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Nucleotide Excision Repair Inhibitors: Still a Long Way to Go

K. Barakat and J. Tuszynski

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1. Introduction

The last few decades have witnessed a new astounding trend emerge in cancer research. The new strategy materialized as a glimmer of hope to improve current standard cancer treatments that target DNA. These DNA-damaging agents induce lesions into the genome, which are aimed at preventing cancer cells from proliferating and invading the surrounding tissue. However, as was shown by many experiments, in response to that cancer cells mobilize DNA repair pathways that tend to remove the induced damage. As a consequence, they exhibit increased resistance towards what would otherwise be an efficacious treatment [1]. These findings have validated DNA repair enzymes as new molecular targets in the context of the battle against cancer [2]. Fortunately, the proof of the concept of targeting DNA repair as a cancer-therapeutic-strategy has been provided by several convincing studies, many of which are advancing through pre-clinical and clinical trials [3]. A particular example of a novel target in such pathways is the nucleotide excision repair (NER) mechanism, which correlates with the induced resistance to platinum treatments [4].

In normal cells, NER removes a broad range of DNA lesions, protecting cell integrity [5]. In cancer cells exposed to DNA damaging agents that distort the DNA helix or form bulky injuries to the genome, NER comes into play and removes the damage, in order to prevent cancer cells from lethal consequences of this damage [5, 6]. A striking example of this mechanism is represented by the use of platinum compounds such as cisplatin, the principal component of many treatments involving solid tumors including testicular, bladder, ovarian, head and neck, cervical, lung and colorectal cancer [7]. It has been demonstrated that NER is the major DNA repair mechanism that removes cisplatin-induced DNA damage, and that resistance to platinum-based therapy correlates with high expression of ERCC1, a major enzymatic element of the NER machinery. In this context, a reasonable way to increase the efficacy of platinum-based

therapy and decrease drug resistance would be to regulate NER by inhibiting the activity of ERCC1 and interacting proteins using yet to be discovered therapeutic compounds [8-11].

The protein ERCC1 forms a heterodimer with XPF. The resulting complex is an endonuclease enzyme that cleaves the 5` end of the damaged DNA strand whereas XPG cleaves it in the 3' position [6]. ERCC1-XPF is recruited to the damage site through a direct interaction between ERCC1 and XPA, an indispensible element of the NER pathway. No cellular function beyond NER has been observed for XPA and competitive inhibition of the XPA interaction with peptide fragments is considered effective at disrupting NER. Furthermore, based on clinical data, cancer patients that have been shown to have low expression levels of either XPA or ERCC1 demonstrate a correlation with a higher sensitivity to cisplatin treatments [12, 13].

This chapter reviews the state-of-the-art efforts that have been made to date to identify inhibitors of the NER pathway. These efforts have been mainly focused on targeting either the ERCC1-XPA or the ERCC1-XPF interactions. We discuss the various methods that were used toward this aim and illustrate the mode of action of the identified inhibitors. We hope that the compiled knowledge in this chapter will help researchers and clinicians in their efforts to develop new drug candidates that can improve the efficacy of and reduce resistance against platinum treatments and other DNA damaging agents as a way to arrest tumor progression.

2. Nucleotide excision repair pathway

The nucleotide excision repair process, shown in Figure 1, occurs as a stepwise mechanism and involves more than 30 different proteins. It is a "cut-and-paste" mechanism that replaces a ~30 nucleotide DNA strand that contains the lesion with a correct base pair sequence. This pathway has been extensively studied so that all the genes that are involved in it have been cloned and expressed as recombinant proteins. The main players within NER include the seven Xeroderma Pigmentosum (XP) complementation groups, XPA to XPG proteins; the Excision Repair Cross Complementing group 1 protein (ERCC1); the human Homolog of yeast RAD23 (hHR23B), the Replication Protein A (RPA), the subunits of Transcription Factor that possess Helicase activity (TFIIH), and the Cockayne Syndrome proteins A and B (CSA and CSB) [14]. Depending on the location of the DNA damage within the genome, one can recognize two NER sub-pathways. First is the transcription-coupled repair (TCR-NER), if the DNA damage is located within the actively transcribed genes of the genome. The second is the global genome repair (GGR-NER), if the damage is located within the whole genome. The two types are thought to be identical except for the initial damage recognition step. The two mechanisms involve five sequential steps [15] described below.

