NATHALIA QUINTERO RUIZ

PERFIL DE MUTAGENICIDADE INDUZIDO PELA LUZ UVA E UVB EM CÉLULAS DE PACIENTES COM XERODERMA PIGMENTOSUM GRUPO C

Tese apresentada ao Programa de Pós-Graduação em Microbiologia do Instituto de Ciência Biomédicas da Universidade de São Paulo, para obtenção do Título de Doutor em Ciências.

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MUTAGENICITY PROFILE INDUCED BY UVA AND UVB LIGHT IN CELLS FROM PATIENTS WITH XERODERMA PIGMENTOSUM GROUP C

Thesis presented to the Graduate Program in Microbiology at the Institute of Biomedical Science of the University of São Paulo, to obtain the title of Doctor of Science.

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CHAPTER 1 – Introduction and Objectives

1.1 Introduction

1.1.1 Ultraviolet light effects

The ultraviolet light (UV) was one of the first environmental mutagen to be discovered. Since all living organisms are constantly exposed to UV via the sunlight, this discovery generated great interest in the scientific community. Experiments using UV light as a mutagen began in the 1930s, with works in Drosophila and maize. Years later, by using microorganisms as a model it was clearly demonstrated that the maximum absorption spectrum of nucleic acids match with the maximum harmful (mutagenic and killing), effects of UV radiation (DeMarini et al. 2020). At present it is already clearly established the relation of UV light to the development of skin cancer, the most common type of cancer around the world (de Gruijl et al. 1993, Armstrong & Kricker 2001, Matsumura & Ananthaswamy 2002). Therefore, in 2009 the UV radiation was included in the list of carcinogenic substances for humans, group 1 (El Ghissassi et al. 2009).

Based on its spectral range and therefore on the energy it contains, the UV radiation can be divided in three main types: i) UVA, ultraviolet radiation type A or long wave (315-400 nm); ii) UVB, ultraviolet radiation type B or medium wave (280-315 nm) and; iii) UVC, ultraviolet radiation type C or short wave (200-280 nm). However, the relative importance of UV types in terms of exposure it is not directly proportional to their energy, since they are differently filtered by the stratospheric ozone layer that protects our planet, which only filters efficiently wavelengths below 300 nm. Thus, while ozone layer is able to filter completely (~100%) or almost completely (~95%) the UVC and UVB wavelengths, respectively. It is not able to filter efficiently the UVA (~ 5%). Although UVB light corresponds to a small fraction of the sunlight spectrum, this wavelength is much more energetic than UVA and it can penetrate the skin to the epidermis. Meanwhile, despite being less energetic, UVA light is epidemiologically more important as it corresponds to 95% of UV from sunlight and it penetrates more deeply in the skin, reaching the dermis (Anderson & Parrish 1981, Frederick et al. 1989, Cadet et al. 2005, Matsumura & Ananthaswamy 2002, Schuch et al. 2012).

The energy contained in the UV light is able to induce a variety of specific structural changes in the DNA double helix by direct or indirect means (Pfeifer et al. 2005). Direct excitation of the molecule mainly causes cyclobutane pyrimidine dimers

(CPDs) and pyrimidine (6,4) pyrimidone (6-4PPs) photoproducts, both generated at dipyrimidine sites and cause large distortions. In CPDs, the adjacent pyrimidines form a cyclobutane ring through a covalent bond between C5 and C6 carbon atoms of the nitrogenous bases (thymine, cytosine or 5-methylcytosine). In the case of 6-4PP, a non-cyclic covalent bond occurs between the C4 and C6 atoms (Rastogi et al. 2010). Although CPDs are formed in a higher proportion than 6-4PPs, their repair is slower, probably because 6-4PPs causes a stronger distortion, making it more easily detected. Therefore, it is believed that CPDs are mainly responsible for the mutations induced by UV light in mammals (Matsumura & Ananthaswamy 2004, Pfeifer et al. 2005). Unrepaired CPDs and 6-4PPs mainly generate transitions of the C>T type, as well as CC>TT tandem transitions and both are known as UVC and UVB signature mutations (Ziegler et al. 1993, Ikehata & Ono 2011).

