



HAL
open science

Role of DNA damage in atherosclerosis - bystander or participant?

Kelly Gray, Martin Bennett

► **To cite this version:**

Kelly Gray, Martin Bennett. Role of DNA damage in atherosclerosis - bystander or participant?. Biochemical Pharmacology, 2011, 82 (7), pp.693. 10.1016/j.bcp.2011.06.025 . hal-00723635

HAL Id: hal-00723635

<https://hal.science/hal-00723635>

Submitted on 12 Aug 2012

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Title: Role of DNA damage in atherosclerosis - bystander or participant?

Authors: Kelly Gray, Martin Bennett

PII: S0006-2952(11)00408-4
DOI: doi:10.1016/j.bcp.2011.06.025
Reference: BCP 10949

To appear in: *BCP*

Received date: 16-5-2011
Revised date: 16-6-2011
Accepted date: 17-6-2011

Please cite this article as: Gray K, Bennett M, Role of DNA damage in atherosclerosis - bystander or participant?, *Biochemical Pharmacology* (2010), doi:10.1016/j.bcp.2011.06.025

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



Role of DNA damage in atherosclerosis - bystander or participant?

Kelly Gray* and Martin Bennett

Division of Cardiovascular Medicine, University of Cambridge

Box 110, Addenbrooke's Centre for Clinical Investigation,

Addenbrooke's Hospital,

Cambridge, CB2 0QQ, UK

Telephone 44 1223 331504

Word Count 7839

Fax 44 1223 331505

Email klg42@cam.ac.uk

* corresponding author

Running title: DNA damage in atherosclerosis

List of non-standard abbreviations:

ApoE^{-/-}: Apolipoprotein E deficient

ATM: Ataxia Telangiectasia Mutated

ATR: ATM- and Rad3-related protein

BER: Base-excision repair

CHK1/2: Checkpoint kinase 1 or Checkpoint kinase 2

CtIP: C-terminal interacting protein

DDR: DNA damage response pathway

DSB: Double Strand Break

γ -H2AX: gamma-phosphorylated form of histone 2A protein

1 HGPS: Hutchinson-Gilford Progeria Syndrome

2 HR: homologous recombination

3
4 ICAM-1: Inter-Cellular Adhesion Molecule 1

5
6 LDL: low-density lipoprotein

7
8 IL-6/8: Interleukin-6/8

9
10 iNOS: Inducible Nitric Oxide Synthase

11
12 IR: Ionising Radiation

13
14 MDC1: Mediator of DNA damage checkpoint protein 1

15
16 MRN: Complex of Nibrin (NBS-1), MRE11 and Rad 50

17
18 MtDNA: mitochondrial DNA

19
20 NBS1: Nijmegen Breakage Syndrome1 or Nibrin

21
22 NER: Nucleotide excision repair

23
24 NHEJ: Non-homologous end joining

25
26 ROS: Reactive oxygen species

27
28 SIPS: Stress induced premature senescence

29
30 SMC: Structural maintenance of chromosomes

31
32 SSBs: Single strand breaks

33
34 UV: Ultra Violet

35
36 VSMCs: vascular smooth muscle cells

37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Introduction

- 1
2 1. Atherosclerosis
- 3
4 2. Evidence for DNA damage in atherosclerosis
- 5
6 3. Causes of DNA damage in atherosclerosis
- 7
8 3.1. *Oxidative Stress (Reactive Oxygen Species)*
- 9
10 3.2. *Epigenetics*
- 11
12 3.3. *Cytotoxic Agents and Radiotherapy*
- 13
14 4. Consequences of DNA damage and Atherosclerosis
- 15
16 4.1. *Growth arrest and Senescence*
- 17
18 4.2. *Cell death*
- 19
20 5. DNA damage response pathway
- 21
22 5.1. *Sensors*
- 23
24 5.2. *Transducers*
- 25
26 5.3. *Effectors*
- 27
28 6. DNA damage syndromes associated with atherosclerosis
- 29
30 6.1. *Ataxia-Telangiectasia (AT)*
- 31
32 6.2. *Werner Syndrome*
- 33
34 6.3. *Hutchinson–Gilford Progeria Syndrome (HGPS)*
- 35
36 7. Therapeutic Options in the prevention of DNA damage
- 37
38 7.1. *Antioxidants*
- 39
40 7.2. *Polyphenols*
- 41
42 7.3. *Statins*
- 43
44 7.3 *ACE Inhibitors*
- 45
46 8. Conclusions and Future Perspectives
- 47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Abstract

1
2 Atherosclerosis leading to cardiovascular disease is the leading cause of death among western
3
4 populations. Atherosclerosis is characterised by the development of a fibrofatty lesion that consists of
5
6 a diverse cell population, including inflammatory cells that create an intensely oxidising environment
7
8 within the vessel. Coupled with normal replication, the local intracellular and extracellular
9
10 environment causes damage to cellular DNA that is recognised and repaired by the DNA damage
11
12 response (DDR) pathway. The role of DNA damage and the resulting deregulation of ‘normal’ cellular
13
14 behaviour and subsequent loss of cell cycle control checkpoints have been widely studied in cancer.
15
16 However, despite the extensive evidence for DNA damage in atherosclerosis, it is only over the past
17
18 two decades that a causative link between DNA damage and atherosclerosis has been hypothesised.
19
20
21 Whilst atherosclerosis is a feature of human disease characterised by defects in DNA damage,
22
23 currently the role of DNA damage in the initiation and progression of atherosclerosis remains highly
24
25 debated, as a ‘chicken and egg’ situation. This review will analyse the evidence for, the causes of, and
26
27 consequences of DNA damage in atherosclerosis, detail the DNA damage response pathway that
28
29 results in these consequences, and highlight therapeutic opportunities in this area. We also outline the
30
31 evidence that DNA damage is a cause of both initiation and progression of atherosclerosis, and not just
32
33 a consequence of disease.
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Introduction

1. Atherosclerosis

Atherosclerosis is a disease associated with remodelling of the arterial intima, in part as a result of an initial protective inflammatory response following lipid uptake into the vessel wall and endothelial injury. Endothelial dysfunction induced by a variety of cardiovascular risk factors (for example, hypercholesterolaemia, diabetes and smoking) promotes infiltration of inflammatory cells such as macrophages, and immune cells also accumulate (lymphocytes, mast cells, dendritic cells). Migrated monocytes are converted to macrophages that subsequently take up oxidized low-density lipoprotein (LDL) present in the extracellular environment to become foam cells, thus forming a fatty streak, one of the earliest lesions in atherosclerosis. Macrophage accumulation, along with subpopulations of migrating T lymphocytes, promote migration and proliferation of vascular smooth muscle cells (VSMCs), resulting in development of a fibrofatty lesion. The release of growth factors and inflammatory cytokines from these various cell types promotes further accumulation of inflammatory cells and deposition of extracellular matrix components causing the lesion to develop into an advanced plaque consisting of a lipid-rich 'necrotic' core covered by a VSMC-rich fibrous cap [1]. Rupture of the fibrous cap leads to thrombosis and artery occlusion, resulting in myocardial infarction [2].

2. Evidence for DNA damage in atherosclerosis

There is increasing evidence that VSMCs and inflammatory cells within atherosclerotic plaques have accumulated DNA damage, and that plaque VSMCs undergo the consequences of DNA damage, including apoptosis and premature senescence [3]. For example, DNA strand breaks and chromosomal damage are present in circulating cells of patients with atherosclerosis; DNA damage correlates with a higher micronucleus index (a marker of genetic instability) compared with healthy controls, and is associated with disease severity [4]. VSMCs and macrophages express markers of DNA damage in plaques, that increase with disease severity, including phosphorylated forms of the Ataxia Telangiectasia Mutated (ATM) and Histone 2A protein X proteins (γ -H2AX)[5]. *In vitro*, plaque-derived VSMCs retain increased DNA damage compared with normal VSMCs, as shown by increased expression of p-ATM and γ -H2AX and a longer tail length on Comet assay, a marker of DNA strand

1 breaks [5]. Similarly, oxidative DNA damage and DDR markers appear in atherosclerotic lesions in
2 animal models after fat feeding and in human plaques, and whilst some markers are reduced by lipid
3 lowering, oxidative DNA damage persists [6, 7].
4
5
6
7

