulate cell proliferation (Hinz et al., 1999).

We already discussed the atheroprotective role of uniformly laminar flow. One pathway of shear stress-mediated atheroprotection consists in the activation of Akt/eNOS leading to increased production of NO (Dimmeler et al., 1999a). Apart from atheroprotective functions, such as vasodilatation, inhibition of platelet aggregation, and inhibition of SMC proliferation, NO inhibits NF-κB activation in EC through the induction and stabilization of IκB-α (Peng et al., 1995). In regions of atherosclerotic lesions, laminar flow is disturbed with layer separation, flow reversal, secondary flows, and shifting and stagnation points. In these regions, the mean shear stress is low and the NF-κB signalling transduction pathways are activated contributing to the development of atherosclerotic lesions by the induction of proatherogenic genes. Constitutive NF-κB activation and VCAM-1 expression were found in EC located in aortic regions of high probability for atherosclerotic lesion development (Hajra et al., 2000). Various risk factors leading to atherosclerosis, including hypertension, have in common the generation of oxidative stress. Cells respond by increasing the levels of antioxidants and altering the intracellular

reduction-oxidation (redox) state. NF-κB may be one of the transcription factors controlled by the intracellular redox status (Li and Karin, 1999).

What is the dual role of NF-κB in the pathogenesis of atherosclerosis? Again, simply stated, it is the right balance between cell death and proliferation in the adaptive response of the vessel. Usually, the activity of NF-κB is very rapid and transient. As we have seen, NF-κB activation is a highly regulated and autoregulated process (NF-κB also activates the *IκB-α* gene, thus replenishing the cytoplasmic pool of its own inhibitor (Rice and Ernst, 1993)). This ensures that the stress-activated cell returns to a quiescent state. Stress-response genes need to be upregulated only on demand for a limited period of time. Pathological and prolonged activation of the NF-κB system, either through persistence of the stimulating agent(s) or through impairment of regulating mechanisms, may contribute to the changes in gene expression that occur during the development of atherosclerosis. What is initially a protective survival signal may in turn become a crucial factor in the development of the disease.

5. Perspectives

In multicellular organisms, survival depends on the function of a diverse set of differentiated cell types. Homeostasis is maintained through a balance between cell proliferation and cell death, the maintenance and renewal of these diverse lineages. Different cell types vary widely in the mechanisms by which they grow, differentiate, and die over the life of an organism. Cell proliferation is a highly regulated and complex process, controlled by numerous checkpoints and communication networks. In recent years, however, cell biologists have recognized that the regulation of cell death is much more than the fatal result from acute cellular injury and is just as complex as the control of cell proliferation. Moreover, proliferation and cell death seem to be highly connected and considerable overlap exists between the components that execute both processes. In rat mesenteric resistance arteries, reduced and elevated blood flow produced, respectively, decrease and increase in SMC number, with signs of dedifferentiation of SMC in both cases (eutrophic and hypertrophic remodelling) (Buus et al., 2001). In both cases, however, increased apoptosis occurred in the medial vessel layer, pointing to the important role of cell death in disease development. Notably, apoptosis often occurs at the very beginning in response to (mechanical) stress, before the adaptive and hyperproliferative response (Mayr et al., 2000; Zou et al., 1998a).

Myocyte loss in heart failure, death of SMC in hypertensive conditions, development of atherosclerotic lesions and plaque instability, SMC death after coronary angioplasty, and apoptosis of SMC and other cells in vein grafts provide some examples, where mechanical stress-induced apoptosis plays a key role in the pathogenesis of the disease. This has raised hope that inhibition or better regulation of apoptosis could prevent or slow the progression of these diseases. In fact, promising results come for example from the beneficial effect of the AT_1 receptor antagonist Losartan, which abolished both, stretch-ATII stimulated p53 activation and apoptosis in myocytes (Leri et al., 1998). Targeting the proaptotic stimulus is one therapeutic approach. The other one consists in the promotion of antiapoptotic signalling pathways, such as gp130 mediated myocyte survival during pressure overload (Hirota et al., 1999). Finally, the proaptotic signalling can be the target of choice, like p38 MAPK, PKCδ, or p53 in the case of vein graft arteriosclerosis

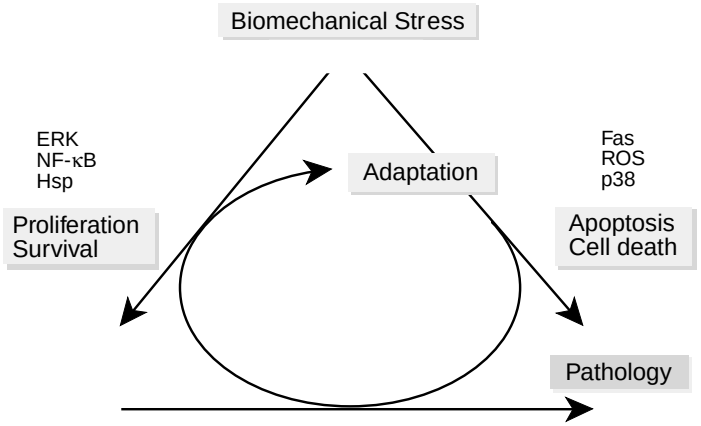

Fig. 6. Schematic representation of biomechanical stress-induced effects in the cardiovascular system. Biomechanical stress stimulates both cell proliferation and apoptosis resulting in a dynamic process of tissue remodelling. Mechanical stress-induced cell death is not just the result of an injury but a highly regulated process and is required for any remodelling that may lead to adaptation. Processes of apoptosis and proliferation are connected in a very complex and concerted way. Overwhelming stress or alterations in the signalling networks, however, may result in pathologic responses, such as heart failure, hypertension related vascular disease, and atherosclerosis

(Leitges et al., 2001; Li et al., 1999a; Mayr et al., 2002). Although being a promising perspective, there are many limitations for such therapeutic concepts. Treatments that increase a cell's resistance to undergo apoptosis bear a considerable risk of teratogenicity. Loss of function of caspase-3, caspase-9, apaf-1, and Bcl-X_L causes embryonic lethality in mice associated with severe brain malformations. Likewise, FADD and caspase-8 deficient mice die *in utero* due to impaired myocardial development and cardiac dilatation (Haunstetter and Izumo, 2000).

