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In vivo effects of DNA lesions in Nucleotide Excision Repair deficient mice

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A molécula de DNA, responsável por carregar informações genéticas, está sob constante estresse químico e físico, proveniente de fontes endógenas e exógenas, que pode levar à formação de lesões no DNA. Para lidar com esses danos, células dispõem de mecanismos de reparo, sendo as lesões que distorcem a molécula de DNA reparadas pela via de Reparo por Excisão de Nucleotídeos (NER). Deficiências em genes da via NER podem levar à doenças humanas, como o Xeroderma Pigmentosum (XP) e a Síndrome de Cockayne (CS), caracterizadas principalmente por um grande aumento na incidência de câncer de pele e por neurodegeneração relacionada à um fenótipo de envelhecimento precoce, respectivamente. Para melhor compreendermos dessas doenças, assim como os efeitos sistêmicos das lesões de DNA relacionadas à via NER, são utilizados modelos de camundongos nocaute (KO), assim como fontes exógenas geradoras de danos no DNA, como a radiação ultravioleta (UVR). Neste trabalho, usamos dois modelos deficientes em NER para estudar os efeitos *in vivo* de lesões relacionadas à via NER, sendo o primeiro um modelo que mimetiza XP e o segundo CS. No primeiro modelo, XPA KO, estudamos o efeito das principais lesões geradas por UVR, os dímeros de pirimidina ciclobutano (CPDs) e os pirimidina (6-4) pirimidona fotoprodutos (6-4PPs) em queratinócitos. Para tanto, utilizamos fotoliases, enzimas capazes de reparar especificamente ou lesões do tipo CPD (CPD-phl) ou 6-4PP (6-4PP-phl). Observamos que em camundongos XPA KO, a remoção de CPDs foi capaz de inibir completamente a proliferação de células epidermais induzidas por UVR, enquanto a remoção de 6-4PPs reduziu, porém não impediu esse efeito. A remoção de lesões do tipo CPD ou 6-4PP foi capaz de diminuir os efeitos de morte celular e extravasamento de leucócitos na pele induzida por UVR em níveis similares, indicando que CPDs têm um maior impacto que 6-4PP sobre o efeito de hiperplasia, enquanto ambos tipos de lesão possuem efeitos similares na indução de apoptose e inflamação por UVR em camundongos XPA KO, tendo os queratinócitos um papel central na regulação desses efeitos. No segundo modelo, estudamos os efeitos de lesões relacionadas ao envelhecimento em camundongos duplo nocaute para os genes CSA/XPA (CX), previamente descrito como tendo morte prematura e neurodegeneração. Apesar de termos encontrado evidências de falhas na barreira hematoencefálica (BBB) nesses animais, não encontramos indícios de disfunção nas células endoteliais. Descobrimos, no entanto, um aumento significativo de marcadores de neuroinflamação, assim como ativação de astrócitos

e microglia, os dois principais tipos celulares relacionados à ativação de inflamação no cérebro, indicando que a neuroinflamação pode estar relacionada à neurodegeneração e defeitos da BBB encontrados neste modelo. As descobertas nesses modelos deficientes em NER podem ajudar a elucidar o papel in vivo das lesões de DNA em relação à resposta de morte e proliferação celular, assim como demonstra novos impactos da DDR sobre a indução de inflamação, com esses efeitos tendo implicações na etiologia de XP e CS, assim como fenômenos associados a danos no DNA como câncer e envelhecimento.

Palavras chave: Danos no DNA. Reparo por excisão de nucleotídeos. Inflamação. Morte celular. Proliferação celular.

ABSTRACT

Kajitani, G.S. In vivo effects of Nucleotide Excision Repair related DNA lesions. Ph.D thesis [(Interunits in Biotechnology)] - Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2018.

The DNA molecule, responsible for carrying genetic information, is under constant chemical and physical stress, both by endogenous and exogenous sources, which may lead to the formation of DNA lesions. These damages are dealt with using several different DNA repair mechanisms, with lesions that distort the DNA molecule are repaired by the Nucleotide Excision Repair (NER) pathway. Deficiencies in the genes related to NER may lead to human syndromes, such as Xeroderma Pigmentosum (XP) and Cockayne Syndrome (CS), characterized mainly by a severely increased skin cancer incidence and premature aging like (progeroid) neurodegeneration, respectively. In order to further study these diseases, as well as the role of NER-related DNA lesions in generating cellular and systemic effects, knockout (KO) mice models are often used, as well as exogenous DNA damaging sources, such as ultraviolet radiation (UVR). In this work, we used two NER deficient models to study in vivo effects of NER-related lesions, the first KO model mimicking XP and the second one CS. In the first model, we studied the effect of the main UVR generated photolesions, cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts (6-4PPs) on keratinocytes, one of the main cell types in the epidermis. In order to study the effect of each lesions, we used photolyases, enzymes that specifically repair either CPD (CPD-phl) or 6-4PP (6-4PP-phl) lesions. We observed that in XPA KO mice, CPD removal in keratinocytes was able to completely inhibit UV induced epidermal cell proliferation and hyperplasia, while the removal of 6-4PPs in keratinocytes reduced, but not abolished these effects. The removal of either CPDs or 6-4PPs in keratinocytes was able to reduce UV induced cell death and leukocyte extravasation on similar levels, indicating that CPDs have a greater impact than 6-4PPs regarding the hyperplasia effect of UV irradiation, and that both types of DNA lesions have similar effects on promoting apoptosis and inflammation in XPA KO mice, with keratinocytes having a central role in regulating these effects. In the second model, we studied the effect of aging related lesions on CSA/XPA double knockout (CX) mice, previously established as exhibiting premature death and neurodegeneration. Although we found evidence of blood brain barrier (BBB) defects in CX mice, we did not find cell autonomous vascular dysfunction. However, we discovered a significant increase of neuroinflammation markers, as well as activation of astrocytes and microglia, the two main endogenous inflammation related cell types of the brain, which indicates that neuroinflammation could play a role in the neurodegenerative and BBB phenotype observed

in this model. The findings in these two NER deficient models help elucidate the *in vivo* role of DNA lesions regarding cell death and proliferation response, as well demonstrating novel impacts of DDR on inflammation induction, with these effects having implications on the etiology of XP and CS, as well as DNA damage associated biological phenomena such as cancer and aging.

Keywords: DNA damage. Nucleotide Excision Repair. Inflammation. Cell death. Cell proliferation.

Chapter 1 - General Introduction

1.1 The DNA molecule - structure and function

The discovery of the structure of the DNA molecule was reported over 60 years ago, on 1953, in a seminal paper by Drs. James Watson & Francis Crick (1) – a breakthrough that rendered them the Nobel prize in Chemistry. Due to its importance to several fields in Biology, Chemistry, Medicine and numerous other areas, that finding is often considered to be one of the most important discoveries of the last century (2). The story of the DNA as the life molecule, however, began many years before the report of its canonical double helix structure, on the 19th century. DNA was first identified in the 1860s, in a research regarding the key components of white blood cells. A substance with different properties to proteins was found, then named as “nuclein”, as it was believed that this substance was present in the cell nucleus (3). Further studies confirmed nuclein as present in the nucleus and revealed its acidic nature (4). The structure of the sugar present in the acid, along with its placement inside the cell and its acidic properties caused this substance to be renamed as “DeoxyriboNucleic Acid” (DNA). The chemical components that make up the DNA molecule were identified, with it being composed of phosphate, sugar and four nitrogenous bases: adenine (A), cytosine (C), guanine (G) and thymine (T), with those components, arranged in the order of phosphate, sugar and base, forming a unit named as “nucleotide” (5). However, in spite of the DNA components being identified, its biological role and chemical structure was still unknown.

Meanwhile, other important biology discoveries were being unraveled. A fibrous structure within the nucleus of cells was discovered, being named “chromatin”, due to its eye catching color after being stained with a basic dye - this structure was later renamed “chromosomes”, with the “chromatin” name being repurposed as the molecular complex of DNA, RNA and proteins that make up the chromosomes in eukaryotic cells (6). Later developments provided evidence for the chromosome theory of inheritance, which postulates that the genetic material is located within the chromosomes, a theory that helped explain the mechanism of the mendelian inheritance laws, unifying cell biology with genetics (7).

The end of the 19th century and the beginning of the 20th century was a period of intense debate about which molecule was responsible for containing genetic information - For a long time, it was believed that proteins were responsible for this function, with the very word protein being coined due to the belief that it had a primary function for cell biology (8). Moreover, the DNA molecule has a much simpler composition than proteins - only four nucleotides, as opposed to more than 20 amino acids, which led many researchers to believe that it could not have a key role in genetic inheritance (9). However, a series of experiments conducted in the middle of the 20th century indicated that, instead of proteins, DNA was responsible for the function of carrying the genetic information (10). Although those results were not conclusive, further discoveries evidenced the role DNA as the genetic material (11).

Further clarification came soon after, with the paper by Watson and Crick on the structure of the DNA molecule, which proposed its structure as a double helix containing two strands of DNA, with base pairing happening on a complementary manner - Adenine pairing with Thymine and Cytosine pairing with Guanine by hydrogen bonds, with the intra strand structure being maintained by bonds between the phosphate groups and the sugar backbone, with these linkages being performed in the third (3') and fifth (5') carbon of the deoxyribose ring (Figure 1.1). This paper was soon followed by another, suggesting that for DNA replication, the two DNA strands would function as the basis for the daughter strands (12), a phenomena that came to be known as the semi-conservative DNA synthesis (13). It is worth noting that, as with all discoveries in science, this discovery would not have been possible without several others preceding it - not only the ones cited beforehand, but also exceptionally the photo 51, taken and interpreted by Rosalind Franklin (14), and the discovery of the 1:1 base pairing ratio of Adenine to Thymine and Cytosine to Guanine by Erwin Chargaff (15).

