Review Article

Interventional Cardiology

Deoxyribonucleic acid repair in atherosclerotic coronary artery disease

Gilpin TR[1], Gabara L[2], Miles EA[2], Curzen NP[2], Mahmoudi M[2*]

[1]Department of Medicine, University of Southampton, Southampton, UK

[2]Coronary Research Group, University Hospital Southampton NHS Foundation Trust, Southampton, UK

*Author for correspondence:
Mahmoudi M, Coronary Research Group, University Hospital Southampton NHS Foundation Trust, Southampton, UK, E-mail: m.mahmoudi@soton.ac.uk

Received date: June 28, 2021
Accepted date: July 12, 2021
Published date: July 19, 2021

Abstract

Despite recent advances in the therapeutic and interventional treatment of coronary artery disease the global prevalence of this disease is increasing and the associated mortality and morbidity remain high. Alongside well-established risk factors such as smoking, hypertension, hypercholesterolaemia and diabetes mellitus, deoxyribonucleic acid (DNA) damage, cascade protein signalling and the associated repair pathways are becoming increasingly recognised as major causative co-factors in the pathogenesis of atherosclerosis. A number of *in vitro* studies have shown defective DNA repair is instrumental in the pathogenesis and progression of atherosclerotic plaques with a positive correlation observed between the level of DNA damage and the severity of the atherosclerotic lesions. In knockdown mouse models of atherosclerosis, the DNA repair signalling cascade has been shown to be amenable to pharmacological manipulation and overexpression of specific repair proteins attenuate atherogenesis. However, to date there is little in-human data which supports these findings. This review will explore the current evidence and understanding of the role of DNA damage and repair in the pathogenesis of atherosclerosis and address possible therapeutic interventions for treatment.

Keywords: Cardiovascular disease • Coronary artery • DNA repair • Atherosclerosis

Introduction

Despite advances in the medical and invasive treatment of Coronary Artery Disease (CAD) the global incidence and mortality remain high [1,2]. Information generated from the latest Global Burden of Disease Study 2019 confirms historical data that cardiovascular disease (CVD) due to atherosclerosis remains the leading cause of overall mortality [2].

Whilst new coronary interventional procedural techniques such as the concordant use of fractional flow reserve assessment of ischaemia [3], intracoronary imaging (intravascular ultrasound (IVUS) and Optical Coherence Tomography (OCT)) have been associated with improvements in clinical outcomes in patients undergoing Percutaneous Coronary Intervention (PCI) [4], there have been very few advances in the medical therapy of CAD in recent years [5]. Progress still needs to be made in the primary prevention of cardiovascular events.

The rapid expansion and improvement in genomic research techniques in recent times has highlighted DNA damage within the atherosclerotic plaque and in circulating leukocytes as key factors in the pathogenesis of atherosclerosis [6-8].

Overview of Deoxyribonucleic Acid (DNA) Damage and the Response Pathways

DNA damage in human cells occurs *via* endogenous and exogenous pathways. Typically,

ISSN 1755-5302

endogenous damage is caused by Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) formed as a result of oxidative stress, metabolic processes and inflammatory responses to a variety of stimuli [9,10]. Exogenous DNA damage primarily occurs as a result of exposure to ionising radiation, ultraviolet radiation and mutagenic chemicals such as organophosphates but can also occur following exposure to toxic metals, mercury being a primary example [11]. The clinical consequences of both pathways include a wide variety of disease processes such as cancer and atherosclerosis.

Of the endogenous and exogenous pathways resulting in DNA damage, endogenous ROS, at both physiological and pathological levels, are the most common cause. Whilst producing adenosine triphosphate (ATP) the mitochondrial electron transport chain, as a double-edged sword, is the primary source for intracellular ROS [12]. Several ROS have been shown to increase the progression of atherosclerosis including superoxide anion, hydrogen peroxide, hydroxyl radicals and oxidised lipids such as oxidised-low density lipoprotein [13,14], acting primarily by disruption of mitochondrial antioxidant pathways [15]. Initially, superoxide and hydrogen peroxide are inert to DNA, but following conversion into hydroxyl radicals *via* the Fenton reaction they can subsequently cause extensive damage to mitochondrial DNA (mtDNA) and nuclear DNA which results in single stand breaks, double strand breaks, glycolytic damage and mispairing [16,17].

In order to protect against the deleterious effects of DNA damage, human cells have developed several pathways collectively known as the DNA Damage Response (DDR) [18]. A number of DDR pathways have been identified to date, which include Base Excision Repair (BER) [19], Double Strand Break Repair (DSBR) subdivided into homologous and non-homologous recombination alternatively known as non-homologous end-joining [20], Nucleotide Excision Repair (NER) [21,22], and mismatch repair (MMR) [23]. BER and DSBR are the most relevant repair mechanisms resulting from oxidative stress in atherosclerosis (Figure 1).

