Review

The regulation of vascular smooth muscle cell apoptosis

Nicola J. McCarthy, Martin R. Bennett*

Unit of Cardiovascular Medicine, Addenbrooke’s Centre for Clinical Investigation, Level 6, Box 110, Addenbrooke’s Hospital, Hills Road, Cambridge CB2 2QQ, UK

Received 23 June 1999; accepted 3 September 1999

Abstract

Apoptosis describes the morphological changes that identify a specific form of regulated cell death. Over recent years, the importance of either aberrant onset or suppression of apoptosis within tissues has become apparent and is associated with the development of several terminal diseases. Here we describe the relevance of apoptosis to the maintenance of vascular homeostasis. Specifically, we address the role of vascular smooth muscle cell death, how this may be regulated at the molecular level and whether any of these molecular mediators will provide targets for intervention in diseases such as atherosclerosis. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Apoptosis; Smooth muscle; Atherosclerosis; Arteries

1. Introduction

Apoptosis or programmed cell death is a process through which multicellular organisms efficiently dispose of cells. Since its initial description as a series of morphological events, much has been discovered about the molecular control of apoptosis. Along with the identification of genes that act to modulate this pathway has come the knowledge that regulation of cell death is critical for the maintenance of tissue homeostasis. Moreover, it is apparent that all cells are programmed to die. Indeed, cell death is their default state, which can be suppressed through the expression or presence of intracellular and extracellular survival factors. It may seem strange that cells can be lost so easily from tissues. However, for long-lived multicellular organisms it makes much biological sense to dispose rapidly of cells that, for whatever reason, may be of no use or potentially harmful. This is now very evident from work on genes that regulate cell cycle. Tumour suppressor genes such as RB and p53 and oncogenes such as c-myc that one would primarily associate with regulation of cell proliferation also induce cell death. Indeed, death and proliferation are two very tightly linked pathways. This paradoxical coupling safeguards the organism against cellular mutations that lead to unrestrained cell growth. A cell may acquire a mutation in a gene that promotes cell growth; however, this will also promote the death of the cell in the absence of survival factors, which are in limited supply in vivo. Thus, diseases that exhibit excess cellular accumulation are likely to have attained lesions that uncouple the link between proliferation and death. Conversely, diseases that exhibit increased cell death are likely to have become more sensitive to apoptotic stimuli possibly due to a defect in survival signalling pathways, or a defect in the regulation of cell cycle control.

Although the above arguments are generally well accepted in areas such as tumour biology, they have not yet been fully investigated in vascular biology. Vascular lesions such as atherosclerosis and restenosis after angioplasty arise in part from excessive accumulation of vascular smooth muscle cells (VSMCs), which may suggest reduced apoptosis in the diseased tissue. When vessels remodel either in physiological or pathological conditions, apoptosis and cell proliferation are intimately coupled. However, vascular atrophy in aneurysm formation is

*Corresponding author. Tel.: +44-1223-762-583; fax: 44-1223-331-505.
E-mail address: mrb@mole.bio.cam.ac.uk (M. Bennett)

0008-6363/00/$ – see front matter © 2000 Elsevier Science B.V. All rights reserved.
PII: S0008-6363(99)00275-8
accompanied by excessive apoptosis. Thus, too little and too much apoptosis within the vessel wall may be deleterious. This review seeks to analyse the evidence for VSMC apoptosis in vascular diseases, and identify potential regulatory pathways involved.

2. Cell proliferation and death in the vascular wall

Vascular smooth muscle cells within the vessel wall are able to proliferate, migrate, synthesise and degrade extracellular matrix upon receiving appropriate stimuli. In the undiseased wall cells are party to all the normal controls that govern cell proliferation and death, such as the presence of survival factors and mitogens, cell to cell contact inhibition and cell–matrix interactions. The normal adult artery shows very low levels of VSMC turnover, thus apoptotic and mitotic indices are low in this tissue [1]. In diseased tissue additional factors are present both locally, such as inflammatory cytokines, inflammatory cells and the presence of modified cholesterol, and systemically, such as blood pressure and flow. These factors can substantially alter the normal balance of cell proliferation and apoptosis, although the degree to which they are altered is dependent upon the vascular disease under study.