Indirect damage into the DNA is generated through photosensitization reactions, which can involve oxygen or other photosensitizer molecules, as NADPH, riboflavin and porphyrin, or even the DNA itself. These result in oxidized bases such as 8-oxoguanine (8oxoG) and thymine glycol (Ravanat et al. 2001, Pfeifer et al. 2005, Rastogi et al. 2010, Sage et al. 2012, Yagura et al. 2017). Apparently, due to the lower reduction potential of guanine, the 8oxoG is the most abundant oxidized base generated; however, it is also rapidly and efficiently repaired (Steenken & Jovanovic 1997). When unrepaired, 8oxoG generates mainly transversions of the G>T (C>A) type, caused by the wrong pairing with an adenine, or A>C transversions, by the erroneous incorporation of oxidized guanine opposite to an adenine (Epe 1991, Cheng et al. 1992, Ikehata & Ono 2011).

Direct damage into the DNA molecule has been traditionally related to UVB wavelength due to its higher energy, while indirect damage has been related to UVA wavelength, because direct absorption of UVA energy by DNA is low (Sutherland & Griffin 1981, Mouret et al. 2006, Nichols & Katiyar 2010). Therefore, the C>T transitions resulting from unrepaired pyrimidine dimers are related to the UVB portion of the solar UV radiation spectrum and the C>A transversions caused by oxidized guanine are attributed mainly to the UVA portion. However, it is controversial whether the mutations caused by UVA are just a consequence of oxidative generated damage, since different studies presented evidence of the CPD and even 6-4PP induction after UVA treatment (Douki et al. 2003, Rochette et al. 2003, Mouret et al. 2006, Schuch et al. 2009, Nichols & Katiyar 2010, Ikehata & Ono 2011, Cortat et al. 2013). Thus, the C>T transitions

have been proposed as a characteristic signature of UV radiation in general, and not specifically of UVB and UVC wavelength (Pfeifer et al. 2005, Sage et al. 2012). This information clearly reveal that the mechanisms by which sunlight induces mutations and skin cancer remains a matter of debate (Sage et al. 2012, Rünger et al. 2012).

1.1.2 Nucleotide Excision Repair (NER): an important and versatile pathway to remove photoproducts from the genome

Genomic DNA is a highly reactive molecule due to its structure, so it is naturally subject to suffer damage, which are chemical alterations of the double helix that challenge the genomic stability and can compromise both replication and transcription. It is estimated that more than 20,000 lesions are induced daily in the DNA as a result of endogenous cell metabolism (Friedberg et al. 2006). Also, physical and chemical exogenous agents such as UV from sunlight, smoke, pollution, and natural or artificial drugs can interact and induce specific damage. Thus, cells have several repair mechanisms to deal with the different types of DNA lesions and try to maintain genomic stability.

Nucleotide excision repair (NER) is a highly conserved and versatile DNA repair pathway, that recognizes and removes a wide range of DNA damage that cause structural distortions in the double helix. NER removes fragments containing lesions in several coordinated steps involving specialized proteins. The first step is damage recognition, followed by unwinding of the double helix around 30 bp at the site of the lesion. Then, cleavage occurs on both sides near the lesion and the damaged chain is excised. After this, the gap is filled by polymerases using the intact chain as a template and ends with a ligation (Sancar 1996, Laat et al. 1999, Menck & Munford 2014).

In eukaryotes, damage recognition can occur by two sub-pathways depending on its locale. DNA damage throughout the genome is recognized by the global genome repair (GGR) sub-pathway. In this case, the primary damage detector is the XPC/HR23B protein complex. The XPE protein is also involved to the damaged DNA binding (DDB) complex and help to improve the efficiency of recognition and removal of some types of lesions, as CPD. On the other hand, DNA lesions in the transcribed strand of actively expressed genes are recognized by the transcription-coupled repair (TCR) sub-pathway. In this case, CSA and CSB proteins recognize lesions that cause blockage of RNA polymerase II during transcription, recruiting NER proteins to

damaged DNA. After damage recognition, the following steps are the same for both sub-pathways (Gillet & Schärer 2006, Menck & Munford 2014).