Apoptosis often represents an immediate response to external stimuli. The following adaptive changes leading to a remodelled structure are beneficial and protective. Overwhelming stress, however, may lead to disturbed remodelling processes (Fig. 6). We should always keep in mind that the first therapeutic approach has to target the underlying cause, for example hypertension and other risk factors in the case of atherosclerotic disorders. In a clinical environment, however, we often have to deal with the consequences. Antiapoptotic treatment should modulate the apoptotic response rather than inhibit apoptosis completely. Complete suppression is not desired, since apoptosis is a physiological condition and required for the normal function and maintenance of a tissue or an organ. The challenge of future therapeutic concepts will be to develop a therapy which is short acting and localized to the target of interest in order to minimize adverse side effects.

Acknowledgements

We thank Manuel Mayr, Yanhua Hu, Chahong Li, Ursula Mayr and Hermann Dietrich, who, together with many other collaborators, contributed to the work summarized in this review.

We also thank Anthony Shaw for critical reading of the manuscript. This work was supported by the Oak Foundation and the British Heart Foundation.

References

Adams, J.W., Sakata, Y., Davis, M.G., Sah, V.P., Wang, Y., Liggett, S.B., Chien, K.R., Brown, J.H., Dorn, G.W., 1998. Enhanced Galphaq signaling: a common pathway mediates cardiac hypertrophy and apoptotic heart failure. Proc. Natl. Acad. Sci. USA 95, 10140–10145.

Andree, H.A., Reutelingsperger, C.P., Hauptmann, R., Hemker, H.C., Hermens, W.T., Willems, G.M., 1990. Binding of vascular anticoagulant alpha (VAC alpha) to planar phospholipid bilayers. J. Biol. Chem. 265, 4923–4928.

Benjamin, I.J., McMillan, D.R., 1998. Stress (heat shock) proteins: molecular chaperones in cardiovascular biology and disease. Circ. Res. 83, 117–132.

Bennett, M.R., Evan, G.I., Schwartz, S.M., 1995. Apoptosis of human vascular smooth muscle cells derived from normal vessels and coronary atherosclerotic plaques. J. Clin. Invest. 95, 2266–2274.

Bennett, M., Macdonald, K., Chan, S.W., Luzio, J.P., Simari, R., Weissberg, P., 1998. Cell surface trafficking of Fas: a rapid mechanism of p53-mediated apoptosis. Science 282, 290–293 (see comments).

Bing, O.H., 1994. Hypothesis: apoptosis may be a mechanism for the transition to heart failure with chronic pressure overload. J. Mol. Cell. Cardiol. 26, 943–948.

Birukov, K.G., Bardy, N., Lehoux, S., Merval, R., Shirinsky, V.P., Tedgui, A., 1998. Intraluminal pressure is essential for the maintenance of smooth muscle caldesmon and filamin content in aortic organ culture. Arterioscler. Thromb. Vasc. Biol. 18, 922–927.

Bombeli, T., Karsan, A., Tait, J.F., Harlan, J.M., 1997. Apoptotic vascular endothelial cells become procoagulant. Blood 89, 2429–2442.

Bombeli, T., Schwartz, B.R., Harlan, J.M., 1999. Endothelial cells undergoing apoptosis become proadhesive for nonactivated platelets. Blood 93, 3831–3838.

Bozkurt, B., Kribbs, S.B., Clubb Jr., F.J., Michael, L.H., Didenko, V.V., Hornsby, P.J., Seta, Y., Oral, H., Spinale, F.G., Mann, D.L., 1998. Pathophysiologically relevant concentrations of tumor necrosis factor-alpha promote progressive left ventricular dysfunction and remodeling in rats. Circulation 97, 1382–1391.

Brand, K., Page, S., Rogler, G., Bartsch, A., Brandl, R., Knuechel, R., Page, M., Kaltschmidt, C., Baeuerle, P.A., Neumeier, D., 1996. Activated transcription factor nuclear factor-kappa B is present in the atherosclerotic lesion. J. Clin. Invest. 97, 1715–1722.

Buus, C.L., Pourageaud, F., Fazzi, G.E., Janssen, G., Mulvany, M.J., De Mey, J.G., 2001. Smooth muscle cell changes during flow-related remodeling of rat mesenteric resistance arteries. Circ. Res. 89, 180–186.

Cattaruzza, M., Dimigen, C., Ehrenreich, H., Hecker, M., 2000. Stretch-induced endothelin B receptor-mediated apoptosis in vascular smooth muscle cells. Faseb. J. 14, 991–998.

Cattaruzza, M., Eberhardt, I., Hecker, M., 2001. Mechanosensitive transcription factors involved in endothelin b receptor expression. J. Biol. Chem. 276, 36999–37003.

Cheng, W., Li, B., Kajstura, J., Li, P., Wolin, M.S., Sonnenblick, E.H., Hintze, T.H., Olivetti, G., Anversa, P., 1995. Stretch-induced programmed myocyte cell death. J. Clin. Invest. 96, 2247–2259.

Chien, S., Li, S., Shyy, Y.J., 1998. Effects of mechanical forces on signal transduction and gene expression in endothelial cells. Hypertension 31, 162–169.

Cho, A., Mitchell, L., Koopmans, D., Langille, B.L., 1997. Effects of changes in blood flow rate on cell death and cell proliferation in carotid arteries of immature rabbits. Circ. Res. 81, 328–337.