8 DNA damage occurs in both genomic and mitochondrial DNA in atherosclerosis. For example,
9 circulating cells in patients with severe coronary atherosclerotic disease exhibit a significantly higher
10 incidence of the common mitochondrial deletion mtDNA4977 [8]. This has prompted investigations
11 into the role of mitochondrial DNA damage and the subsequent association with cellular metabolic
12 dysfunction in atherosclerosis. Mitochondrial DNA damage appears to be an early event in
13 atherosclerosis [9], and is also a feature of mice lacking the DNA damage protein ATM. ATM
14 haploinsufficiency in Apolipoprotein E deficient (ApoE^{-/-}) mice results in accelerated atherosclerosis
15 in addition to symptoms of metabolic syndrome [10, 11]. VSMCs and macrophages from ATM^{+/-} cells
16 exhibited increased nuclear DNA damage, defects in the DNA repair pathway, reduced proliferation
17 and increased apoptosis [11]. In addition many of the tissues analysed had an increased frequency of a
18 5kB deletion in the mitochondrial DNA and reduced mitochondrial oxidative phosphorylation [11].
19 Whilst these studies still demonstrate only an association between MtDNA damage and atherosclerosis,
20 they demonstrate that mitochondrial DNA damage is sufficient in these models to induce
21 mitochondrial dysfunction, that may result in a metabolic syndrome phenotype, thereby promoting
22 atherosclerosis [10].
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44

45 **3. Causes of DNA damage in atherosclerosis**

46 Genetic damage is caused by both insults from the extrinsic environment, in addition to reactive
47 species generated from normal cell metabolism. If left unrepaired DNA damage creates errors during
48 DNA replication. As such it has been estimated that a single cell can incur up to 10⁴ DNA changes per
49 day [12]. The activation of the DDR and subsequent downstream repair pathway is a highly evolved,
50 complex network of tightly regulated post-translation modifications and protein-protein interactions.
51 Whilst the DDR shares many common components (see below), the major stimuli inducing DNA
52 damage in atherosclerosis are oxidative stress due to reactive oxygen species (ROS), specific risk
53
54
55
56
57
58
59
60
61
62
63
64
65

1 factors for cardiovascular disease such as diabetes, and extrinsic stimuli, including drug treatment or
2 radiotherapy.
3

4 5 6 **3.1 Oxidative Stress (Reactive Oxygen species)** 7

8
9 DNA damage is manifested in a variety of forms including single strand breaks, double strand breaks,
10 base modification (8-oxo-G) and mis-pairing, all of which need to be successfully repaired to avoid
11 accumulation of mutations, cell cycle arrest and apoptosis. Although DNA damage in atherosclerosis
12 may be caused by environmental factors (Ultra-violet or ionising radiation), most DNA lesions appear
13 to be due to either physiological or pathological levels of reactive oxidant species (ROS), including
14 hydrogen peroxide, superoxide anion and lipid peroxides. ROS are generated during normal
15 metabolism by cytoplasmic and mitochondrial enzymes, including nicotinamide adenine dinucleotide
16 (phosphate) oxidase, xanthine oxidase, lipoxygenase, or the uncoupling of nitric oxide synthase. In
17 their native form superoxide and hydrogen peroxide species are inert to DNA, although when
18 converted into hydroxyl radicals by the Fenton reaction they induce extensive damage to both nuclear
19 and mitochondrial DNA including DNA single and double strand breaks, glycolytic damage and mis-
20 repairing. The cell has evolved a number of antioxidant mechanisms relying on enzymes such as
21 superoxide dismutases (SODs), catalase and glutathione peroxidase to efficiently scavenge and
22 remove ROS from the cellular environment (**Figure 1**). Increased oxidative stress is also a major
23 feature of Type II diabetes, a major risk factor for atherosclerosis [13]. For example, circulating
24 mononuclear cells from patients with type II diabetes exhibit increased production of reactive oxygen
25 species [14] and enhance lipid peroxidation [15] resulting in DNA damage [16] and mitochondrial
26 dysfunction [17].
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50

51 Oxidative stress is also associated with accelerated telomere shortening and subsequently reduced
52 telomere length [18, 19], leading to premature cellular senescence. Telomere attrition is linked with
53 vascular disease and vascular ageing [19, 20], in both circulating cells and in VSMCs in plaques
54 themselves[19], and is also associated with increased oxidative DNA damage. For example,
55 lymphocyte DNA from patients with type II diabetes exhibit increased susceptibility to oxidative DNA
56
57
58
59
60
61
62
63
64
65

1 damage [18] and telomere length in monocytes isolated from type II diabetic patients is inversely
2 correlated to the levels of oxidative DNA [21]. Whilst the functional consequences of either oxidative
3 DNA damage or telomere shortening in leukocytes is not known, plaques are characterised by
4 macrophages showing both oxidative DNA damage and apoptosis [19], potentially contributing to
5 plaque instability and ongoing inflammation.
6
7
8
9

10 11 12 13 **3.2 Epigenetics**

14 Epigenetic regulation, particularly DNA methylation and histone modification, of vascular genes and
15 growth factors is observed in the development and progression of atherosclerosis. For example, DNA
16 hypomethylation occurs in monocytes, VSMCs and the plaques of patients with atherosclerosis [22]
17 and additional studies using ApoE^{-/-} mice have shown that DNA hypomethylation represents a
18 significant risk factor associated with susceptibility to atherosclerosis [23]. DNA damage in vascular
19 cells can result from exposure to oxidative stress, and under normal circumstances there are a variety
20 of antioxidant enzymes that remove these damaging reactive oxygen species from the environment.
21 Recent studies have shown that inflammatory cytokines can alter the expression of mediators of
22 oxidative stress such as inducible nitric oxide synthase (iNOS) by causing changes to the chromatin
23 structure of the promoter [24]; the resultant increase in the expression of nitric oxide (catalysed by
24 iNOS) causes VSMC apoptosis that is associated with increased plaque instability. Furthermore DNA
25 hypomethylation of the antioxidant enzyme superoxide dismutase (SOD) gene results in reduced
26 expression [25]. These gene expression changes likely force the cellular redox balance towards that of
27 a highly oxidising environment, that may potentially increase cellular DNA damage and enhance the
28 progression of atherosclerotic lesions.
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50

51 **3.3 Cytotoxic Agents and Radiotherapy**

52 One of the most direct demonstrations of the link between DNA damage and atherosclerosis comes
53 from studies of both chemotherapy and radiotherapy. Both cytotoxic drugs and radiotherapy target the
54 processes and machinery involved in regulating DNA and cell replication. Chemotherapeutics such as
55 the alkylating agent cyclophosphamide [26] or the antibiotic-based drugs (e.g. anthracyclines) [27]
56
57
58
59
60
61
62
63
64
65

1 prevent cell division by introducing new bonds into the DNA structure or by intercalating into base
2 pairs in the DNA minor groove respectively. These agents also induce the generation of ROS that
3 increase the levels of DNA damage and mitochondrial injury; although the exact mechanism for this
4 event is unknown it is thought to be p53-dependent [28]. The topoisomerase inhibitors, a third group
5 of cytotoxic reagents, cause cell death before damaged DNA can undergo repair in a cell cycle-
6 dependent manner [29].
7
8
9
10
11
12
13
14

15 Similarly, radiotherapy works on the principle that radiation directly induces single and double strand
16 breaks, resulting in growth arrest and apoptosis. For both indirect and direct ionizing radiation,
17 damage is induced through hydroxyl radicals resulting from the ionization of water, or by through
18 exposing DNA to free radicals. In cancer, the increased proliferative ability of tumour cells results in
19 incomplete DNA repair after radiotherapy, with consequent damage accumulation through multiple
20 divisions.
21
22
23
24
25
26
27
28
29
30