2.1. Remodelling

Vessel wall remodelling defines a condition in which alterations in luminal size can occur through processes that do not necessarily require large changes in overall cell number or tissue mass. Thus, redistribution of cells, either towards or away from the lumen, through processes such as selective cell proliferation/apoptosis or matrix synthesis/degradation can significantly alter lumen dimensions. Physiological remodelling occurs in closure of the ductus arteriosus due to the reduction in blood flow [2], and reduction in lumen size of infra-umbilical arteries after birth [3,4]. Surgical reduction in flow also results in compensatory reduction in VSMC numbers by apoptosis [5,6]. Remodelling also occurs in primary atherosclerosis, after angioplasty and in angioplasty restenosis. Although apoptosis undoubtedly occurs in all of these conditions (see below), the role of VSMC apoptosis in determining the outcome of remodelling is unclear.

A further example of vessel remodelling accompanied by VSMC apoptosis comes from studies examining both development and regression of vessel hypertrophy/hyperplasia in hypertension. Hypertension is associated with VSMC apoptosis in cells from SHR rats [7] and in SHR rats as hypertension develops [8,9]. In addition, relief of either systemic or pulmonary hypertension results in apoptosis of VSMCs in the affected artery, with evidence that some antihypertensives are more potent than others [9–11]. In particular, angiotensin converting enzyme inhibitors, angiotensin II receptor antagonists and calcium channel blockers can modify the contribution of apoptosis, independently of the blood pressure fall [9,10].

2.2. Arterial injury

Acute arterial injury, such as that occurring at angioplasty, is followed by rapid induction of medial cell apoptosis, at least in animal models. Thus, in rat or rabbit vessels, balloon overstretch injury results in medial cell apoptosis from 30 min–4 h after injury [12–14]. In pigs, apoptotic cells occur within the media at 6 h with peaks in the media, adventitia, and neointima at 18 h, 6 h, and 7 days after PTCA, respectively [15]. Although we have no direct evidence, the consistency of this response in animal models suggest that human vessels may behave similarly. Repair of the vessel after injury is also associated with VSMC apoptosis, both in the media and in the intima, and in the rat occurs 8–21 days after injury [16]. In humans, restenosis after angioplasty has been reported to be associated with either an increase [17], or decrease [18] in VSMC apoptosis. The role of VSMC apoptosis in either the initial injury or the remodelling process in restenosis is still unclear in human vessels.

2.3. Aneurysm formation

The commonest form of arterial aneurysm in humans is associated with advanced atherosclerosis, and is characterised by a loss of VSMCs from the vessel media, with fragmentation of elastin and matrix degradation, leading to progressive dilatation and eventually rupture. Apoptosis of VSMCs is increased in aortic aneurysms [19–21] compared with normal aorta and is associated with an increase in expression of a number of pro-apoptotic molecules, such as death receptors and p53 [19,21]. Macrophages and T-lymphocytes are found in aneurysmal lesions, suggesting that inflammatory mediators released by these cells may increase the loss of cells from these areas. Moreover, the production of tissue metalloproteinases by macrophages may accelerate cell death by degrading the extracellular matrix from which VSMCs derive survival signals (see below).

2.4. Atherosclerosis

Rupture of atherosclerotic plaques is associated with a thinning of the VSMC-rich fibrous cap overlying the core. Rupture occurs particularly at the shoulder regions of plaques, which are noted for their lack of VSMCs and the presence of macrophages and other inflammatory cells. Unsurprisingly then, apoptotic VSMC are evident in advanced human plaques [17,22,23], including the shoulder regions, prompting the suggestion that VSMC apoptosis may hasten plaque rupture. Indeed, there is evidence of increased VSMC apoptosis in unstable versus stable angina lesions [18]. However, there is no direct evidence of the
effect of apoptosis per se in the advanced human lesion. Most apoptotic cells in histological sections are found in advanced lesions next to the lipid core [24] and it is still not clear how many of these apoptotic cells are macrophages or VSMC. Loss of macrophages from atherosclerotic lesions is likely to promote plaque stability rather than rupture, since macrophages can promote VSMC apoptosis by both direct interactions [25] and by release of cytokines [26]. Of interest, apoptosis also occurs in early stages of atherosclerosis induced by cholesterol feeding in animals, at the fatty streak stage of before morphological evidence of lesion formation [27]. Again, the effect of apoptosis at this early stage of lesion development is unknown.