Condorelli, G., Morisco, C., Stassi, G., Notte, A., Farina, F., Sgaramella, G., de Rienzo, A., Roncarati, R., Trimarco, B., Lembo, G., 1999. Increased cardiomyocyte apoptosis and changes in proapoptotic and antiapoptotic genes bax and bcl-2 during left ventricular adaptations to chronic pressure overload in the rat. Circulation 99, 3071–3078.

Dartsch, P.C., Voisard, R., Bauriedel, G., Hofling, B., Betz, E., 1990. Growth characteristics and cytoskeletal organization of cultured smooth muscle cells from human primary stenosing and restenosing lesions. Arteriosclerosis 10, 62–75.

Darzynkiewicz, Z., Bruno, S., Del Bino, G., Gorczyca, W., Hotz, M.A., Lassota, P., Traganos, F., 1992. Features of apoptotic cells measured by flow cytometry. Cytometry 13, 795–808.

Davies, P.F., Remuzzi, A., Gordon, E.J., Dewey Jr., C.F., Gimbrone Jr., M.A., 1986. Turbulent fluid shear stress induces vascular endothelial cell turnover in vitro. Proc. Natl. Acad. Sci. USA 83, 2114–2117.

Dhalla, A.K., Singal, P.K., 1994. Antioxidant changes in hypertrophied and failing guinea pig hearts. Am. J. Physiol. 266, H1280–1285.

Dhalla, A.K., Hill, M.F., Singal, P.K., 1996. Role of oxidative stress in transition of hypertrophy to heart failure. J. Am. Coll. Cardiol. 28, 506–514.

Di Lisa, F., Blank, P.S., Colonna, R., Gambassi, G., Silverman, H.S., Stern, M.D., Hansford, R.G., 1995. Mitochondrial membrane potential in single living adult rat cardiac myocytes exposed to anoxia or metabolic inhibition. J. Physiol. 486 (Pt 1), 1–13.

Diep, Q.N., Li, J.S., Schiffrin, E.L., 1999. In vivo study of AT(1) and AT(2) angiotensin receptors in apoptosis in rat blood vessels. Hypertension 34, 617–624.

Dietrich, H., Hu, Y., Zou, Y., Huemer, U., Metzler, B., Li, C., Mayr, M., Xu, Q., 2000. Rapid development of vein graft atheroma in ApoE-deficient mice. Am. J. Pathol. 157, 659–669.

Dimmeler, S., Haendeler, J., Rippmann, V., Nehls, M., Zeiher, A.M., 1996. Shear stress inhibits apoptosis of human endothelial cells. FEBS Lett. 399, 71–74.

Dimmeler, S., Haendeler, J., Nehls, M., Zeiher, A.M., 1997a. Suppression of apoptosis by nitric oxide via inhibition of interleukin-1beta-converting enzyme. (ICE)-like and cysteine protease protein (CPP)-32-like proteases. J. Exp. Med. 185, 601–607.

Dimmeler, S., Rippmann, V., Weiland, U., Haendeler, J., Zeiher, A.M., 1997b. Angiotensin II induces apoptosis of human endothelial cells. Protective effect of nitric oxide. Circ. Res. 81, 970–976.

Dimmeler, S., Assmus, B., Hermann, C., Haendeler, J., Zeiher, A.M., 1998. Fluid shear stress stimulates phosphorylation of Akt in human endothelial cells: involvement in suppression of apoptosis. Circ. Res. 83, 334–341.

Dimmeler, S., Fleming, I., Fisslthaler, B., Hermann, C., Busse, R., Zeiher, A.M., 1999a. Activation of nitric oxide synthase in endothelial cells by Akt- dependent phosphorylation. Nature 399, 601–605.

Dimmeler, S., Fleming, I., Fisslthaler, B., Hermann, C., Busse, R., Zeiher, A.M., 1999b. Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. Nature 399, 601–605.

Dimmeler, S., Hermann, C., Galle, J., Zeiher, A.M., 1999c. Upregulation of superoxide dismutase and nitric oxide synthase mediates the apoptosis-suppressive effects of shear stress on endothelial cells. Arterioscler. Thromb. Vasc. Biol. 19, 656–664.

Eliceiri, B.P., 2001. Integrin and growth factor receptor crosstalk. Circ. Res. 89, 1104–1110.

Fadok, V.A., Voelker, D.R., Campbell, P.A., Cohen, J.J., Bratton, D.L., Henson, P.M., 1992. Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages. J. Immunol. 148, 2207–2216.

Fadok, V.A., Bratton, D.L., Rose, D.M., Pearson, A., Ezekewitz, R.A., Henson, P.M., 2000. A receptor for phosphatidylserine-specific clearance of apoptotic cells (see comments). Nature 405, 85–90.

Feuerstein, G.Z., Young, P.R., 2000. Apoptosis in cardiac diseases: stress- and mitogen-activated signaling pathways. Cardiovasc. Res. 45, 560–569.

Fukumoto, S., Nishizawa, Y., Hosoi, M., Koyama, H., Yamakawa, K., Ohno, S., Morii, H., 1997. Protein kinase C delta inhibits the proliferation of vascular smooth muscle cells by suppressing G1 cyclin expression. J. Biol. Chem. 272, 13816–13822.

Gavrieli, Y., Sherman, Y., Ben-Sasson, S.A., 1992. Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J. Cell. Biol. 119, 493–501.

Geng, Y.J., Libby, P., 1995. Evidence for apoptosis in advanced human atheroma. Colocalization with interleukin-1 beta-converting enzyme. Am. J. Pathol. 147, 251–266.

Girard, P.R., Nerem, R.M., 1995. Shear stress modulates endothelial cell morphology and F-actin organization through the regulation of focal adhesion-associated proteins. J. Cell. Physiol. 163, 179–193.

Gobe, G., Browning, J., Howard, T., Hogg, N., Winterford, C., Cross, R., 1997. Apoptosis occurs in endothelial cells during hypertension-induced microvascular rarefaction. J. Struct. Biol. 118, 63–72.

Goethe, J.W.v, 1817. Zur Morphologie, Jena.