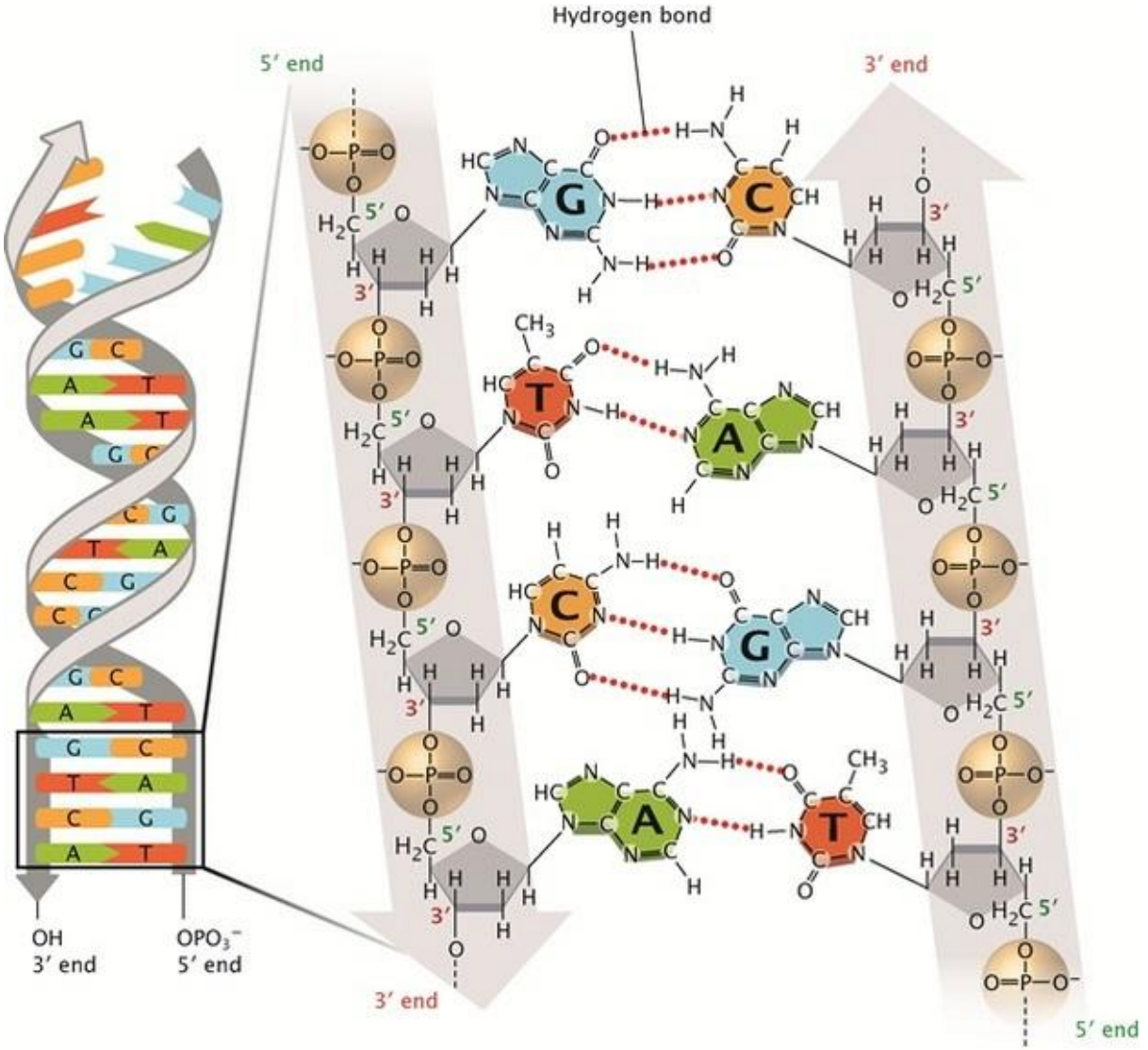

Figure 1.1. Structure of the DNA molecule. The double helix with 3' and 5' ends, alongside each DNA strand chemical structure and base pairing model. Adapted from Pray, 2008 (16).

After the confirmation of Watson and Crick suggestions on the DNA structure and replication mechanism, alongside the identification of its function as a master blueprint for coding proteins [17], DNA was solidified as the molecule that holds the genetic information. The finding of the DNA as the genetic molecule holds great importance in the life sciences, being considered a landmark that paved the way for numerous subsequent discoveries and opening up the field of molecular biology and its subfields, such as molecular biotechnology, gene editing, RNA biology being opened up afterwards.

The canonical structure of the DNA double helix, alongside the canonical A-T and C-G base-pairing, however, are prone to several spontaneous or environmental induced modifications. This was also a surprising discovery - because of the

importance of this molecule, it was assumed that it ought to be extraordinarily stable in order to maintain a high degree of fidelity required of a master blueprint (17). However, it was soon discovered that this was not the case.

1.2 DNA damage and Repair

After DNA was established as the molecule responsible for carrying the genetic information, it was generally thought that it was be incredibly chemically stable in order to maintain the integrity of the information held within it. Although that holds true in some states, such as in frozen and/or fossilized DNA (18), DNA is also an incredibly dynamic molecule and subject to constant change and stress when under life conditions - a finding that was something of a surprise at the time of its discovery (17). Francis Crick himself later recognized the importance of DNA maintenance through mechanisms such as DNA repair (19):

"we totally missed the possible role of …(DNA) repair although… I later came to realize that DNA is so precious that probably many distinct repair mechanisms would exist"

In physiological conditions, DNA is under constant stress, being subjected to several different kinds of chemical and physical stresses capable of inducing various types of modifications on the DNA structure (20). These stresses may be caused by the external environment, but may also be generated spontaneously by cell metabolism, such as reactive oxygen species (ROS) formation during cellular respiration. Moreover, the DNA replication and transcription machinery themselves can create stressful situations capable of destabilizing the DNA structure and generating mismatched base pairing, DNA breaks and other forms of chemical modifications. These modifications are broadly considered to be DNA lesions (17). Each and every primary component of DNA - bases, sugar and phosphodiester bonds are liable to being damaged. Amongst those modifications, there are lesions such as single and double DNA strand breaks, base modifications such as alkylation, methylation, and oxidation, non-canonical base pairings, base dimerization, intra or interstrand crosslinks, protein-DNA crosslinks, RNA:DNA structures and non-canonical DNA structures, such as G-quadruplex structures. All of these different

kinds of modifications have distinct effects on DNA and cell metabolism, involving distinct but often overlapping pathways in order to deal with these types of damage (21,22).

Much like the discovery of DNA itself, knowledge on these lesions, as well as the pathways responsible for dealing with them, collectively termed the DNA Damage Response (DDR), was built in a non-sequential manner. In fact, DNA damaging agents, such as, X-rays, were found to be mutagenic before the establishment of the DNA as the life molecule (23). Moreover, publication of Alexander Hollaender in 1939 had identified that the UVR induced mutagenesis in fungi was coincident of that of nucleic acids, also indicating that nucleic acids were the components of genes (24). The first DNA repair mechanism, the light dependent “reactivation” of viruses - thereafter named photoreactivation, was also found before 1953 (25). Obviously, since the DNA was not yet established as the genetic molecule, these discoveries were not linked to it at the time, but further findings helped elucidating the molecular structure and mechanism behind these lesions and their repair, such as the discovery of the photolyases enzymes in 1958 (26), responsible for the photoreactivation mechanism first discovered in 1949. Studies on many distinct models done by numerous different groups have had roles on building the DDR field, with Figure 1.2 containing some of the main discoveries in a timeline.