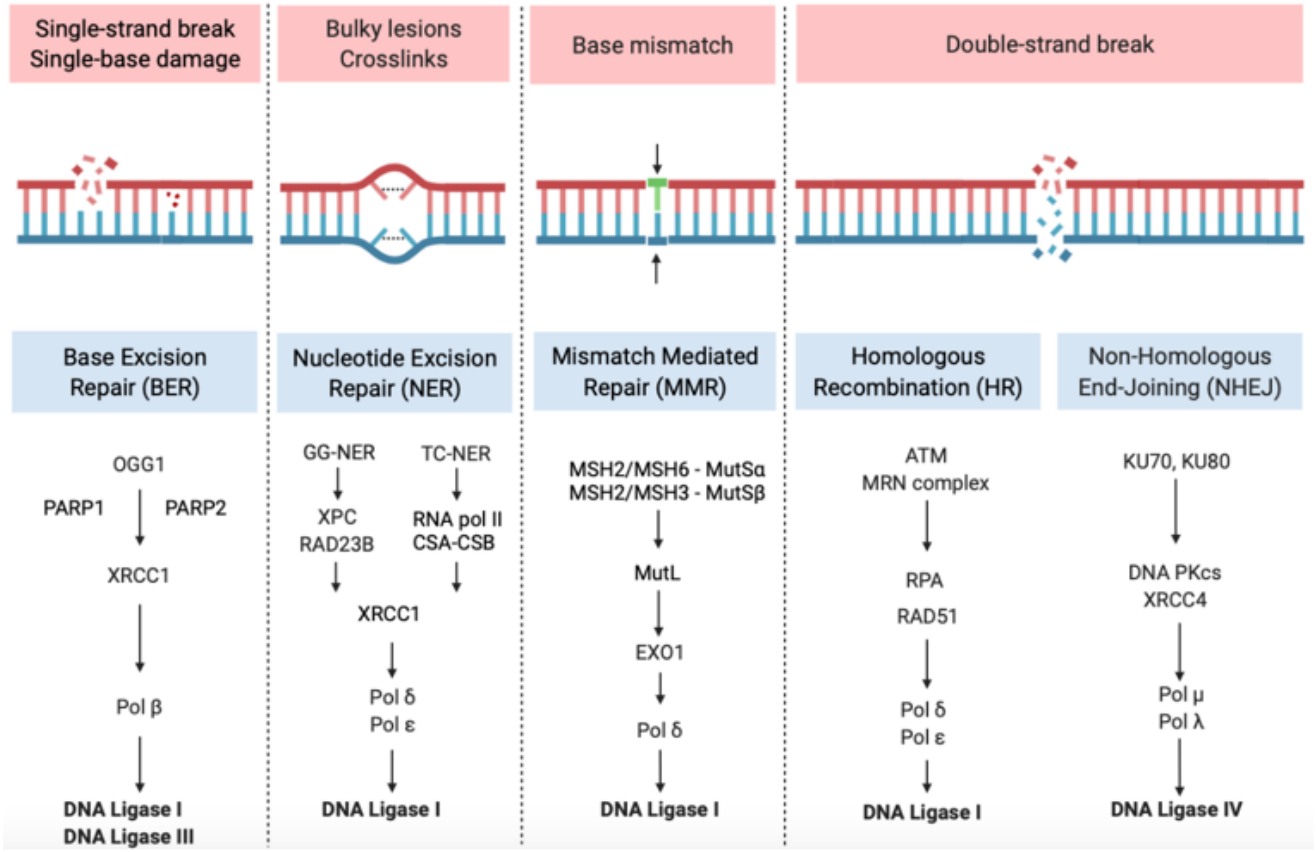

Figure 1: The major repair pathways for DNA damage-the DDR. Type of DNA damage sustained (top, pink), commonly employed repair pathway for correction (middle, blue) and the sensory, transducer and effector cascades (bottom). Adapted from Lord, et al. The DNA damage response and cancer therapy and Biorender, DNA repair pathways [56,57].

The repair mechanisms are broadly governed by a series of modulatory and recruiting proteins acting in three stages. Sensor proteins detect the initial damage to DNA [24,25], transducer/adaptor proteins initiate a signalling cascade [26], which in turn results in effector proteins enacting the final stage of the pathway, cell cycle arrest, delay, DNA repair or apoptosis [27].

DNA repair pathways

Base Excision Repair (BER): When damage occurs as a result of oxidative stress, alkylation and abasic single base damage that does not result in significant distortion to the integrity of the DNA helix, base excision repair may correct the issue [28]. Chromatin remodelling of the damaged bases results in the recruitment of a variety of DNA glycosylases which in turn remove the distorted lesions forming an Abasic Site (AP) [29]. DNA glycosylases are either monofunctional, possessing only glycosylase activity, commonly uracil glycosylases, for example N-methylpurine DNA Glycosylase (MPG), or are bifunctional possessing additional β-lyase activity: Examples include Nth-like DNA glycosylase-1 (NTHL1) and Nei-like DNA glycosylase 1 (NEIL1) [30]. It is important to note that some are able to act as both monofunctional or bifunctional glycosylases, for example the ubiquitous 8-oxoguanine DNA glycosylase (OGG1) [31].

Monofunctional glycosylases initiate short-patch repair in which a 5' phosphodiester incision is made by apurinic endonuclease-1 (APE-1) to the AP site. The single gap is filled by DNA polymerase-β (Pol-β) and then ligated by either DNA ligase I (LIG1) or the DNA ligase III (LIG3) X-Ray repair Cross-Complementing protein 1 (XRCC1) complex [32]. Long-patch repair is started as the result of bifunctional glycosylases, under the action of poly (ADP-ribose) polymerase 1 (PARP1) and poly (ADP-ribose) polymerase 2 (PARP2) [33]. Following the initial DNA glycosylase incision, the resultant AP site is customised by the 3' phosphodiesterase activity of APE-1, after which DNA polymerase δ/ε (Pol δ/ε) begin synthesising in a strand displacement manner, creating a flap of typically 2-10 nucleotides, this is finally ligated by LIG1 [28,34].

Double Strand Break Repair (DSBR): Double strand DNA breaks have a significantly more detrimental effect on cellular longevity and DNA integrity. They commonly arise as a result of exposure to ROS, ionising radiation, V(D)J recombination and immunoglobulin class switching processes [35]. The repair of double strand breaks is primarily *via* two pathways, Homologous Recombination (HR) and Non-Homologous End-Joining (NEHJ) [28].

In NHEJ the repair process is governed by the tumour suppressor p53 binding protein 1 (53BP1). It is responsible for recruiting many of the other repair components and, importantly, facilitating the joining of the two break ends [36]. The Ku heterodimer (Ku70 and Ku80) moves rapidly to stabilise the break and prevent end resection and, in turn, recruits DNA-dependent protein kinase-catalytic subunit (DNA-PKcs) [37,38]. Ku subsequently moves inwards on the DNA whilst, simultaneously, X-ray Repair Cross-Complementing protein 4 (XRCC4) stabilises the whole complex by tethering the ends together [39]. The groups which are blocking the strand ends are resected and the resultant gaps are filled by DNA polymerase μ (Pol μ) and DNA polymerase λ (Pol λ). DNA ligase 4 (LIG4) completes the NHEJ procedure by joining the ends [40,41].