Gorczyca, W., Gong, J., Darzynkiewicz, Z., 1993. Detection of DNA strand breaks in individual apoptotic cells by the in situ terminal deoxynucleotidyl transferase and nick translation assays. Cancer Res. 53, 1945–1951.

Green, D.R., Reed, J.C., 1998. Mitochondria and apoptosis. Science 281, 1309–1312.

Gurtu, V., Kain, S.R., Zhang, G., 1997. Fluorometric and colorimetric detection of caspase activity associated with apoptosis. Anal. Biochem. 251, 98–102.

Hajra, L., Evans, A.I., Chen, M., Hyduk, S.J., Collins, T., Cybulsky, M.I., 2000. The NF-kappa B signal transduction pathway in aortic endothelial cells is primed for activation in regions predisposed to atherosclerotic lesion formation. Proc. Natl. Acad. Sci. USA 97, 9052–9057.

Hamet, P., Kong, D., Pravenec, M., Kunes, J., Kren, V., Klir, P., Sun, Y.L., Tremblay, J., 1992. Restriction fragment length polymorphism of hsp70 gene, localized in the RT1 complex, is associated with hypertension in spontaneously hypertensive rats. Hypertension 19, 611–614.

Hamet, P., Richard, L., Dam, T.V., Teiger, E., Orlov, S.N., Gaboury, L., Gossard, F., Tremblay, J., 1995. Apoptosis in target organs of hypertension. Hypertension 26, 642–648.

Han, D.K., Haudenschild, C.C., Hong, M.K., Tinkle, B.T., Leon, M.B., Liau, G., 1995. Evidence for apoptosis in human atherogenesis and in a rat vascular injury model. Am. J. Pathol. 147, 267–277.

Hashimoto, T., Mosser, R.D., Tremblay, J., Hamet, P., 1991. Increased accumulation of hsp70 messenger RNA due to enhanced activation of heat-shock transcription factor in spontaneously hypertensive rats. J. Hypertension Suppl. 9, S170–171.

Haunstetter, A., Izumo, S., 2000. Toward antiapoptosis as a new treatment modality. Circ. Res. 86, 371–376.

Hengartner, M.O., 2000. The biochemistry of apoptosis. Nature 407, 770–776.

Hinz, M., Krappmann, D., Eichten, A., Heder, A., Scheidereit, C., Strauss, M., 1999. NF-kappaB function in growth control: regulation of cyclin D1 expression and G0/G1-to-S-phase transition. Mol. Cell. Biol. 19, 2690–2698.

Hirota, H., Chen, J., Betz, U.A., Rajewsky, K., Gu, Y., Ross Jr., J., Muller, W., Chien, K.R., 1999. Loss of a gp130 cardiac muscle cell survival pathway is a critical event in the onset of heart failure during biomechanical stress. Cell 97, 189–198.

Hishikawa, K., Luscher, T.F., 1997. Pulsatile stretch stimulates superoxide production in human aortic endothelial cells. Circulation 96, 3610–3616.

Horiuchi, M., Hayashida, W., Kambe, T., Yamada, T., Dzau, V.J., 1997. Angiotensin type 2 receptor dephosphorylates Bcl-2 by activating mitogen-activated protein kinase phosphatase-1 and induces apoptosis. J. Biol. Chem. 272, 19022–19026.

Hu, Y., Cheng, L., Hochleitner, B.W., Xu, Q., 1997. Activation of mitogen-activated protein kinases (ERK/JNK) and AP-1 transcription factor in rat carotid arteries after balloon injury. Arterioscler. Thromb. Vasc. Biol. 17, 2808–2816.

Hu, Y., Bock, G., Wick, G., Xu, Q., 1998. Activation of PDGF receptor alpha in vascular smooth muscle cells by mechanical stress. Faseb. J. 12, 1135–1142.

Hu, Y., Zou, Y., Dietrich, H., Wick, G., Xu, Q., 1999. Inhibition of neointima hyperplasia of mouse vein grafts by locally applied suramin. Circulation 100, 861–868.

Intengan, H.D., Schiffrin, E.L., 2000. Structure and mechanical properties of resistance arteries in hypertension: role of adhesion molecules and extracellular matrix determinants. Hypertension 36, 312–318.

Intengan, H.D., Schiffrin, E.L., 2001. Vascular remodeling in hypertension: roles of apoptosis, inflammation, and fibrosis. Hypertension 38, 581–587.

Isner, J.M., Kearney, M., Bortman, S., Passeri, J., 1995. Apoptosis in human atherosclerosis and restenosis. Circulation 91, 2703–2711.

Itoh, G., Tamura, J., Suzuki, M., Suzuki, Y., Ikeda, H., Koike, M., Nomura, M., Jie, T., Ito, K., 1995. DNA fragmentation of human infarcted myocardial cells demonstrated by the nick end labeling method and DNA agarose gel electrophoresis. Am. J. Pathol. 146, 1325–1331.

Izumo, S., Nadal-Ginard, B., Mahdavi, V., 1988. Protooncogene induction and reprogramming of cardiac gene expression produced by pressure overload. Proc. Natl. Acad. Sci. USA 85, 339–343.

Jacobson, M.D., Weil, M., Raff, M.C., 1997. Programmed cell death in animal development. Cell 88, 347–354.

Kaiser, D., Freyberg, M.A., Friedl, P., 1997. Lack of hemodynamic forces triggers apoptosis in vascular endothelial cells. Biochem. Biophys. Res. Commun. 231, 586–590.

Karin, M., 1999. The beginning of the end: IkappaB kinase (IKK) and NF-kappaB activation. J. Biol. Chem. 274, 27339–27342.

Kerr, J.F., Wyllie, A.H., Currie, A.R., 1972. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br. J. Cancer 26, 239–257.