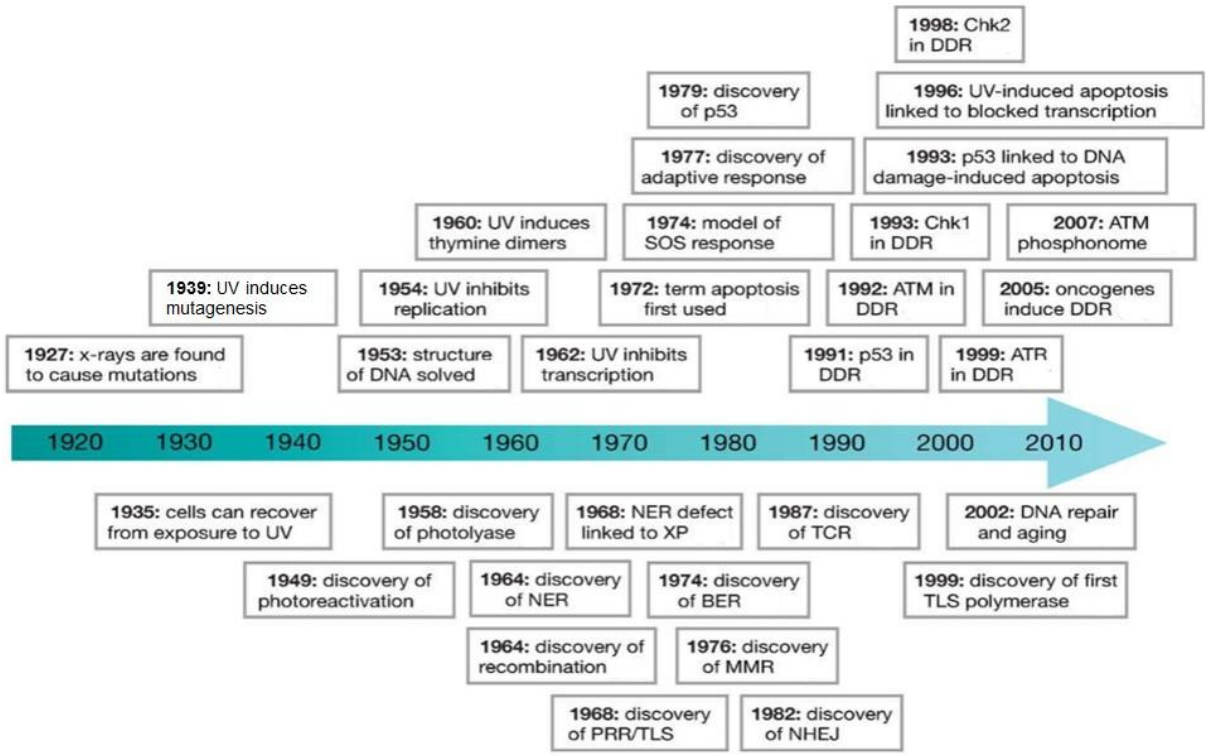

Figure 1.2. Timeline of major discoveries regarding the DNA damage response. Findings on effects of DNA damage are shown above the time arrow, and below it are the findings on DNA repair and tolerance mechanisms. Adapted from Ljungman, 2010 (27).

Considering the multitude of these sorts of damage, it is not surprising that organisms possess several molecular pathways able to deal with them. Some of main DNA repair pathways and the types of DNA lesions they are associated with are represented in figure 1.3. Notably, although there are established types of DNA repair generally considered to be the canonical DNA repair pathways, new pathways and kinds of damage are still being discovered, alongside their effects (28). Moreover, besides DNA repair, cells have other ways to deal with harmful agents, such as antioxidant defenses (29), physical blockage of damaging agents (30), and other molecular stress response mechanisms to better deal with or tolerate DNA damages (31).

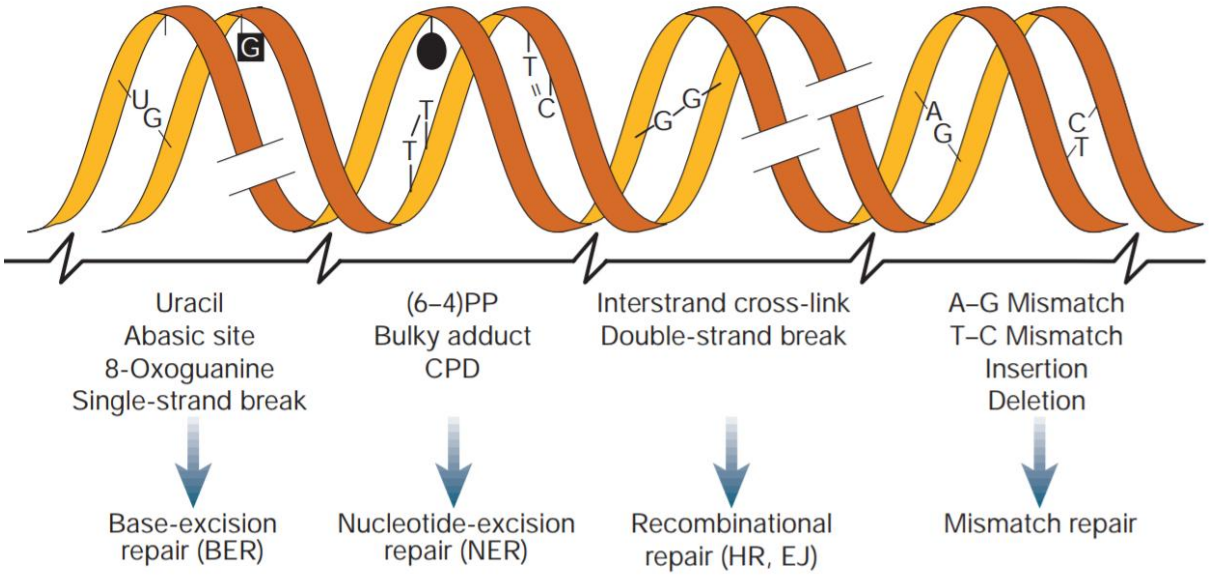

Figure 1.3. DNA lesions and repair pathways. Representation of the DNA molecule and damages that may occur to it, with the respective repair pathways responsible for dealing with these lesions displayed below them. Hoeijmakers, 2001 (32).

In this work, we will focus on a specific DNA repair pathway, responsible for repairing a wide array DNA helix distorting lesions, the Nucleotide Excision Repair (NER) pathway.

1.3 Nucleotide Excision Repair - Associated damages and diseases

The Nucleotide Excision Repair (NER) pathway was first hinted at in 1958, when an *E. coli* strain sensitive to ultraviolet (UV) irradiation was discovered (33). At the time, it was already known that UV radiation (UVR) had genotoxic and mutagenic properties (27), with this *E. coli* strain being a valuable and attractive tool for studying how organisms deal with genetic damage (34). By using methods for the measurement of DNA synthesis in this bacteria, it was found that DNA replication in the UVR sensitive strain was inhibited by minute amounts of UV irradiation when compared to a regular strain, linking UVR mediated DNA damage (also known as photolesions) to the blockage of DNA synthesis (35). It was then postulated that the recovery of DNA synthesis in the non-sensitive bacterial strand must have been due to some sort of repair of the DNA damage, with this kind of damage then rightfully presumed to be pyrimidine dimers (34,35). Furthermore, this repair mechanism was able to operate independently of light - an important observation, considering that

another, light dependent repair system (photoreactivation), had been discovered beforehand (36). It was then hypothesized that this damaged DNA was excised by a lesion removal pathway, with a new strand being synthesized to replace it. This was later confirmed, with small DNA fragments containing thymine dimers being present in UV irradiated bacterial cell cultures (37) and with the discovery of a non-semiconservative mode of DNA replication, with this unscheduled DNA synthesis following UV irradiation involving short, single-stranded DNA sections (38). This pathway that repairs lesions that distort the DNA double helix, including photolesions, and involves the enzymatic excision of chemically altered nucleotides later came to be known as the Nucleotide Excision Repair pathway.

The NER pathway was subsequently further characterized in *E. coli*, with the related proteins and their functions in the pathway being identified. This helped to better elucidate the molecular mechanisms of the NER pathway, uncovering the types of lesion it is capable of repairing and revealing the necessary steps for the lesion removal and repair, with these being the recognition of the lesion, dual excision of the DNA fragment containing the lesion, resynthesis of the excised section and ligation of the newly synthesized fragment to the pre-existing DNA strand (39).

The *E. coli* proteins involved in the NER pathway are generally conserved within prokaryotes, as well as the mechanism itself. Surprisingly, despite the eukaryote NER genes not being related to the prokaryote ones, the mechanism itself is strikingly similar, containing the same steps, although the eukaryotic NER is significantly more complex, involving more than 30 different proteins, as opposed to the 7 proteins in *E. coli* (40).

NER genes are generally conserved in Eukarya (41), with most of the studies regarding its mechanism being performed in humans and mice models, due to their biomedical significance. The eukaryotic NER (hereafter referred to simply as NER), pathway contains two subpathways for lesion recognition, the Transcription-Coupled Repair (TC-NER) and Global Genome Repair (GG-NER), with the signal for TC-NER being the stalling of a RNA polymerase (RNA pol I and II), with this subpathway being exclusive to transcriptionally active genes. Cockayne Syndrome proteins A and B

(CSA and CSB) and UV-stimulated scaffold protein A (UVSSA) proteins are involved in the removal of the arrested RNA pol II and signaling for the excision complex. The human GG-NER scans the genome for DNA lesions by utilizing the Xeroderma Pigmentosum C (XPC)-human RAD 23 homolog B (hHR23B) complex with the aid of another protein complex, the UV-DDB, composed of a dimer containing DDB1-DDB2 (DDB2 also being known as XPE), with this complex acting upon specific types of damage, such as the photolesion cyclobutane pyrimidine dimer (CPD). The excision complex is then recruited to the lesion site after the damage is recognized by either subpathway (31,42).

The excision complex involves a large number of proteins, with the XPA protein having a key, scaffolding function for the assembly of other NER factors. Another central player during this step is the transcription factor II H (TFIIH) complex, which contains several subunits required for NER, including the helicases XPB and XPD and the endonucleases XPG and the excision repair cross complementing 1 (ERCC1)-XPF complex. XPB functions in a 5'-3' direction, while XPD acts in 3'-5', thereby unwinding the DNA molecule, with replication protein A (RPA) proteins being recruited to stabilize single stranded DNA. The ERCC1-XPF endonuclease then cleaves the damaged strand 5' to the lesion site, leading to the initiation of the synthesis of a new DNA strand by polymerase δ, κ or Pol ε assisted by the proliferating cell nuclear antigen (PCNA) protein, followed by 3′ incision by XPG. Finalizing the NER, DNA ligase I or DNA ligase IIIα/XRCC1 then ligates the newly synthesized strand to the previous DNA (31,42). A simplified model of the human NER is shown below in figure 1.4.