Homologous recombination results in a very high quality repair and acts *via* pathways utilising DNA strand invasion and template directed DNA repair synthesis [42]. The governing complex MRN, consisting of the double strand break repair protein MRE11 (MRE11), DNA repair protein RAD50 (RAD50) and Nibrin (NBS1), recognises the double strand break and recruits ataxia-telangiectasia mutated (ATM) and histone acetyltransferase KAT5 (TIP60) [43]. An intermediate recruitment cascade occurs resulting in replication protein A (RPA) and RAD51 initiating strand invasion allowing 3'-OH priming synthesis *via* DNA polymerases δ, κ and ν [44,45].

Nucleotide Excision Repair (NER): Nucleotide excision repair is often employed in helix distorting damage that is classically a result of ultraviolet radiation [46]. In a similar fashion to DSBR, NER employs two distinct pathways to repair the damage; global genome NER (GG-NER) and transcription-coupled NER (TC-NER) [47]. In TC-NER damaged lesions are excised from transcribed stands following exposure as a result RNA polymerase II reverse translocation which is stimulated by a complex of DNA excision repair protein-8 (ERCC8) and DNA excision repair protein-6 (ERCC6) also known as the CSA-CSB complex [48]. In GG-NER the damage sensor Xeroderma pigmentosum complementation group C (XPC)-UV excision repair protein RAD23 homolog B (RAD23B) complex scans the genome for transient single-stranded DNA (ssDNA) caused by disrupted base pairing due to the lesion, thus exposing the damage [49]. The excision of the damaged nucleotide and finishing process is shared by both the GG-NER and TC-NER pathways and culminates in a myriad of DNA polymerases, typically δ and ε, completing the gap filling and LIG1 or XRCC1-LIG3 implementing ligation [50]. Whilst there is currently limited evidence of increased NER activity in atherosclerosis the importance of NER was shown in two NER defective mouse models ERCC1$^{d/-}$ and XPR^{TTD}. Specifically, increased vascular cell senescence, stiffness, hypertension and impaired vasomotor function were all noted [21].

Mismatch Repair (MMR): The MMR pathway is an evolutionarily conserved pathway which typically repairs base mismatches that occurred during replication and insertion-deletion loops that have resulted from strand slippage events [51]. It is further utilised in numerous other cellular processes such as DNA-damage signalling, apoptosis, meiotic and mitotic recombination and microsatellite instability [52]. Chromatin modification is the initial step in the MMR pathway and allows cascade signalling proteins access to the DNA lesion in order to initiate repair [53]. In humans base mismatches and short, meaning single or double insertion/deletion loops, are recognised by the DNA mismatch repair protein Msh2 (MSH2)/MSH6 heterodimer, otherwise known as MutSα, with longer loops recognised by the MutSβ heterodimer (MSH2/MSH3) [54]. Several MutL complexes are recruited, with MutLα, in particular, having a central role since it controls the cessation of mismatched-provoked excision along with influencing the 3' nick-directed digestion by Exonuclease 1 (EXO1) [55-57]. The final steps in the pathway involve various DNA polymerases and LIGI1 synthesising and sealing the gap.

Evidence for DNA Damage in Atherosclerosis

The pathogenesis of atherosclerosis is a multifaceted process and DNA damage has been shown to be a key component in the process at both micro and macros levels [58]. Several studies have shown that cells in the systemic circulation and those found within the mature atherosclerotic plaque contain increased levels of DNA damage compared to controls without significant atherosclerotic disease [59-62].

DNA base damage

As mentioned previously, DNA bases can be particularly vulnerable to damage by ROS, in particular guanine due to its low redox potential, which results in base modification to 7,8-dihydro-8-oxoguanine (8-oxoG) [63]. The consequence of this can be G:C and T:A transverse mutations [64]. Martinet, et al. found high levels of cytoplasmic and nuclear immunoreactivity for 8-oxoG in atherosclerotic Vascular Smooth Muscle Cells (VSMC), endothelial cells and, importantly, in monocytes. This was not replicated in surrounding normal media or arteries [60,61]. In a recent landmark study from Shah, et al. the key BER enzyme OGG1 was found to have significantly reduced activity resulting in defective oxidative DNA repair in human VSMCs [65]. 8-oxoG repair in VSMCs from human carotid plaques was compared to age and sex matched controls (normal aortic tissue), plaque VSMC had reduced nuclear 8-oxoG activity, as shown by fluorescently labelled 8-oxoG containing molecular beacon assay. Following isolated *in vitro* OGG1 knockdown of exon 1 and 7 in rat VSMCs (confirmed by normal expression of other BER enzymes NEIL1 and NTH), oxidative damage was induced by tert-butyl hydroperoxide (t-BHP). It was found that t-BHP increased intracellular 8-oxoG significantly in OGG1 knockdown VSMCs compared to controls, thus confirming OGG1 is a major BER enzyme repairing 8-oxoG. $OGG1^{-/-}$ mice had more extensive atherosclerosis compared to controls following a lipid rich diet, as expected, but importantly, correction of OGG1 levels resulted in improved BER and a subsequent reduction in atherosclerotic burden. This indicates that oxidative DNA damage directly promotes atherogenesis [65].

Telomere attrition

Shortened telomeres have been shown to be present in atherosclerotic VSMC and contribute to the pathogenesis of ischaemic heart disease [66]. Telomere shortening and reduced telomere lengths have been observed in many previous studies in a variety of cell lines known to be instrumental in the pathogenesis of atherosclerosis. Reduced telomere length was found in atherosclerotic endothelial cells [67] and cultured sub-endothelial progenitor cells from patients with coronary artery disease [68]. Brouilette, et al. showed that circulating leukocytes also show reduced telomere length in patients with ischaemic heart disease and previous myocardial infarction compared with controls [69], a finding further replicated by Spyridopoulos, et al. [70,71]. Reduced telomere length is inversely correlated to cardiovascular disease risk in patients with subclinical atherosclerotic disease, and furthermore reduced telomere length is also found to be more prevalent in men [72]. This has important clinical consequences. In the landmark study published [73], Brouilette, et al. reported that statin treatment in those deemed at higher risk of adverse cardiovascular events, based on their telomere length, yielded a reduction in clinical events compared to controls. This finding was deemed to be independent of blood lipid concentrations or inflammation as there was no difference in LDL, HDL, triglycerides, CRP or fibrinogen levels between the groups. They suggested another potential protective mechanism of statins is *via* increased expression of the telomere capping protein Telomeric Repeat-binding Factor 2 (TRF2) [73,74].

