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Role of DNA damage in atherosclerosis - bystander or participant?

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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

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HGPS: Hutchinson-Gilford Progeria Syndrome

HR: homologous recombination

ICAM-1: Inter-Cellular Adhesion Molecule 1

LDL: low-density lipoprotein

IL-6/8: Interleukin-6/8

iNOS: Inducible Nitric Oxide Synthase

IR: Ionising Radiation

MDC1: Mediator of DNA damage checkpoint protein 1

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

MtDNA: mitochondrial DNA

NBS1: Nijmegen Breakage Syndrome1 or Nibrin

NER: Nucleotide excision repair

NHEJ: Non-homologous end joining

ROS: Reactive oxygen species

SIPS: Stress induced premature senescence

SMC: Structural maintenance of chromosomes

SSBs: Single strand breaks

UV: Ultra Violet

VSMCs: vascular smooth muscle cells

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Abstract

Atherosclerosis leading to cardiovascular disease is the leading cause of death among western populations. Atherosclerosis in characterised by the development of a fibrofatty lesion that consists of a diverse cell population, including inflammatory cells that create an intensely oxidising environment within the vessel. Coupled with normal replication, the local intracellular and extracellular environment causes damage to cellular DNA that is recognised and repaired by the DNA damage response (DDR) pathway. The role of DNA damage and the resulting deregulation of 'normal' cellular behaviour and subsequent loss of cell cycle control checkpoints have been widely studied in cancer. However, despite the extensive evidence for DNA damage in atherosclerosis, it is only over the past two decades that a causative link between DNA damage and atherosclerosis has been hypothesised. Whilst atherosclerosis is a feature of human disease characterised by defects in DNA damage, currently the role of DNA damage in the initiation and progression of atherosclerosis remains highly debated, as a 'chicken and egg' situation. This review will analyse the evidence for, the causes of, and consequences of DNA damage in atherosclerosis, detail the DNA damage response pathway that results in these consequences, and highlight therapeutic opportunities in this area. We also outline the evidence that DNA damage is a cause of both initiation and progression of atherosclerosis, and not just a consequence of disease.

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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

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

DNA damage occurs in both genomic and mitochondrial DNA in atherosclerosis. For example, circulating cells in patients with severe coronary atherosclerotic disease exhibit a significantly higher incidence of the common mitochondrial deletion mtDNA4977 [8]. This has prompted investigations into the role of mitochondrial DNA damage and the subsequent association with cellular metabolic dysfunction in atherosclerosis. Mitochondrial DNA damage appears to be an early event in atherosclerosis [9], and is also a feature of mice lacking the DNA damage protein ATM. ATM haploinsufficiency in Apolipoprotein E deficient ($ApoE^{-/-}$) mice results in accelerated atherosclerosis in addition to symptoms of metabolic syndrome [10, 11]. VSMCs and macrophages from $ATM^{+/-}$ cells exhibited increased nuclear DNA damage, defects in the DNA repair pathway, reduced proliferation and increased apoptosis [11]. In addition many of the tissues analysed had an increased frequency of a 5kB deletion in the mitochondrial DNA and reduced mitochondrial oxidative phosphorylation [11]. Whilst these studies still demonstrate only an association between MtDNA damage and atherosclerosis, they demonstrate that mitochondrial DNA damage is sufficient in these models to induce mitochondrial dysfunction, that may result in a metabolic syndrome phenotype, thereby promoting atherosclerosis [10].