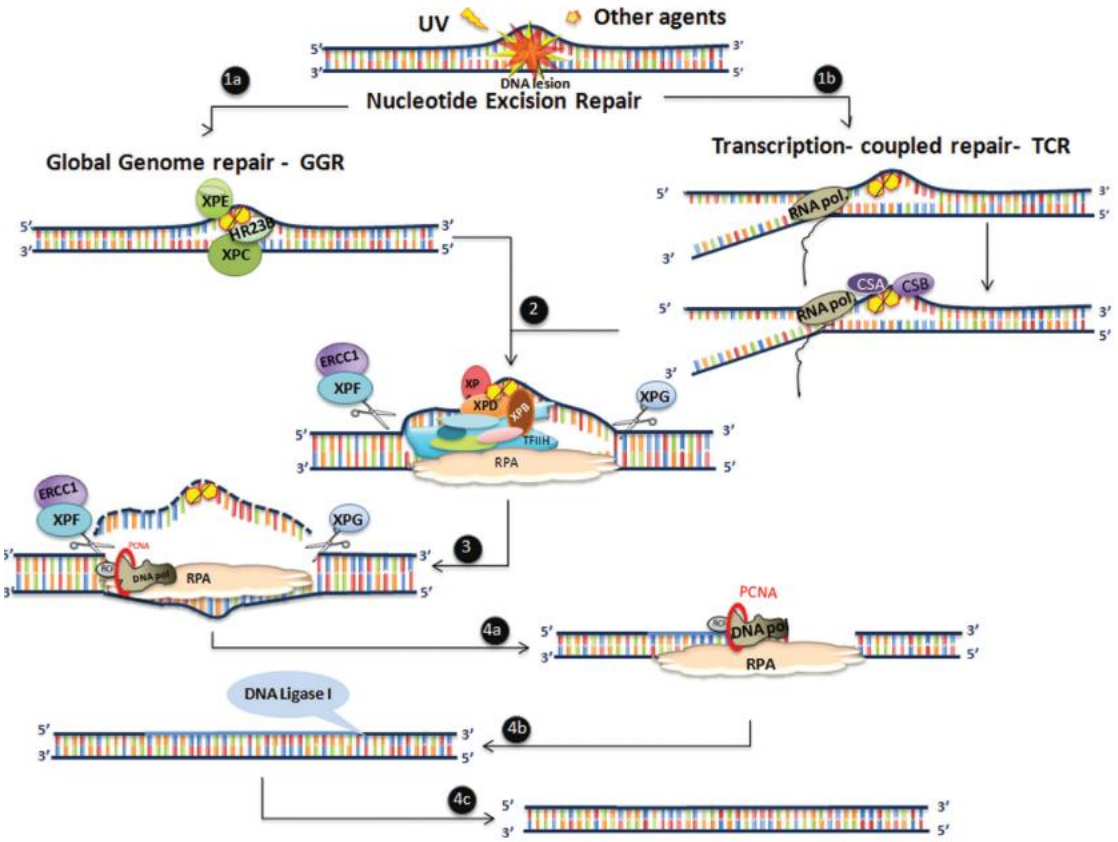

Figure 1.4. The Nucleotide Excision Repair pathway. A simplified model displays the two recognition subpathways, TC-NER and GG-NER and the steps following lesion recognition - the formation of the excision complex, removal of DNA fragment containing the lesion, resynthesis and ligation of the new DNA. Menck & Munford, 2014 (31).

Mutations in NER related genes may result in a plethora of different disorders, as shown in figure 1.5 X. These include Xerodema Pigmentosum (XP), Cockayne Syndrome (CS), Trichothiodistropy (TTD), UV-Sensitive Syndrome (UVSS), Cerebro-oculo-facio-skeletal syndrome (COFS), and combinations of these phenotypes (43). The symptoms, affected organs and age of onset of these diseases vary a lot, though they usually include cancer and/or progeroid (early aging-like) features, such as neurodegeneration. In fact, these two features are also commonly associated with diseases related to other, non-NER repair pathways, which serves to demonstrate the importance of DNA repair systems to cancer and aging. A review of these genetic diseases and their relative DNA repair pathways can be found in (44). In this work, we will focus on two of the most important NER-related disorders, Xeroderma Pigmentosum and Cockayne Syndrome.

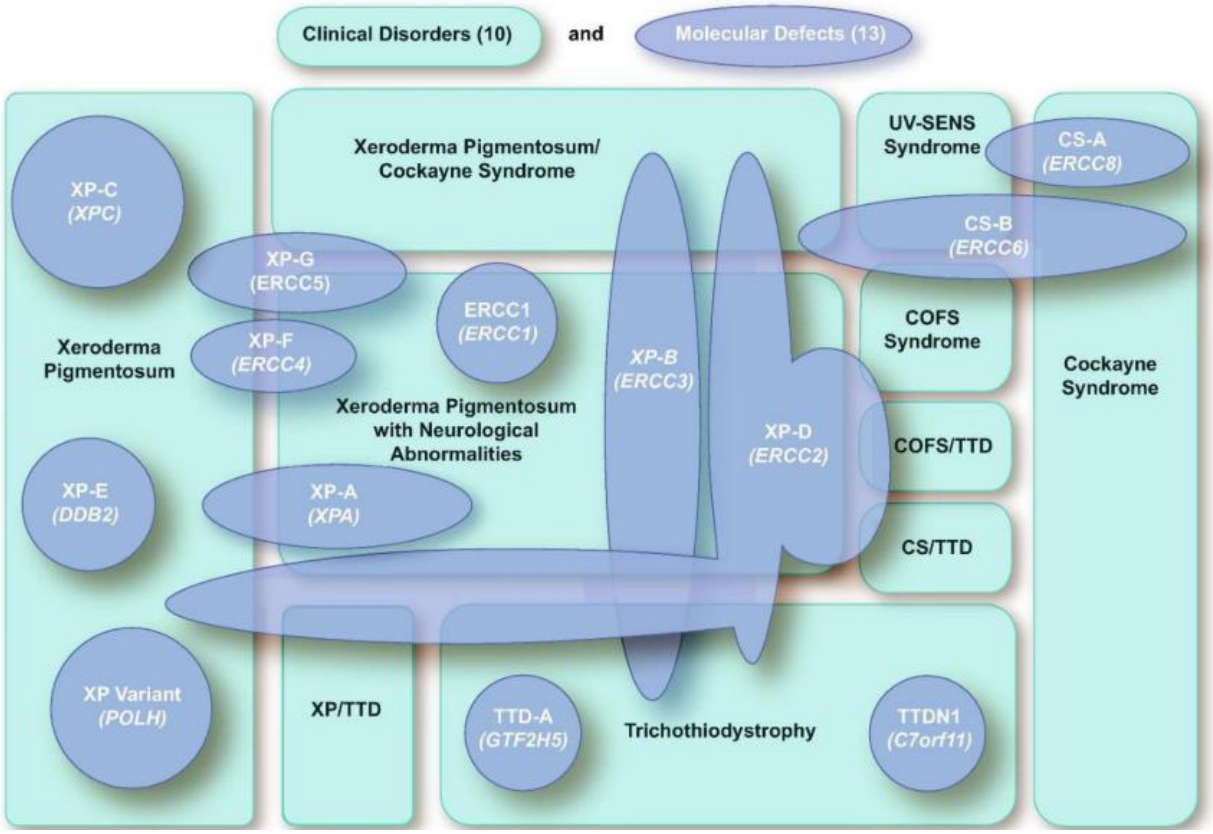

Figure 1.5. NER related disorders. Overview of syndromes caused by mutations in NER genes, and how the clinical phenotypes may overlap. DiGiovanna & Kraemer, 2012 (43).

1.3.1 Xeroderma Pigmentosum, photolesions and skin cancer

Xeroderma Pigmentosum is a rare, autosomal recessive disease with an estimated incidence of one per one million people, depending on the population (45). It manifests clinically as increased cancer risk of sun exposed areas, such as skin and mucous membranes of the eyes and mouth, having >2,000-fold increase in the incidence of skin cancers before the age of 20, photosensitivity, with pronounced burning and blistering of the skin after sun exposure, actinic damage to the skin (46). Unrelated to the sun-exposure, some XP patients also express progressive neurologic degeneration, with cerebral atrophy and primary neuronal degeneration with around 25% of XP patients having neurological symptoms, which include hearing loss, swallowing difficulties and mental retardation (43).

Our current understanding of XP starts on the 19th century in 1874, when Dr. Moriz Kaposi first described XP patients as having a dry, thin and wrinkled

appearance of their skin, alongside its checkered pigmentation, with these characteristics being responsible for the coining of the term XP, with Xeroderma meaning “dry skin”. On the 20th century, with the advancement of cell culture techniques, alongside many other discoveries related to molecular biology, including DNA repair, it was found that cells from XP patients presented hypersensitivity to UVR, and that these cells had a deficiency in the NER pathway, being unable to correctly remove photolesions from the genome (43).

The many XP NER related genes were initially found due to cell fusion studies, based on complementation of genes and then repair resynthesis measurements (unscheduled DNA synthesis), which demonstrated the presence of seven XP complementation groups (XP-A to XP-G). These genes were later cloned and identified. These cellular models paved the way for unveiling the mechanisms of the human NER pathway by way of the function of the proteins encoded by the *XPA-XPG* genes, and the order that they act for the repairing of the lesions (31). A variant complementation group was also found, named XP-V, described as having functional NER, but are defective for the *POLH* gene, which codifies for the DNA polymerase eta (pol η) protein, first described to participate in a postreplication repair process, then found to be a polymerase that takes part in a DNA damage tolerance mechanism, now known as Translesion Synthesis (TLS). Thus, the pol η does not repair the damaged strand, but rather bypass the lesion. The TLS mechanism acts using several different DNA TLS polymerases, with each having a different preferential substrate, with pol η having a higher affinity and replication fidelity for pyrimidine dimers formed by UVR (47). XP-V patients generally have similar, although milder symptoms to the XPA-XPG patients, including high skin cancer predisposition and photosensitivity. However, in contrast to many of the NER-deficient patients, they do not exhibit any form of neurological abnormalities, with UVR-induced photolesions being related to the skin symptoms of all types of XP patients (48).

