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# DNA damage-dependent mechanisms of ageing and disease in the macro- and microvasculature

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ABSTRACT

A decline in the function of the macro- and micro-vasculature occurs with ageing. DNA damage also accumulates with ageing, and thus DNA damage and repair have important roles in physiological ageing. Considerable evidence also supports a crucial role for DNA damage in the development and progression of macrovascular disease such as atherosclerosis. These findings support the concept that prolonged exposure to risk factors is a major stimulus for DNA damage within the vasculature, in part via the generation of reactive oxygen species. Genomic instability can directly affect vascular cellular function, leading to cell cycle arrest, apoptosis and premature vascular cell senescence. In contrast, the study of age-related impaired function and DNA damage mechanisms in the microvasculature is limited, although ageing is associated with microvessel endothelial dysfunction. This review examines current knowledge on the role of DNA damage and DNA repair systems in macrovascular disease such as atherosclerosis and microvascular disease. We also discuss the cellular responses to DNA damage to identify possible strategies for prevention and treatment.

## 1. Introduction

The vasculature of healthy individuals undergoes several important changes with increasing age, which increase the likelihood of developing cardiovascular disease. Both the onset and progression of vascular disease are related to age, in part because of common manifestations. For example, ageing is associated with endothelial dysfunction (Celermajer et al., 1994; Van Der Loo et al., 2000), arterial stiffening and remodelling (Lakatta and Levy, 2003), impaired angiogenesis (Rivard et al., 1999), defective vascular repair (Weinsaft and Edelberg, 2001), and an increasing prevalence of atherosclerosis (Eggen and Solberg, 1968; Erusalimsky and Kurz, 2005). The reasons for these associations are still unclear, but it is plausible that organismal ageing and vascular disease share common cellular mechanisms. One process that has been increasingly linked to both ageing and the development of vascular pathology is DNA damage (Fuster and Andrés, 2006; Minamino et al., 2002). Here, we review current knowledge on the role of DNA damage and DNA repair systems in atherosclerosis and microvascular disease, and discuss the cellular response to DNA damage to identify possible strategies for prevention and treatment.

## 2. DNA damage pathways and repair mechanisms

DNA damage is manifested in a variety of forms including single strand breaks, double strand breaks, base modification (e.g. 7,8-dihydro-8-oxoguanine (8-oxoG)) and mispairing, all of which need to be successfully repaired to avoid accumulation of mutations, cell cycle arrest and apoptosis. DNA damage activates a variety of DNA repair mechanisms, collectively termed the DNA-damage response (DDR). Many of these DNA repair mechanisms are activated in vascular disease, which repair the DNA, stall the cell cycle to allow repair to occur, or trigger apoptosis or cell senescence to prevent propagation of damaged DNA (Fig. 1).

### 2.1. Oxidative damage and base excision repair

DNA bases are particularly susceptible to oxidation mediated by reactive oxygen species (ROS) (Neeley and Essigmann, 2006). The low redox potential of guanine makes this base particularly vulnerable and leads to a plethora of oxidised guanine products (Neeley and Essigmann, 2006). The most thoroughly studied guanine oxidation product is 8-oxoG (Neeley and Essigmann, 2006), and the presence of 8-oxoG is often used as a cellular biomarker to indicate the extent of oxidative stress. 8-oxoG is a highly mutagenic miscoding lesion that

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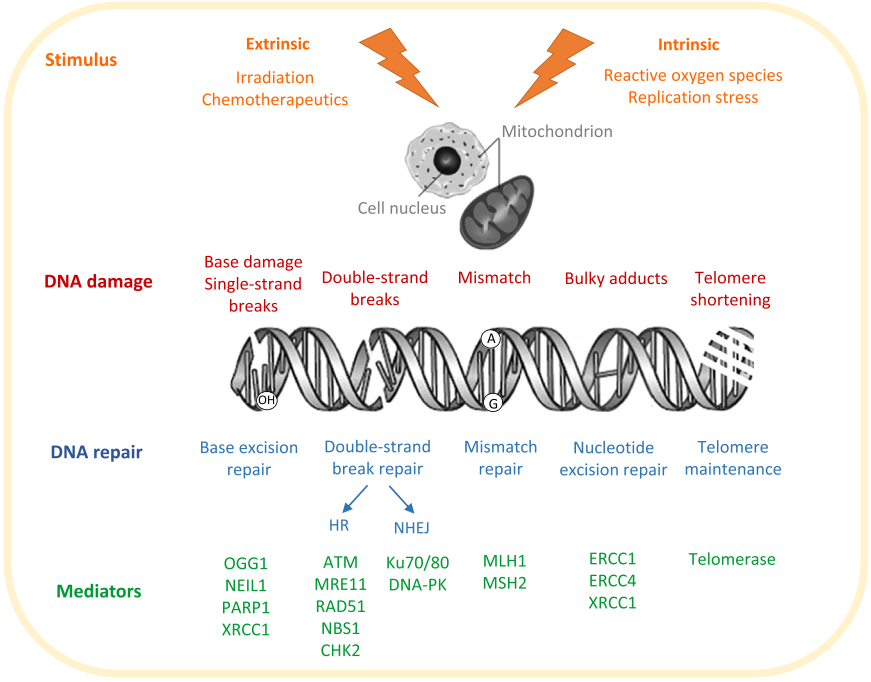