Once the lesion is recognized, the double helix is unwinded by the XPB (3´-5´) and XPD (5´-3´) helicases, which are part of the transcription factor IIH (TFIIH), a multiprotein complex. Then, the replication protein A (RPA) complex and XPA protein bind to protect the DNA single strand and ensure the stability and proper assembly of proteins involved in repair. A region of around 30 bp containing the lesion is cleaved by the ERCC1-XPF and XPG endonucleases, at the 5´ and the 3´ of the lesion, respectively. The next step is to fill the gap by the coordinated action of the DNA polymerases delta (δ) or epsilon (ε), with the help of several accessory factors of replication (as the RPA, RFC and PCNA proteins) by using the complementary strand as a template and the 3'-hydroxyl extremity generated by the ERCC1-XPF as a primer. Finally, the ligation of the 3´ recently synthesized fragment with the 5´ extremity (generated by XPG) is performed by the DNA ligase I or III and the repair process is completed in an error free manner (A general scheme of the NER mechanism is summarized in figure 1.1.) (Gillet & Schärer 2006, Menck & Munford 2014).

1.1.3 XPC protein

The main function of the XPC protein is to detect a variety of DNA lesions throughout the GGR sub-pathway of NER (Sugasawa et al. 1998). During this recognition process the double helix is thermodynamically destabilized, which is essential for the subsequent recruitment of NER factors in the pre-incision complex (Sugasawa et al. 1998, Min & Pavletich 2007).

The *XPC* gene encodes a protein containing 940 amino acid residues with a predicted mass of 125 kDa, it is located on the short arm of chromosome 3 at position 25.1 (3p25.1, NCBI gene ID:7508) (Legerski et al. 1994). The XPC protein was cloned by Legerski & Peterson in 1992 and then purified by Masutani and co-workers in 1994. Different research revealed that this protein shares limited similarity with the homologous product of the yeast DNA repair gene *RAD4*, and form a complex with the centrin 2 protein and the human homolog of the yeast ubiquitin-domain repair factor RAD23, designated HR23B (58KDa). The Centrin 2 is not essential in the NER repair pathway, but potentiates the damage recognition by XPC protein, while the HR23B is essential in this repair mechanism since it promotes the XPC protein stability. The XPC protein also has high affinity for ssDNA, is highly hydrophilic and has acid and basic

domains, suggesting a possible interaction with chromosomal proteins and DNA. *In vitro*, it was established that the C-terminal portion of the XPC protein is responsible for the interactions with DNA, RAD23B, Centrin 2 and TFIIH, while the N-terminal portion of the protein interacts with XPA (Legerski & Peterson, 1992, Masutani et al. 1994, Ng et al. 2003, Bunick et al. 2006, Nishi et al. 2005).