2.5. Effect of VSMC apoptosis

The effect of VSMC apoptosis is clearly context-dependent. Thus, VSMC apoptosis in advanced atherosclerotic plaques would be expected to promote plaque rupture (although there is as yet no direct evidence that it does so), and medial atrophy in aneurysm formation (where there is more evidence of such). In neointima formation post injury, VSMC apoptosis of both the intima and media can limit neointimal formation [13,14,28] at a defined time point (although long term studies have not been performed to ensure that the neointima is not simply delayed). It is not yet known whether such inhibition of neointimal formation in an animal model can translate into suppression of restenosis following angioplasty or stenting, but the evidence suggesting that most of angioplasty restenosis occurs through remodelling [29] and the near total failure of anti-proliferative therapy to inhibit restenosis does not augur well.

Therapeutic induction of apoptosis in the vessel wall may also be limited by important sequelae. In contrast to the prevailing notion that apoptotic deaths are effectively silent (that is, they do not elicit an immune response) a number of deleterious effects of apoptotic cells have emerged within the vasculature. First, the exposure of phosphatidylserine on the surface of apoptotic cells provides a potent substrate for the generation of thrombin and activation of the coagulation cascade [30,31]. Apoptotic cells release membrane-bound microparticles into the circulation which remain pro-coagulant and are increased in patients with unstable versus stable coronary syndromes [32,33]. Although apoptotic cells are not the only source of circulating microparticles, such micro-particles may contribute to the increased pro-coagulant state in these syndromes. Apoptotic VSMCs can also release both mitogens (bFGF) and pro-inflammatory cytokines such as MCP-1 resulting in recruitment of monocytes [34], both of which may abrogate any direct reduction in neointima formation. Indeed, in some studies massive induction of apoptosis in intimal VSMCs may reduce cell density but not overall neointimal size or increase lumen dimensions [34]. The demonstration that apoptosis releases active molecules rather negates the idea of apoptosis as a ‘silent’ mechanism of deleting cells. However, apoptotic cells that are not rapidly phagocytosed will begin to release inflammatory cytokines as they undergo secondary necrosis. This release of inflammatory cytokines will result in the recruitment of monocytes and macrophages to the surrounding area. This may well occur to allow phagocytosis of a large number of apoptotic cells that cannot be efficiently disposed of by surrounding healthy smooth muscle cells [35]. In atherosclerosis, inefficient phagocytosis of dead cells may occur due to the presence of modified LDL, which hampers phagocytosis of apoptotic cells [36]. Thus, it may be that death is initially silent in atherosclerotic lesions but as corpses mount up and professional phagocytes are impeded by modified LDL, more inflammatory cells may be recruited. Moreover, soluble death ligands such as Fas-L are released from the surface of macrophage/macrophages as they phagocytose, resulting in bystander death of adjacent neutrophils or monocytes [37], enhancing the deficit of professional phagocytic cells. There exists therefore the potential for the death and subsequent phagocytosis of apoptotic cells in atherosclerotic plaques to trigger further apoptosis, setting up a positive feedback loop.

3. Regulation of VSMC apoptosis

3.1. Apoptosis via death receptors

Many stimuli can trigger apoptosis in cells, but in vascular disease it is likely that specific alterations within the VSMC itself elicit sensitivity to a particular stimulus that is disease-associated. Thus, remodelling may trigger apoptosis following reduction in blood flow, and the major stimulus may therefore be flow-dependent stimuli such as nitric oxide or shear stress. In contrast, apoptosis in atherosclerosis or aneurysm formation may be due to the surrounding influences of inflammatory cells that express death ligands on their surface or secrete pro-apoptotic cytokines. The external triggers for apoptosis in many of these disease states have been reviewed recently [38,39]. Here, we will review the potential machinery by which an apoptotic stimulus may be transmitted and executed within VSMCs by comparing the data from VSMC models with other well established cell models of apoptosis.