**Fig. 1. Types of DNA damage that accumulate during ageing and vascular disease and their associated DNA repair pathways.** Extrinsic or intrinsic insults can stimulate a variety of DNA lesions, from single-strand breaks to telomere attrition. Key mediator proteins involved in each DNA damage repair mechanism are shown. Both nuclear DNA and mitochondrial DNA undergo age-associated genomic alterations. Excessive DNA damage or insufficient DNA repair favours the ageing process and can be a cause or consequence of vascular disease. HR, homologous recombination; NHEJ, non-homologous end joining. Adapted from (Lord and Ashworth, 2012).

can lead to G:C to T:A transversion mutations (Grollman and Moriya, 1993).

The base excision repair pathway is the primary mechanism for repair of oxidative base lesions, such as 8-oxoG and formamidopyrimidines (4,6-diamino-5-formamidopyrimidine, FapyG)(Fig. 1). Base excision repair is a critical process for genomic maintenance, as highlighted by the severe phenotypes seen in animals deficient in base excision repair function, including premature ageing and metabolic defects (Vartanian et al., 2006). Base excision repair involves the concerted effort of several repair proteins that recognize and excise specific DNA damage, eventually replacing the damaged moiety with a normal nucleotide and restoring the DNA back to its original state (Hoeijmakers, 2001). Base excision repair facilitates the repair of damaged DNA via two general pathways – which involve either the replacement of one (short-patch base excision repair) or more nucleotides (long-patch base excision repair) at the lesion site (David et al., 2007). The initial step in base excision repair uses DNA glycosylases, which cleave the N-glycosyl bond between the sugar and the base, thus releasing the damaged base to form an abasic site, also termed apurinic/apyrimidinic (AP) site. The efficient repair of abasic sites is critical because they are highly mutagenic (Loeb, 1985). There are several different glycosylases specific for certain lesions. Individual glycosylases may recognize more than one type of damage, and each specific modification may be recognised by more than one type of glycosylase, giving a degree of redundancy in the process. For example, 8-oxoguanine DNA glycosylase (OGG1) has strong specificity for 8-oxoG lesions, whereas Nei-like DNA glycosylase 1 (NEIL1) efficiently removes FapyG lesions (Parsons et al., 2005). OGG1 and NEIL1 are classified as bifunctional glycosylases as they possess both glycosylase and AP lyase activity, namely base excision and an incision 3' to the AP site can occur, resulting in a single strand break.

### 2.2. Single strand breaks and repair

DNA single-strand breaks are among the most frequent DNA lesions, arising directly from damage to the deoxyribose moieties or indirectly as intermediates of base excision repair (Beckman and Ames, 1997; Lindahl, 1993). Left unrepaired, single-strand breaks are a major threat to genetic stability and cell survival, accelerating mutation rates and increasing levels of chromosomal aberrations (Caldecott, 2008). The pathways for single-strand break repair in mammalian cells involve a number of co-ordinated, sequential reactions responsible for damage detection, end processing, gap filling and ligation. Three excision repair pathways exist to repair single stranded DNA damage: base excision repair, nucleotide excision repair, and DNA mismatch repair. While the base excision repair pathway, as discussed, recognizes specific non-bulky lesions in DNA, nucleotide excision repair removes bulky DNA adducts induced by UV, and the DNA mismatch repair pathway targets mismatched base pairs (Caldecott, 2008)(Fig. 1).

Poly (ADP-ribose) polymerase 1 (PARP1) is one of the key proteins in single-strand break repair, as it is a nick sensor and also binds to single-strand breaks, short gaps in duplex DNA, double-strand breaks and other abnormal DNA structures, and initiates the efficient repair of single-strand breaks (Lan et al., 2004; Okano et al., 2003). After activation of PARP at single-strand breaks, the X-ray repair cross-complementing protein 1 (XRCC1) accumulates at poly(ADP-ribose) (pAR) sites (Lan et al., 2004; Okano et al., 2003). XRCC1 is thought to act as a scaffold protein for other repair factors and has been shown to physically interact with several enzymes known to be involved in the repair of single-strand breaks, including DNA ligase IIIα, DNA polymerase β, APE1, polynucleotide kinase/phosphatase, PARP-1 and OGG1 (Marsin et al., 2003; Masson et al., 1998; Schreiber et al., 2002). Key mediator proteins involved in nucleotide excision repair include excision repair cross complementing-group (ERCC)-1 and ERCC4, and the DNA mismatch repair pathway is mediated by MutL homolog 1 (MLH1) and MutS homolog 2 (MSH2) (Caldecott, 2008) (Fig. 1).

### 2.3. Double strand breaks and recombinational repair

Double-strand breaks are produced by ROS, ionising radiation, and chemicals that generate ROS. DNA double-strand breaks are generated