Reactive oxygen species and mitochondrial DNA (mtDNA) damage

In addition to nuclear DNA damage, mtDNA damage can occur by similar deleterious mechanisms. The mitochondrial deletion mtDNA 4977 has been shown to result in mitochondrial dysfunction [75], with circulating leukocytes in patients who have severe coronary artery disease showing a greater degree of this deletion compared to controls [76]. However, a causal link was not established.

More concrete evidence from Ballinger, et al. found higher levels of mtDNA damage in aortas of patients undergoing surgery for severe atherosclerotic vascular disease compared to normal aorta from transplant donors at the time of organ harvesting, high mtDNA damage was also found in aortas from apolipoprotein E knockdown ($ApoE^{-/-}$) mice suggesting a possible causal link [15]. Increased mitochondrial reactive species generation which is as a direct result of a deficiency in the protective mitochondrial superoxide dismutase (SOD2) antioxidant enzyme increases mtDNA damage and accelerates further atherosclerosis development, perpetuating the destructive cycle [15,77].

Yu, et al. again looked at $ApoE^{-/-}$ mice that were also deficient for mitochondrial polymerase-γ (polG) proof reading activity. They found $ApoE^{-/-}/polG^{-/-}$ mice (possessing high levels of mtDNA damage and oxidative phosphorylation) had increased atherosclerosis and impaired proliferation and apoptosis of VSMCs [78]. Furthermore, $ApoE^{-/-}/polG^{-/-}$ monocytes had increased apoptosis and inflammatory cytokine release, known to perpetuate atherosclerosis. In the human population of the study the authors found increased mtDNA damage in atherosclerotic plaques compared with normal vessels. Furthermore, leucocyte mtDNA damage was associated with higher risk, vulnerable plaques as characterised on virtual histology-IVUS during coronary angiography [78]. It is important to note that high risk coronary atherosclerotic plaques such as thin capped fibroatheroma have been shown to be associated with a higher frequency of major adverse cardiovascular events, as elegantly described in the PROSPECT trial [79]. This suggests a possible causative link between leucocyte mtDNA damage and major adverse cardiovascular events that warrants further investigation.

Human studies

The most recent key piece of research addressing DNA damage in atherosclerosis is the DECODE I study from Shah, et al. [80]. A total of 89 patients, who were scheduled to undergo PCI for stable angina or Non-ST Elevation Myocardial Infarction (NSTEMI), were prospectively enrolled. DNA ligase I, III and IV activity were measured in PBMCs as a marker of total DNA repair activity. The presence of healed coronary plaque rupture (which has been shown in previous autopsy and *ex vivo* studies to be related to sudden death in an Acute Coronary Syndrome (ACS) population [81,82] was assessed using intracoronary OCT imaging during angiography. The presence of healed coronary plaque was greater in individuals with higher level of DNA ligase activity and had a positive and direct correlation. The authors postulated that in advanced and diffuse atherosclerosis the DNA damage and repair signalling cascade may contribute to the development and progression of atherosclerotic plaque formation directly.

Consequences of DNA Damage

DNA repair

The assembly and disassembly of the DDR factors facilitates DNA repair by the pathways described earlier. Disassembly of DDR factors is key to allow return of normal cellular function and replication. Several pathways share a common end process, for example DNA-dependent protein kinase catalytic subunit (DNA-PKcs) autophosphorylation mediates its dissociation from double strand breaks and E3 ubiquitin-protein ligase RNF8 ubiquitination allows the removal of Ku from the damaged site [83-85].

The choice of DNA repair pathway has been shown not only to be a consequence of the type of damage but also the stage of the cell cycle at which the lesion occurs. HR repair requires substantially resected DSB DNA; Cyclin-Dependent Kinases (CDKs) control the C-terminal Interacting Protein (CtIP) nucleolytic function in a cell cycle dependent manner, and CtIP is phosphorylated in S and G2 phase of the cell cycle. This increases its activity and interaction with breast cancer type 1 susceptibility protein (BRCA1) [86,87]. Contrary to this, DSB resection may be stalled by ATM-dependent phosphorylation of 53BP1, preventing BRCA1 migration to the DSB during G1 [88]. This suggests a 'choice' exists between the fast, more error prone NHEJ, acting during G1, and the more accurate HR, acting during S and G2 with 53BP1 and BRCA1 antagonising each other, influencing the repair undertaken [89,90].

Growth arrest, cell senescence and inflammation

The normal cell cycle is delayed by DNA damage checkpoints, thereby allowing adequate time for lesion repair. However, if the employed repair process is unsuccessful then irreversible growth arrest results. Cellular growth inhibition typically occurs during G1 and is controlled by the p16/pRB and p53/p21 pathways [91,92]. Senescence is typically recognised by the expression series of markers including Senescence-associated beta Galactosidase (SaβG) [93]. More recently, studies have shown that there are between 40 and 80 secretory intracellular signaling factors released as a consequence of senescence and are colloquially known as the Senescence-Associated Secretory Phenotype (SASP) [94]. The DDR is crucial for the regulation of SASP and controlling the rate of cellular senescence. It has been observed that a reduction in key regulatory factors (ATM, NBS1 and checkpoint kinase 2 (*Chk2*)) responding to DDR also reduces many SASP factors including interleukin (IL)-6 and IL-8 [95].

Many of the SAPS factors released during senescence can themselves promote and perpetuate the cycle of atherosclerosis through inflammation and recruitment of modulatory immune cells [96]. Further to this, the autocrine and paracrine function SAPS results in further recruitment of inflammatory matrix metalloproteinases (MMPs) from non-senescent neighbouring cells, such as MMP-1, MMP-3 and MMP-10 [97,98]. These matrix metalloproteinases are known to contribute to extracellular matrix degradation and further damage to the vascular wall [99].

