Mercer, J.R., and Bennett, M. (2006) The role of p53 in atherosclerosis. Cell Cycle, 5 (17). pp. 1907-1909. ISSN 1538-4101

Copyright © 2006 Landes Bioscience

A copy can be downloaded for personal non-commercial research or study, without prior permission or charge

The content must not be changed in any way or reproduced in any format or medium without the formal permission of the copyright holder(s)

When referring to this work, full bibliographic details must be given

http://eprints.gla.ac.uk/80568/

Deposited on: 3 June 2013

Enlighten – Research publications by members of the University of Glasgow
http://eprints.gla.ac.uk

[Cell Cycle 5:17, 1907-1909, 1 September 2006]; ©2006 Landes Bioscience

Evaluation

The Role of p53 in Atherosclerosis

John Mercer

Martin Bennett*

Division of Cardiovascular Medicine; University of Cambridge; Addenbrooke's Hospital; Cambridge; UK

*Correspondence to: Professor Martin Bennett; British Heart Foundation Professor of Cardiovascular Sciences; Division of Cardiovascular Medicine; University of Cambridge; Box 110; Addenbrooke's Hospital; Cambridge; CB2 2QQ, UK; Tel.: +44.1223.331504; Fax: +44.1223.331505; Email: mrb@mole.bio.cam.ac.uk

Original manuscript submitted: 07/04/06
Manuscript accepted: 07/12/06

Previously published online as a *Cell Cycle* E-publication:
http://www.landesbioscience.com/journals/cc/abstract.php?id=3166

KEY WORDS
p53, apoptosis, atherosclerosis, cell senescence

ABBREVIATIONS

Ad-p53	adenovirus vector encoding p53
ApoE	apolipoprotein E
JNK	c-Jun NH(2)-terminal kinase
LDL	Low density lipoprotein
MDM2	mouse double minute 2
VSMCs	vascular smooth muscle cells

ACKNOWLEDGEMENTS

J.M. and M.R.B. are supported by BHF grants RG/04/001 and CH/2000003.

ABSTRACT

Although the role of the tumor suppressor gene p53 is well known in cancer, recent studies have highlighted a fundamental role for p53 in regulating cells in the advanced atherosclerotic plaque, the major cause of heart attacks and stroke. In particular, p53 is activated in the complex environment of the plaque, in part by DNA damage within the lesion, and regulates growth arrest, cell senescence and apoptosis of vascular smooth muscle cells (VSMCs). The role of endogenous p53 has been determined using p53 knockout in mice developing advanced atherosclerosis, using bone marrow transplant to separate effects on blood cells from vessel wall cells. These studies have produced apparently contradictory and surprising results. In particular, recent studies have identified a role for endogenous p53 in protection of VSMCs from apoptosis, trans-differentiation of bone marrow stromal cells into VSMCs in atherosclerosis, and altering the mode of cell death in the plaque.

INTRODUCTION

Heart attacks and stroke are the commonest cause of death in the Western world, and by 2020 are predicted to be the commonest cause of death worldwide. Both diseases are clinical manifestations of atherosclerosis, the thickening of the innermost layer of the artery wall. Atherosclerotic plaques consist of an accumulation of vascular smooth muscle cells (VSMCs), inflammatory cells (macrophages, T lymphocytes, dendritic cells and mast cells) underlying a dysfunctional endothelium, together with extracellular lipid, collagen and matrix. Recruitment of circulating inflammatory cells is an early event in atherosclerosis, which triggers reactive proliferation and migration of VSMCs of the vessel wall. There is also increasing evidence that circulating cells can transdifferentiate into VSMCs that comprise native atherosclerotic plaques and a variety of other vascular pathologies.[1,2] Most atherosclerosis is clinically silent, and consequences of atherosclerosis rarely occur before the development of advanced lesions. Many advanced plaques comprise a VSMC-rich fibrous cap overlying a lipid and macrophage rich necrotic core, and it is the relative proportion of these components that determine the clinical manifestations of the plaque. For example, unstable plaques (that are prone to rupture) contain a higher proportion of inflammatory cells and lipid, and a lower proportion of VSMCs than stable lesions. In particular, the fibrous cap of advanced plaques is thinned from loss of VSMCs, and these thin-cap fibroatheromata are the commonest lesions that rupture to produce fatal heart attacks.