Kim, H.S., Hwang, K.K., Seo, J.W., Kim, S.Y., Oh, B.H., Lee, M.M., Park, Y.B., 2000. Apoptosis and regulation of Bax and Bcl-X proteins during human neonatal vascular remodeling. Arterioscler. Thromb. Vasc. Biol. 20, 957–963.

Knowlton, A.A., Eberli, F.R., Brecher, P., Romo, G.M., Owen, A., Apstein, C.S., 1991. A single myocardial stretch or decreased systolic fiber shortening stimulates the expression of heat shock protein 70 in the isolated, erythrocyte-perfused rabbit heart. J. Clin. Invest. 88, 2018–2025.

Kockx, M.M., 1998. Apoptosis in the atherosclerotic plaque: quantitative and qualitative aspects. Arterioscler. Thromb. Vasc. Biol. 18, 1519–1522.

Ku, D.N., Giddens, D.P., Zarins, C.K., Glagov, S., 1985. Pulsatile flow and atherosclerosis in the human carotid bifurcation. Positive correlation between plaque location and low oscillating shear stress. Arteriosclerosis 5, 293–302.

Kubota, T., McTiernan, C.F., Frye, C.S., Slawson, S.E., Lemster, B.H., Koretsky, A.P., Demetris, A.J., Feldman, A.M., 1997. Dilated cardiomyopathy in transgenic mice with cardiac-specific overexpression of tumor necrosis factor-alpha. Circ. Res. 81, 627–635.

Kuznetsov, G., Nigam, S.K., 1998. Folding of secretory and membrane proteins. N. Engl. J. Med. 339, 1688–1695.

Landry, D.B., Couper, L.L., Bryant, S.R., Lindner, V., 1997. Activation of the NF-kappa B and I kappa B system in smooth muscle cells after rat arterial injury. Induction of vascular cell adhesion molecule-1 and monocyte chemoattractant protein-1. Am. J. Pathol. 151, 1085–1095.

Lehoux, S., Tedgui, A., 1998. Signal transduction of mechanical stresses in the vascular wall. Hypertension 32, 338–345.

Leitges, M., Mayr, M., Braun, U., Mayr, U., Li, C., Pfister, G., Ghaffari-Tabrizi, N., Baier, G., Hu, Y., Xu, Q., 2001. Exacerbated vein graft arteriosclerosis in protein kinase Cdelta-null mice. J. Clin. Invest. 108, 1505–1512.

Leri, A., Claudio, P.P., Li, Q., Wang, X., Reiss, K., Wang, S., Malhotra, A., Kajstura, J., Anversa, P., 1998. Stretch-mediated release of angiotensin II induces myocyte apoptosis by activating p53 that enhances the local renin-angiotensin system and decreases the Bcl-2-to-Bax protein ratio in the cell. J. Clin. Invest. 101, 1326–1342.

Leri, A., Fiordaliso, F., Setoguchi, M., Limana, F., Bishopric, N.H., Kajstura, J., Webster, K., Anversa, P., 2000a. Inhibition of p53 function prevents renin-angiotensin system activation and stretch-mediated myocyte apoptosis. Am. J. Pathol. 157, 843–857.

Leri, A., Liu, Y., Li, B., Fiordaliso, F., Malhotra, A., Latini, R., Kajstura, J., Anversa, P., 2000b. Up-regulation of AT(1) and AT(2) receptors in postinfarcted hypertrophied myocytes and stretch-mediated apoptotic cell death. Am. J. Pathol. 156, 1663–1672.

Levine, G.N., Chodos, A.P., Loscalzo, J., 1995. Restenosis following coronary angioplasty: clinical presentations and therapeutic options. Clin. Cardiol. 18, 693–703.

Li, N., Karin, M., 1999. Is NF-kappaB the sensor of oxidative stress? Faseb. J. 13, 1137–1143.

Li, C., Xu, Q., 2000. Mechanical stress-initiated signal transductions in vascular smooth muscle cells. Cell Signal 12, 435–445.

Li, P.F., Dietz, R., von Harsdorf, R., 1997a. Reactive oxygen species induce apoptosis of vascular smooth muscle cell. FEBS Lett. 404, 249–252.

Li, Z., Bing, O.H., Long, X., Robinson, K.G., Lakatta, E.G., 1997b. Increased cardiomyocyte apoptosis during the transition to heart failure in the spontaneously hypertensive rat. Am. J. Physiol. 272, H2313–2319.

Li, C., Hu, Y., Mayr, M., Xu, Q., 1999a. Cyclic strain stress-induced mitogen-activated protein kinase (MAPK) phosphatase 1 expression in vascular smooth muscle cells is regulated by Ras/Rac-MAPK pathways. J. Biol. Chem. 274, 25273–25280.

Li, P.F., Maasch, C., Haller, H., Dietz, R., von Harsdorf, R., 1999b. Requirement for protein kinase C in reactive oxygen species-induced apoptosis of vascular smooth muscle cells. Circulation 100, 967–973.

Li, C., Hu, Y., Sturm, G., Wick, G., Xu, Q., 2000. Ras/Rac-Dependent activation of p38 mitogen-activated protein kinases in smooth muscle cells stimulated by cyclic strain stress. Arterioscler. Thromb. Vasc. Biol. 20, E1–9.

Lusis, A.J., 2000. Atherosclerosis. Nature 407, 233–241.

Lytle, B.W., Loop, F.D., 2001. Superiority of bilateral internal thoracic artery grafting: it's been a long time comin. Circulation 104, 2152–2154.

Malik, N., Francis, S.E., Holt, C.M., Gunn, J., Thomas, G.L., Shepherd, L., Chamberlain, J., Newman, C.M., Cumberland, D.C., Crossman, D.C., 1998. Apoptosis and cell proliferation after porcine coronary angioplasty. Circulation 98, 1657–1665.

Mallat, Z., Tedgui, A., 2000. Apoptosis in the vasculature: mechanisms and functional importance. Br. J. Pharmacol. 130, 947–962.