Apoptosis

Cellular apoptosis may occur if DNA damage cannot be fully repaired [100]. Whilst attempting to repair damage, the DDR simultaneously activates pro-apoptotic and growth arrest pathways, with the degree of damage present dictating the dominant pathway [16]. As previously described in homologous recombination of DSBR, ATM signalling results in Replication Protein A (RPA) and RAD51 initiating strand invasion [44,45]. However, simultaneously ATM, and to a lesser extent ataxia telangiectasia and Rad3-related protein (ATR), trigger transcriptional activation of tumour protein p53 *via* checkpoint kinases 1 (*Chk1*) and 2. P53 in turn upregulates pro-apoptotic genes (Bax, Puma, Bid [101]) resulting in the release of cytochrome c which, along with activating actor 1 (APF-1) and caspase 9, result in cell death *via* caspase 3 activation [102]. Apoptotic bodies are formed within the intimal space following cellular morphological changes such as nuclear fragmentation and chromatin condensation and these are subsequently phagocytosed by nearby cells [103,104].

Clarke, et al. showed that chronic apoptosis of VSMCs accelerates atherogenesis and progression of atherosclerosis in established plaques [105]. The group had previously shown that smooth muscle protein 22α-human diphtheria toxin receptor (SM22α-hDTR) mice have VSMC specific apoptosis in both normal arteries and within plaque, conditionally induced by the transgenic expression of the hDTR from the minimal SM22α promoter [106] (Table 1). SM22α-hDTR/ApoE$^{-/-}$ mice atheroma was also noted to have increased high risk features characterised by a larger necrotic core, fibrous cap thinning as compared to ApoE$^{-/-}$ controls, which has also been noted in human studies [79] suggesting a possible causal link between the rate of VSMC apoptosis and atherosclerotic events which warrants further investigation (Figure 2).

Table 1: A summary of the most notable studies addressing the evidence for DNA damage in atherosclerosis.

Authors	Title	Journal	Study population	Findings
Martinet, et al. [60]	Oxidative DNA damage and repair in experimental atherosclerosis are reversed by dietary lipid lowering	Circulation Research	Male New Zealand white rabbits fed cholesterol rich diet for 24 weeks	Induced atherosclerotic plaques had increased 8-oxoG levels seen on immunohistochemistry, this was highest within the most superficial layer of the plaques, correlating with numerous macrophage derived foam cells. DNA strand breaks were higher with upregulation of *XRCC1, PARP-1, p53* and phospho-p53. These breaks normalised after 4 weeks of dietary lipid lowering.
Martinet, et al. [61]	Elevated levels of oxidative DNA damage and DNA repair enzymes in human atherosclerotic plaques.	Circulation	Human atherosclerotic plaques (carotid endarterectomy specimens)	Increased immune reactivity against 8-oxo-dG in carotid plaques compared to adjacent inner intima and normal mammary arteries, this was present in all cell types including macrophages, vascular smooth muscle cells and endothelial cells. This was associated with concordant upregulation of PARP-1 and p53.
Ballinger, et al. [15]	Mitochondrial integrity and function in atherogenesis.	Circulation	ApoE$^{-/-}$ murine model and human aortic samples (obtained during surgery, normal aortic tissue harvested form transplant donors at organ harvesting)	mtDNA damage directly correlated with the extent of atherosclerosis in human arterial specimens and ApoE$^{-/-}$ mice. Furthermore, the mtDNA damage preceded atherosclerosis formation in ApoE$^{-/-}$ mice. ApoE$^{-/-}$ mice deficient in manganese superoxide dismutase showed early mtDNA damage and accelerated atherogenesis at arterial bifurcations.
Brouilette, et al. [69]	White cell telomere length and risk of premature myocardial infarction.	Arteriosclerosis, Thrombosis, and Vascular Biology	Human circulating leukocytes in patients with premature myocardial infarction (<50 years age)	Age and sex adjusted mean terminal restriction length of telomeres in circulating leukocytes of patients who suffered premature myocardial infarction was significantly shorter than controls. This finding was independent of any other coronary artery disease risk factor, suggesting biological age may play a role in the aetiology of CAD.