The regulation of apoptosis within the cell can be simplified into two major pathways (Figs. 1 and 2). First, membrane-bound death receptors of the tumour necrosis receptor family (TNF-R), such as Fas (CD95), TNF-R1, death receptor (DR)-3, DR4 and DR5 bind their trimerised ligands causing receptor aggregation, and subsequent recruitment of a number of adapter proteins through protein: protein interactions [40] (see Fig. 1). For example, binding of agonistic anti-Fas monoclonal antibody or its
natural ligand Fas-ligand to its cognate receptor induces receptor trimerisation, with subsequent recruitment of adapter molecules such as FADD and RIP to the receptor

complex [41–43]. In turn, FADD and RIP recruit cell death cysteine proteases (caspases) such as caspase 8 (FLICE) and caspase 2 to the complex [44]. Within the

Fig. 1. Schematic of Fas death signalling pathways. Fas, the prototypic member of the TNF death receptor family binds to its cognate ligand. Recruitment of the adapter molecule FADD and procaspase 8 results in activation of the latter. Caspase 8 activation directly activates downstream caspases (3, 6 and 7) which results in DNA fragmentation and cleavage of cellular proteins. This pathway is thought to occur in type I cells and does not involve mitochondrial pathways. Caspase 8 activation also results in cleavage of Bid, which translocates and interacts with other Bcl-2 family members (see Fig. 2).

Fig. 2. Schematic of mitochondrial death signalling pathways. Anti-apoptotic members of the Bcl-2 family, such as Bcl-2 and Bcl-X are located on the mitochondrial outer membrane. Here they act to prevent the release of apoptogenic factors from the inner mitochondrial space. Binding of the pro-apoptotic proteins Bid (after cleavage by caspase 8) or Bad (after dephosphorylation) to Bcl-2 mitigates the protective effect of Bcl-2 and triggers release of cytochrome C. Cytochrome C, in concert with the adapter protein apaf-1 and caspase 9 activates caspase 3 and the downstream caspase cascade. Stimuli such as growth factor withdrawal, deregulated oncogene (c-myc, E1A) expression, activation of p53 and Fas activation in type II cells act through this mitochondrial pathway.
complex of Fas, FADD and caspase 8 (known as the death-inducing signalling complex (DISC)), caspase 8 becomes proteolytically activated by oligomerisation [45]. This facilitates the subsequent activation of terminal effector caspases such as caspases 3, 6 and 7 [46–50] responsible for cleavage of intracellular substrates required for cellular survival, architecture and metabolic function [51,52]. Caspase activation is also responsible for many of the hallmarks of apoptosis, such as DNA fragmentation, chromatin condensation and apoptotic body formation [53–55]. The major active caspases in Fas-mediated apoptosis are caspases 8, 3, 6 and 7 [55], with stepwise appearance of active caspases suggesting a caspase cascade. Cells in which caspase 8 is expressed in abundance, recently termed type I cells, use this pathway of direct caspase 3 activation. Moreover, in these cells, Fas mediated cell death cannot be inhibited by anti-apoptotic factors such as Bcl-2 and Bcl-XL since the pathway does not require amplification by pro-apoptotic factors released by mitochondria [56].