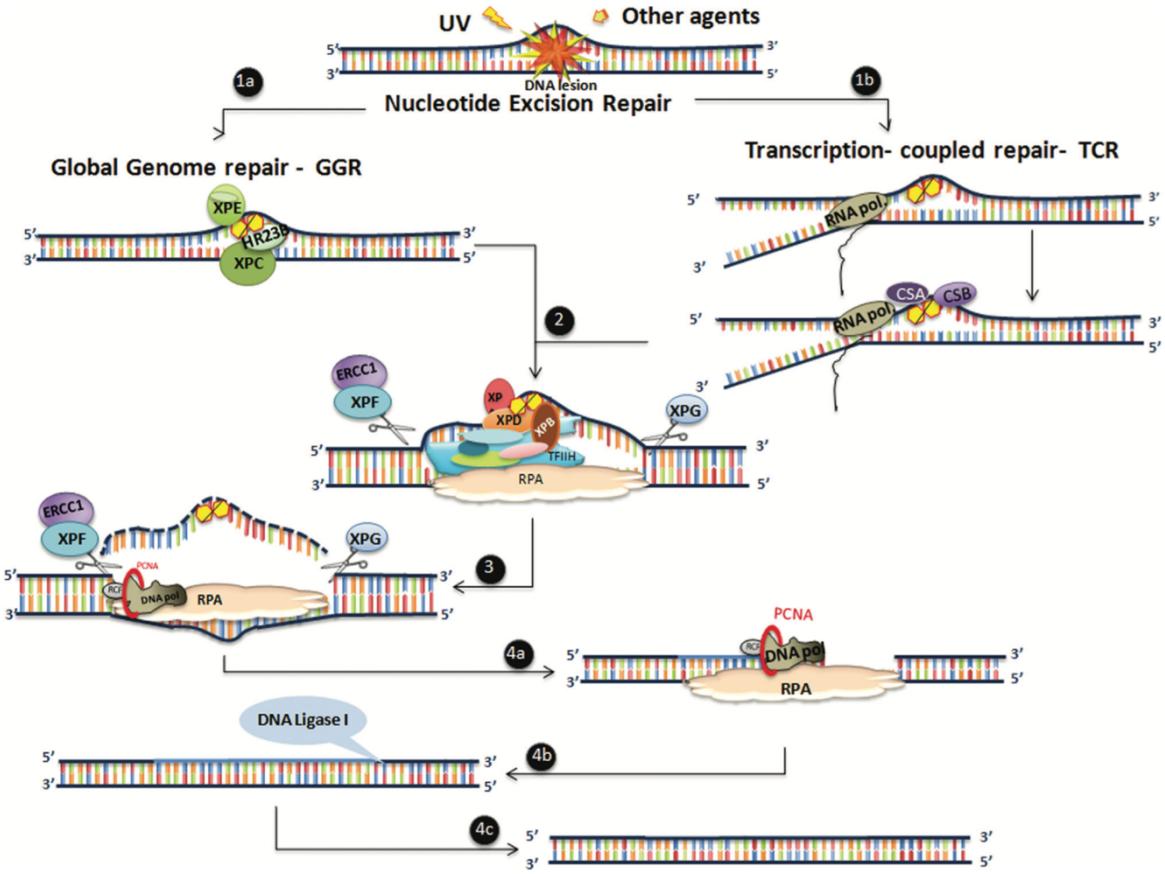

Figure 1.1. Nucleotide excision repair (NER) pathway: Schematic representation of the NER steps for DNA lesion removal in an error-free manner: 1) damage recognition can occur by the Global Genome Repair (GGR) sub-pathway or the Transcription Coupled Repair (TCR) sub-pathway, depending on the damage localization. 2) opening of the double strand of DNA, 3) excision of the region containing the lesion, 4) synthesis of a new error-free strand (figure from Menck & Munford 2014).

Several studies presented evidence of additional roles for the XPC-HR23B protein complex, either as auxiliary protein in repair routes other than NER or in cellular processes not related to DNA repair. For example, XPC-HR23B complex can act as a co-factor in the base excision repair (BER) pathway, since it interacts with the human glycosylases OGG1 and TDG, which participate in the repair of 8oxoG and G/T mismatches, respectively (Shimizu et al. 2003, D´Errico et al. 2006). On the other hand, XPC protein also could act as transcriptional co-activator at the promoters of

inducible genes in the absence of exogenous genotoxic attack and on embryonic stem (ES) cells (Le May et al. 2010, Fong et al. 2011). Additionally, XPC protein also seems to have an important role in cellular metabolic processes, since in his absence the balance between the mitochondrial respiratory complex I and II (CI and CII) is altered: the CI activity is diminished as long as the CII activity is increased (Mori et al. 2017).

For the year 2010, forty-five different mutations had been characterized in the XPC gene sequence of xeroderma pigmentosum (XP) patients (for more details see Li et al. 1993, Cleaver 1999, Soufir et al. 2010). The study model used in this work is the human skin fibroblast XP4PA-SV40 (Daya-Grosjean et al. 1987). This cell line is homozygous for the most common mutation reported, a deletion of two nucleotides (TG) in exon 9 of the *XPC* gene (c.1643_1644delTG) that leads to a frameshift mutation and a premature stop codon (p.Val548AlafsX572), resulting in an inactive protein (Li et al. 1993, Ben Rekaya et al. 2009, Soufir et al. 2010). Thus, the XP4PA-SV40 cell line is deficient on the GGR sub-pathway of the NER repair mechanism. As a result, unrepaired lesions can block the DNA replication and transcription processes, leading to fork collapse, DNA breaks and genomic instability, which may result in cell death and increased mutagenesis, what may result in the development of cancer (Setlow 1974, Pfeifer et al. 2005, Sugasawa 2008, Ikehata & Ono 2011, Menck & Munford 2014, Bowden et al. 2015).