UVR is electromagnetic radiation often defined having a wavelength range of 100 to 400 nm and can be subdivided into UVA (from 315 to 400 nm), UVB (280–315 nm) and UVC (100-280 nm). Broader definitions may also include smaller wavelengths down to 10 nm (classified as either Vacuum UV or Extreme UV), though

these are less commonly used for biological models, as they require vacuum for transmission and do not reach the biosphere (49). Although UVC is blocked by the ozone layer, it is often used as a UVR model for biological processes due to its ease of use and its characteristics as being “clean” source of DNA damage, as it directly causes the same DNA photolesions as UVB, a more physiologically relevant DNA damaging source (50). Sunlight is responsible for Earth’s environmental UVR, as the electromagnetic spectrum emitted by the Sun encompasses X-rays, UVR, visible light and infrared light, with UVA and UVB being the only bands of UVR capable of passing through the ozone layer and reaching the biosphere. Though UVB is partially blocked by the ozone layer, it is biologically significant due to its properties of directly generating DNA photolesions (51).

UVR is capable of damaging the DNA molecule both directly and indirectly, due to its absorption by numerous cellular components, including DNA, proteins, RNA and other organic compounds (52,53). The type of molecule affected by UVR depends on the UV wavelength, with nucleic acid, such as DNA and RNA, being very effective in absorbing UVB, and chromophores and proteins such as collagen, elastin and melanin generally having a higher absorbance for UVA (54). Though in this work we will focus on the direct DNA lesions caused by UVB, UVR is also capable of generating DNA damage by indirect intracellular mechanisms through the production of reactive oxygen species (ROS) after photosensitized chromophores react with oxygen molecules (54). ROS oxidize DNA in a plethora of ways, often reacting with DNA bases and in some cases, breaking the phosphate-sugar bond, thus generating DNA strand breaks (55). These ROS are mainly induced by UVA irradiation, with UVB playing a minor role in generating them (56).

The direct UVR induced DNA damage, the pyrimidine dimers, are formed after UVR is absorbed by thymine or cytosine, which generates covalent bonds between that pyrimidine with an adjacent pyrimidine of the same DNA strand via photochemical reaction. The main DNA photolesions formed by UVR absorption are the Cyclobutane Pyrimidine Dimers (CPDs) and the Pyrimidine (6-4) Pyrimidone photoproducts (6-4PPs). These lesions are not only chemically different (as represented in figure 1.6), with covalent bonds forming between different carbons of

the pyrimidines (57), but also differ in their formation rate, with CPDs being 3 to 5 more frequently generated than 6-4PPs (58,59).

The sites of formation also differ between these two kinds of lesions, 6-4PPs having a smaller generation at either CT or CC sites than CPDs, and 6-4PPs being formed in a generally less uniform manner than CPDs at nucleosomes, CPDs generally being formed once every 10 nucleotides at this site. 6-4PPs are also more liable to being further changed by UVA, which may induce the formation of a 6-4PP variant, the Dewar photoproducts (Dewar-PPs) (57). Furthermore, CPDs and 6-4PPs cause a different distortion on the DNA molecule, 6-4PPs being able to warp DNA in a more pronounced way in comparison the CPDs. This, in turn, affects its repair rate by NER, being able to more easily recognize the more distorted DNA, with rate of repair of 6-4PPs being significantly higher in NER proficient mammalian cells (60).

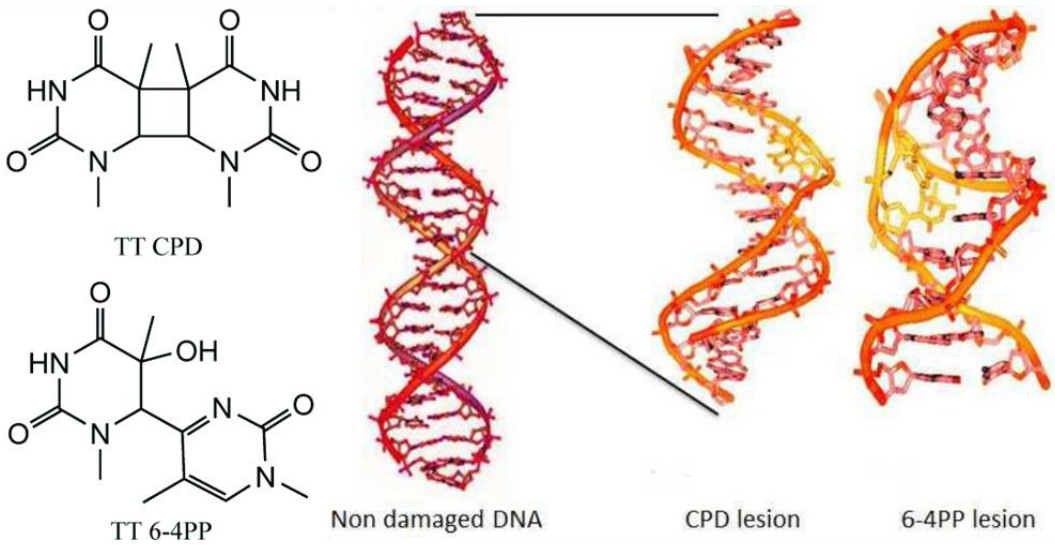

Figure 1.6. UV-induced DNA lesions. Molecular structure of CPD and 6-4PP lesions between thymines and the distortions they cause to the DNA double helix structure. Adapted from Rastogi et al., 2010 (57).

Although NER is generally thought to be found in all species, it is not the only pathway organisms rely to deal with photolesions. Photolyases, enzymes of the first discovered DNA repair mechanism (the photoreactivation), are found throughout all nodes of the evolutionary tree of life, including bacteria, archaea, plants, fungi, animal. They were lost in some groups however, such as placental mammals (which includes mice and humans) (61). The photorepair by photolyases is performed in a

light-dependent manner using a single enzyme in a process named direct repair. These enzymes bind to the DNA lesion, then through a flavin adenine dinucleotide (FAD) group, present in the enzyme, absorbs UVA/blue light (310–500 nm), enabling an electron transfer from the FAD chromophore to the DNA damage. The covalent bonds that make up the lesion are broken, followed by an electron transfer back to the FAD group (62). There are different kinds of photolyases, those mainly being CPD or 6-4PP photolyases, with CPD photolyases being more common throughout evolution. Interestingly, these photolyases are highly specific, with CPD photolyases repairing specifically CPDs and 6-4PP photolyases repairing 6-4PPs, which in turn makes them valuable tools for understanding the role of each lesion, with the removal of one photolesion enabling the observation of the effects of the remaining lesion (63).

UV induced lesions have a high biological significance due to their genotoxic and mutagenic properties, with NER deficient disorders, such as Xeroderma Pigmentosum and Cockayne syndrome further evidencing the importance of NER related DNA damages, with these lesions having a very important role regarding tumorigenesis and activation of other biological processes, such as inflammation and cell death (64,65).

1.3.2 Cockayne Syndrome, endogenous lesions and aging

Cockayne Syndrome is another rare, recessive, autosomal NER related genetic disease, characterized by photosensitivity, developmental abnormalities, such as cachetic dwarfism and arrested sexual development, and most notably premature aging-like (progeroid) features, including kyphosis, thin hair, fat loss, osteoporosis and neurological symptoms, which may have contributions of both abnormal development and progressive degeneration (66). CS neurodegeneration differs from XP neurodegeneration, CS patients displaying dysmyelination, vascular abnormalities, cerebellar ataxia, microcephaly, brain calcification and mainly purkinje cell death, as opposed to XP, which displays cerebral atrophy and primary neuronal degeneration. CS neurodegeneration features often result in progressive hearing and visual loss, ataxia, psychomotor delay and mental retardation (67,68). Moreover, CS patients, unlike XP patients, do not have an increase in mutation rate nor in cancer

incidence (69). CS is a highly heterogeneous disease, being divided into 3 subtypes, depending on the severity and age of onset of the symptoms. The most common genes associated with CS are the TC-NER *csa* and *csb* genes, though variant forms of the syndrome, such as XP/CS may also be caused due to mutations in *xpb*, *xpd* and *xpg* genes (70).

Although CS was first described in 1936 by Edward Cockayne (71), its first connection to DNA repair deficiency only surfaced in 1977, with initial studies revealing that although CS cells had the unscheduled DNA synthesis associated with NER, they still were hypersensitive to UVR. It was later revealed that these cells had a slow recovery of RNA synthesis after UV irradiation, which suggested an issue with transcription of the damaged strand, leading to the hypothesis and later comprehension that these patients had an impaired TC-NER (72). These findings explain the photosensitivity in CS patients, with it being caused by a failure to correctly repair UV induced photolesions in a similar way to XP. However, whether the deficiencies in TC-NER directly contribute and are alone able to explain the progeroid features of the disease is still an ongoing debate (73–75).