However, in some cells caspase 8 is not abundantly expressed and cannot activate caspase 3 and other downstream caspases directly. Instead, in these cells termed type II cells [56], caspase 8 activation causes cleavage of proteins of the bcl-2 family such as bid [57] (see Fig. 2). Bcl-2 family members are characterised as either pro-apoptotic (Bax, Bid, Bik, Bak) or anti-apoptotic (Bcl-2, Bcl-XL). Activation of pro-apoptotic Bcl-2 family members causes their translocation to mitochondria, where they interact with anti-apoptotic members that are components of the mitochondrial membrane. This interaction causes changes in voltage-dependent mitochondrial channels and releases mitochondrial mediators of apoptosis such as cytochrome c [58]. In turn the association of cytochrome c with an adapter molecule apaf-1 and caspase 9 activates caspase 3, and the caspase cascade. Thus, death induced by Fas signalling may or may not be inhibitable by Bcl-2 family members suggesting that high levels of expression of anti-apoptotic Bcl-2 family members do not automatically correlate with suppression of cell death. The classification of human VSMCs into type I or type II cells has yet to be made, however, the kinetics of cell death in response to anti-Fas antibodies suggests that they are type II cells.

Fas-induced apoptosis can also be blocked by expression of several intracellular proteins, including FLIPs (FLICE inhibitory protein) and IAPs (inhibitor of apoptosis) (see Fig. 1). FLIPs are similar to caspase molecules, having the same pro-domain structure as caspase 8, but not the active caspase site within the C-terminus. The pro-domain of caspase 8 has two protein; protein interaction motifs called death effector domains (DEDs) that are also found in FADD. These DEDs facilitate the binding of FADD to caspase 8 and the binding of FLIP to FADD. Caspase 8 is activated upon binding to FADD via a series of cleavage reactions. FLIP undergoes the first of these cleavages, but not the second, preventing the activation of caspase 8 [59]. In contrast, IAPs can inhibit the enzymatic activity of downstream caspases [60–62] (see below), or they can mediate anti-apoptotic signalling pathways through the activation of nuclear transcription factor B (NF-κB) [63].

Human VSMCs express both Fas and TNF-R1 [64], and given the widespread occurrence of TRAIL-Rs are also likely to express these receptors. T-lymphocytes and macrophages within the atherosclerotic plaque express CD95 ligand and TNF-α, and interaction between membrane-bound ligands on T-cells and receptors on VSMC may induce the death of VSMC. VSMCs can also express Fas-L and TNF-α, which exist as membrane-bound or soluble forms. The soluble forms are cleaved from the membrane-bound forms by a metalloproteinase of the ADAM family. Interestingly, a recent study of the tissue inhibitors of metalloproteinases (TIMPs), indicates that TIMP-3 can induce VSMC apoptosis in vitro and in vivo [65], which may be acting by stabilising expression of the more potent membrane forms of death ligands on the VSMC surface. Although overexpression of FADD can induce VSMC apoptosis in vitro and in vivo [34], soluble ligand binding to death receptors is a very weak inducer of apoptosis in VSMCs [26,66], and does not induce apoptosis in the absence of ‘priming’ of the cell, usually with cycloheximide. Some of this resistance can be explained by the observation that death receptors are sequestered intracellularly in VSMCs [66], and priming may be associated with increased surface expression. Physiologically, increased death receptor expression can be achieved via combination of cytokines such as IL-1-β, IFN-γ and TNF-α [64,67,68], possibly via NO and p53 stabilisation [26,68–70], or via direct activation of p53 [66]. Free radicals and NO can also induce apoptosis which may be independent of p53 but associated with caspase 3 activation [71–76], but not associated with death receptor signalling. In contrast, efficient death receptor-mediated apoptosis of VSMCs can be achieved by high level expression of death ligands such as Fas-L [28], possibly by increasing expression of membrane-bound forms of these ligands. Thus, DNA damage induced by nitric oxide, anoxia or free radical formation for instance may stabilise p53, effectively priming VSMCs to death receptor mediated apoptosis.

Irrespective of the local environment, VSMCs derived from atherosclerotic plaques show increased rates of apoptosis in culture compared with cells from normal vessels, reflecting an intrinsic sensitivity to apoptosis [77]. This appears to be a stable property, and is part of the phenotype of plaque VSMCs that includes slow proliferation [78]. The classiﬁcation of VSMCs associated with death receptor expression is likely to reﬂect differences in expression of pro- and anti-apoptotic molecules, speciﬁcally those regulating signalling from survival cytokines, cell:cell and cell:matrix interactions.
interactions and members of the bcl-2 family. Indeed, Insulin-like growth factor 1 (IGF-1) is a potent survival factor for normal VSMCs, although IGF-1 cannot completely inhibit plaque VSMC apoptosis in vitro after serum withdrawal [77]. Similarly, inhibition of bFGF binding induces VSMC apoptosis in vitro [80,81] and in vivo [82], possibly by induction of the oncogene mdm2 which inactivates p53 [83].