3. Causes of DNA damage in atherosclerosis

Genetic damage is caused by both insults from the extrinsic environment, in addition to reactive species generated from normal cell metabolism. If left unrepaired DNA damage creates errors during DNA replication. As such it has been estimated that a single cell can incur up to 10^4 DNA changes per day [12]. The activation of the DDR and subsequent downstream repair pathway is a highly evolved, complex network of tightly regulated post-translation modifications and protein-protein interactions. Whilst the DDR shares many common components (see below), the major stimuli inducing DNA damage in atherosclerosis are oxidative stress due to reactive oxygen species (ROS), specific risk

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

3.1 Oxidative Stress (Reactive Oxygen species)

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

Oxidative stress is also associated with accelerated telomere shortening and subsequently reduced telomere length [18, 19], leading to premature cellular senescence. Telomere attrition is linked with vascular disease and vascular ageing [19, 20], in both circulating cells and in VSMCs in plaques themselves[19], and is also associated with increased oxidative DNA damage. For example, lymphocyte DNA from patients with type II diabetes exhibit increased susceptibility to oxidative DNA

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

3.2 Epigenetics

Epigenetic regulation, particularly DNA methylation and histone modification, of vascular genes and growth factors is observed in the development and progression of atherosclerosis. For example, DNA hypomethylation occurs in monocytes, VSMCs and the plaques of patients with atherosclerosis [22] and additional studies using ApoE$^{-/-}$ mice have shown that DNA hypomethylation represents a significant risk factor associated with susceptibility to atherosclerosis [23]. DNA damage in vascular cells can result from exposure to oxidative stress, and under normal circumstances there are a variety of antioxidant enzymes that remove these damaging reactive oxygen species from the environment. Recent studies have shown that inflammatory cytokines can alter the expression of mediators of oxidative stress such as inducible nitric oxide synthase (iNOS) by causing changes to the chromatin structure of the promoter [24]; the resultant increase in the expression of nitric oxide (catalysed by iNOS) causes VSMC apoptosis that is associated with increased plaque instability. Furthermore DNA hypomethylation of the antioxidant enzyme superoxide dismutase (SOD) gene results in reduced expression [25]. These gene expression changes likely force the cellular redox balance towards that of a highly oxidising environment, that may potentially increase cellular DNA damage and enhance the progression of atherosclerotic lesions.

3.3 Cytotoxic Agents and Radiotherapy

One of the most direct demonstrations of the link between DNA damage and atherosclerosis comes from studies of both chemotherapy and radiotherapy. Both cytotoxic drugs and radiotherapy target the processes and machinery involved in regulating DNA and cell replication. Chemotherapeutics such as the alkylating agent cyclophosphamide [26] or the antibiotic-based drugs (e.g. anthracyclines) [27]

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

Similarly, radiotherapy works on the principle that radiation directly induces single and double strand breaks, resulting in growth arrest and apoptosis. For both indirect and direct ionizing radiation, damage is induced through hydroxyl radicals resulting from the ionization of water, or by through exposing DNA to free radicals. In cancer, the increased proliferative ability of tumour cells results in incomplete DNA repair after radiotherapy, with consequent damage accumulation through multiple divisions.

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

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The mechanism of action of vascular disease induced by cytotoxic chemotherapy and ionizing radiation is also becoming clearer. Both modalities induce DNA damage and cell death in endothelial cells and VSMCs, with subsequent endothelial dysfunction and inflammation, resulting in cell senescence, apoptosis, thrombosis formation, mitochondrial dysfunction and fibrosis, all of which promote atherosclerosis. Irradiation exposure has been shown induce the expression of adhesion molecules (e.g. I-CAM-1) and inflammatory cytokines, including interleukin-6 and interleukin-8 (IL-6 and IL-8) [34] causing radiation- induced DNA damage and mitotic death of endothelial cells. In larger vessels this has been proposed to lead to endothelial denudation, an initiator of lesion development [35]. In ApoE$^{-/-}$ mice, ionising radiation had been shown to result in the accumulation of macrophages in atherosclerotic lesions, accelerated plaque development and increased susceptibility to intraplaque haemorrhage [36], the latter representing a feature of advanced atherosclerosis.