Mallat, Z., Tedgui, A., Fontaliran, F., Frank, R., Durigon, M., Fontaine, G., 1996. Evidence of apoptosis in arrhythmogenic right ventricular dysplasia. N. Engl. J. Med. 335, 1190–1196.

Mathur, A., Hong, Y., Kemp, B.K., Barrientos, A.A., Erusalimsky, J.D., 2000. Evaluation of fluorescent dyes for the detection of mitochondrial membrane potential changes in cultured cardiomyocytes. Cardiovasc. Res. 46, 126–138.

Mayr, M., Xu, Q., 2001. Smooth muscle cell apoptosis in arteriosclerosis. Exp. Gerontol. 36, 969–987.

Mayr, M., Li, C., Zou, Y., Huemer, U., Hu, Y., Xu, Q., 2000. Biomechanical stress-induced apoptosis in vein grafts involves p38 mitogen-activated protein kinases. Faseb. J. 14, 261–270.

Mayr, U., Mayr, M., Li, C., Wernig, F., Dietrich, H., Hu, Y., Xu, Q., 2002. Loss of p53 Accelerates Neointimal Lesions of Vein Bypass Grafts in Mice. Circ. Res. 90, 197–204.

McCarthy, N.J., Bennett, M.R., 2000. The regulation of vascular smooth muscle cell apoptosis. Cardiovasc. Res. 45, 747–755.

Metzler, B., Hu, Y., Dietrich, H., Xu, Q., 2000. Increased expression and activation of stress-activated protein kinases/c-Jun NH(2)-terminal protein kinases in atherosclerotic lesions coincide with p53. Am. J. Pathol. 156, 1875–1886.

Miller, C.E., Donlon, K.J., Toia, L., Wong, C.L., Chess, P.R., 2000. Cyclic strain induces proliferation of cultured embryonic heart cells. In Vitro Cell Dev. Biol. Anim. 36, 633–639.

Morawietz, H., Ma, Y.H., Vives, F., Wilson, E., Sukhatme, V.P., Holtz, J., Ives, H.E., 1999. Rapid induction and translocation of Egr-1 in response to mechanical strain in vascular smooth muscle cells. Circ. Res. 84, 678–687.

Morimoto, R.I., 1993. Cells in stress: transcriptional activation of heat shock genes. Science 259, 1409–1410.

Moss, N.S., Benditt, E.P., 1975. Human atherosclerotic plaque cells and leiomyoma cells. Comparison of in vitro growth characteristics. Am. J. Pathol. 78, 175–190.

Motwani, J.G., Topol, E.J., 1998. Aortocoronary saphenous vein graft disease: pathogenesis, predisposition, and prevention. Circulation 97, 916–931.

Narula, J., Haider, N., Virmani, R., DiSalvo, T.G., Kolodgie, F.D., Hajjar, R.J., Schmidt, U., Semigran, M.J., Dec, G.W., Khaw, B.A., 1996. Apoptosis in myocytes in end-stage heart failure. N. Engl. J. Med. 335, 1182–1189.

Narula, J., Pandey, P., Arbustini, E., Haider, N., Narula, N., Kolodgie, F.D., Dal Bello, B., Semigran, M.J., Bielsa-Masdeu, A., Dec, G.W., Israels, S., Ballester, M., Virmani, R., Saxena, S., Kharbanda, S., 1999. Apoptosis in heart failure: release of cytochrome c from mitochondria and activation of caspase-3 in human cardiomyopathy. Proc. Natl. Acad. Sci. USA 96, 8144–8149.

Newby, A.C., George, S.J., 1996. Proliferation, migration, matrix turnover, and death of smooth muscle cells in native coronary and vein graft atherosclerosis. Curr. Opin. Cardiol. 11, 574–582.

Noon, J.P., Walker, B.R., Webb, D.J., Shore, A.C., Holton, D.W., Edwards, H.V., Watt, G.C., 1997. Impaired microvascular dilatation and capillary rarefaction in young adults with a predisposition to high blood pressure. J. Clin. Invest. 99, 1873–1879.

Olivetti, G., Abbi, R., Quaini, F., Kajstura, J., Cheng, W., Nitahara, J.A., Quaini, E., Di Loreto, C., Beltrami, C.A., Krajewski, S., Reed, J.C., Anversa, P., 1997. Apoptosis in the failing human heart. N. Engl. J. Med. 336, 1131–1141.

Pahl, H.L., 1999. Activators and target genes of Rel/NF-kappaB transcription factors. Oncogene 18, 6853–6866.

Peng, H.B., Libby, P., Liao, J.K., 1995. Induction and stabilization of I kappa B alpha by nitric oxide mediates inhibition of NF-kappa B. J. Biol. Chem. 270, 14214–14219.

Perlman, H., Maillard, L., Krasinski, K., Walsh, K., 1997. Evidence for the rapid onset of apoptosis in medial smooth muscle cells after balloon injury. Circulation 95, 981–987.

Pimentel, D.R., Amin, J.K., Xiao, L., Miller, T., Viereck, J., Oliver-Krasinski, J., Baliga, R., Wang, J., Siwik, D.A., Singh, K., Pagano, P., Colucci, W.S., Sawyer, D.B., 2001. Reactive oxygen species mediate amplitude-dependent hypertrophic and apoptotic responses to mechanical stretch in cardiac myocytes. Circ. Res. 89, 453–460.

Post, G.R., Brown, J.H., 1996. G protein-coupled receptors and signaling pathways regulating growth responses. Faseb. J. 10, 741–749.

Price, R.J., Skalak, T.C., 1994. Circumferential wall stress as a mechanism for arteriolar rarefaction and proliferation in a network model. Microvasc. Res. 47, 188–202.

Ranjan, V., Xiao, Z., Diamond, S.L., 1995. Constitutive NOS expression in cultured endothelial cells is elevated by fluid shear stress. Am. J. Physiol. 269, H550–555.