Evidence suggesting the critical role of bcl-2 family members in regulating VSMC apoptosis has come from both in vitro and in vivo studies. Human VSMCs express low levels of Bcl-2 [77,84], but Bax is seen particularly in atherosclerotic plaques, both in human and animal models of atherosclerosis and injury [84–86]. In addition, spontaneous and growth-factor withdrawal-induced apoptosis can be inhibited by overexpression of bcl-2 [77]. In vivo, rat VSMCs express minimal Bcl-2, but high levels of Bcl-X can be found after injury [86]. Indeed, inhibition of Bcl-X can dramatically induce apoptosis of VSMCs after balloon injury [13] and differences in expression of Bcl-X may account for differences in sensitivity to apoptosis after injury of intimal versus medial VSMCs [14]. The reduced levels of VSMCs apoptosis seen after cholesterol lowering in rabbit models of atherosclerosis is also associated with a loss of Bax immunoreactivity [85], arguing for a pro-apoptotic role of this protein in VSMC apoptosis. However, it should be noted that excessive reliance on immunocytochemistry of one member of the bcl-2 family to ascertain a role for that protein in vivo should be avoided. Although Bcl-X is upregulated after injury, in rats it is the short Bcl-x, or pro-apoptotic form that appears to predomi-
nate [86].

Regulation of sensitivity to apoptosis in VSMCs is also mediated by expression of IAP proteins [87] and individual caspases [79,88], and it is likely that there are marked differences in expression of multiple proteins which reg-
ulate VSMC apoptosis of individual VSMCs in response to specific stimuli. This may underlie observations that despite (apparently) the same stimulus for apoptosis, VSMC apoptosis in the either the normal or diseased vessel wall is highly localised.

3.2. Cell proliferation and apoptosis

Considerable evidence suggests that cell proliferation and apoptosis are linked. Thus, growth arrest often causes resistance to apoptosis in response to specific signals [89], and in some cases cells may undergo apoptosis at defined points in the cell cycle [90]. Considerable overlap also exists between the components that execute both pro-
cesses. For example, activation of the mitogen-activated protein kinase cascade can promote apoptosis as well as proliferation [91,92] and activation or cleavage of cell cycle proteins occurs in apoptosis as it does in mitosis [93,94]. Cell proliferation and apoptosis can also be induced by the same mediator, but either outcome requires different molecular pathways. For example, the transcription factor E2F-1 induces S-phase entry by transcriptional activation of target genes, but induces apoptosis most likely via transcriptional repression [95,96]. In addition, there is marked heterogeneity in the ability of E2F family members to promote cell proliferation and apoptosis. For example, despite E2F-2 and E2F-3 also inducing S-phase entry, neither are capable of inducing apoptosis [97].

In VSMCs, deregulated expression of a number of proto-
oncogenes, such as c-myc or the adenovirus gene product E1A, promotes both apoptosis and cell proliferation simultaneously, implying that the functional separation of these processes lies downstream of expression of both proteins [98,99]. Whilst the activity of E1A is partly due to its binding to the retinoblastoma gene product RB, releasing the E2F family of transcription factors and allowing induction of S-phase genes, the mechanism of c-myc-induced cell proliferation and apoptosis is largely un-
known.

The tumour suppressor gene RB arrests cells in G1 of the cell cycle when in its active, hypophosphorylated form. Loss of this gene would be predicted to facilitate proliferation. However, RB deficient animals are not viable due to massive cell death in the haematopoietic and nervous systems. This evidence suggests that genes that act to regulate cell cycle are ‘booby-trapped’. That is, when mutated or lost the ability to confer autonomous growth is dampened by the ability to also induce death or profound growth arrest. This suggests a cell with a proliferative lesion is likely to be much more sensitive to death signals and a cell that has enhanced survival capacity is subject to profound cell cycle arrest [100]. Thus, exposure of VSMC to inflammatory cytokines and other cytokines such as platelet derived growth factor [101] and bFGF initiates a mitogenic signal within the VSMC, but if this is not balanced by the presence of a survival signal cells enter apoptosis and not proliferation.