p53 expression and phosphorylation is increased in advanced human plaques, in association with oxidative DNA damage and activation of DNA repair pathways.[3-5] The inability to find p53 mutations in atherosclerosis[3] together with colocalization with MDM2 and p21[4,6] suggests that detected p53 is wild type and is transcriptionally active. Thus, the major effects of p53 in atherosclerosis are likely to be due to augmentation of wild type p53 activity, including growth arrest, senescence and apoptosis. Indeed, plaque VSMCs show impaired proliferation compared with cells from normal vessels and early senescence,[7] coupled with expression of typical pRB-mediated growth arrest proteins, including the p53 target p21. In vitro, inhibition of p53 and pRB bypasses the growth arrest and cell senescence of plaque VSMCs, demonstrating that premature senescence of human plaque VSMCs is determined by pRB and p53.[7] In vitro, plaque VSMCs have increased sensitivity to p53-induced apoptosis.[8] This is partly due to impaired protection in plaque VSMCs, but p53 can also sensitize cells to death receptor signaling.[9]

©2006 LANDES BIOSCIENCE. DO NOT DISTRIBUTE.

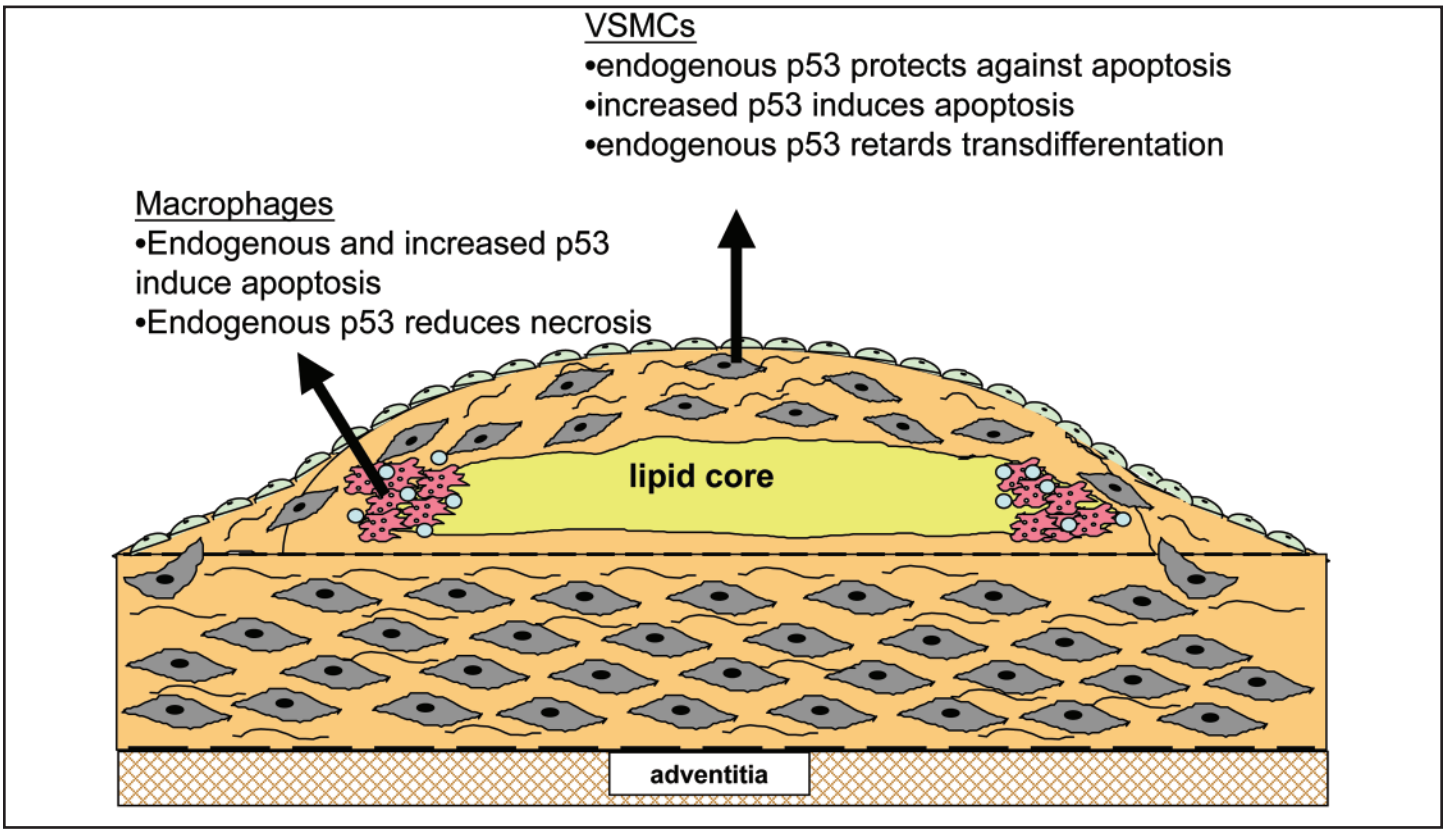

Figure 1. Schematic of an advanced atherosclerotic plaque showing a VSMC-rich fibrous cap separating a lipid rich necrotic core from the vessel lumen. Plaque macrophages are indicated as red cells in the shoulder region of the plaque, the most frequent site of plaque rupture. Whilst endogenous p53 protects VSMCs from apoptosis, in part by promoting DNA repair, increased p53 above basal levels induces apoptosis. Endogenous p53 also inhibits transdifferentiation of bone marrow-derived stromal cells into VSMCs. In contrast, endogenous and increased p53 promote macrophages apoptosis; loss of p53 in macrophages may lead to secondary necrosis.