1.1.4 Xeroderma pigmentosum (XP), a syndrome related to defects in the NER repair pathway

The cellular risk of accumulate potentially oncogenic mutations depends on the frequency with which DNA lesions occur and the ability of cells to deal with such lesions. Thus, to safeguard genomic integrity it is necessary to maintain a balance between damage induction and the efficient functioning of the multiple DNA repair pathways, damage tolerance processes and cell cycle checkpoints (Kawanishi et al. 2001; Laval et al. 1998; Peltomäki 2001, Giglia-Mari et al. 2011). In this context, the importance of cellular responses to DNA damage is exemplified by various syndromes caused by mutations in genes that participate in any of the response pathways. Some of the clinical features include neurological abnormalities, premature aging and predisposition to cancer, among others (For detailed information, please refer to: Hoeijmakers 2009, Ghosal & Chen 2013, Bukowska & Karwowski 2018). Defects in the NER system, for example, can lead to a phenotypic diversity of disorders such as

XP, trichothiodystrophy (TTD) and Cockayne syndrome (CS) (Menck & Munford 2014, Bukowska & Karwowski 2018).

XP is a rare autosomal recessive and hereditary disease that promotes high sunlight sensitivity, especially by the ultraviolet (UV) spectrum. Individuals who suffer from XP have strong pigmentation, skin dryness, ten thousand more chance to develop skin cancer, ten times more chance to develop internal tumors and a short lifetime in relation to the unaffected population. Additionally, in approximately 25-30% of cases, patients also exhibit progressive neurological abnormalities and in 40% of cases ophthalmologic pathologies (Taylor 1994, Sugasawa 2008, Cleaver et al. 2009, Menck & Munford 2014, Bowden et al. 2015, Bukowska & Karwowski 2018). This syndrome was originally documented by the dermatologists Ferdinand von Hebra and Moriz Kaposi in 1874, but only in 1968 the molecular defects were identified (Cleaver 1968). By means of cell fusion experiments, seven complementation groups that affect genes encoding proteins (XPA to XPG) associated to the NER pathway were established. In addition, there is a variant group (XPV), that is NER proficient but deficient in DNA polymerase η (pol eta), involved with translesion synthesis (TLS) of DNA damage (De Weerd-Kastelein et al. 1972, Cleaver et al. 2009, DiGiovanna et al. 2012).

XP patients have been identified around the world and in all ethnic groups. The XP estimated incidence in population range from 1:450,000 in Western Europe, 1: 250,000 in USA and 1: 20,000 In Japan and North Africa (Ben Rekaya et al. 2009, Menck & Munford 2014). In Brazil, there are not official estimates, however, in the northwest region of Goiás (municipality of Faina, in the village of Araras), one of the highest frequencies of XP in the world is found, 1:410 inhabitants of Faina (Munford and Castro et al. 2017). This high frequency of the disease is explained by a high rate of consanguineous marriage and endogamy on some of those populations (Ben Rekaya et al. 2009, Munford and Castro et al. 2017). The complementation group C of XP patients (XPC) appears to be the most prevalent type of classical XP in the world. The most common clinical features include strong photosensitivity, early mortality mainly due to a high accumulation of skin cancers in body-exposed areas to sunlight (including basal cell carcinoma, squamous cell carcinoma, and malignant melanoma) and ophthalmological sings. The vast majority of patients do not have neurologic abnormalities so early diagnosis and full protection from sun-exposure are crucial (Soufir et al. 2010).

As previously mentioned the *XPC* gene most common mutation c.1643_1644delTG (p.Val548AlafsX572) result in an incomplete protein unable to recognize cellular DNA damage on the non-transcribed regions of the genome (Li et al. 1993, Soufir et al. 2010), so XPC patients are specifically deficient in GGR, since they are unable to recognize the damage generated in the DNA. This mutation was mainly identified in XPC patients with severe clinical XP symptoms and is the most prevalent type in Western and Southern Europe and North Africa (Ben Rekaya et al. 2009, Soufir et al. 2010). Locals for which haplotype analysis suggest a founder effect for this mutation, estimated in 50 generations or 1250 years (Ben Rekaya et al. 2009, Soufir et al. 2010). This mutation was also detected on USA, Honduras and Brazil (Khan et al. 2006, Leite et al. 2009, Soufir et al. 2010, Santiago et al. 2020).

1.1.5 Analysis of somatic mutation patterns in cancer genomes: from fingerprints to Mutational Signatures

Cancer is an aggressive and silent disease that causes about one in six deaths worldwide, being the second leading cause of death after cardiovascular diseases (American Cancer Society 2018). Efforts and resources have focused on prevention, early diagnosis and to improve treatment. However, its basic biology has not been still fully understood (Greaves 2015). Until now, it has been well established that the accumulation of mutations is related to cancer development and can contribute to the proliferation and survival of cancer cells (Tomasetti et al. 2015).