The phenotype (low proliferation, early senescence, increased apoptosis) of VSMCs in atherosclerotic plaques does not fit the above arguments. However, it may be that plaque VSMC’s have a complex set of lesions in both cell cycle and apoptotic pathways. Moreover, there are many different signals impinging on the plaque VSMC that may significantly alter their sensitivity to cell death stimuli. Although we cannot at present explain the phenotype of plaque VSMCs in terms of existing models, there is good evidence that these cells are senescent. Populations of plaque VSMCs show increased hypophosphorylated RB (the active form) which may account for their slow proliferation and senescence. However, whenever these cells are stimulated to proliferate, for example by virus gene products or E2F-1, cells undergo massive apoptosis, which is, in part, mediated by p53 [102]. Of interest, p53 alone does not induce apoptosis in either normal human or rat VSMCs in vitro or in vivo unless the cells are primed to die [89,103,104], or massive expression is used via
adenovirus vectors [105]. However, stabilisation of p53 may sensitise the cells to other apoptotic triggers such as Fas ligand [66]. In vivo, plaque VSMCs show increased expression of p53 and p21, the cell cycle inhibitor expressed upon stabilisation of p53 [19,106]. Moreover, cells which express p53 and p21 do not express proliferative markers such as Ki67, suggesting that these cells are non-proliferative [106]. Excessive p53 activation is an attractive hypothesis to account for the biological properties of plaque VSMCs; however, basal expression and activity of p53 in plaque and normal VSMCs is similar [89] and p53 deficient animal models of atherosclerosis exhibit enhanced cell proliferation, but do not show statistically significant changes in the number of apoptotic cells found with the lesions compared to controls. On the surface this indicates that the effect of p53 in VSMC biology in atherosclerosis may be growth arrest rather than induction of apoptosis [107]. However, it should be noted that this study examined only early atherosclerotic plaques up to 17 weeks of age in ApoE mice, and did not discriminate between apoptosis in VSMCs or macrophages. Therefore from this study it is not possible to determine the role of p53 in VSMC death in vivo. In addition, the majority of proliferating cells in these lesions appeared to be macrophages and T-lymphocytes. A formal demonstration of an increase in VSMC proliferation in these p53-deficient animals was not made. It is thus still possible that a combination of profound growth arrest and lack of survival signals, complexed with proliferative stimuli generated by inflammatory cytokines or mitogens may lead to the sensitisation of VSMCs to apoptotic triggers.

4. Summary

There is now much evidence for VSMC apoptotic cell death occurring in the vasculature in both physiological and pathological contexts. These deaths are regulated by a known body of proteins that serve to either induce or protect against apoptosis. We are now beginning to understand the complex biology observed in lesions such as atherosclerosis and to identify potential pro-apoptotic factors that may lead to the loss of cells from the vasculature. Sensitivity to apoptosis is determined by expression of cell death receptors and ligands, and by multiple protein species below receptor level. In addition, sensitivity to apoptosis is determined by the presence and response to survival cytokines, mitogens, and local cell and matrix interactions, and by the growth status of the cell. However, much of this research has been carried out in vitro where it is not possible to study all of the physiological signals received by cells in vivo. Future studies in animal models should help to identify which of the pro and anti-apoptotic factors that are effective in vitro are also relevant in vivo. Moreover, a closer examination of the population dynamics of vascular cells within the vessel wall will aid the understanding of the timing and triggers of VSMC apoptosis in disease.

References


[74] Nishio E, Watanabe Y. NO induced apoptosis accompanying the induced apoptosis requires DNA binding but not transactivation and participation of c-Myc-induced apoptosis by Ras signalling through PI3K and PKB. Nature 1997;385:544–548.