Whilst what p53 does in vitro in VSMCs appears straightforward, and is similar to other mesenchymal cells, the role of p53 in VSMC proliferation and apoptosis in atherosclerosis is more controversial. p53 expression is negatively correlated with markers of cell proliferation in human atherosclerosis,[4] suggesting that p53 inhibits cell proliferation in vivo. Indeed, adenovirus expression of p53 (Ad-p53) reduces cell proliferation in the rat carotid artery[10] or migration in the human saphenous vein[11] and conversely, antisense oligonucleotides to p53 increase proliferation.[12] p53 expression is associated with increased apoptosis in vivo and in vitro.[13-15] Ad-p53 induces VSMC apoptosis and plaque rupture in a collar model of atherosclerosis in ApoE knockout mice that develop accelerated atherosclerosis,[16] and apoptosis in human saphenous vein intima and media.[11]

These studies all suggest that p53 inhibits cell proliferation and promotes VSMC apoptosis in atherosclerosis. However, direct in vivo evidence from separate studies is contradictory, and in particular, the effect of p53 deficiency on different cell types is unclear.[17-19] Mice deficient for p53 crossed onto a variety of atherosclerosis-prone mice (ApoE$^{-/-}$, ApoE*3-Leiden, or LDL-R$^{-/-}$) develop accelerated atherosclerosis compared with p53$^{+/+}$ mice. However, two studies from the same group found that p53$^{-/-}$ mice show increased cell proliferation but no change in apoptosis,[17,19] whereas the third study demonstrated reduced apoptosis, but no change in cell proliferation.[18] Although these studies have demonstrated effects of p53 on macrophages by bone marrow transplant,[18,19] none of the studies identified the lineage of the proliferating/dying cells. In addition, none of the studies examined the specific role of p53 knockout in VSMCs, as transplants were performed of p53$^{+/+}$ or p53$^{-/-}$ cells into p53$^{+/+}$ mice, so that VSMCs derived from the vessel wall were all p53$^{+/+}$. Finally, the effects of p53 were not examined in more advanced lesions, where p53 is more expressed than in early plaques.