Several studies have attempted to connect specific agents with particular molecular mutations that occur in the carcinogenic processes (or carcinogenesis) (Vogelstein & Kinzler 1992). The first studies were performed by analyzing somatic mutations in single oncogenes or in tumor suppressor genes, and revealed that a mutational process generated by a particular agent leads to specific molecular fingerprints. The first link was suggested for the G>T mutation at the codon 249 of the p53 gene of hepatocellular carcinomas from patients exposed to aflatoxin B1, but which is rarely found in tumors of other organs (Vogelstein & Kinzler 1992). However, although this strategy revealed valuable information that allowed to clarify some questions related to mutagenesis by establishing fingerprints for diverse mutagens, it is not enough to understand the complexity of the final catalog of mutations observed, since multiple mutational processes could be involved during the tumor development (Petljak and Alexandrov 2016).

Then, the analysis of somatic mutation patterns found in the cancer genome was extended from a single gene to a group of genes. However, it was with the development and widespread use of the next generation sequencing (NGS) technology that the analyses of the exome and whole-genome sequences of tumors became possible. Several studies using this high-throughput technology confirmed patterns previously obtained in pioneering studies within p53 and, naturally, also revealed new patterns of characteristic mutations in different types of cancer, indicating that diverse mutational processes operate on different tissues (Rubin & Green 2009, Petljak and Alexandrov 2016).

A large amount of cancer genomics data has been generated in the last decade by NGS, giving access to the 'mutational record' of a cancer genome. However, in addition to accessing and the ability to read the "mutational record", it is necessary to be able to organize and understand these data, which was made possible by the development of advanced mathematical models and computational tools (Petljak and Alexandrov 2016).

The concept of mutational signatures emerged in 2012 and refers to patterns generated by the mutational processes that cells during the tumor development suffer/endure, defined by the mechanisms of DNA damage (originated both from endogenous sources and exogenous factors) and DNA repair involved. Thus, the diversity of somatic mutations on cancers can be explained by one or more mutational signatures depending on the amount of mutational processes, and the strength and the duration of exposure to each one (Nik-Zainal et al. 2012). The mathematical algorithm used to extract mutational signatures, as well as the systematic computational framework that can be freely used to establish them, were published in 2013 (Alexandrov et al. 2013a).

The method developed by Alexandrov (2013a) is based on a matrix factorization algorithm and takes into consideration the six possible types of single base substitutions in a trinucleotide context (*i.e.* the mutated base and the immediately 5'and 3' sequence context, generating 96 different possible combinations). A total of 30 independent mutational signatures were established by applying this method and after analyzing 12.000 cancer genomic data of 40 different cancer types (Alexandrov et al. 2013b). Recently, this number was updated to 67 signatures (Alexandrov et al. 2020). The patterns of these mutational signatures and information about them, including prevalence in different cancer types and possible etiology, can be found in the

catalogue of somatic mutations in cancer, COSMIC, database (available at: https://cancer.sanger.ac.uk/cosmic/signatures/SBS/). The mutational signatures analysis has been established as a very useful analytical tool that constitutes a breakthrough in the cancer research, since it is used to identify novel mutational signatures and to study the processes involved in different cancers and patients (Bayati et al. 2020).

1.2 Objectives

1.2.1 General objective

- Establish and compare the mutagenicity profile, type and frequency of mutations, induced by UVA and UVB light in cells from XP-C patients.

1.2.2 Specific objectives

- Verify human cell survival after UVA and UVB irradiation;
- Determine the effects of UVA and UVB irradiation on the cell cycle profile of XP-C cells using flow cytometry;
- Evaluate the induction of mutations in XP-C cells submitted to different doses of UVA and UVB irradiation;
- Identify the type and frequency of mutations in XP-C cells using exome sequencing of the isolated clones of irradiated cells.

CHAPTER 5 – General discussion and conclusions

Since the discovery of UV irradiation as an environmental mutagen, numerous studies attempt to understand its effects by using different biological models (DeMarini et al. 2020). Each one with a particular focus but all with the intuition of trying to put the pieces together and understand the consequences of exposure to UV irradiation in human health as a whole. This work seeks to provide information about the mutagenic effects of the UV irradiation spectrum that reaches the Earth's surface, UVA and UVB. To get this, we took advantage from the NGS technology that allowed to access point mutations generated in the exome of human cells, and therefore revealed a more global approach of the mutagenic effects of UV irradiation in the human genome. Also, the use of cells deficient in the main repair pathway that recognizes and remove UV induced damages, the NER pathway, allows increasing the sensitivity of the model to detect the mutagenic effect of both UVA and UVB light wavelengths.