31 Although chemo- and radiotherapies are widely used in the treatment of cancer, both modalities have
32 side effects due to extensive DNA damage to 'off-target' healthy cells, including those in the
33 vasculature [30, 31]. Indeed, there is now strong causative evidence between DNA damage-inducing
34 treatments for cancer and cardiovascular disease, particularly atherosclerosis. For example, large
35 clinical cohort studies have assessed the risk of cardiovascular disease in 5-year survivors treated for
36 testicular cancer between 1965-1995 using chemotherapy and radiotherapy compared with surgical
37 strategies. Non-surgical patients had an up to 2-fold increased risk of myocardial infarction compared
38 to the surgical cohort [32]. Although the cellular effects of these treatments could not be assessed, it
39 was proposed that long-term exposure of the endothelium to circulating platinum, and lowered
40 testosterone levels may have been instrumental in the development of metabolic syndrome and
41 associated cardiovascular disease. A similar trend was observed in a 1474-strong cohort of patients
42 treated for Hodgkin Lymphoma, with a 3-5 fold increase in the incidence of CVD in patients
43 compared with the general population; this was proposed to be as a result of damage to the vascular
44 endothelium of irradiated vessels [33].
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2 The mechanism of action of vascular disease induced by cytotoxic chemotherapy and ionizing
3 radiation is also becoming clearer. Both modalities induce DNA damage and cell death in
4 endothelial cells and VSMCs, with subsequent endothelial dysfunction and inflammation, resulting
5 in cell senescence, apoptosis, thrombosis formation, mitochondrial dysfunction and fibrosis, all of
6 which promote atherosclerosis. Irradiation exposure has been shown induce the expression of
7 adhesion molecules (e.g. I-CAM-1) and inflammatory cytokines, including interleukin-6 and
8 interleukin-8 (IL-6 and IL-8) [34] causing radiation- induced DNA damage and mitotic death of
9 endothelial cells. In larger vessels this has been proposed to lead to endothelial denudation, an
10 initiator of lesion development [35]. In ApoE^{-/-} mice, ionising radiation had been shown to result in
11 the accumulation of macrophages in atherosclerotic lesions, accelerated plaque development and
12 increased susceptibility to intraplaque haemorrhage [36], the latter representing a feature of
13 advanced atherosclerosis.
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31

32 **4. Consequences of DNA damage in Atherosclerosis**

33 ***4.1 Growth arrest and Senescence***

34 Normal cell division is regulated by a four-stage cycle that is able to verify the efficacy of the cellular
35 DNA before replication occurs. This inhibits reproduction of damaged DNA to prevent accumulation
36 of mutated proteins within the cell. DNA synthesis begins with production of specific enzymes
37 required for replication in G₁, followed by replication of chromosomes during S phase. Major
38 checkpoints exist throughout the cell cycle, particularly in G₁ and G₂, that are activated following
39 DNA damage [37]. DNA damage activates the DDR (see below) with transient growth arrest to allow
40 DNA repair to occur, preventing propagation of damaged DNA. Repeated or excessive DNA damage
41 can also induce replicative senescence, a state of irreversible growth arrest [38]. In vascular cells,
42 senescence is characterised by activation of G₁/S restriction point proteins, and there is now increasing
43 evidence that human plaque VSMCs show impaired cell proliferation [3] and multiple features of cell
44 senescence *in vivo* and *in vitro* [19].
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

4.2. Cell death

Oxidative stress and accumulation of DNA damage without efficient repair can cause the cell to undergo apoptosis, a process of programmed cell death. As described above, both macrophage and VSMC apoptosis is a feature of advanced atherosclerotic plaques, as is DNA damage in cells derived from plaques and mouse models of atherosclerosis [5, 9]. Oxidative DNA damage is induced in plaques that positively correlates with the expression of DNA damage markers and p53, a major regulator of macrophage and VSMC apoptosis within the plaque [7]. Whilst macrophage apoptosis can promote formation of the necrotic core and inflammation, VSMC cell death results in multiple features of plaque vulnerability, including a thinned cap, a larger necrotic core, and inflammation, and an overall acceleration of plaque growth [39, 40]. Thus, DNA damage and subsequent apoptosis appears to be a major component of the pathology associated with atherosclerosis.

5. DNA damage response (DDR) pathway

Environmental (UV, ionising radiation) and physiological (iNOS, ROS) stress stimuli induce cellular DNA damage that is sensed by internal mediators to initiate the DNA damage response (DDR). The DNA damaging agent, type of DNA damage and position in the cell cycle dictates the specific cellular response to the lesion. Single strand breaks (SSBs) and damage to individual bases is repaired by base-excision repair (BER) and larger adducts by nucleotide excision repair (NER) [41]. The two main mechanisms that facilitate DSB repair are reviewed elsewhere [42], but consist of non-homologous end joining (NHEJ) that takes place at any stage in the cell cycle, and homologous recombination (HR) that is restricted to the S- and G₂- phase of the cell cycle and relies on 5'-3' resection of the break to produce single-strand tails [43].

The DDR consists of a complex network of proteins that undergo post-translational modification to regulate the activation and functionality of specific response mediators that interact to efficiently repair the damage. These proteins can be divided into sensors, transducers and effectors (**Figure 2**).

5.1 Sensors

1
2 Sensor proteins are recruited to sites of DNA damage and orchestrate formation of multi-protein
3
4 complexes to activate the DDR. For example, nibrin (NBS1), as one of the initial sensors of double
5
6 strand breaks, forms a trimeric complex with Mre11 and Rad50 (MRN) (reviewed by Rupnik *et al*
7
8 2008 [44]). NBS-1 acts as a molecular scaffold that tethers the complex to the specific site of DNA
9
10 damage. Mre11 is a single-strand specific endonuclease and double-strand specific exonuclease that is
11
12 phosphorylated upon DNA damage; interaction with Rad50 regulates its nuclease activity due to
13
14 binding to the DNA and tethering the ends in close proximity to allow repair of damaged DNA. This
15
16 complex translocates to the nucleus and binds at DSBs to form distinct foci with an additional sensor
17
18 protein histone 2A (H2AX) that is phosphorylated at Ser¹³⁹ following DNA damage [45].
19
20
21
22
23

24 DNA single strand breaks are detected by the protein Rad9 which forms a checkpoint complex at the
25
26 break with the proteins Rad1 and HUS1 (9-1-1 complex) that precedes the recruitment of Rad17 to the
27
28 site of damage [46]. This recruits and activates ATM- and Rad3-related (ATR) protein, that
29
30 phosphorylates transducer proteins such as Chk1 to initiate the downstream signalling of mediators
31
32 including TopBP1 [47], Cdc25C and p53 (Reviewed by Sancar *et al* [48] (**Figure 2**)).
33
34
35
36
37

5.2 Transducers

38
39 DNA damage sensors including MRN and H2AX triggers a cascade of phosphorylation events that
40
41 activate downstream transducer proteins, that ultimately lead to induction of effector proteins that stall
42
43 the cell cycle to carry out DNA repair. DNA repair is first characterised by the recruitment of C-
44
45 terminal interacting protein (CtIP) an endonuclease that cooperates with the MRN complex [49, 50]
46
47 and mediator of DNA damage checkpoint protein 1 (MDC1) that acts as a scaffold for the recruitment
48
49 of other DNA repair proteins such as Ataxia Telangiectasia Mutated protein (ATM), breast cancer
50
51 protein 1 (BRCA1) and p53 binding protein (53BP1) [51, 52]. There is crossover and cooperation
52
53 between the ATM/ATR pathways in the DNA damage response to facilitate efficient repair of DNA
54
55 lesions with up to 25 ATM and ATR substrates having been identified [53]. ATM exists as an
56
57 inactive monomer whose activation occurs in two ways, by autophosphorylation of Ser¹⁹⁸¹ [54], or by
58
59
60
61
62
63
64
65

1 binding NBS-1 at the site of the DNA break to release the active monomer. A cyclical process is
2 activated that involves the phosphorylation of H2AX that recruits MDC1 to stabilise ATM at the DSB,
3
4 ATM can then further activate H2AX molecules [55]. Activated ATM phosphorylates multiple
5
6 downstream effectors including checkpoint kinase 2 (Chk2), p53, TLK1/2 and Cdc25A.
7
8
9

10 **5.3 Effectors**

11 ***Cell Cycle Arrest***

12 Both the ATM and ATR-regulated pathways initiate cell cycle arrest at either the G₁/S or the G₂/M
13
14 phase to facilitate the efficient repair of DNA damage. Transient growth arrest occurs until the DNA
15
16 damage has been adequately repaired; the blocks to the replication machinery are then removed
17
18 allowing a return to normal function. Cell cycle arrest is initiated by ATR/ATM-mediated activation
19
20 of the checkpoint kinases Chk1 and Chk2 respectively that phosphorylate the Cdc25 phosphatase on
21
22 multiple residues to provide binding sites for the 14-3-3 proteins, Rad24 and Rad25. This prevents
23
24 entry of Cdc25 into the nucleus blocking interaction with its substrate Cyclin E-Cdk2 [56]. Following
25
26 DNA damage ATM activation also phosphorylates ‘the guardian of the genome’ p53 at residues Ser¹⁵
27
28 and Ser²⁰ leading to accumulation of this protein in the nucleus [57, 58]. Active p53 induces the
29
30 cyclin-dependent kinase inhibitor p21 that inhibits the CyclinD1-Cdk2 and CyclinE-Cdk4 kinase
31
32 complexes preventing the cell from moving to the next stage of the cell cycle [59].
33
34
35
36
37
38
39
40
41