Reers, M., Smith, T.W., Chen, L.B., 1991. J-aggregate formation of a carbocyanine as a quantitative fluorescent indicator of membrane potential. Biochemistry 30, 4480–4486.

Reusch, P., Wagdy, H., Reusch, R., Wilson, E., Ives, H.E., 1996. Mechanical strain increases smooth muscle and decreases nonmuscle myosin expression in rat vascular smooth muscle cells. Circ. Res. 79, 1046–1053.

Rice, N.R., Ernst, M.K., 1993. In vivo control of NF-kappa B activation by I kappa B alpha. Embo J. 12, 4685–4695.

Rich, T., Allen, R.L., Wyllie, A.H., 2000. Defying death after DNA damage. Nature 407, 777–783.

Rizzoni, D., Rodella, L., Porteri, E., Rezzani, R., Guelfi, D., Piccoli, A., Castellano, M., Muiesan, M.L., Bianchi, R., Rosei, E.A., 2000. Time course of apoptosis in small resistance arteries of spontaneously hypertensive rats. J. Hypertension 18, 885–891.

Ross, R., 1993. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 362, 801–809.

Ross, R., 1999. Atherosclerosis–an inflammatory disease. N. Engl. J. Med. 340, 115–126.

Ross, R., Wight, T.N., Strandness, E., Thiele, B., 1984. Human atherosclerosis. I. Cell constitution and characteristics of advanced lesions of the superficial femoral artery. Am. J. Pathol. 114, 79–93.

Sadoshima, J., Izumo, S., 1997. The cellular and molecular response of cardiac myocytes to mechanical stress. Annu. Rev. Physiol. 59, 551–571.

Sata, M., Sugiura, S., Yoshizumi, M., Ouchi, Y., Hirata, Y., Nagai, R., 2001. Acute and chronic smooth muscle cell apoptosis after mechanical vascular injury can occur independently of the Fas-death pathway. Arterioscler. Thromb. Vasc. Biol. 21, 1733–1737.

Schlaepfer, D.D., Hauck, C.R., Sieg, D.J., 1999. Signaling through focal adhesion kinase. Prog. Biophys. Mol. Biol. 71, 435–478.

Schwartz, S.M., deBlois, D., O'Brien, E.R., 1995. The intima. Soil for atherosclerosis and restenosis. Circ. Res. 77, 445–465.

Shattil, S.J., Ginsberg, M.H., 1997. Integrin signaling in vascular biology. J. Clin. Invest. 100, S91–95.

Sheng, Z., Knowlton, K., Chen, J., Hoshijima, M., Brown, J.H., Chien, K.R., 1997. Cardiotrophin 1 (CT-1). Inhibition of cardiac myocyte apoptosis via a mitogen-activated protein kinase-dependent pathway. Divergence from downstream CT-1 signals for myocardial cell hypertrophy. J. Biol. Chem. 272, 5783–5791.

Shyy, J.Y., Chien, S., 1997. Role of integrins in cellular responses to mechanical stress and adhesion. Curr. Opin. Cell. Biol. 9, 707–713.

Siwik, D.A., Tzortzis, J.D., Pimental, D.R., Chang, D.L., Pagano, P.J., Singh, K., Sawyer, D.B., Colucci, W.S., 1999. Inhibition of copper-zinc superoxide dismutase induces cell growth, hypertrophic phenotype, and apoptosis in neonatal rat cardiac myocytes in vitro. Circ. Res. 85, 147–153.

Slomp, J., Gittenberger-de Groot, A.C., Glukhova, M.A., Conny van Munsteren, J., Kockx, M.M., Schwartz, S.M., Koteliansky, V.E., 1997. Differentiation, dedifferentiation, and apoptosis of smooth muscle cells during the development of the human ductus arteriosus. Arterioscler. Thromb. Vasc. Biol. 17, 1003–1009.

Smith, P.G., Tokui, T., Ikebe, M., 1995. Mechanical strain increases contractile enzyme activity in cultured airway smooth muscle cells. Am. J. Physiol. 268, L999–1005.

Sorger, P.K., 1991. Heat shock factor and the heat shock response. Cell 65, 363–366.

Steller, H., 1995. Mechanisms and genes of cellular suicide. Science 267, 1445–1449.

Sudhir, K., Wilson, E., Chatterjee, K., Ives, H.E., 1993. Mechanical strain and collagen potentiate mitogenic activity of angiotensin II in rat vascular smooth muscle cells. J. Clin. Invest. 92, 3003–3007.

Tea, B.S., Der Sarkissian, S., Touyz, R.M., Hamet, P., deBlois, D., 2000. Proapoptotic and growth-inhibitory role of angiotensin II type 2 receptor in vascular smooth muscle cells of spontaneously hypertensive rats in vivo. Hypertension 35, 1069–1073.

Teiger, E., Than, V.D., Richard, L., Wisnewsky, C., Tea, B.S., Gaboury, L., Tremblay, J., Schwartz, K., Hamet, P., 1996. Apoptosis in pressure overload-induced heart hypertrophy in the rat. J. Clin. Invest. 97, 2891–2897.

Tricot, O., Mallat, Z., Heymes, C., Belmin, J., Leseche, G., Tedgui, A., 2000. Relation between endothelial cell apoptosis and blood flow direction in human atherosclerotic plaques. Circulation 101, 2450–2453.

Udelsman, R., Blake, M.J., Stagg, C.A., Li, D.G., Putney, D.J., Holbrook, N.J., 1993. Vascular heat shock protein expression in response to stress. Endocrine and autonomic regulation of this age-dependent response. J. Clin. Invest. 91, 465–473.

Urbich, C., Walter, D.H., Zeiher, A.M., Dimmeler, S., 2000. Laminar shear stress upregulates integrin expression: role in endothelial cell adhesion and apoptosis. Circ. Res. 87, 683–689.

van Heerde, W.L., Robert-Offerman, S., Dumont, E., Hofstra, L., Doevendans, P.A., Smits, J.F., Daemen, M.J., Reutelingsperger, C.P., 2000. Markers of apoptosis in cardiovascular tissues: focus on Annexin V. Cardiovasc. Res. 45, 549–559.