This situation appears to have been clarified by two recent studies. In the first, we examined atherosclerosis in advanced lesions of p53$^{-/-}$/ApoE$^{-/-}$ mice vs. ApoE$^{-/-}$ mice expressing wild type p53. Cell lineage of proliferating and apoptotic cells was examined. p53$^{-/-}$/ApoE$^{-/-}$ mice showed increased aortic plaque formation, with increased rates of cell proliferation and reduced rates of apoptosis in brachiocephalic artery plaques. Although most proliferating cells were monocyte/macrophages, apoptotic cells were both vascular smooth muscle cells (VSMCs) and macrophages. Transplant of p53$^{+/+}$ bone marrow to p53$^{-/-}$/ApoE$^{-/-}$ mice reduced aortic plaque formation and cell proliferation in brachiocephalic plaques, but also markedly reduced apoptosis. These findings indicate that the major effect of endogenous p53 in atherosclerosis is to limit proliferation, but also protect against apoptosis.

We studied VSMCs in culture to examine this potential anti-apoptotic effect. Indeed, p53$^{-/-}$ VSMCs showed enhanced apoptosis following serum deprivation, UV irradiation and etoposide treatment, and was associated with activation of DNA damage pathways. Reexpression of p53 using a conditional allele rescued apoptosis and inhibited the DNA damage response. We also examined the ability of p53 to alter the trans-differentiation of bone marrow stromal cells into cells that resemble VSMCs. Endogenous

p53 inhibited the trans-differentiation of stromal cells, and also inhibited their apoptosis. In contrast, $p53^{-/-}$ macrophages showed the predicted resistance to apoptosis compared with $p53^{+/+}$ macrophages.

More recently, macrophage specific p53 deficiency has been achieved using cre-lox technology, to examine the role of endogenous p53 in atherosclerosis in $ApoE^{-/-}$ mice.[20] Surprisingly, p53 deficiency in macrophages reduced apoptosis but increased necrosis in these lesions.

These findings identify novel anti-apoptotic functions of endogenous p53 in VSMCs and stromal cells that differentiate into VSMCs. The protective effect of endogenous p53 in fibroblasts has been noted before, and mapped to the C-terminal 38 amino acids.[21,22] Although this activity did not require transcriptional activity, its mechanism is currently unclear. We find that endogenous p53 inhibits DNA damage signaling in VSMCs, consistent with published studies that demonstrate a role in repair of single strand breaks, nucleotide excision repair and homologous recombination.[23-26] In addition, p53 protects the genome from oxidation by reactive oxygen species (ROS), a major cause of DNA damage in atherosclerosis. In the absence of severe stresses, relatively low levels of p53 are sufficient for upregulation of several genes with antioxidant products, associated with a decrease in intracellular ROS.[27] Downregulation of p53 results in excessive oxidation of DNA,[27] which may trigger the activation of the DNA damage response pathways we observe in $p53^{-/-}$ cells, ultimately promoting apoptosis. p53 can also prevent the release of pro-apoptotic molecules from the mitochondria, in part by suppressing JNK activity.[28] Our studies also suggest that p53 regulates the differentiation of stromal cells into VSMCs, at least in vitro. Again, the mechanism of this effect is unclear, and it remains possible that it is secondary to the enhanced ability of stromal cells from $p53^{-/-}$ mice to survive and proliferate in culture in conditions that promote differentiation.

At last it appears we can draw some firm conclusions from these studies (Fig. 1). First, global deficiency of p53 increases atherosclerosis formation. This appears to be due to an increase in proliferation of VSMCs. Secondly, p53 deficiency protects macrophages from apoptosis, but may promote macrophage necrosis, so that overall death rates of macrophages may not change. Thirdly, p53 deficiency in VSMCs appears to be actively protective; this may be due in part to its ability to promote DNA repair and turn off the response to DNA damage.