4. Consequences of DNA damage in Atherosclerosis

4.1 Growth arrest and Senescence

Normal cell division is regulated by a four-stage cycle that is able to verify the efficacy of the cellular DNA before replication occurs. This inhibits reproduction of damaged DNA to prevent accumulation of mutated proteins within the cell. DNA synthesis begins with production of specific enzymes required for replication in G_1, followed by replication of chromosomes during S phase. Major checkpoints exist throughout the cell cycle, particularly in G_1 and G_2, that are activated following DNA damage [37]. DNA damage activates the DDR (see below) with transient growth arrest to allow DNA repair to occur, preventing propagation of damaged DNA. Repeated or excessive DNA damage can also induce replicative senescence, a state of irreversible growth arrest [38]. In vascular cells, senescence is characterised by activation of G_1/S restriction point proteins, and there is now increasing evidence that human plaque VSMCs show impaired cell proliferation [3] and multiple features of cell senescence *in vivo* and *in vitro* [19].

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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 was 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 G_2- 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)**.

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5.1 Sensors

Sensor proteins are recruited to sites of DNA damage and orchestrate formation of multi-protein complexes to activate the DDR. For example, nibrin (NBS1), as one of the initial sensors of double strand breaks, forms a trimeric complex with Mre11 and Rad50 (MRN) (reviewed by Rupnik *et al* 2008 [44]). NBS-1 acts as a molecular scaffold that tethers the complex to the specific site of DNA damage. Mre11 is a single-strand specific endonuclease and double-strand specific exonuclease that is phosphorylated upon DNA damage; interaction with Rad50 regulates its nuclease activity due to binding to the DNA and tethering the ends in close proximity to allow repair of damaged DNA. This complex translocates to the nucleus and binds at DSBs to form distinct foci with an additional sensor protein histone 2A (H2AX) that is phosphorylated at Ser^{139} following DNA damage [45].

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

5.2 Transducers

DNA damage sensors including MRN and H2AX triggers a cascade of phosphorylation events that activate downstream transducer proteins, that ultimately lead to induction of effector proteins that stall the cell cycle to carry out DNA repair. DNA repair is first characterised by the recruitment of C-terminal interacting protein (CtIP) an endonuclease that cooperates with the MRN complex [49, 50] and mediator of DNA damage checkpoint protein 1 (MDC1) that acts as a scaffold for the recruitment of other DNA repair proteins such as Ataxia Telangiectasia Mutated protein (ATM), breast cancer protein 1 (BRCA1) and p53 binding protein (53BP1) [51, 52]. There is crossover and cooperation between the ATM/ATR pathways in the DNA damage response to facilitate efficient repair of DNA lesions with up to 25 ATM and ATR substrates having been identified [53]. ATM exists as an inactive monomer whose activation occurs in two ways, by autophosphorylation of Ser^{1981} [54], or by

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

5.3 Effectors

Cell Cycle Arrest

Both the ATM and ATR-regulated pathways initiate cell cycle arrest at either the G_1/S or the G_2/M phase to facilitate the efficient repair of DNA damage. Transient growth arrest occurs until the DNA damage has been adequately repaired; the blocks to the replication machinery are then removed allowing a return to normal function. Cell cycle arrest is initiated by ATR/ATM-mediated activation of the checkpoint kinases Chk1 and Chk2 respectively that phosphorylate the Cdc25 phosphatase on multiple residues to provide binding sites for the 14-3-3 proteins, Rad24 and Rad25. This prevents entry of Cdc25 into the nucleus blocking interaction with its substrate Cyclin E-Cdk2 [56]. Following DNA damage ATM activation also phosphorylates 'the guardian of the genome' p53 at residues Ser^{15} and Ser^{20} leading to accumulation of this protein in the nucleus [57, 58]. Active p53 induces the cyclin-dependent kinase inhibitor p21 that inhibits the CyclinD1-Cdk2 and CyclinE-Cdk4 kinase complexes preventing the cell from moving to the next stage of the cell cycle [59].