Vermes, I., Haanen, C., Steffens-Nakken, H., Reutelingsperger, C., 1995. A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V. J. Immunol. Methods 184, 39–51.

Vogt, C., 1842. Untersuchungen ueber die Entwicklungsgeschichte der Geburtshelferkroete (Alytes obstetricans).

Wang, C.Y., Mayo, M.W., Korneluk, R.G., Goeddel, D.V., Baldwin Jr., A.S., 1998a. NF-kappaB antiapoptosis: induction of traf1 and traf2 and c-iap1 and c-iap2 to suppress caspase-8 activation. Science 281, 1680–1683.

Wang, Y., Huang, S., Sah, V.P., Ross Jr., J., Brown, J.H., Han, J., Chien, K.R., 1998b. Cardiac muscle cell hypertrophy and apoptosis induced by distinct members of the p38 mitogen-activated protein kinase family. J. Biol. Chem. 273, 2161–2168.

Wang, Y., Su, B., Sah, V.P., Brown, J.H., Han, J., Chien, K.R., 1998c. Cardiac hypertrophy induced by mitogen-activated protein kinase kinase 7, a specific activator for c-Jun NH2-terminal kinase in ventricular muscle cells. J. Biol. Chem. 273, 5423–5426.

Wang, C.Y., Guttridge, D.C., Mayo, M.W., Baldwin Jr., A.S., 1999. NF-kappaB induces expression of the Bcl-2 homologue A1/Bfl-1 to preferentially suppress chemotherapy-induced apoptosis. Mol. Cell. Biol. 19, 5923–5929.

Wilson, E., Mai, Q., Sudhir, K., Weiss, R.H., Ives, H.E., 1993. Mechanical strain induces growth of vascular smooth muscle cells via autocrine action of PDGF. J. Cell. Biol. 123, 741–747.

Wollert, K.C., Taga, T., Saito, M., Narazaki, M., Kishimoto, T., Glembotski, C.C., Vernallis, A.B., Heath, J.K., Pennica, D., Wood, W.I., Chien, K.R., 1996. Cardiotrophin-1 activates a distinct form of cardiac muscle cell hypertrophy. Assembly of sarcomeric units in series VIA gp130/leukemia inhibitory factor receptor-dependent pathways. J. Biol. Chem. 271, 9535–9545.

Wyllie, A.H., 1980. Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. Nature 284, 555–556.

Xu, Q., 2000a. Biomechanical-stress-induced signaling and gene expression in the development of arteriosclerosis. Trends Cardiovasc. Med. 10, 35–41.

Xu, Y., 2000b. How blood flows. Science 290, 1291.

Xu, Q., Wick, G., 1996. The role of heat shock proteins in protection and pathophysiology of the arterial wall. Mol. Med. Today 2, 372–379.

Xu, Q., Li, D.G., Holbrook, N.J., Udelsman, R., 1995. Acute hypertension induces heat-shock protein 70 gene expression in rat aorta. Circulation 92, 1223–1229.

Xu, Q., Fawcett, T.W., Udelsman, R., Holbrook, N.J., 1996. Activation of heat shock transcription factor 1 in rat aorta in response to high blood pressure. Hypertension 28, 53–57.

Xu, Q., Fawcett, T.W., Gorospe, M., Guyton, K.Z., Liu, Y., Holbrook, N.J., 1997a. Induction of mitogen-activated protein kinase phosphatase-1 during acute hypertension. Hypertension 30, 106–111.

Xu, Q., Hu, Y., Kleindienst, R., Wick, G., 1997b. Nitric oxide induces heat-shock protein 70 expression in vascular smooth muscle cells via activation of heat shock factor 1. J. Clin. Invest. 100, 1089–1097.

Xu, Q., Schett, G., Li, C., Hu, Y., Wick, G., 2000. Mechanical stress-induced heat shock protein 70 expression in vascular smooth muscle cells is regulated by Rac and Ras small G proteins but not mitogen-activated protein kinases (see comments). Circ. Res. 86, 1122–1128.

Yamada, T., Horiuchi, M., Dzau, V.J., 1996. Angiotensin II type 2 receptor mediates programmed cell death. Proc. Natl. Acad. Sci. USA 93, 156–160.

Yasukawa, H., Hoshijima, M., Gu, Y., Nakamura, T., Pradervand, S., Hanada, T., Hanakawa, Y., Yoshimura, A., Ross Jr., J., Chien, K.R., 2001. Suppressor of cytokine signaling-3 is a biomechanical stress-inducible gene that suppresses gp130-mediated cardiac myocyte hypertrophy and survival pathways. J. Clin. Invest. 108, 1459–1467.

Yokoyama, T., Nakano, M., Bednarczyk, J.L., McIntyre, B.W., Entman, M., Mann, D.L., 1997. Tumor necrosis factor-alpha provokes a hypertrophic growth response in adult cardiac myocytes. Circulation 95, 1247–1252.

Zhang, D., Gaussin, V., Taffet, G.E., Belaguli, N.S., Yamada, M., Schwartz, R.J., Michael, L.H., Overbeek, P.A., Schneider, M.D., 2000. TAK1 is activated in the myocardium after pressure overload and is sufficient to provoke heart failure in transgenic mice. Nat. Med. 6, 556–563.

Zou, Y., Dietrich, H., Hu, Y., Metzler, B., Wick, G., Xu, Q., 1998a. Mouse model of venous bypass graft arteriosclerosis. Am. J. Pathol. 153, 1301–1310.

Zou, Y., Hu, Y., Metzler, B., Xu, Q., 1998b. Signal transduction in arteriosclerosis: mechanical stress-activated MAP kinases in vascular smooth muscle cells (review). Int. J. Mol. Med. 1, 827–834.