In conclusion, it is clear that in a complex tissue such as the atherosclerotic plaque, the effects of both loss of p53 and p53 overexpression depend upon the cell type under study. We cannot simply extrapolate the results from tumor cell lines which contain multiple mutations (such as oncogene activation) in addition to loss of p53, or embryonic fibroblasts, which have proliferative potential far above adult human mesenchymal cells, to explain how p53 functions in human atherosclerosis. p53 can promote growth arrest, cell senescence and apoptosis in atherosclerosis, in line with its conventional properties. However, endogenous p53 can also regulate trans-differentiation, protect against apoptosis and alter the mode of cell death within the plaque. Only by selectively altering p53 expression in different components of the plaque do these more subtle activities emerge.

References

1. Sata M, Saiura A, Kunisato A, Tojo A, Okada S, Tokuhisa T, Hirai H, Makuuchi M, Hirata Y, Nagai R. Hematopoietic stem cells differentiate into vascular cells that participate in the pathogenesis of atherosclerosis. Nat Med 2002; 8:403-9.
2. Caplice NM, Bunch TJ, Stalboerger PG, Wang S, Simper D, Miller DV, Russell SJ, Litzow MR, Edwards WD. Smooth muscle cells in human coronary atherosclerosis can originate from cells administered at marrow transplantation. Proc Natl Acad Sci USA 2003; 100:4754-9.
3. Iacopetta B, Wysocki S, Norman P, House A. The *p53* tumor suppressor gene is overexpressed but not mutated in human atherosclerotic tissue. Int J Oncol 1995; 7:399-402.
4. Ihling C, Menzel G, Wellens E, Monting JS, Schaefer HE, Zeiher AM. Topographical association between the cyclin-dependent kinases inhibitor p21, p53 accumulation, and cellular proliferation in human atherosclerotic tissue. Art Thromb Vasc Biol 1997; 17:2218-24.
5. Martinet W, Knaapen M, De Meyer G, Herman A, Kockx M. Elevated levels of oxidative DNA damage and DNA repair enzymes in human atherosclerotic plaques. Circ Res 2002; 106:927-32.
6. Ihling C, Haendeler J, Menzel G, Hess RD, Fraedrich G, Schaefer HE, Zeiher AM. Coexpression of p53 and MDM2 in human atherosclerosis: Implications for the regulation of cellularity of atherosclerotic lesions. J Pathol 1998; 185:303-12.
7. Bennett MR, Macdonald K, Chan SW, Boyle JJ, Weissberg PL. Cooperative interactions between RB and p53 regulate cell proliferation, cell senescence, and apoptosis in human vascular smooth muscle cells from atherosclerotic plaques. Circ Res 1998; 82:704-12.
8. Bennett MR, Littlewood TD, Schwartz SM, Weissberg PL. Increased sensitivity of human vascular smooth muscle cells from atherosclerotic plaque to p53-mediated apoptosis. Circ Res 1997; 81:591-9.
9. Bennett M, Macdonald K, Chan SW, Simari R, Luzio J, Weissberg P. Cell surface trafficking of Fas: A rapid mechanism of p53-mediated apoptosis. Science 1998; 282:290-3.
10. Scheinman M, Ascher E, Levi GS, Hingorani A, Shirazian D, Seth P. *p53* gene transfer to the injured rat carotid artery decreases neointimal formation. J Vasc Surg 1999; 29:360-9.
11. George SJ, Angelini GD, Capogrossi MC, Baker AH. Wild-type *p53* gene transfer inhibits neointima formation in human saphenous vein by modulation of smooth muscle cell migration and induction of apoptosis. Gene Ther 2001; 8:668-76.