42 ***DNA Repair***

43 The Structural Maintenance of Chromosomes (SMC1) protein is phosphorylated on Ser⁹⁶⁶ by active
44
45 ATM in an NBS-1-dependent manner resulting in S-phase checkpoint activation. SMC1 forms a
46
47 heterodimer with SMC3 to form the ‘cohesion complex’ creating a structural change that coordinates
48
49 cohesion between sister chromatids during DNA replication. This complex has also been shown to be
50
51 required for post-replicative DSB DNA repair [60] Further studies in animal models have investigated
52
53 the role of DNA repair proteins involved in NHEJ including XRCC4, DNA ligase IV, DNA-PK and
54
55 Ku80 in p53-deficient mice. Double knockout mice (p53/XRCC4 and p53/DNA ligase IV double null)
56
57 exhibit increased chromosomal instability due to defective NHEJ and cell cycle checkpoint capability
58
59
60
61
62
63
64
65

1 demonstrating the importance of these functions and associated proteins in maintaining genome
2 integrity [61].
3
4
5

6 *Apoptosis*

7
8 If the damage to DNA is too substantial and cannot be satisfactorily repaired by DDR proteins, the cell
9 may be forced to undergo apoptosis, a process that serves to prevent further replication of mutated
10 DNA. DNA damage-mediated cell death is primarily thought to be regulated by the ATM-mediated
11 phosphorylation of p53 that activates pro-apoptotic genes including Puma and Bax that induce
12 permeabilisation of the outer membrane of the mitochondria to allow release of cytochrome c from the
13 mitochondrial intermembrane space. Cytochrome c activates and induces oligomerisation of the
14 protein Apaf-1 that results in the formation of the apoptosome, a complex that recruits and activates
15 caspase-9 subsequently inducing the executioner caspase cascade [62].
16
17
18
19
20
21
22
23
24
25
26
27

28 *Senescence*

29
30 Alternatively, the accumulation of DNA damage without efficient repair can lead irreversible arrest of
31 the cell cycle. Two types of senescence have been identified; Stress-induced premature senescence
32 (SIPS) is a process induced by exposure of cells to cytotoxic stress that induces a DNA damage
33 response and cell cycle arrest [63]. In contrast replicative senescence defines a situation where a cell
34 has exhausted its replicative potential, and routinely occurs within cell populations. Replicative arrest
35 appears to be mediated mostly by telomere shortening. As cells divide their telomeres shorten,
36 reaching a critical limit that results in activation of a DDR comprising the same signalling pathways as
37 described above [38]. Both types of senescence induce growth arrest, but are also associated with pro-
38 inflammatory activity, predominantly due to secretion of senescence-associated inflammatory
39 cytokines, that might also be important in atherosclerosis. The causes and effects of cellular ageing
40 and senescence in vascular cells has been recently reviewed [64].
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57

58 **6. DNA damage syndromes associated with atherosclerosis**

59
60
61
62
63
64
65

1 A variety of inherited defects in the DNA repair pathway exist, many characterised by inefficient
2 repair of DNA damage, leading to the accumulation of mutations. Whilst this group of diseases is
3 frequently associated with an increased risk of cancer, some are also associated with increased risk of
4 cardiovascular disease, including Ataxia-Telangiectasia (AT), Werner Syndrome and Hutchinson–
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

A variety of inherited defects in the DNA repair pathway exist, many characterised by inefficient repair of DNA damage, leading to the accumulation of mutations. Whilst this group of diseases is frequently associated with an increased risk of cancer, some are also associated with increased risk of cardiovascular disease, including Ataxia-Telangiectasia (AT), Werner Syndrome and Hutchinson–Gilford Progeria Syndrome (HGPS). The manifestation of vascular disease in these syndromes, often at a very young age, is one of the strongest indications that DNA damage and inefficient repair is directly causal in atherosclerosis, in the absence of any other risk factors for atherosclerosis.

6.1 *Ataxia-Telangiectasia (AT)*

Ataxia-Telangiectasia (AT) is an autosomal recessive disorder caused by mutation in the ATM gene. The defective protein leads to an inability to recognise and repair damage to DNA and telomeres. Patients with AT exhibit increased radiosensitivity and chromosomal instability culminating in greater susceptibility to cancer and metabolic syndrome; the latter is a condition associated with atherosclerosis and ATM heterozygosity has been shown to be associated with a 0.5-2% increased risk of death from cardiovascular disease [65]. Studies using haploinsufficient ATM mice [10, 11] have also shown features common to the metabolic syndrome, including glucose intolerance and insulin resistance. Furthermore $ATM^{+/-}/ApoE^{-/-}$ mice transplanted with bone marrow from $ATM^{-/-}/ApoE^{-/-}$ mice and fed a western diet, showed lesions sizes 80% greater than those observed in control subjects. One study also showed that activation of ATM activity pharmacologically reduced lesion size in the aorta by ~25% [10]. These studies provide strong evidence in support of a protective role for ATM in reducing atherosclerotic plaque development.

6.2 *Werner Syndrome*

Werner Syndrome is caused by a loss-of-function mutation in the Werner Syndrome ATP-dependent helicase (WRN), a protein that has helicase, ATPase, exonuclease and single strand annealing functions. Transgenic mouse models to study the pathophysiology associated with Werner Syndrome have been developed using either a knockout, or functional mutant approach. Cells from WRN knockout mice exhibit reduced proliferative ability, with increased susceptibility to DNA damage

1 inducing agents [66]. Similarly mice lacking the functional helicase domain of the WRN protein have
2 genomic instability, telomere attrition and loss of their proliferative capacity. In addition these mice
3
4 showed abnormal levels of visceral fat and high fasting cholesterol, culminating in the development of
5
6 insulin resistance and high blood glucose [67]. The absence of a functional WRN protein leads to the
7
8 development of a phenotype characterised by severe cardiac fibrosis. Humans with Werner syndrome
9
10 demonstrate premature ageing, including osteoporosis, type I diabetes, atherosclerosis and cancer [68].
11
12
13
14
15

16 **6.3 Hutchinson–Gilford Progeria Syndrome (HGPS)**

17
18
19
20 HGPS is a syndrome associated with premature ageing caused by defects in the gene encoding Lamin
21
22 A, a structural scaffold in the nuclear lamina, that results in the translation of a mutated protein,
23
24 progerin. The inability of this protein to become posttranslationally modified means it that enters the
25
26 nuclear envelope but cannot insert into the lamina, causing defects in the morphology of the nucleus.
27
28 This causes alterations in the organisation of chromatin, gene expression and consequently a failure to
29
30 repair DNA damage. Fibroblasts isolated from HGPS patients with advanced atherosclerosis show
31
32 increased DNA damage [69] and undergo premature senescence compared with normal donors [70]. In
33
34 early-passage VSMCs ectopically expressing prelamin A, and in aged-VSMCs with accumulated
35
36 prelamin A, the presence of this protein was shown to activate the DNA damage signalling pathway
37
38 and dysregulate mitosis resulting in the early onset of senescence in these cells [71]. HGPS patients
39
40 die at a young age (<20), with vascular disease a major feature of the HGPS pathology.
41
42
43
44
45

46 **7. Therapeutic Options in the prevention of DNA damage**

47
48 The evidence presented above indicates that DNA damage may be a major causal factor in both the
49
50 initiation and progression of human atherosclerosis. As such, it should be a target for therapy.
51
52 Effectively management of atherosclerosis is currently based on reduction in exposure to risk factors
53
54 (e.g. smoking, cholesterol levels, diabetes), with little if any specific therapy. Similarly, whilst risk
55
56 factor reduction should reduce DNA damage, animal models have shown that damage is highly
57
58 persistent in established plaques. There is thus the potential for treatments that directly augment DNA
59
60
61
62
63
64
65

1 repair in atherosclerosis. To date, there have been no therapies trialled that specifically promote DNA
2 repair in atherosclerosis, although both reduction in DNA damage and augmenting repair may result
3 from current classes of pharmacological agents, including anti-oxidants, Statins and angiotensin II
4 converting enzyme (ACE) inhibitors.
5
6
7
8
9