It was initially assumed that TC-NER dysfunction was the main cause of CS, as it was hypothesized that endogenous lesions, unrepaired by TC-NER and accumulated throughout CS patients lifespan were responsible for dysregulation of transcription and eventual cell death. Though it was a reasonable proposition at the time of its inception, emerging new data are now challenging this hypothesis (74). One of the main arguments against it is the recent discovery of the molecular mechanism of UV-Sensitive Syndrome (UVsS), a genetic syndrome whose patients also lack TC-NER due to mutations in either *uvssa*, *csa* or *csb* genes, but are only photosensitive for UV-induced photolesions and so far do not display any signs of progeroid or neurodegenerative features (76,77). And although the mutations in CSA or CSB that cause UVsS are different from those of CS patients, the outcome regarding their TC-NER deficiency of UV induced lesions is the same (74).

Although there are types of endogenous DNA lesions that are substrates for NER but not other repair pathways, such as cyclopurine deoxynucleosides (78), some DNA-protein crosslinks (79) and DNA lesions resulting from lipid peroxidation

such as the malondialdehydedeoxyguanosine adduct M1G (80), the main endogenous lesions hypothesized to cause CS have yet to be identified, which further brings speculation as to what is the molecular cause of CS. Other possible explanations for the molecular causes of CS are mitochondrial and metabolic alterations, as well as transcriptional abnormalities (81). CS proteins are involved in the regulation of these processes, with CS proteins being detected in mitochondria, having a role in the autophagy of damaged mitochondria (82) and CS cells having an altered fatty acid oxidation rate (83). Moreover, RNA metabolism is heavily altered in CS cells, with both RNA pol II and recently RNA pol I being implicated in this transcriptional alterations. Finally, a more recent theory implicates the persistence of protein complexes, such as NER machinery (75) or the transcription factors at damaged sites (84) as a main feature of NER-related neurodegeneration.

In spite of these more recent speculations as to the molecular cause of CS, the canonical explanation still refers to endogenous DNA damage having a central role in this disease and its early aging-like features, as well as aging in general, a theory also supported by several other evidences, including, but not limited to, different mouse models. In a similar way to the human progeroid diseases, these models have a deficiency in one or more NER related genes, such as the $Ercc1^{-/\Delta}$, $Csb^{m/m}/Xpa^{-/-}$, $Csb^{m/m}/Xpc^{-/-}$, $Xpd^{TTD}/Xpa^{-/-}$, $Xpg^{-/-}$ and $Csa^{-/-}/Xpa^{-/-}$ mice (85). All of these mice models display similar characteristics that generally mimic those of progeroid diseases, with decreased lifespan, smaller body size, lipodystrophy and neurodegeneration. Though shortened lifespan alone can be a misleading point regarding premature aging, as it is sensitive to artefacts regarding genetics and environmental sources of variation, the aforementioned and other age-related pathologies and additional aging parameters points to DNA damage having a causal role in progeroid features and being related to aging in general (86), with the mechanisms that links DNA damage to aging and cancer being discussed in the following topic.

1.4 Cellular and Molecular effects of NER-related DNA damage

DNA damage can have several different effects depending on the type of lesion and repair pathway involved (87). As previously mentioned, NER-related DNA damage are capable of distorting the structure of the DNA double helix, with the main effect being the stalling of RNA and DNA polymerases, thereby having an effect on transcription and replication (88). The stalling of these processes and persistence of lesions have major implications for cells, being able to activate a series of cellular reactions to genomic stress, with these effects collectively being called the DNA damage response (DDR) (89).

Cellular DDR often involves the signaling of the damage through different kinases, such as Ataxia Telangiectasia Mutated (ATM) and Ataxia Telangiectasia and Rad3 Related (ATR), which are in turn able to activate several other responses, including cell cycle arrest, DNA repair, transcriptional changes and programmed cell death. ATM and ATR have been shown to phosphorylate over 400 different substrates in order to deal with genomic stress (90). Among these, we highlight the tumor suppressor protein p53, a highly versatile protein with several different post-translational modification sites capable of regulating a plethora of cellular processes, including the DDR-related aforementioned ones, such as apoptosis and cell cycle arrest (91). The importance of p53 is further demonstrated by its significance to carcinogenesis regulation, with about 50% of tumors having a p53 mutation (92). DNA damage, DDR and its players therefore have a profound effect on cell homeostasis, with these molecular and cellular aspects having a large influence on the two previously discussed topics of cancer and aging. Amongst these aspects, we will focus on mutagenesis and carcinogenesis, cell death, senescence and inflammation.

1.4.1 Mutagenesis, carcinogenesis and cell proliferation

Mutagenesis has been shown to be the main cause of tumorigenesis, especially when occurring in tumor suppressor genes (such as p53) (93) or proto-oncogenes (such as some ras genes) (94). Mutations may occur spontaneously, due to replicational errors inserted by a replicative DNA polymerase (such as DNA pol ε).

These spontaneous mutation are generally uncommon, however, since the error rate of these polymerases are exceptionally low, estimated to be in the magnitude of 1×10^{-6} to 1×10^{-8} (95). Bulky DNA lesions and their causative sources, such as UVR, are able to severely increase the mutational rate due to their effect of blocking the DNA replication machinery. In order to deal with this arrest, other, more error-prone polymerases, capable of bypassing the damage through translesion synthesis (TLS) are recruited to the lesion site (31). TLS polymerases have a generally higher mutation rate, depending on the substrate having a rate of $1x10^{-2}$, over 4 orders of magnitude higher than replicative polymerases with proofreading activity (96).

The TLS process allows the cell to continue replicating its DNA, thereby impeding more severe effects, such as a DNA double strand break caused by replication stress (97,98). The mutational load and type of mutation varies with the type of damage and TLS polymerase recruited to deal with the lesion, with UVR generally producing a C>T transition mutation due to the action of error-prone TLS polymerases (99). Moreover, in order to properly occur a mutation, two rounds of DNA synthesis are necessary: one to first insert an erroneous base pairing and the other to fixate the mutation by inserting the base complementing to the lately acquired one (17). Mutations may or may not have effects on protein conformation, depending on where and how they happen, with the most prominent effects being either gain or loss of function (100).

As previously stated, mutations in tumor suppressor or proto-oncogenes are responsible for the process of tumorigenesis. These genes generally have a role regarding cell proliferation or processes that regulate it, such as cell death or growth arrest, though others have been recently implicated as well, such as genes that regulate cell metabolism and inflammation, with several hallmarks of cancer having been established (101). Still, as cancer is majorly a cell proliferation disease, the most traditional proto-oncogenes are generally growth factor receptors (such as EGFR) and related genes, as the proteins encoded by those genes have a major role in activating complex signaling cascades that result in activation of the cell cycle, such as MAP kinases and/or ERK.

Importantly, cell proliferation can also be activated as a part of DDR depending on the cell type, and may have important physiological roles such as a protective or restorative function, depending on the context. The signaling cascades that contribute to cell proliferation are often in a tightly regulated balance in order to impede tumorigenesis process, with organisms having a plethora of failsafe mechanisms to deal with this possibility. One of these mechanisms is the programmed cell death, with the balance of cell proliferation and cell death, amongst other factors often being implicated in the balance between cancer and aging related disorders (64) (figure 1.7).

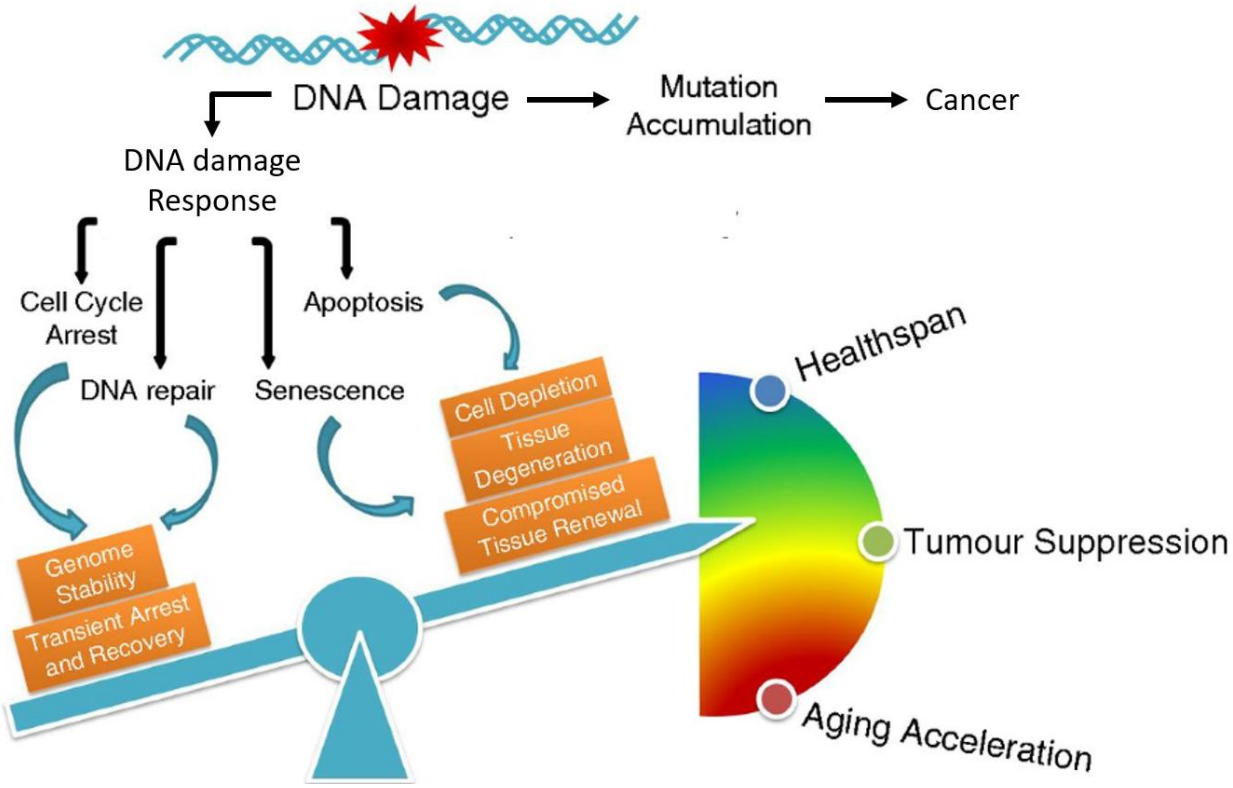

Figure 1.7. DNA damage, cancer and aging. Possible consequences of genomic damage, with repair failure affecting tumorigenesis by the accumulation of mutations. Another possible negative consequence is the triggering of senescence or apoptosis, which contributes to aging. Adapted from Ou & Schumacher, 2018 (102).