12. Matsushita H, Morishita R, Aoki M, Tomita N, Taniyama Y, Nakagami H, Shimozato T, Higaki J, Kaneda Y, Ogihara T. Transfection of antisense *p53* tumor suppressor gene oligodeoxynucleotides into rat carotid artery results in abnormal growth of vascular smooth muscle cells. Circulation 2000; 101:1447-52.
13. Li ZH, Lakatta EG. Localized apoptosis and enhanced expression of p53 and interleukin-1 beta converting enzyme (ICE) proteins in human aortic atherosclerotic lesions. Atherosclerosis 1997; 134:299.
14. Hayashi S, Morishita R, Higaki J, Tomita N, Ogihara T. Inhibition of growth of human aortic vascular smooth muscle cells by a phosphodiesterase type 3 inhibitor, cilostazol, through p53- mediated apoptosis. Atherosclerosis 1997; 134:257.
15. Kinscherf R, Claus R, Wagner M, Gehrke C, Kamencic H, Hou D, Nauen O, Schmiedt W, Kovacs G, Pill J, Metz J, Deigner HP. Apoptosis caused by oxidized LDL is manganese superoxide dismutase and p53 dependent. Faseb Journal 1998; 12:461-7.
16. von der Thusen JH, van Vlijmen BJ, Hoeben RC, Kockx MM, Havekes LM, van Berkel TJ, Biessen EA. Induction of atherosclerotic plaque rupture in *apolipoprotein E-/-* mice after adenovirus-mediated transfer of *p53*. Circulation 2002; 105:2064-70.
17. Guevara N, Kim HS, Antonova E, Chan L. The absence of p53 accelerates atherosclerosis by increasing cell proliferation in vivo. Nature Med 1999; 5:335-9.
18. van Vlijmen BJ, Gerritsen G, Franken AL, Boesten LS, Kockx MM, Gijbels MJ, Vierboom MP, van Eck M, van De Water B, van Berkel TJ, Havekes LM. Macrophage p53 deficiency leads to enhanced atherosclerosis in *APOE*3-Leiden* transgenic mice. Circ Res 2001; 88:780-6.
19. Merched AJ, Williams E, Chan L. Macrophage-specific p53 expression plays a crucial role in atherosclerosis development and plaque remodeling. Arterioscler Thromb Vasc Biol 2003; 23:1608-4.
20. de Winther M. Macrophage function in atherogenesis. Immmunology 2005; 116:59, (Abstract).
21. Lassus P, Ferlin M, Piette J, Hibner U. Anti-apoptotic activity of low levels of wild type p53. EMBO J 1996; 15:4566-73.
22. Lassus P, Bertrand C, Zugasti O, Chambon JP, Soussi T, MathieuMahul D, Hibner U. Anti-apoptotic activity of p53 maps to the COOH-terminal domain and is retained in a highly oncogenic natural mutant. Oncogene 1999; 18:4699-709.
23. Bakalkin G, Yakovleva T, Selivanova G, Magnusson K, Szekely L, Kiseleva E, Klein G, Terenius L, Wiman K. p53 binds single stranded DNA ends and catalyzes renaturation and strand transfer. Proc Natl Acad Sci USA 1994; 91:413-7.
24. Tron VA, Trotter MJ, Ishikawa T, Ho VC, Li G. p53-dependent regulation of nucleotide excision repair in murine epidermis in vivo. J Cutan Med Surg 1998; 3:16-20.
25. Linke SP, Sengupta S, Khabie N, Jeffries BA, Buchhop S, Miska S, Henning W, Pedeux R, Wang XW, Hofseth LJ, Yang Q, Garfield SH, Sturzbecher HW, Harris CC. p53 interacts with hRAD51 and hRAD54, and directly modulates homologous recombination. Cancer Res 2003; 63:2596-605.
26. Kumari A, Schultz N, Helleday T. p53 protects from replication-associated DNA double-strand breaks in mammalian cells. Oncogene 2004; 23:2324-9.
27. Sablina AA, Budanov AV, Ilyinskaya GV, Agapova LS, Kravchenko JE, Chumakov PM. The antioxidant function of the *p53* tumor suppressor. Nat Med 2005; 11:1306-13.
28. Lo PK, Huang SZ, Chen HC, Wang FF. The prosurvival activity of p53 protects cells from UV-induced apoptosis by inhibiting c-Jun NH2-terminal kinase activity and mitochondrial death signaling. Cancer Res 2004; 64:8736-45.