10 ***7.1 Antioxidants***

11 The excess production of ROS within the diseased vessel and the resulting DNA damage, adds weight
12 to the use of antioxidants as a potent therapeutics, both as prevention and as a means to reduce plaque
13 progression. However, published studies give contradictory outcomes across the many clinical trials
14 with antioxidants including vitamin C [72], vitamin E [73] selenium [74] or folic acid [75]. Whilst the
15 agents and doses used may not have been optimal, meta-analyses of these trials demonstrate that
16 antioxidants in their current form and dosage have limited effect as a treatment for atherosclerosis [76,
17 77].
18
19
20
21
22
23
24
25
26
27
28
29
30

31 **7.2 Polyphenols**

32 Polyphenols including quercetin and theaflavin derived from fruit and vegetables are naturally
33 occurring organic chemicals composed of a number of phenol subunits that form a complex ring
34 structure. Studies have shown that populations with polyphenol-rich diets show evidence of a trend
35 toward protection from the development of cardiovascular diseases (reviewed in [78]). Due to the
36 number of processes involved in the development of atherosclerosis, including oxidative stress,
37 inflammation and endothelial dysfunction, it has been proposed that polyphenols exert their protective
38 effects by preventing one or more of these processes. A recent study investigated the role of specific
39 dietary polyphenols in regulating the development of atherosclerosis in ApoE^{-/-} mice. These results
40 showed that of the polyphenols examined (quercetin, epicatechin, theaflavin, sesamin, chlorogenic
41 acid), there was a reduction in the atherosclerosis observed in these mice and this occurred through
42 inhibition of inflammation, increased production of nitric oxide, and the induction of heme oxygenase
43 [79]. Whilst this study suggests that polyphenols represent candidates for an effective chemo-
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 prevention strategy, they have limited bio-availability and rapid secretion; extensive dose response
2 studies would be needed for this approach to succeed in the clinic.
3
4
5

6 **7.3 Statins**

7
8 Statins are a class of drugs that primarily act to inhibit the activity of HMG-CoA reductase; inhibition
9 of this enzyme reduces circulating low-density lipoprotein (LDL) levels by decreasing the production
10 of cholesterol in the liver. This effectively results in lower levels of oxidised LDL in the vessel,
11 reducing both the production of damaging ROS, and the subsequent oxidative DNA damage caused by
12 these molecules. As additional benefit to their lipid-lowering effects, statins have been shown to
13 participate in modulating other processes involved in the development of atherosclerosis, including
14 improving endothelial function, modulating the inflammatory response, maintaining plaque stability
15 and preventing the formation of thrombus in the vessel [80, 81].
16
17
18
19
20
21
22
23
24
25
26
27
28

29 Importantly, statins have also been shown to reduce DNA damage both *in vitro* and *in vivo* [5, 82],
30 protecting cells against telomere shortening. Whilst some of these effects may be due to reduced DNA
31 damage, there is also evidence that Statins directly accelerate DNA repair, by regulating DDR proteins
32 levels and activity. For example, VSMCs treated with atorvastatin exhibit increased DNA repair by
33 regulating the expression of NBS-1 [5].
34
35
36
37
38
39
40
41

42 **7.4 ACE Inhibitors**

43 Angiotensin converting enzyme (ACE) inhibitors reduce the activity of the renin-angiotensin-
44 aldosterone system, blocking the conversion of angiotensin I to angiotensin II and the enzyme
45 responsible for the degradation of bradykinin, a vasodilator. Bradykinin has been shown to protect
46 endothelial cells from superoxide-induced senescence through inhibition of DNA damage [83].
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

8. Conclusions and Future Perspectives

There is extensive evidence that DNA damage occurs in atherosclerosis, that increases as the disease progresses. The consequences of DNA damage, including growth arrest, senescence, and apoptosis, are all increased in plaques compared with normal vessels. Atherosclerosis is increased following both chemotherapy and radiotherapy, and human DDR syndromes are associated with both DNA damage and increased atherosclerosis. Thus, DNA damage may be a major causal factor in both the initiation and progression of atherosclerosis, and represents a novel target for therapeutics in cardiovascular disease.

The DNA damage response is a complex and highly evolved pathway that relies on post-translational modifications of proteins that sense and repair the DNA lesions caused by various environmental and physiological insults. The DDR is also utilised following multiple DNA damage-inducing stimuli, including the normal senescence of cells. Current cardiovascular therapeutics may affect the DDR, but this is not their primary target, and their aim would be to augment DNA repair. In contrast, most therapeutics that directly target the DDR focus on inhibiting key DDR enzymes to promote DNA damage in cancer cells, promoting selective apoptosis. This dilemma is a common feature of modern treatment regimes. For example, as described above, current cancer treatments increase atherosclerosis. Whilst treatments based on accelerating DNA repair may protect normal tissues during chemo- or radiotherapy, prolonged treatment, for example in atherosclerosis, runs the risk of promoting cancer.

A potential solution to this problem comes from the inherited human DDR syndromes that are associated with premature cancer and /or accelerated atherosclerosis. Whilst some DDR syndromes predispose to atherosclerosis, many do not. Identifying both how the DNA is damaged and understanding how the impaired DNA damage response promotes atherosclerosis in these individuals may identify pathways aimed at preventing DNA damage-regulated initiation of atherosclerosis, without allowing cell replication with damaged DNA that predisposes to cancer.

