Structural studies of Nucleotide Excision Repair for drug development targeting protein-DNA interactions

DNA is the molecule that encodes hereditary information required for survival and development of most organisms. The integrity of DNA is continually challenged by thousands of insults each day that may occur spontaneously or caused by environmental agents. Given the potentially devastating effects of genomic instability, cells have evolved an intricate series of mechanisms that maintain genomic integrity. One of the most versatile DNA repair mechanisms is nucleotide excision repair (NER). This repair system can remove a remarkably wide variety of lesions such as UV induced photoproducts and chemical adducts.

While the efficiency of NER is essential for maintaining genomic stability, it presents a problem for cancer treatment. Chemotherapy has been the mainstay of cancer therapy for decades and includes drugs, such as cisplatin, that induce covalent crosslinks between DNA bases and promote cell death. However, the efficacy of chemotherapeutic drugs is highly influenced by cellular NER capacity that removes lesions and enables tumor cells to survive. Determining the molecular mechanism of NER and the means for its regulation has both clinical implication and scientific importance.

Here, we set out to resolve the human defense against UV radiation and carcinogens at the molecular level. We studied the rate-limiting step of NER, the DNA incision made by XPF/ERCC1 and the role of XPA and RPA proteins in this process.

We have been successful in reconstituting the quaternary macromolecular complex, consisting of three proteins and DNA. This required expression and purification of each protein component separately, a laborious task because the subunits of XPF/ERCC1 heterodimer and XPA heterotrimer were to be co-expressed and purified to homogeneity. Thanks to the unique expertise of collaborators, DNA molecules were synthesized to imitate the DNA intermediates formed at the pre-incision step of NER. Several biophysical assays were used to demonstrate that XPF/ERCC1, the catalytic component of the system, was active against these DNA molecules. This, in turn, allowed to screen 60.000 compounds and obtained hits against XPF/ERCC1 catalytic function. For some of the hits, we were able to correlate their effect with the DNA binding sites we identified on XPF/ERCC1. This is the first systematic effort to identify specific XPF/ERCC1 inhibitors, that have yet to be validated with additional assays, in order to exploit DNA-repair deficiency in cancer treatment. The advancements were made possible by a wealth of techniques available at CEITEC MU (NMR, SAXS, ITC, AUC, FA, CE).

Now the researchers have everything in their hands to describe the NER machine at the molecular level. The mechanistic insights will assist to make connections among recessive mutations, functions and disease, and are expected to pave the way for developing specific NER inhibitors.

Platinum-based agents have been the most commonly prescribed chemotherapeutics in the treatment of a wide variety of solid tumors including lung, head, neck, and ovarian, cancers for many years. While the therapy is initially effective, resistance to cis-platin is often acquired that limits the effectiveness of the treatment. Resistance to chemotherapy is believed to cause treatment failure in over 90% of patients with metastatic cancer. XPF/ERCC1 specific inhibitors could be used in combination settings, because repair of platinum adducts relies on XPF/ERCC1 cellular activity.

The novel inhibitors identified here, could support an application to pursue lead compound development and assess compound activity in cancer lines and animal models. We expect XPF/ERCC1 inhibition to be effective in sensitizing cells to cis-platin in ERCC1 positive tumours (high levels of ERCC1). For ERCC1 negative tumours (low levels of ERCC1) it has a great potential to impact overall survival.

In the long term, the preliminary findings of the present work have the potential to impact those individuals diagnosed with cancer that will receive chemotherapy and improve their survival rate.

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