Epidemiologically, it is important to study UVA since it corresponds to 95% of the UV-sunlight spectrum and penetrates deep in the dermis, which increases the amount and cellular types that it reaches. Also for a long time it was considered harmless and little studied compared to UVC and UVB, so less information is available (Sage et al. 2012). Meanwhile, studying UVB is also of great importance because although proportionally UVB corresponds to only a small fraction of the UV spectrum, it is much more energetic and generates 10^3 times more direct damage than UVA (Kuluncsics et al. 1999, Cadet et al. 2005). Therefore, small increases in UVB levels that reaches the Earth´s surface due to stratospheric ozone depletion translates into large effects on different ecosystems and consequently in a wide range of life forms (de Gruijl & van der Leun 2000). The implementation of Montreal protocol has improved the ozone levels by controlling the production of diverse ozone depleting substances (Williamson et al. 2014). However, to reach pre-industrial levels of UVB it is still a challenge, as UV incidence is also affected by the Earth´s complex climate systems and by non-stratospheric factors (Williamson et al. 2014, Chipperfield et al. 2017). For those reasons, it is important to pay special attention to the consequences of both UVA and UVB exposure in human health, especially its effect on skin cancer (Bais et al. 2015).

According to the literature, XPC deficient cells are more sensitive to UVB and UVC irradiation than proficient cells (Feraudy et al. 2010, Dupuy et al. 2013, Andrade-

Lima 2015). Our data confirm this sensitivity to UVB light and reveal, for the first time, an elevated sensitivity also to UVA light, since XP-C cells showed a dose-dependent decrease on cell survival experiments, where higher doses were cytotoxic. In both cases, the XPC deficient cells were more sensitive than its isogenic control, COMP cells, obtained by complementation of the XP-C cells, in this work, with a lentiviral vector carrying the *XPC* gene.

Based on cell survival experiments, irradiation doses for mutagenesis were determined, 60 kJ/m^2 for UVA and 120 J/m^2 for UVB. These doses can be considered environmentally relevant, as they correspond approximately to twenty minutes and one minute and a half, respectively, of the sunlight exposure at midday during summer in a tropical latitude (Schuch et al. 2012). At these doses, UVA and UVB irradiation induces genotoxic stress in XP-C cells, as indicated by the increased levels of γH2AX after irradiation, while in COMP cells the increase recorded was statistically irrelevant. This relationship between phosphorylation of H2AX as a consequence of UVB and UVC irradiation has been previously reported (Limoli et al. 2002, Revet et al. 2011, Quinet et al. 2014, Andrade-Lima et al. 2015). High levels of sub-G1 content in UVA and UVB irradiated XP-C cells were also detected, which indicates an increase in apoptosis levels.

Our data showed that UVB-irradiation but not UVA-irradiation causes an S / G2 arrest in XP-C cells. Previously an accumulation in S-phase 24 h after UVB-irradiation had already been reported. And similar to our results, cells that do not die by apoptosis recover and continue cycling (Andrade-Lima et al. 2015). Additionally, it was reported an arrest at late S and/or G2 phases, depending on the UVC dose used (Quinet et al. 2014). These results suggest that although UVA generates sufficient damage to activate DDR it is not enough to affect cell cycle progression, at least not at the doses employed. An accumulation of single-stranded DNA (ssDNA) after UVC irradiation indicates that this response may be due to the persistence of 6-4PP lesions (Quinet et al. 2014). Also XP-V cells irradiated with double the dose of UVA used in this work suffer replication fork stalling and cell cycle arrest in the S-phase (Moreno et al. 2019). Then, the arrest in the cell cycle could be related to the amount of pyrimidine dimers caused by each type of UV radiation that were not repaired.

The deficiency in the XPC protein is responsible for the sun-sensitivity phenotype of the XP-C cells as demonstrated in the survival and cell cycle experiments. XP-C cells were more sensitive to UVA and UVB irradiation when

compared to control cells. This higher sensitivity of XP-C cells is certainly due to its incapacity to recognize and remove the UV- induced DNA damage in the global genome (not affecting TCR repair responsible for the repair in actively transcribed regions). Thus, the XP-C + UV light model allows to study directly the relationship between DNA damage, mutation and cancer, which could help to clarify the mechanisms involved in this process.