Spyridopoulos, et al. [70]	Telomere gap between granulocytes and lymphocytes is a determinant for hematopoietic progenitor cell impairment in patients with previous myocardial infarction	Arteriosclerosis, Thrombosis, and Vascular Biology	Human circulating leukocytes and mononuclear bone marrow cells in patients with coronary artery disease and previous myocardial infarction	Telomere erosion was noted at the bone marrow level with age and number of affected vessels as independent predictors (P=0.025 and P=0.029 respectively). This was associated with bone marrow functionality with reduced SDF and VEGF specific migration of mononuclear bone marrow cells and reduced mean telomere lengths in patients with CAD. Lymphocytes demonstrated significant telomere length shortening between mononuclear bone marrow cells and peripheral blood in patients with CAD.
Mahmoudi, et al. [71]	Statins use a novel Nijmegen breakage syndrome-1 dependent pathway to accelerate DNA repair in vascular smooth muscle cells	Circulation Research	C57B1-6 mice. Male New Zealand white rabbits fed cholesterol rich diet for 9 months.	Telomere erosion was noted at the bone marrow level with age and number of affected vessels as independent predictors (P=0.025 and P=0.029 respectively). This was associated with bone marrow functionality with reduced SDF and VEGF specific migration of mononuclear bone marrow cells and reduced mean telomere lengths in patients with CAD. Lymphocytes demonstrated significant telomere length shortening between mononuclear bone marrow cells and peripheral blood in patients with CAD.
Mahmoudi, et al. [71]	Statins use a novel Nijmegen breakage syndrome-1 dependent pathway to accelerate DNA repair in vascular smooth muscle cells	Circulation Research	C57B1-6 mice. Male New Zealand white rabbits fed cholesterol rich diet for 9 months. Human aortic VSMC.	Human atherosclerotic VSMCs were noted to have increased levels of double strand DNA breaks and increased activity of ATM/H2AX mediated repair pathways *in vivo* and *in vitro*. Statin treatment did not reduce oxidative stress or DNA damage but accelerated DNA repair pathways *via* NBS-1 and *Hdm2*. Furthermore, statins were noted to reduce VSMC senescence and telomere attrition, accelerate DNA repair and reduce apoptosis *in vivo* following irradiation.
Yu, et al. [78]	Mitochondrial DNA damage can promote atherosclerosis independently of reactive oxygen species through effects on smooth muscle cells and monocytes and correlates with higher-risk plaques in humans	Circulation	$ApoE^{-/-}$ and $PolG^{-/-}/ApoE^{-/-}$ murine model and human circulating leukocytes who underwent VH IVUS.	mtDNA damage in the vascular wall and circulating monocytes is present in $ApoE^{-/-}$ mice prior to the formation of atherosclerotic plaque. $PolG^{-/-}/ApoE^{-/-}$ mice had increased atherosclerosis with impaired proliferation and apoptosis of vascular smooth muscle cells. Human atherosclerotic plaque showed increased mtDNA damage compared to normal vessels and higher leukocyte mtDNA damage was associated with higher-risk plaques.
Shah, et al. [65]	Defective base excision repair of oxidative DNA damage in vascular smooth muscle cells promotes atherosclerosis	Circulation	$OGG^{-/-}$ and SM22α-SIRTex4/ex4 murine model and human atherosclerotic plaques (carotid endarterectomy samples and normal aorta from patients undergoing aortic valve replacement).	Human plaque VSMC have defective nuclear 8oxoG base excision repair and reduced acetylation of OGG1. p300 and SIRT-1 were identified as the OGG1 acetyl transferase and deacetylase regulators. Reduction in oxidative damage improved OGG1 activity and thus reduces plaque development, highlighting the detrimental effects of 8oxoG on VSMC function.
Shah, et al. [80]	DNA damage and repair in patients with coronary artery disease: correlation with plaque morphology using optical coherence tomography (DECODE study)	Cardiovascular Revascularization Medicine	Human circulating peripheral blood mononuclear cells in those with stable angina and NSTEMI	Genes involved in double strand break repair and nucleotide excision repair differed between patients with stable angina and NSTEMI compared to controls. GTSE1, DDB1, MLH3 and XRCC1 expression were higher in patients with stable angina, and this correlated with a high degree of fibrocalcific plaque seen on OCT. In NSTEMI patients, ATM and XPA expression was strongly corelated with fibrous plaques.

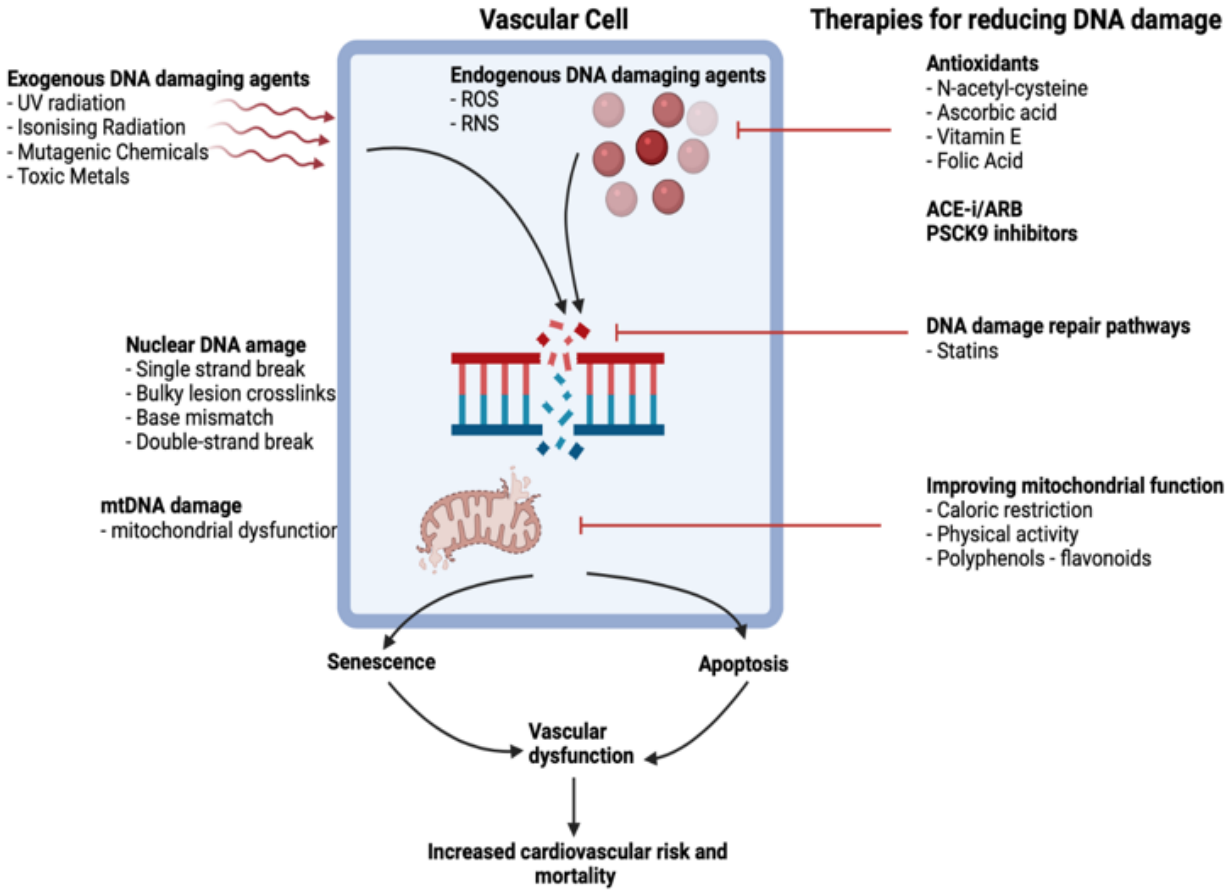

Figure 2: Cellular manifestations of exogenous and endogenous DNA damage with atherosclerosis. Causes of DNA damage (left), cellular and systemic consequences (centre) and therapeutic agents and their targets (right). Adapted from Oh, et al. [107].