References

- 1
2
3 [1] Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature*
4 1993;362:801-9.
5
6
7
8 [2] Newby D, Cousins F, Myatt L, Lyall F. Heme oxygenase expression in cultured
9 human trophoblast cells during in vitro differentiation: effects of hypoxia. *Placenta*
10 2005;26:201-9.
11
12
13 [3] Bennett MR, Evan GI, Schwartz SM. Apoptosis of human vascular smooth muscle
14 cells derived from normal vessels and coronary atherosclerotic plaques. *Journal of*
15 *Clinical Investigation* 1995;95:2266-74.
16
17
18 [4] Botto N, Rizza A, Colombo MG, Mazzone AM, Manfredi S, Masetti S, et al.
19 Evidence for DNA damage in patients with coronary artery disease. *Mutat Res*
20 2001;493:23-30.
21
22
23 [5] Mahmoudi M, Gorenne I, Mercer J, Figg N, Littlewood T, Bennett M. Statins use a
24 novel Nijmegen breakage syndrome-1-dependent pathway to accelerate DNA repair in
25 vascular smooth muscle cells. *Circ Res* 2008;103:717-25.
26
27
28 [6] Martinet W, Knaapen MW, De Meyer GR, Herman AG, Kockx MM. Elevated levels
29 of oxidative DNA damage and DNA repair enzymes in human atherosclerotic plaques.
30 *Circulation* 2002;106:927-32.
31
32
33 [7] Martinet W, Knaapen MW, De Meyer GR, Herman AG, Kockx MM. Oxidative DNA
34 damage and repair in experimental atherosclerosis are reversed by dietary lipid
35 lowering. *Circ Res* 2001;88:733-9.
36
37
38 [8] Botto N, Berti S, Manfredi S, Al-Jabri A, Federici C, Clerico A, et al. Detection of
39 mtDNA with 4977 bp deletion in blood cells and atherosclerotic lesions of patients
40 with coronary artery disease. *Mutat Res* 2005;570:81-8.
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
- [9] Ballinger SW, Patterson C, Knight-Lozano CA, Burow DL, Conklin CA, Hu Z, et al. Mitochondrial integrity and function in atherogenesis. *Circulation* 2002;106:544-9.
- [10] Schneider JG, Finck BN, Ren J, Standley KN, Takagi M, Maclean KH, et al. ATM-dependent suppression of stress signaling reduces vascular disease in metabolic syndrome. *Cell Metab* 2006;4:377-89.
- [11] Mercer JR, Cheng KK, Figg N, Gorenne I, Mahmoudi M, Griffin J, et al. DNA damage links mitochondrial dysfunction to atherosclerosis and the metabolic syndrome. *Circ Res* 2010;107:1021-31.
- [12] Lindahl T, Nyberg B. Rate of depurination of native deoxyribonucleic acid. *Biochemistry* 1972;11:3610-8.
- [13] Dandona P, Aljada A. A rational approach to pathogenesis and treatment of type 2 diabetes mellitus, insulin resistance, inflammation, and atherosclerosis. *Am J Cardiol* 2002;90:27G-33G.
- [14] Orié NN, Zidek W, Tepel M. Increased intracellular generation of reactive oxygen species in mononuclear leukocytes from patients with diabetes mellitus type 2. *Exp Clin Endocrinol Diabetes* 2000;108:175-80.
- [15] Ahmed FN, Naqvi FN, Shafiq F. Lipid peroxidation and serum antioxidant enzymes in patients with type 2 diabetes mellitus. *Ann N Y Acad Sci* 2006;1084:481-9.
- [16] Song F, Jia W, Yao Y, Hu Y, Lei L, Lin J, et al. Oxidative stress, antioxidant status and DNA damage in patients with impaired glucose regulation and newly diagnosed Type 2 diabetes. *Clin Sci (Lond)* 2007;112:599-606.
- [17] Garcia-Ramirez M, Francisco G, Garcia-Arumi E, Hernandez C, Martinez R, Andreu AL, et al. Mitochondrial DNA oxidation and manganese superoxide dismutase activity in peripheral blood mononuclear cells from type 2 diabetic patients. *Diabetes Metab* 2008;34:117-24.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
- [18] Sampson MJ, Astley S, Richardson T, Willis G, Davies IR, Hughes DA, et al. Increased DNA oxidative susceptibility without increased plasma LDL oxidizability in Type II diabetes: effects of alpha-tocopherol supplementation. *Clin Sci (Lond)* 2001;101:235-41.
- [19] Matthews C, Gorenne I, Scott S, Figg N, Kirkpatrick P, Ritchie A, et al. Vascular smooth muscle cells undergo telomere-based senescence in human atherosclerosis: effects of telomerase and oxidative stress. *Circ Res* 2006;99:156-64.
- [20] Yamada N. Telomere shortening, atherosclerosis, and metabolic syndrome. *Intern Med* 2003;42:135-6.
- [21] Sampson MJ, Winterbone MS, Hughes JC, Dozio N, Hughes DA. Monocyte telomere shortening and oxidative DNA damage in type 2 diabetes. *Diabetes Care* 2006;29:283-9.
- [22] Pogribny IP, Beland FA. DNA hypomethylation in the origin and pathogenesis of human diseases. *Cell Mol Life Sci* 2009;66:2249-61.
- [23] Lund G, Andersson L, Lauria M, Lindholm M, Fraga MF, Villar-Garea A, et al. DNA methylation polymorphisms precede any histological sign of atherosclerosis in mice lacking apolipoprotein E. *J Biol Chem* 2004;279:29147-54.
- [24] Chan GC, Fish JE, Mawji IA, Leung DD, Rachlis AC, Marsden PA. Epigenetic basis for the transcriptional hyporesponsiveness of the human inducible nitric oxide synthase gene in vascular endothelial cells. *J Immunol* 2005;175:3846-61.
- [25] Laukkanen MO, Mannermaa S, Hiltunen MO, Aittomaki S, Airenne K, Janne J, et al. Local hypomethylation in atherosclerosis found in rabbit ec-sod gene. *Arterioscler Thromb Vasc Biol* 1999;19:2171-8.
- [26] Colvin OM. An overview of cyclophosphamide development and clinical applications. *Curr Pharm Des* 1999;5:555-60.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
- [27] Von Hoff DD, Layard MW, Basa P, Davis HL, Jr., Von Hoff AL, Rozenzweig M, et al. Risk factors for doxorubicin-induced congestive heart failure. *Ann Intern Med* 1979;91:710-7.
- [28] Velez JM, Miriyala S, Nithipongvanitch R, Noel T, Plabplueng CD, Oberley T, et al. p53 Regulates Oxidative Stress-Mediated Retrograde Signaling: A Novel Mechanism for Chemotherapy-Induced Cardiac Injury. *PLoS One* 2011;6:e18005.
- [29] Larsen AK, Escargueil AE, Skladanowski A. Catalytic topoisomerase II inhibitors in cancer therapy. *Pharmacol Ther* 2003;99:167-81.
- [30] Curigliano G, Mayer EL, Burstein HJ, Winer EP, Goldhirsch A. Cardiac toxicity from systemic cancer therapy: a comprehensive review. *Prog Cardiovasc Dis* 2010;53:94-104.
- [31] Taylor CW, Nisbet A, McGale P, Darby SC. Cardiac exposures in breast cancer radiotherapy: 1950s-1990s. *Int J Radiat Oncol Biol Phys* 2007;69:1484-95.
- [32] van den Belt-Dusebout AW, Nuver J, de Wit R, Gietema JA, ten Bokkel Huinink WW, Rodrigus PT, et al. Long-term risk of cardiovascular disease in 5-year survivors of testicular cancer. *J Clin Oncol* 2006;24:467-75.
- [33] Aleman BM, van den Belt-Dusebout AW, De Bruin ML, van 't Veer MB, Baaijens MH, de Boer JP, et al. Late cardiotoxicity after treatment for Hodgkin lymphoma. *Blood* 2007;109:1878-86.
- [34] Van Der Meeren A, Squiban C, Gourmelon P, Lafont H, Gaugler MH. Differential regulation by IL-4 and IL-10 of radiation-induced IL-6 and IL-8 production and ICAM-1 expression by human endothelial cells. *Cytokine* 1999;11:831-8.
- [35] Reidy MA. A reassessment of endothelial injury and arterial lesion formation. *Lab Invest* 1985;53:513-20.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
- [36] Stewart FA, Heeneman S, Te Poele J, Kruse J, Russell NS, Gijbels M, et al. Ionizing radiation accelerates the development of atherosclerotic lesions in ApoE^{-/-} mice and predisposes to an inflammatory plaque phenotype prone to hemorrhage. *Am J Pathol* 2006;168:649-58.
- [37] Elledge SJ. Cell cycle checkpoints: preventing an identity crisis. *Science* 1996;274:1664-72.
- [38] Blackburn EH. Switching and signaling at the telomere. *Cell* 2001;106:661-73.
- [39] Clarke MC, Littlewood TD, Figg N, Maguire JJ, Davenport AP, Goddard M, et al. Chronic apoptosis of vascular smooth muscle cells accelerates atherosclerosis and promotes calcification and medial degeneration. *Circ Res* 2008;102:1529-38.
- [40] Clarke M, Bennett M. The emerging role of vascular smooth muscle cell apoptosis in atherosclerosis and plaque stability. *Am J Nephrol* 2006;26:531-5.
- [41] Friedberg EC. DNA damage and repair. *Nature* 2003;421:436-40.
- [42] Hartlerode AJ, Scully R. Mechanisms of double-strand break repair in somatic mammalian cells. *Biochem J* 2009;423:157-68.
- [43] Pardo B, Gomez-Gonzalez B, Aguilera A. DNA repair in mammalian cells: DNA double-strand break repair: how to fix a broken relationship. *Cell Mol Life Sci* 2009;66:1039-56.