UVA and UVB light increased the mutation frequency (SNVs per million base pair sequenced) on XP-C cells from 1.4 to 5.2 and 8.1, respectively. However, there were no significant changes in the irradiated controls. Interestingly, the absence of the XPC protein also leads to an significant increase in the basal mutation frequency of the deficient cells compared to the complemented ones, from 0.9 to 1.4. This seems to be explained by a significant increase in the C>T transition, that maybe related to the spontaneous deamination of cytosine, one of the most common mutation in human cells. The presence of COSMIC´s signature 1 in all of our experimental groups supports this idea.

The data clearly demonstrate that for both types of UV light evaluated, the mainly induced mutations were the C>T transitions. Also, it was observed a significant increase in the CC>TT tandem mutations, considered as the UV light hallmark. By using the mutation spectrum and motif analysis, it was demonstrated that more than 95% of the C>T mutations induced by UVA or UVB occur preferentially in potential sites for the pyrimidine dimer formation, within the TCN and CCN sequence context and with a predilection for the non-transcribed strand, which is consistent with these cells being able to remove pyrimidine dimers by TC-NER. Interestingly, for cells with functional NER, this mutation was mainly found at CC dimers, without preference for mutation at the first or the second cytosine. In XP-C cells, without functional GG-NER, the pyrimidine 3' of the dimer was the most mutated and TC was the most mutagenic dimer. Then, the analysis of the sequence context where the C>T transition occurs exhibits an enrichment bias for pyrimidine-rich sequence context, revealing almost exactly the same logo for both the UVA and the UVB light, C(T/C)(T/C)C(Y)NC. The difference is in the +1 position, so for UVA is C followed by T, while for UVB is T followed by C, however in both cases the preference is for a pyrimidine base. Finally, our data point out that a unique dose of UVA- or UVB-irradiation recapitulate experimentally the typical mutation pattern of skin cancer, signature 7 of the COSMIC catalog, established by the analysis of more than 12.000 genomic data of 40 different

cancer types (Alexandrov et al. 2013b, 2020).

The C>A (G>T) transversion was the second most increased type of mutation in XP-C cells after both UVA- and UVB-irradiation. This base substitution is known as the mutagenic hallmark of the 8oxoG, generated by oxidation of guanines (Epe 1991, Cheng et al. 1992). However, after exploring these mutations within the RGR motif it was not possible to relate them with oxidized guanines. It is important to remark that the RGR motif was established in *E. coli* and maybe it is not the best one to explore mutations induced by oxidized guanine in eukaryotes. Thus, the induced mutations in G base should be analyzed more carefully, in fact according to the logo analyses it could be interesting to explore a motif that consider a purine either on the 3' or 5' of the oxidized guanine. Additionally, mutations in the other four types of base substitutions were also significantly increased by UVA- and UVB- irradiation, and could be consequences of base oxidation processes. Interestingly, this work indicates that the induction of C>G transversions is specific for UVA, while T>G transversions seems to be specific for UVB. Probably, this result is related to the oxidative damage induced by each type of light.

The results together, provide evidence that pyrimidine dimers are the main type of lesion contributing both to UVA and UVB induced mutagenesis in NER deficient cells and support the idea that both types of UV light generates the same mutational signature: the C>T transition at C-containing pyrimidine dimers, considered the UV signature since it is commonly found in skin cancer, but not in other types of cancers. Other types of mutations (including the more common C>A transversions) were also detected, probably due to lesions induced by oxidative stress. The data evidenced that not only UVB light but also UVA are highly mutagenic and in XPC patients this is exacerbated. Thus, this work discloses the UVA light participation in the high sunlight sensitivity and the elevated rate of skin carcinogenesis, in the complementation C group patients. The information generated in this work may be used for comparison with the mutational profiles of skin tumors obtained from XP patients, but also from the general population, since it has been suggested that mutations or loss of the XPC gene may be an early event during skin carcinogenesis (Feraudy et al. 2010). Thus, it is expected that these findings may help to understand the mutational processes of skin tumors in general. Finally, our results highlight the importance of photoprotection against solar UV radiation and other artificial sources of UVA radiation, since UVA is clearly not innocuous.

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