Therapies to Reduce DNA Damage in Atherosclerosis

Antioxidant therapy

It is well established that an attenuated response to oxidative stress agents occurs with ageing [108-110]. Collins, et al. showed the mechanism through which older low density lipoprotein receptor null ($LDLR^{-/-}$) mice have accelerated vascular injury is a consequence of impaired response to oxidative stress. $LDLR^{-/-}$ 12-month-old male mice (middle aged) were noted to express greater aortic levels of the key antioxidant genes, glutathione peroxidase-1 and -4, catalase, superoxide dismutase-2 and uncoupling protein-2, compared to young mice. Interestingly, once young mice were fed a high fat diet the aortic expression of these genes significantly increased, but this was not replicated in middle aged $LDLR^{-/-}$ mice. The inability to mount an antioxidant response was due to a reduction in the vascular expression of 2 key regulatory transcriptional pathways: DJ-1 and forkhead box, subgroup O family. The group found that treatment of the mice with the antioxidant apocynin reduced oxidative stress and attenuated atherosclerosis [111]. There are many other historical studies supporting these findings, in which cholesterol rich animal models have atherosclerosis regression in response to antioxidative agents [112]. Unfortunately, to date, randomised controlled trials assessing several major dietary antioxidants including ascorbic acid, vitamin E, folic acid, selenium have shown no clinical benefit in humans and, thus, antioxidant therapy does not feature in any established guideline for the treatment of epicardial atherosclerotic coronary artery disease [113-118].

However, antioxidant therapy has shown promise in the treatment of end organ damage as a result of microvascular dysfunction. Free radical scavengers such as ascorbic acid and N-acetyl-cysteine yielded benefit in patients with microvascular disease typically caused by diabetes mellitus [119]. Interestingly low level endogenous Nitric Oxide (NO) in inducible Nitric Oxide Synthase (iNOS) transfected cultured B6 mouse fibroblasts resulted in protection from DNA single-strand break formation and micronuclei induction by hydrogen peroxide (H2O2) [120]. Higher concentrations of NO (>1 mM) resulted in moderate to severe endonuclease sensitive oxidative base damage. However, as

a double-edged sword, NO was also noted to selectively inhibit the repair of oxidative DNA base modifications [120,121]. Modulation of NO levels may provide a therapeutic target to reduce DNA damage.

It is important to note that the evidence to suggest that antioxidant therapy can reduce atherosclerosis in animal models is predominantly in a young adult population, whereas in human randomised clinical trials the population tend to be older age groups with more mature coronary artery disease [122].

Polyphenols

Polyphenols are naturally occurring organic compounds, abundant in plants and fruit, and have been found to have atheroprotective effects in humans [123,124]. Flavonoids are a commonly occurring plant polyphenol, and epidemiological and cohort studies have shown those with a higher dietary intake of flavonoids have a lower incidence of coronary artery disease, as well as a lower cardiovascular mortality [125-128]. To explore the biological consequences of the action of flavonoids further Weisel, et al. administered anthocyanin rich juice to healthy male adult humans in comparison with polyphenol depleted juice in the control arm. They observed a decrease in oxidative DNA damage, and an increase of glutathione levels and glutathione status in the anthocyanin juice group which returned to baseline in a wash out period [129]. However, there was no reduction in nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) binding activity in those consuming anthocyanin juice, as has been previously seen in diabetic patients consuming flavonoids [130]. NF-κB DNA binding activity is a key regulatory process in the expression of many genes related to inflammation [131].

Caloric reduction, dietary modification and exercise

A causal link between nutritional status, diet and the development of atherosclerosis has been clearly established [132]. Furthermore, dietary manipulation by Caloric Restriction (CR) has been shown to exert beneficial effects on endothelial function *in vivo* [133,134]. This occurs primarily by the reduction in inflammation and reduction of oxidative stress through the upregulation of antioxidant expression, thus resulting in improved endothelial physiology and preservation of mitochondrial function [135,136]. Sirtuins are an important class of Nicotinamide Adenine Dinucleotide (NAD^+)-dependant deacetylases and adenosine diphosphate-ribosyltransferases [64] and have a similar structure to the Saccharomyces cerevisiae Sir2 protein [137]. In B6D2F1 mice models, CR increased sirtuin-1 (*SIRT1*), corresponding to an improvement of endothelial function [136]. Dietary supplementation with compounds mimicking the effects of CR, without sacrificing balanced nutrition, may also have therapeutic benefits.

SIRT1 inducers such as resveratrol, quercetin, berberine, curcumin and fisetin are important in the treatment of cardiovascular disease acting to improve DNA repair by higher oxidative stress responses though amplified mitochondrial function [138,139]. Resveratrol (a stilbenoid), is a naturally occurring phenol found in the skin of grapes, raspberries, and peanuts and can increase *SIRT1* expression [140]. Resveratrol has been shown to increase aortic elasticity and reduce endothelial apoptosis in C57BL/6NIA mice and closely mimics dietary or caloric restriction [137]. Human studies have replicated this finding, so that both CR and resveratrol both increased plasma concentration of *SIRT1*, although any biological/clinical consequences of this pathway are yet to be observed in humans [141]. Conversely, Sirtuin inhibitors such as cambinol and Ex-527 may interfere with and corrupt CVD prevention therapeutics [142-144]. Typically, the diet of developed populations is much higher in Sirtuin inducers, with the diet of developing nations higher in Sirtuin inhibitors [145], this presents a public health challenge that if addressed may have an impact of the global burden of CVD [146].

Physical activity and aerobic exercise are potentially powerful contributors in the primary prevention of cardiovascular disease [147]. High intensity exercise has been proven to not only reduce oxidative stress through upregulation of key antioxidants such as superoxide dismutase, but also to be directly anti-atherogenic [148,149]. Recently, in a mtDNA mutator mouse model, forced endurance exercise mice were found to have high levels of full length mtDNA content and fewer mtDNA point mutations in comparison to sedentary controls. Furthermore, increased mitochondrial oxidative capacity and lower levels of DNA fragmentation were noted in several tissues including myocardial specimens, suggesting the presence of lower levels of caspase driven apoptosis in forced endurance exercise mice [150]. In elite athletes, intermittent hypoxic exposure, using a GO2 altitude hypoxiactor, in combination with physical activity, was found to increase NO and Heat Shock Protein 27 (*HSP27*), enhancing NO bioavailability and endothelial function [151].