- [44] Rupnik A, Grenon M, Lowndes N. The MRN complex. *Curr Biol* 2008;18:R455-7.
- [45] Rogakou EP, Pilch DR, Orr AH, Ivanova VS, Bonner WM. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *J Biol Chem* 1998;273:5858-68.
- [46] Rauen M, Burtelow MA, Dufault VM, Karnitz LM. The human checkpoint protein hRad17 interacts with the PCNA-like proteins hRad1, hHus1, and hRad9. *J Biol Chem* 2000;275:29767-71.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
- [47] Makiniemi M, Hillukkala T, Tuusa J, Reini K, Vaara M, Huang D, et al. BRCT domain-containing protein TopBP1 functions in DNA replication and damage response. *J Biol Chem* 2001;276:30399-406.
- [48] Sancar A, Lindsey-Boltz LA, Unsal-Kacmaz K, Linn S. Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. *Annu Rev Biochem* 2004;73:39-85.
- [49] Eid W, Steger M, El-Shemerly M, Ferretti LP, Pena-Diaz J, Konig C, et al. DNA end resection by CtIP and exonuclease 1 prevents genomic instability. *EMBO Rep* 2010;11:962-8.
- [50] Yun MH, Hiom K. CtIP-BRCA1 modulates the choice of DNA double-strand-break repair pathway throughout the cell cycle. *Nature* 2009;459:460-3.
- [51] Stewart GS, Wang B, Bignell CR, Taylor AM, Elledge SJ. MDC1 is a mediator of the mammalian DNA damage checkpoint. *Nature* 2003;421:961-6.
- [52] Stucki M, Clapperton JA, Mohammad D, Yaffe MB, Smerdon SJ, Jackson SP. MDC1 directly binds phosphorylated histone H2AX to regulate cellular responses to DNA double-strand breaks. *Cell* 2005;123:1213-26.
- [53] Matsuoka S, Ballif BA, Smogorzewska A, McDonald ER, 3rd, Hurov KE, Luo J, et al. ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. *Science* 2007;316:1160-6.
- [54] So S, Davis AJ, Chen DJ. Autophosphorylation at serine 1981 stabilizes ATM at DNA damage sites. *J Cell Biol* 2009;187:977-90.
- [55] Stucki M, Jackson SP. gammaH2AX and MDC1: anchoring the DNA-damage-response machinery to broken chromosomes. *DNA Repair (Amst)* 2006;5:534-43.
- [56] Karlsson-Rosenthal C, Millar JB. Cdc25: mechanisms of checkpoint inhibition and recovery. *Trends Cell Biol* 2006;16:285-92.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
- [57] Banin S, Moyal L, Shieh S, Taya Y, Anderson CW, Chessa L, et al. Enhanced phosphorylation of p53 by ATM in response to DNA damage. *Science* 1998;281:1674-7.
- [58] Jabbur JR, Huang P, Zhang W. DNA damage-induced phosphorylation of p53 at serine 20 correlates with p21 and Mdm-2 induction in vivo. *Oncogene* 2000;19:6203-8.
- [59] He G, Siddik ZH, Huang Z, Wang R, Koomen J, Kobayashi R, et al. Induction of p21 by p53 following DNA damage inhibits both Cdk4 and Cdk2 activities. *Oncogene* 2005;24:2929-43.
- [60] Yazdi PT, Wang Y, Zhao S, Patel N, Lee EY, Qin J. SMC1 is a downstream effector in the ATM/NBS1 branch of the human S-phase checkpoint. *Genes Dev* 2002;16:571-82.
- [61] Zhu C, Mills KD, Ferguson DO, Lee C, Manis J, Fleming J, et al. Unrepaired DNA breaks in p53-deficient cells lead to oncogenic gene amplification subsequent to translocations. *Cell* 2002;109:811-21.
- [62] Green DR. Apoptotic pathways: ten minutes to dead. *Cell* 2005;121:671-4.
- [63] Herbig U, Jobling WA, Chen BP, Chen DJ, Sedivy JM. Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and p21(CIP1), but not p16(INK4a). *Mol Cell* 2004;14:501-13.
- [64] Costopoulos C, Liew TV, Bennett M. Ageing and atherosclerosis: Mechanisms and therapeutic options. *Biochem Pharmacol* 2008;75:1251-61.
- [65] Su Y, Swift M. Mortality rates among carriers of ataxia-telangiectasia mutant alleles. *Ann Intern Med* 2000;133:770-8.
- [66] Wang L, Ogburn CE, Ware CB, Ladiges WC, Youssoufian H, Martin GM, et al. Cellular Werner phenotypes in mice expressing a putative dominant-negative human WRN gene. *Genetics* 2000;154:357-62.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
- [67] Massip L, Garand C, Turaga RV, Deschenes F, Thorin E, Lebel M. Increased insulin, triglycerides, reactive oxygen species, and cardiac fibrosis in mice with a mutation in the helicase domain of the Werner syndrome gene homologue. *Exp Gerontol* 2006;41:157-68.
- [68] Burtner CR, Kennedy BK. Progeria syndromes and ageing: what is the connection? *Nat Rev Mol Cell Biol* 2010;11:567-78.
- [69] Liu Y, Rusinol A, Sinensky M, Wang Y, Zou Y. DNA damage responses in progeroid syndromes arise from defective maturation of prelamin A. *J Cell Sci* 2006;119:4644-9.
- [70] Scaffidi P, Misteli T. Lamin A-dependent nuclear defects in human aging. *Science* 2006;312:1059-63.
- [71] Ragnauth CD, Warren DT, Liu Y, McNair R, Tajsic T, Figg N, et al. Prelamin A acts to accelerate smooth muscle cell senescence and is a novel biomarker of human vascular aging. *Circulation* 2010;121:2200-10.
- [72] Cook NR, Albert CM, Gaziano JM, Zaharris E, MacFadyen J, Danielson E, et al. A randomized factorial trial of vitamins C and E and beta carotene in the secondary prevention of cardiovascular events in women: results from the Women's Antioxidant Cardiovascular Study. *Arch Intern Med* 2007;167:1610-8.
- [73] Dietrich M, Jacques PF, Pencina MJ, Lanier K, Keyes MJ, Kaur G, et al. Vitamin E supplement use and the incidence of cardiovascular disease and all-cause mortality in the Framingham Heart Study: Does the underlying health status play a role? *Atherosclerosis* 2009;205:549-53.
- [74] Ravn-Haren G, Bugel S, Krath BN, Hoac T, Stagsted J, Jorgensen K, et al. A short-term intervention trial with selenate, selenium-enriched yeast and selenium-enriched milk: effects on oxidative defence regulation. *Br J Nutr* 2008;99:883-92.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
- [75] Miller ER, 3rd, Juraschek S, Pastor-Barriuso R, Bazzano LA, Appel LJ, Guallar E. Meta-analysis of folic acid supplementation trials on risk of cardiovascular disease and risk interaction with baseline homocysteine levels. *Am J Cardiol* 2010;106:517-27.
- [76] Bleys J, Miller ER, 3rd, Pastor-Barriuso R, Appel LJ, Guallar E. Vitamin-mineral supplementation and the progression of atherosclerosis: a meta-analysis of randomized controlled trials. *Am J Clin Nutr* 2006;84:880-7; quiz 954-5.
- [77] Katsiki N, Manes C. Is there a role for supplemented antioxidants in the prevention of atherosclerosis? *Clin Nutr* 2009;28:3-9.
- [78] Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, et al. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *Am J Med* 2002;113 Suppl 9B:71S-88S.
- [79] Loke WM, Proudfoot JM, Hodgson JM, McKinley AJ, Hime N, Magat M, et al. Specific dietary polyphenols attenuate atherosclerosis in apolipoprotein E-knockout mice by alleviating inflammation and endothelial dysfunction. *Arterioscler Thromb Vasc Biol* 2010;30:749-57.
- [80] Martinez-Gonzalez J, Badimon L. Influence of statin use on endothelial function: from bench to clinics. *Curr Pharm Des* 2007;13:1771-86.
- [81] Wassmann S, Laufs U, Baumer AT, Muller K, Ahlbory K, Linz W, et al. HMG-CoA reductase inhibitors improve endothelial dysfunction in normocholesterolemic hypertension via reduced production of reactive oxygen species. *Hypertension* 2001;37:1450-7.
- [82] Manfredini V, Biancini GB, Vanzin CS, Dal Vesco AM, Cipriani F, Biasi L, et al. Simvastatin treatment prevents oxidative damage to DNA in whole blood leukocytes of dyslipidemic type 2 diabetic patients. *Cell Biochem Funct* 2010;28:360-6.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
- [83] Oeseburg H, Iusuf D, van der Harst P, van Gilst WH, Henning RH, Roks AJ. Bradykinin protects against oxidative stress-induced endothelial cell senescence. *Hypertension* 2009;53:417-22.
- [84] Fiordaliso F, Cuccovillo I, Bianchi R, Bai A, Doni M, Salio M, et al. Cardiovascular oxidative stress is reduced by an ACE inhibitor in a rat model of streptozotocin-induced diabetes. *Life Sci* 2006;79:121-9.
- [85] Madamanchi NR, Vendrov A, Runge MS. Oxidative stress and vascular disease. *Arterioscler Thromb Vasc Biol* 2005;25:29-38.
- [86] Warnholtz A, Nickenig G, Schulz E, Macharzina R, Brasen JH, Skatchkov M, et al. Increased NADH-oxidase-mediated superoxide production in the early stages of atherosclerosis: evidence for involvement of the renin-angiotensin system. *Circulation* 1999;99:2027-33.