DNA Repair

The Structural Maintenance of Chromosomes (SMC1) protein is phosphorylated on Ser^{966} by active ATM in an NBS-1-dependent manner resulting in S-phase checkpoint activation. SMC1 forms a heterodimer with SMC3 to form the 'cohesion complex' creating a structural change that coordinates cohesion between sister chromatids during DNA replication. This complex has also been shown to be required for post-replicative DSB DNA repair [60] Further studies in animal models have investigated the role of DNA repair proteins involved in NHEJ including XRCC4, DNA ligase IV, DNA-PK and Ku80 in p53-deficient mice. Double knockout mice (p53/XRCC4 and p53/DNA ligase IV double null) exhibit increased chromosomal instability due to defective NHEJ and cell cycle checkpoint capability

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demonstrating the importance of these functions and associated proteins in maintaining genome integrity [61].

Apoptosis

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

Senescence

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

6. DNA damage syndromes associated with atherosclerosis

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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

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

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6.3 Hutchinson–Gilford Progeria Syndrome (HGPS)

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

7. Therapeutic Options in the prevention of DNA damage

The evidence presented above indicates that DNA damage may be a major causal factor in both the initiation and progression of human atherosclerosis. As such, it should be a target for therapy. Effectively management of atherosclerosis is currently based on reduction in exposure to risk factors (e.g. smoking, cholesterol levels, diabetes), with little if any specific therapy. Similarly, whilst risk factor reduction should reduce DNA damage, animal models have shown that damage is highly persistent in established plaques. There is thus the potential for treatments that directly augment DNA

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

7.1 Antioxidants

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

7.2 Polyphenols

Polyphenols including quercetin and theaflavin derived from fruit and vegetables are naturally occurring organic chemicals composed of a number of phenol subunits that form a complex ring structure. Studies have shown that populations with polyphenol-rich diets show evidence of a trend toward protection from the development of cardiovascular diseases (reviewed in [78]). Due to the number of processes involved in the development of atherosclerosis, including oxidative stress, inflammation and endothelial dysfunction, it has been proposed that polyphenols exert their protective effects by preventing one or more of these processes. A recent study investigated the role of specific dietary polyphenols in regulating the development of atherosclerosis in ApoE$^{-/-}$ mice. These results showed that of the polyphenols examined (quercetin, epicatechin, theaflavin, sesamin, chlorogenic acid), there was a reduction in the atherosclerosis observed in these mice and this occurred through inhibition of inflammation, increased production of nitric oxide, and the induction of heme oxygenase [79]. Whilst this study suggests that polyphenols represent candidates for an effective chemo-

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

7.3 Statins

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

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

7.4 ACE Inhibitors

Angiotensin converting enzyme (ACE) inhibitors reduce the activity of the renin-angiotensin-aldosterone system, blocking the conversion of angiotensin I to angiotensin II and the enzyme responsible for the degradation of bradykinin, a vasodilator. Bradykinin has been shown to protect endothelial cells from superoxide-induced senescence through inhibition of DNA damage [83]. Studies using a rat model of induced diabetes showed that ACE inhibitors are able to reduce the production of DNA damage inducing ROS, that cause endothelial dysfunction and subsequent cardiovascular remodelling [84] thereby modulating a key process in the development and progression of atherosclerosis.

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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.

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

Under normal condition the cell is able to efficiently use enzymes that remove the oxidants produced as a result of normal metabolic processes. However, ROS are produced to excess in atherosclerosis , overwhelming the antioxidant system [85]. There is evidence to show that the diseased vessel wall and adjoining plaque has increased levels of ROS generated by residing cells, and it is these ROS that are responsible for causing DNA damage events [86].

Figure 2. The DSB DNA damage response

DNA damage responsible for the initiation of DSBs associated with cancer and atherosclerosis occur through different stimuli. However the same DNA damage response pathways are activated to repair this damage and the efficacy of this repair regulates disease pathogenesis.

