Nucleotide excision repair: from molecular mechanisms to patient phenotypes

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Appendix

Summary

The human body consists of around 37 trillion cells. Each of these cells contains six billion base pairs of DNA. Our DNA is continuously exposed to endogenous and exogenous agents capable of inflicting damage to the DNA. The sources of DNA damage include solar UV light, environmental chemicals, food-borne mutagens, and reactive metabolites that generate a wide variety of structurally diverse genomic DNA lesions. If not repaired properly, DNA damage starts to accumulate which eventually leads to disease development. Therefore, maintaining genomic integrity is of great importance.

Solar UV irradiation induces up to 100,000 DNA damages per cell per day. The two major types of DNA damage triggered by UV light are cyclobutane pyrimidine dimers (CPDs) and 6-4 pyrimidine-pyrimidone photoproducts (6-4PPs). These bulky lesions are repaired by nucleotide excision repair (NER). The NER pathway is initiated by two different damage-recognition mechanisms. While transcription-coupled repair (TCR) preferentially removes DNA lesions from actively transcribed DNA strands, global genome repair (GGR) removes lesions from the rest of the genome. The key damage-recognition factors in this process are known and NER has been reconstituted *in vitro* on naked DNA. However, it is becoming more clear that regulatory factors play an important role as well, to facilitate the repair process in the chromatin context of the living cell. In this thesis, we describe regulatory factors and their role in NER, in order to get a better understanding of the events that take place during DNA damage recognition.

In chapter 1 we describe how NER leaves a mark on chromatin to enable efficient DNA damage-detection in nucleosomes. Generally, DNA lesions in the genome are recognized by the GGR-specific damage-recognition factors XPC and DDB2. However, genomic DNA is tightly wrapped around histones, which creates a barrier for DNA repair proteins to access DNA lesions buried in nucleosomal DNA. The emerging view is that a tight interplay between XPC and DDB2 is regulated by post-translational modifications (PTMs) on the damage factors themselves as well as on chromatin containing DNA lesions. The choreography between XPC and DDB2, their interconnection with PTMs, such as ubiquitylation, SUMOylation, methylation, poly(ADP-ribos)ylation and acetylation, and the functional links with chromatin remodelling activities regulate not only the initial recognition of DNA lesions in nucleosomes, but also the downstream recruitment and necessary displacement of GG-NER factors

as repair progresses.

In chapter 2 we provide evidence for a molecular bookmarking system that primes chromatin containing CPDs for efficient repair. The lesion-recognition factor XPC initiates repair of helix-destabilizing DNA lesions, but binds poorly to lesions such as CPDs that do not destabilize DNA. We identify the poly-(ADP-ribose) polymerases PARP1 and PARP2 as constitutive interactors of XPC. The biochemical interaction between these proteins results in the PARylation of XPC at UV lesions and an XPC-dependent stimulation of the poly-(ADP-ribose) response. This enables the recruitment of the poly-(ADP-ribose)-regulated chromatin remodeler ALC1. Notably, we found that both PARP2 and ALC1 are required for the efficient clearing of CPD lesions.

In chapter 3 and chapter 4 we explore the role of high-mobility group (HMG) proteins HMGN, HMGB and HMGA at UV-induced DNA lesions. Repair of UV lesions in genomic DNA poses a challenge, as the DNA is tightly wrapped around histones, forming an obstacle for the binding of DNA repair factors in chromatin. Proteins that modulate DNA accessibility could stimulate the recruitment of repair proteins to genomic DNA lesions. The HMG family proteins are known to modulate DNA accessibility and are therefore interesting candidates to study.

In chapter 3 we studied the role of HMGN in human TCR. TCR is a sub-pathway of NER that removes DNA lesions from the transcribed strand of active genes. Previously, studies in mice have revealed that HMGN1 is required to enhance the repair of UV-induced lesions in transcribed genes. Whether HMGN also has a role in human TCR remains unclear. We started by studying the role of HMGN in human TCR, by performing functional repair assays in human cells. Using different approaches we showed that neither HMGN1, nor the related HMGN2 is required for human TCR. The functional difference between mice and humans might be partially explained by species-specific genetic differences.

In chapter 4 we studied the landscape of proteins that either associates with or dissociates from UV-irradiated chromatin. By employing chromatin mass spectrometry (CHROMASS) in *Xenopus laevis* (African clawed frog) egg extract, we observed that HMGN and HMGA proteins dissociate while HMGB showed a strong association with UV-irradiated chromatin. To further study the role of HMGB at UV lesions, we established an efficient method to individually deplete all HMGB-type isoforms in order to characterize the functional relevance of their recruitment during the response to UV-induced DNA lesions. Future studies could reveal the functional relevance of these UV-induced chromatin interactions.

In chapter 5 we describe two patients with novel ERCC1 mutations that impede DNA damage repair and cause liver and kidney dysfunction.

Together with XPF, ERCC1 forms a multifunctional endonuclease that is involved in NER, inter-strand crosslink (ICL) repair and double-strand break (DSB) repair. Genomic sequencing identified a deletion and a missense variant (R156W) within ERCC1 that disrupts a salt bridge below the XPA-binding pocket. Patient-derived fibroblasts carrying the R156W point mutation show dramatically reduced protein levels of both ERCC1 and XPF. Moreover, ERCC1 R156W has a weaker interaction with essential NER and ICL repair proteins compared to wildtype ERCC1. Altogether, the R156W mutation in ERCC1 has a severe effect on NER and considerably impacts ICL repair, together resulting in a unique phenotype combining short stature, photosensitivity and progressive liver and kidney dysfunction.

In chapter 6 the scientific findings of this thesis are discussed in a broader context, providing a basis for further discussions and future perspectives. This thesis improves our understanding of the regulatory factors in NER. In particular, it highlights how different regulatory factors work together in this process. Moreover, we show that a novel ERCC1 mutation can cause liver and kidney defects. With our research publications we aim to increase the awareness of this unique phenotype. This becomes all the more relevant since a third patient with a similar ERCC1 mutation was meanwhile indentified.