Figure 1. Generation of reactive oxygen species (Adapted from www.cvphysiology.com)

1
2 Under normal condition the cell is able to efficiently use enzymes that remove the oxidants produced
3
4 as a result of normal metabolic processes. However, ROS are produced to excess in atherosclerosis ,
5
6 overwhelming the antioxidant system [85]. There is evidence to show that the diseased vessel wall and
7
8 adjoining plaque has increased levels of ROS generated by residing cells, and it is these ROS that are
9
10 responsible for causing DNA damage events [86].
11
12
13
14
15
16
17

Figure 2. The DSB DNA damage response

18
19
20 DNA damage responsible for the initiation of DSBs associated with cancer and atherosclerosis occur
21
22 through different stimuli. However the same DNA damage response pathways are activated to repair
23
24 this damage and the efficacy of this repair regulates disease pathogenesis.
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

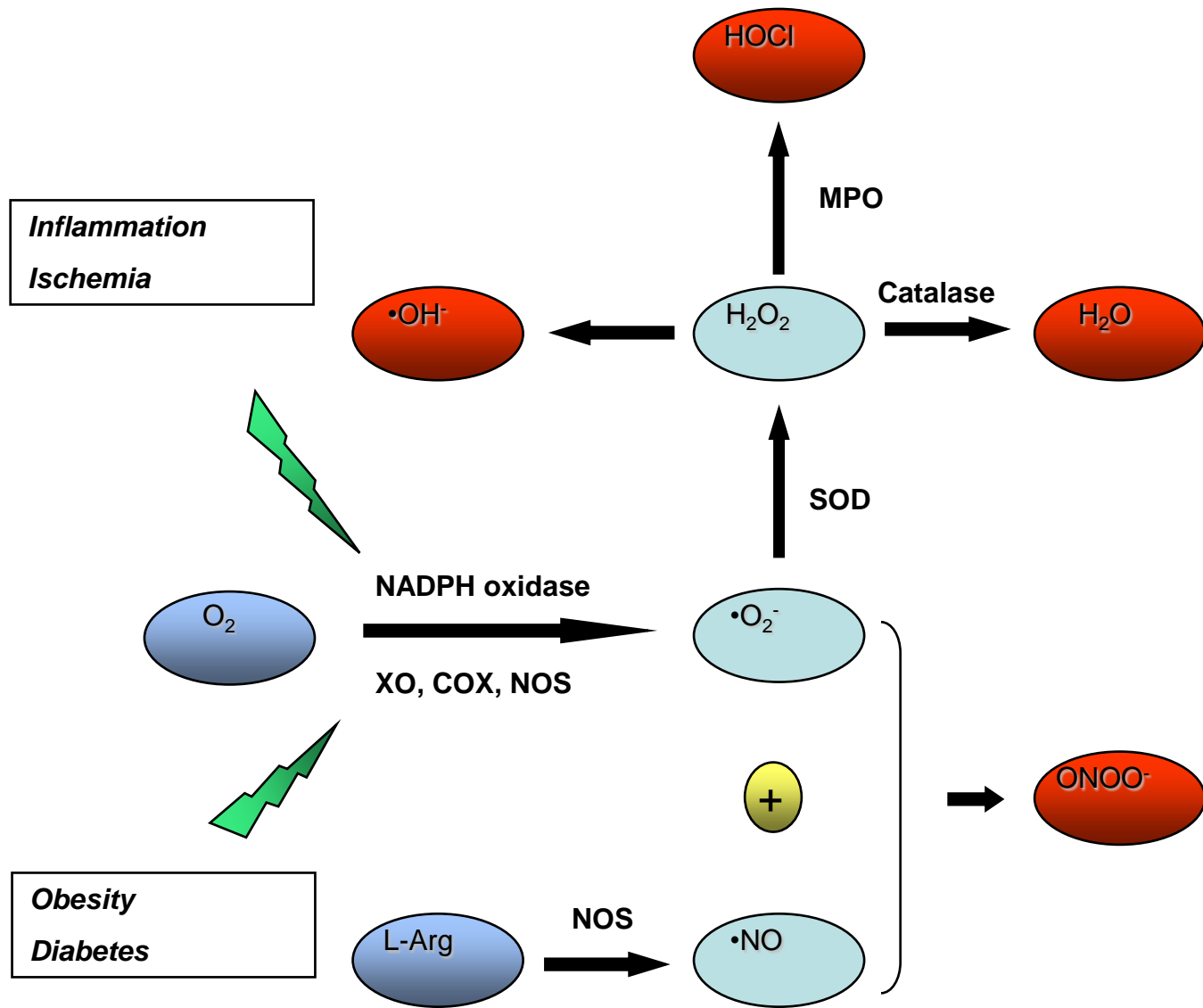


Figure 1

ATHEROSCLEROSIS

Oxidative Stress
Endothelial Dysfunction
Inflammation

CANCER

Ionizing radiation
UV
Oncogenic transformation

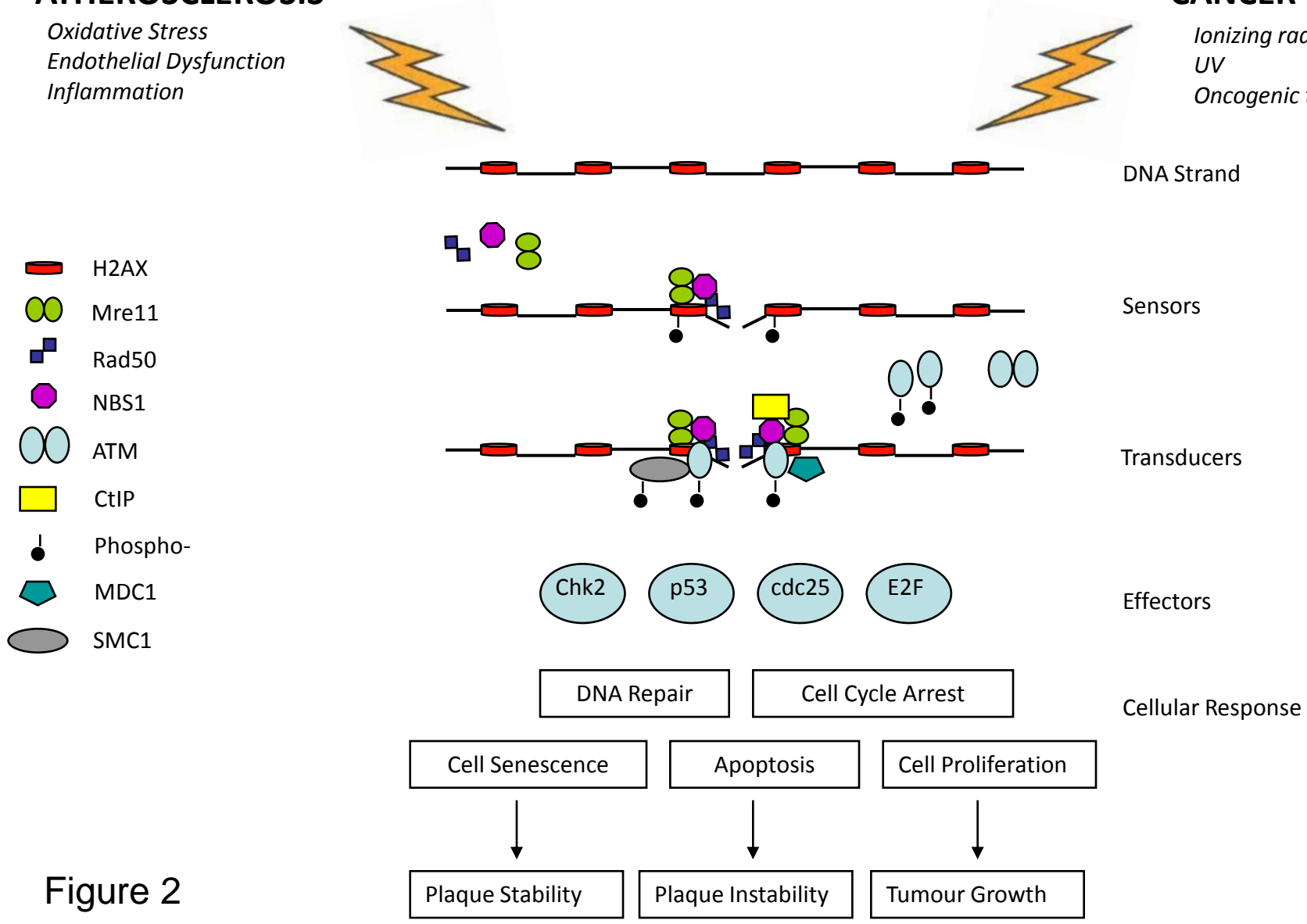


Figure 2

ATHEROSCLEROSIS

Oxidative Stress
Endothelial Dysfunction
Inflammation

CANCER

Ionizing radiation
UV
Oncogenic transformation