1.4.2 DNA damage induced cell death

Cell death has been implicated in several different biological processes, including, but not limited to, development, anti-tumorigenesis, inflammation initiation and resolution, several different disorders and aging. The type of cell death is important to further understand these phenomena, with cell death mechanisms being intrinsic or extrinsic, programmed or accidental. Regarding DNA damage associated cell death, the main type is apoptosis, a programmed and regulated cell death mechanism in which morphological changes, such as rounding-up of the cell, reduction of cellular volume, chromatin condensation, nuclear fragmentation and plasma membrane blebbing (103). Due to modifications in the plasma membrane structure, such as the exposure of the membrane lipid phosphatidylserine, macrophages are able to recognize and phagocyte *in vivo* apoptotic cells, thereby maintaining tissue homeostasis, as the cell contents do not spill into the tissue and activate other molecular signals, such as pro-inflammatory pathways (104).

Apoptosis is orchestrated through the activation of several specific caspases, a family of protease enzymes that, during apoptotic cell death, initiate and execute this program by producing a cascade reaction, cleaving hundreds of substrates in the process, destabilizing cell organelles and activating DNases, thus producing DNA fragmentation and cell death (105). DNA damage activates mainly the intrinsic apoptosis pathway, mediated by overactivation of p53, permeabilization of the mitochondrial membrane and activation of caspases, such as initiator caspases 8 and 9 and effector caspase 3. Importantly, DNA damage induced apoptosis signaling is often counter balanced by pro-survival factors, such as EGF signaling, NF-κB and Bcl-2, with cell fate being determined by the amount, site and type of lesion, as well as the cell capacity to repair it (106). For instance, in NER proficient cells, CPD lesions are a more significant apoptosis inducer than 6-4PPs, while in NER deficient cells, both lesions are able to induce apoptotic cell death in a similar proportion, despite CPDs having a higher formation rate than 6-4PPs. Furthermore, the cell type and epigenetic background also influence the impact of DNA damage on cell death, with keratinocytes having a higher survival rate than fibroblasts when exposed to UVB irradiation (107,108) and some neurons being more resistant than others to oxidatively generated damage depending on the brain region (109).

Cell death has key roles in maintaining organism homeostasis in numerous different ways, with DNA damage induced apoptosis being a key regulator of tumorigenesis (101). However, programmed cell death pathways have also been implicated in normal aging and progeroid syndromes, by promoting immune system decline, sarcopenia and neurodegenerative diseases (110). DNA lesions and their responses are an important regulator of these processes (111), with the level of signaling and of DNA damage possibly leading the cell to its demise. Another possibility that also impacts aging and disease and can be a resultant of damaged DNA is cell senescence (112).

1.4.3 Senescence and inflammation

Cell senescence, unlike cell death or mutagenesis, is a concept still under heavy debate as to its exact classification and hallmarks (113). While initially introduced to describe age-related changes in aging eukaryotic cell cultures, especially the exhaustion of their mitotic potential due to an intrinsic mechanism (114), cell senescence has grown into a field of its own, with the accumulation of senescent cells being a hallmark of both cancer (101) and aging (112), and with the concept of what is a senescence state being further expanded upon, including characteristics other than permanent cell cycle arrest, such as resistance to apoptosis induction, deregulation of cell metabolism and an abnormal secretory phenotype, with senescent cell generally secreting molecules such as IL-1 α, IL-1B, IL-6 and IL-8 through the activation of NF-κB, a pro-survival, anti-apoptotic, pro-inflammatory mechanism. The senescence associated secretory phenotype (SASP) is, however, a very heterogeneous characteristic of senescent cells, depending on the cell type and context, often secreting anti-inflammatory, immunosuppressive factors as well, such as IL-10 and IL-13 through other types of signaling, such as the Jak2/Stat3 pathway (113).

The mechanisms of the induction of cell senescence have also been expanded upon, with DNA damage caused by endogenous and exogenous sources having been shown to induce this state, with persistent DDR being considered a hallmark among senescent cells and having the involvement of cell cycle arrest

proteins such as p21 and, notably, $p16^{InkA}$ (113). Additionally, DNA repair deficient progeroid models have been shown to accumulate early senescent cells depending on the tissue, with these cells having a significant impact on organismal health (115,116). Recently, the induction of apoptosis specifically in senescent cells has been shown to rescue several of the progeroid phenotypes in a mouse model (117). The main hypothesis regarding this health improvement involves the decrease of pro-inflammatory SASP and subsequent decrease of basal, non-pathogenic induced (sterile) inflammation, as inflammation as a process has been implicated in several different aging-associated disorders, including cancer, cardiovascular and neurodegenerative diseases, as well as a decrease in lifespan (118).

Inflammation, much like cell senescence, is a concept that has recently been expanded upon (119). It is generally defined as a response of the innate immune system to stimulation by invading pathogens or endogenous damage signals involving several different cell types, such as immune, vascular, and tissue specific immuno-competent cells, such as Langerhans cells and keratinocytes in skin tissue (120,121), and astrocytes and microglia in the brain (122). Though it is classically and most often associated with defense against pathogens and/or tissue damage and repair, new data and insights have revealed a more multifaceted concept with interactions to other systems, such as metabolism and nucleic acid damage. Both lesions in DNA and RNA have been shown to elicit some pro-inflammatory cell and molecular responses through different mechanisms, such as activation of IL-1α (123), of the protein complex known as the inflammasome (124), the transcription factor NF-κB (125) and toll-like receptors (126), with these non-pathogen related (sterile) inflammation mechanisms being novel concepts, with its underlying structures and effects currently being established.

Importantly, although cell senescence and inflammation may have a negative impact on organismal health especially when under chronic situations, these two processes are also very important for tissue homeostasis, with senescence being an important anti-tumorigenesis mechanism, as it arrests cell cycle, impeding proliferation and mutagenesis of damaged cells (127), and inflammation having major roles regulating defense against pathogens and tumorigenesis (128), as well as driving tissue remodeling and restoration (129). Thus, much like cell death and

proliferation, the complexity of these biological processes must be considered when studying and developing strategies to improve organismal health.

1.5 Final considerations

DNA damage and its responses have important effects on numerous biological processes. NER deficient models, such as the ones used in this work (namely Xpa and Csa knockout mice) are important tools in order to study the role of different NER-related lesions on DNA damage induced effects such as cell proliferation, cell death, senescence and inflammation.

This work expands upon these concepts in two fronts: In Chapter 2, we investigate the role of UV induced CPD and 6-4PP lesions on keratinocyte responses of NER deficient, $Xpa^{-/-}$ knockout mice, a model for Xeroderma Pigmentosum. Meanwhile, in Chapter 3, we examine the impact of aging related effects on neurovascular dysfunction and inflammation in $Xpa^{-/-}/Csa^{-/-}$ double knockout mice, a model for Cockayne Syndrome. Chapter 4 integrates and discusses the results of the previous chapters and their biological implications.

1.6 Objectives

- Evaluate the specific effect of CPD and 6-4PP lesions generated by UVB irradiation regarding the induction of hyperplasia, cell proliferation, cell death and inflammation using NER deficient, Xpa knockout mice expressing photolyases in keratinocytes.

- Investigate the relationship between accumulation of NER-related DNA lesions and neuroinflammation and neurovascular dysfunction using a progeroid, Csa/Xpa double knockout mouse model.

Chapter 5 - General Discussion and Conclusions

The effects of DNA damage are numerous and depend on a number of factors, such as number, site and type of lesions, as well as cell type and *in vivo* context. In this work, we used two NER-deficient models, the Xpa and the Csa/Xpa knockout mice, in order to further understand the *in vivo* effects of NER-related lesions. Though these models differ phenotypically in several different ways, with the Xpa KO mice resembling Xeroderma Pigmentosum patients (141) and the Csa/Xpa (CX) knockout mice mimicking Cockayne Syndrome (166), they have a defect in the same molecular pathway, as well as similarities regarding higher sensitivity to DNA damaging agents (167,213). In this chapter, we will explore some of the general overlaps of these models regarding cellular and systemic effects of DNA damage - namely, cell death and induction of inflammation.

Apoptotic cell death is a known DNA damage response (DDR) mechanism that participates in the maintenance of tissue homeostasis by inhibiting tumorigenesis, clearing out dysfunctional cells and regulating inflammation (105,214). A high level of apoptosis, however, may directly or indirectly be detrimental to organism health, contributing to the aging and dysfunction of various organs, including the skin (215) and the brain (212), the two main organs studied in this thesis. Although the balance between this cell death and pro-survival pathways, as well as the amount of damage necessary to tip the balance in favor of cell death varies with cell type and context, the pathway used for this effect is generally the same for many cell types (216).