Statins

The role of 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-Co-A) reductase inhibitors, known as statins, has been well established in the primary and secondary prevention of coronary artery disease [152,153]. Outside of their main mechanisms of actions (inhibiting cholesterol synthesis and increasing low density lipoprotein uptake), the pleotropic effects of statins are wide ranging

and include: Maintenance of atherosclerotic plaque stability [154], modulation of inflammatory response [155], reduction of TNFα-induced apoptosis [156], and improvement of endothelial function by restoration of nitric oxide availability [157]. Statins have been shown to be instrumental in the prevention of DNA damage and stabilisation of cellular genetic material and reduction of oxidative stress through down regulation of NAD(P)H oxidase subunits and upregulation of catalase expression [158-160]. Statins have also been shown to provide increased telomere protection by an increase in the expression of the telomere capping protein TRF2 [74] in human Peripheral Blood Mononuclear Cells (PBMC). A cross-sectional study of individuals on statin therapy revealed increased telomerase activity and increased telomere length in the PBMCs of subjects compared to controls not taking statin therapy [161]. Lovastatin, whilst not commonly used in clinical practice in many countries, was found to protect primary human endothelial cells from ionising radiation-induced DNA damage by increased p53 expression and ATM/ATR-regulated activation of *Chk1* [162]. A further protective mechanism of statins against DSB was identified by Mahmoudi et al., who reported atorvastatin accelerated DNA repair *via Hdm2* phosphorylation, NBS1 stabilisation and faster ATM and H2A histone family member X (H2AX) phosphorylation, attenuating DNA damage, telomere length reduction and senescence, suggesting an atherosclerotic plaque stabilisation effect [62].

PCSK9 inhibitors

Pro-protein Convertase Subtilisin/Kexin type 9 (*PCSK9*) inhibitors have been shown to be effective at lowering of LDL in patients already taking statin therapy, and have shown promise in the secondary prevention of future events in those with previous acute coronary syndromes [163,164], and also the prevention of the development of complex coronary disease requiring high risk intervention [165]. Wu et al. found that knockdown of *PCSK9* in human umbilical vein endothelial cells resulted in reduced oxidised Low-Density Lipoprotein (ox-LDL)-induced apoptosis, thus suggesting *PCSK9* can impair endothelial cell survival [166]. The myriad of typical inflammatory stimuli found within atherosclerotic plaque, such as TNFα, oxLDL and lipopolysaccharides, stimulated *PCSK9 via* the NF-κB signalling pathway [167]. The same study found that lectin-like ox-LDL receptor-1 (*LOX-1*), one of the major scavenger receptors responsible for the removal of ox-LDL [168] stimulates *PCSK9* and vice versa, and thereby suggested that their cross-talk is accentuated in the inflammatory state. This suggests that *PCSK9* inhibitors may inhibit atherogenesis in hypercholesterolaemic states by altering *LOX-1* expression [167]. Further research into the molecular consequences of *PCSK9* inhibition and its effect on DNA integrity and stability is now needed.

ACE inhibitors

Angiotensin Converting Enzyme-Inhibitors (ACE-I) exert their cardiovascular effects by inhibiting the conversion of angiotensin I to angiotensin II within the renin-angiotensin-aldosterone system (RASS), thus reducing the degradation of bradykinin, a potent vasodilator. Angiotensin II has several deleterious effects, including the production of ROS from VSMCs resulting in DNA damage [169]. The ROS formed as result of angiotensin II cause Stress-Induced Premature Senescence (SIPS) and premature replicative senescence due to accelerated telomere attrition [170,171]. It is likely that both SIPS and replicative senescence occur simultaneously in vascular cells, and furthermore they also share common signalling and termination pathways involving stabilisation of p53, increased p21 expression and resultant hypophosphorylation of Rb protein mediating growth arrest [171,172]. Higher levels of bradykinin, as a result of ACE inhibition, reduces superoxide-induced endothelial cell senescence, subsequently reducing DNA damage in VSMCs *via* bradykininB2 receptor mediated nitric oxide release [173,174]. Previous studies have shown that ACEI and ARB are consistently associated with improvement in endothelial function. Both ACE-I and ARB cause a reduction in DNA damage inducing ROS and therefore they can directly modulate the pathogenesis of atherosclerosis [175,176].

Future Perspectives

The role of DNA damage in the development and progression of coronary artery atherosclerosis is increasingly well understood. There is a firm evidence base for the existence of DNA damage within both nuclear and mitochondrial DNA, and that the damage itself perpetuates the cycle of the development of atherosclerosis. The consequences of DNA damage range from successful repair *via* DSBR, BER, NER and mismatch repair or, by contrast, result in cellular senescence, apoptosis and inflammation which further drives the pathogenesis of atherosclerotic plaques. However, the exact mechanisms by which DNA damage drives this process are not fully understood.

The development of a novel biomarker (outside of the assessment of conventional risk factors for the development of atherosclerosis) that could highlight those at higher risk would facilitate the initiation of appropriate evidence-based lifestyle modification

interventions and targeted primary prevention therapeutics proven to reduce the likelihood of adverse cardiovascular events and mortality in the future [5]. The DDR is a candidate pathway to yield such a biomarker.

Alteration of the DNA damage response pathway in mouse models has been shown to reverse or stall the process of atherosclerosis, importantly modulating high risk features such as a thin capped fibrous and fragmented cap [177].

Conclusion

Currently, there is limited human data exploring DNA damage and gene expression in atherosclerosis. The prevention of DNA damage, and the augmentation of the DDR, are important targets for future therapeutics. Statins have proven benefit in reducing DNA damage and newer treatments, such as *PCSK9* inhibitors, may have similar beneficial effects. Successful modulation of the DDR may help reduce the morbidity and mortality, together with the global economic burden associated with atherosclerotic coronary artery disease. The potential clinical and financial benefits of appropriate biomarkers and then therapeutic tools that could identify the high risk and modify their subsequent trajectory are enormous.

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