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Figures

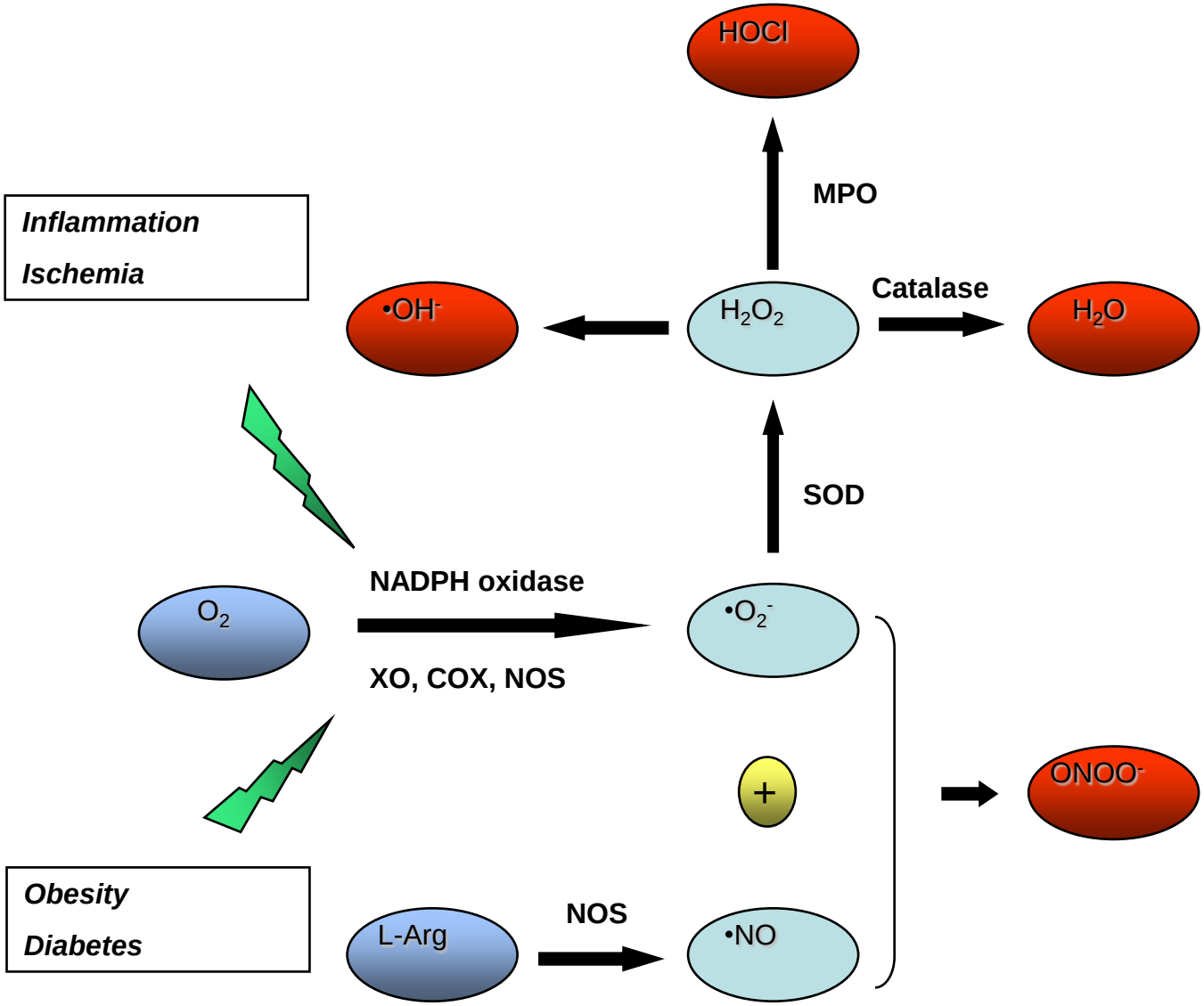


Figure 1

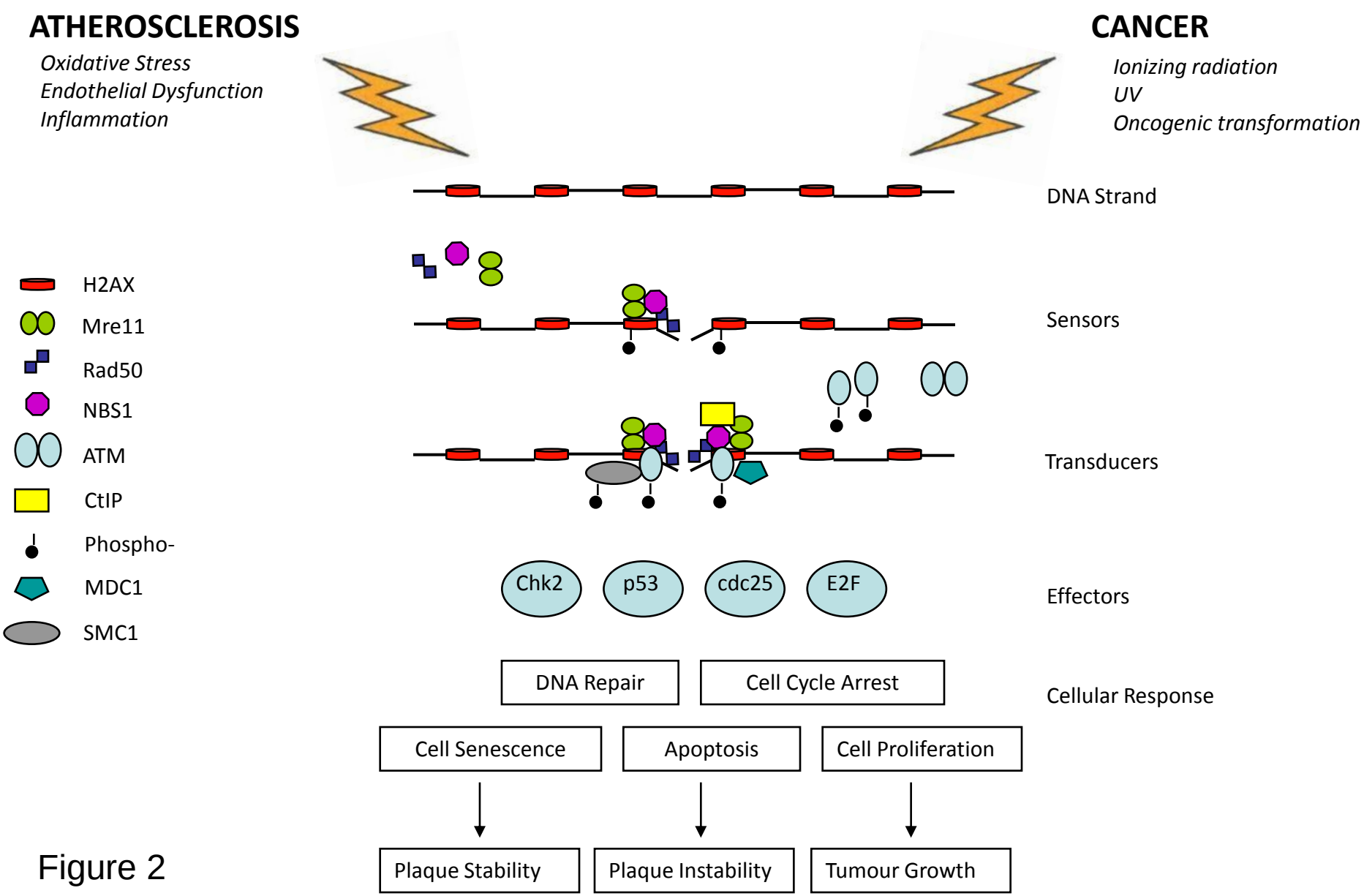
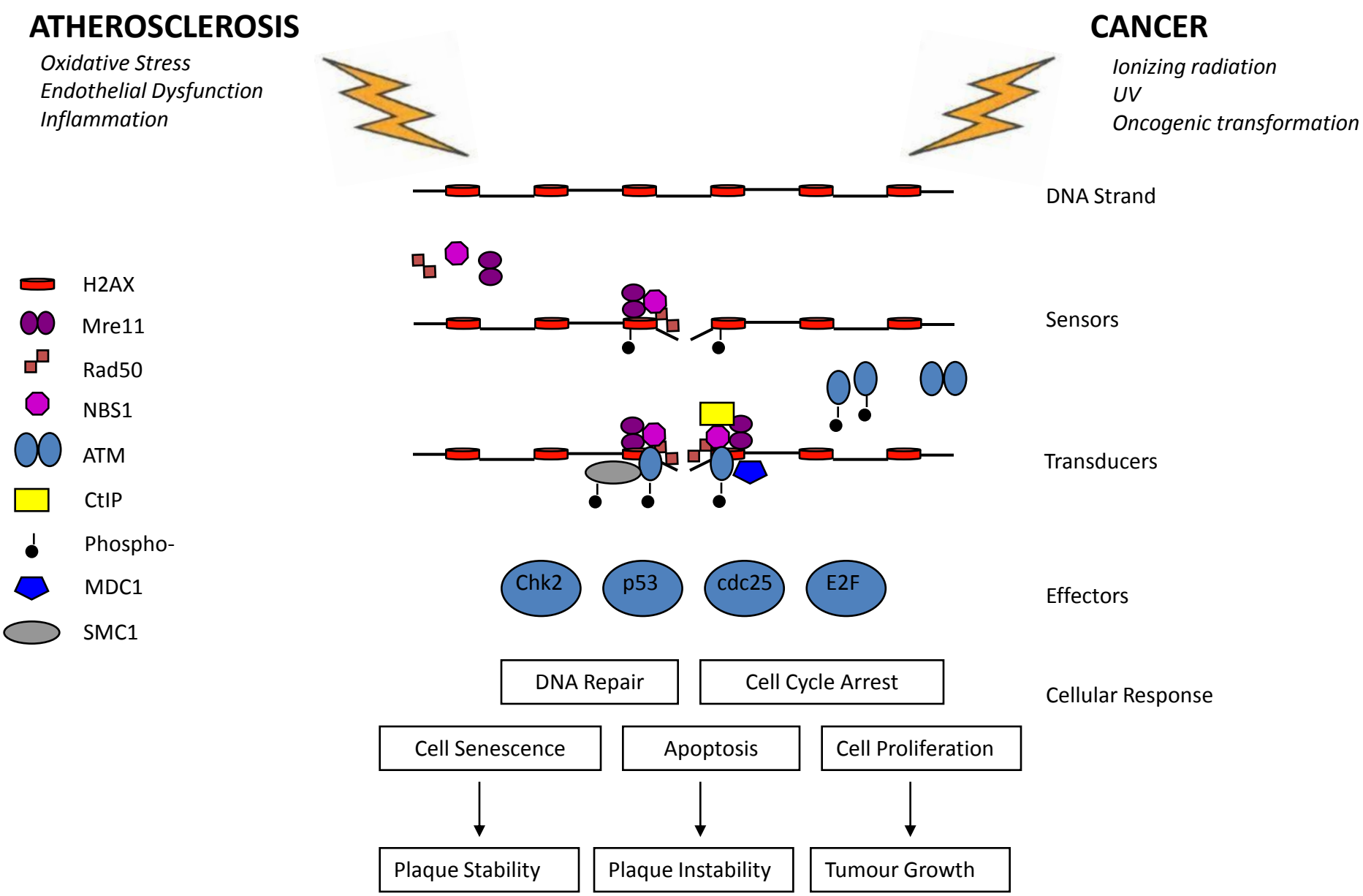

ATHEROSCLEROSIS
Oxidative Stress
Endothelial Dysfunction
Inflammation
CANCER
Ionizing radiation
UV
Oncogenic transformation
DNA Strand
Sensors
Transducers
Effectors
Cellular Response
H2AX
Mre11
Rad50
NBS1
ATM
CtIP
Phospho-
MDC1
SMC1
Chk2
p53
cdc25
E2F
DNA Repair
Cell Cycle Arrest
Cell Senescence
Apoptosis
Cell Proliferation
Plaque Stability
Plaque Instability
Tumour Growth

Figure 2

*Graphical Abstract