The main source for skin cell DNA damage under physiological conditions is ultraviolet radiation (UVR), an electromagnetic radiation considered to be the most ubiquitous exogenous source of DNA damage of our environment (130). In chapter 2, we describe the *in vivo* role of the two main DNA lesions generated by UVR, cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts (6-4PPs) on Xpa knockout mice expressing either CPD or 6-4PP photolyases in keratinocytes, and have observed that similarly to previous *in vitro* results (88), removal of either CPD or 6-4PP on a NER deficient background resulted in a reduction of apoptosis, indicating that both lesions contribute to the induction of

this effect. On the opposite side of apoptosis, we have UV-induced cell proliferation in epidermis, with both photolesions contributing to this process, but with CPDs having a more prominent role in this process on NER deficient mice. On NER proficient models, CPDs are markedly more important than 6-4PPs for both processes, possibly due to rapid repair of 6-4PPs (88,140).

Apoptosis has been extensively studied on NER deficient progeroid models, especially regarding its effect on neurodegenerative diseases, with $Ercc1^{-/\Delta}$, $Xpg^{-/-}$ and $Csb^{m/m}/Xpa^{-/-}$ mice having been described as displaying increased apoptosis in certain regions of the brain (168,213,217), with an alleviation of DNA damage in these models being correlated to a decrease in neuronal cell death and an improvement in neurological function (212). CX mice, the model used in chapter 3, has also been observed to have age dependent neuronal cell death (167). Furthermore, $Csb^{m/m}/Xpa^{-/-}$neuron-specific knockout mice also display a severe age dependent increase in neuronal cell death. Interestingly, although these mice display some similar phenotypes to its whole body counterpart, the neurodegenerative phenotype can only be observed at a much later life stage than every other whole body NER progeroid mouse model (169). Thus, while cell-autonomous neuronal apoptosis induced by endogenous DNA damage is important to the neurodegenerative aspects of progeroid models, it is not the only factor involved in this process. Another component to progeroid mice neurodegeneration might involve neuroglia regulated neuroinflammation, which has been implicated in numerous neurodegenerative disorders by mechanisms such as synapse elimination (174) and induction of oligodendrocyte and neuronal cell death (177).

In the previous chapters of this thesis, we speculate upon and bring evidences to the hypothesis of an interplay between DNA damage and inflammation, with DNA damage being related to the induction of proteins related to inflammation such as ICAM-1 and NF-κB, and participating in processes of neutrophil, astrocyte and microglial activation, thereby having a major role in UV-induced inflammation and neuroinflammation. Although the exact pro-inflammatory pathways have yet to be fully elucidated, we propose that DNA damage may be directly or indirectly driving these effects, with DNA damage induced inflammation being a general phenomenon

in many immune-related cell types, including keratinocytes (124), adipocytes (218), fibroblasts (219), and microglia (220).

As shown and discussed in chapter 2, UV-induced inflammation is linked to both CPD and 6-4PP photolesions, as the removal of either lesion was able to reduce inflammation markers in NER deficient XPA mice skin, and CPD removal was able to decrease UV induced inflammation in NER proficient mice. Several molecular pathways have been previously implicated for the signaling of this effect (65), such as the activation of the pro-inflammatory transcription factor NF-κB through a DDR mediated mechanism (221). Another possible mechanism for DNA damage induced sterile inflammation was described in (123), with the cytokine IL-1α appearing to have a role as a DNA damage sensor, being recruited at CPD containing sites, and with IL-1α$^{-/-}$ mice having a reduced neutrophil infiltration response in the skin after UV irradiation. The NLRP3 inflammasome protein complex, related to IL-1α and NF-κB, has also been shown to be activated by UV-induced DNA damage (124). These pro-inflammatory pathways have been proposed to be a type of UV-induced DNA damage response, with NER having a major role in regulating the pathways involved in this process (222). Moreover, UV induced inflammation has also been shown to have a role in tumorigenesis alongside UV photolesions themselves (223), with the inhibition of inflammation resulting in a decrease in cancer incidence after chronic UV irradiation in both NER proficient and deficient mice (224,225).

Regarding the progeroid related neuroinflammation, although activation of every previously mentioned pathway (NF-κB, IL-1α and NLRP3) has been linked to neurodegeneration (226–228), there have been so far fewer direct evidences of NER-related DNA damage activating a neuroinflammation signaling pathway. This is difficulted due to a lack of usable tools to specifically repair aging related DNA damage, in contrast to UV induced photolesions, which can be repaired by photolyases (63). Additionally, neuroinflammation is an emerging concept and novel area of study, still requiring and developing more precise markers to better elucidate this multifactorial process (229). Regardless, considering the amount of evidence, including this thesis chapter 3, showing that deficiency in NER generates a neuroinflammation phenotype *in vivo* (168,213), alongside other evidences showing this effect *in vitro* (171,220), it is reasonable to hypothesize that this is a general

effect caused by DNA damage in certain nervous system cells. Whether the signaling pathways of its activation and execution are similar to the UV induced ones remains to be determined.

Besides the aforementioned inflammation pathways inducible directly by DNA damage, there are other indirect ways DNA lesions can stimulate pro-inflammatory pathways, especially by causing cell senescence. One of the hallmarks of cell senescence is activation of the senescence associated secretory phenotype (SASP), partially induced by the overexpression of the pro-survival protein NF-κB, which in turn also activates the expression of generally pro-inflammatory cytokines, such as IL1, IL-6 and IL-8 (113). Surprisingly, we did not find senescence of endothelial cells in the CX model, in contrast to previous findings using the Ercc1$^{-/\Delta}$ model (115), which shows that although similar, these segmental progeroid models can have fundamentally different phenotypes in some cell types. We also found pronounced adipose tissue senescence in our model, which may contribute to general organismal inflammation and/or priming of immune cells. The adipose tissue SASP, however, may also differ between progeroid models, as there was no evidence of adipose tissue specific inflammation in CX mice (83), again unlike the Ercc1$^{-/\Delta}$ model (230).

The general findings of this thesis regarding the *in vivo* effects of NER-related DNA damages are summarized in figure 5.1. Briefly, UVR induced photolesions (CPD and 6-4PP) in keratinocytes of XPA KO, NER deficient mice are related to induction of cell death, inflammation and cell proliferation (CPDs having a more prominent role than 6-4PPs in cell proliferation). Regarding NER progeroid mice, although we found no evidence of a cell autonomous endothelial vascular dysfunction in the CX model, we report that these mice have an increase of neuroinflammation markers and a blood brain barrier defect. These observations on the consequences of NER-related DNA damage have implications on the etiology of the NER associated diseases Xeroderma Pigmentosum and Cockayne Syndrome as well as the biological processes related to these diseases – respectively, cancer and aging-related neurodegeneration.

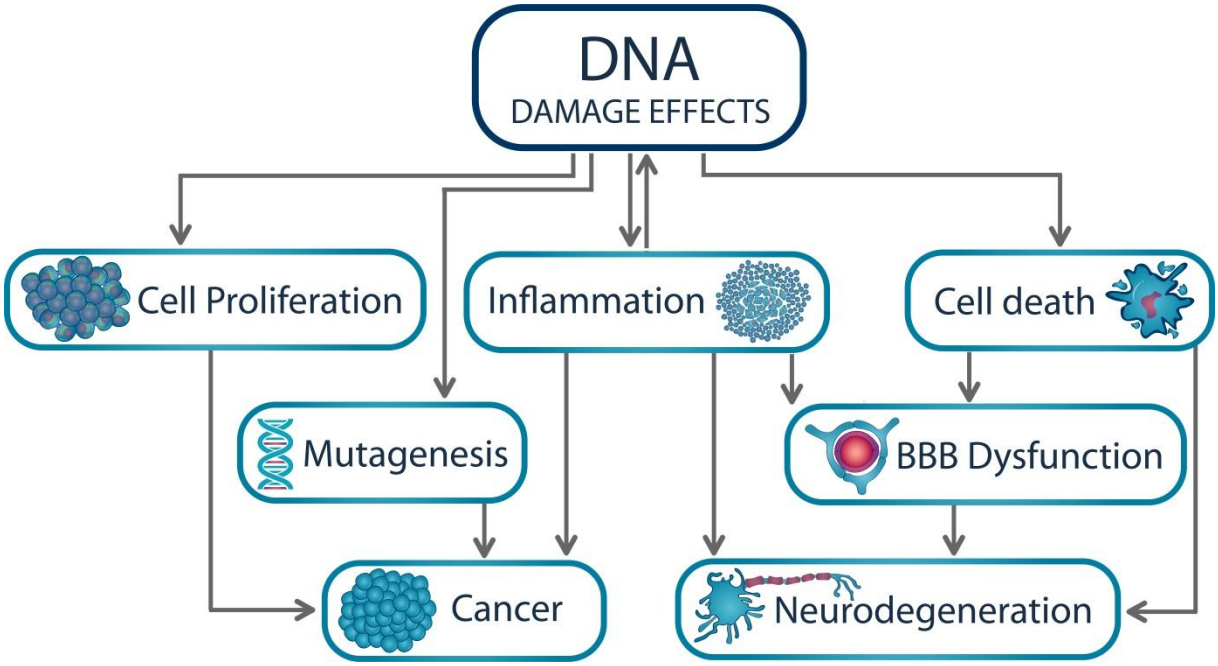

Figure 5.1. Conclusions summary. Working model of the studied DNA damage effects and how they relate to processes that affect organism health, namely Cancer and Neurodegeneration.

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