The foremost step is the detection of the damage. As mentioned above, the recognition step is the only difference between TCR and GGR. In the GGR subpathway, the XPC-hHR23B-XPE complex continuously scans the genome for bulky DNA damage until it recognizes a lesion and, consequently, initiates the rest of the NER sequence. On the other hand, a stalled RNAPII and Cockayne syndrome proteins, CSA and CSB, recognize the damage and activate the TCR-NER pathway. Once the damage is recognized the second step starts by recruiting the TFIIH

complex in order to unwind the DNA helix surrounding the lesion. TFIIH is composed of two major sub-complexes. The core is formed from the association of a large number of proteins including XPB, XPD, p62, p52, p44, p34 and p8. The rest of TFIIH is the cdk-activating kinase sub-complex, which contains cdk7, cyclin H and MAT1. Interestingly, TFIIH possesses both 3′-5′ and 5′-3′ helicase activities through the two ATP-dependent helicases XPB and XPD, respectively [16]. It opens the DNA structure forming a ~30 base pair bubble around the lesion. The two proteins RPA and XPA stabilize the opened DNA structure and recruit the two endonucleases that are necessary for the subsequent incision step. The interaction of XPA with the 34-kDa subunit of RPA (RPA34) activates XPA to recruit the other components of NER.

The Damaged strand-incision is the rate-limiting step for the whole pathway. The two endonucleases XBG and XPF-ERCC1 cut the two ends of the strand that contains the damage. The correct location of XPA is crucial for the recruitment of the XPF-ERCC1 heterodimer endonuclease. XPG cuts the 3′ end of the damage, while XPF-ERCC1 cuts the 5′ end [17]. The damaged strand is then released. DNA polymerases fill the single strand gap using the complementary intact strand as a template and DNA ligase I closes the 3′ nick as a final step [15].

3. ERCC1 over-expression correlates with cisplatin resistance

ERCC1 is a 33-kDa protein that forms a tight heterodimer endonuclease complex with XPF. As described above, this endonuclease cleaves the DNA strand at the phosphodiester bonds on the 5′ side of the damage. It is important to mention that the ERCC1-XPF complex has additional functions in other DNA repair pathways including inter-strand cross-link repair, double-strand break repair, and homologous recombination. Many studies have shown considerable correlation between resistance to cisplatin and the over-expression of ERCC1 [19]. This profoundly significant conclusion has been reached from several independent clinical trial investigations on ovarian [20], colorectal [21], and non–small cell lung cancer [22]. For example, a study on ~750 patients who suffer from late stages of lung cancer revealed that patients with low levels of ERCC1 and who received platinum therapy had better survival rates than those with the same levels of the protein but did not receive the platinum treatment [23]. A more recent study on 444 patients who experienced non-small lung cancer concluded that non-platinum-containing chemotherapy is more effective than platinum-based therapy on patients with high ERCC1 levels [24]. Very recently, Stefanie and coworkers [25] performed a retrospective study investigating the correlation of ERCC1 expression with patients' survival in ovarian cancer after platinum-based treatment. Their work revealed that patients with ERCC1-negative ovarian cancer had significantly better survival rates than those with ERCC1-positive ovarian cancer. They concluded that ERCC1 protein over-expression is a marker for poor survival of high-grade ovarian cancer even in patients operated on who had residual disease. All of these investigations lead to the conclusion that ERCC1 is not only a gene that is usually activated in patients subjected to platinum-based therapy but it may also act as a predictive criterion for identifying those patients who could benefit from platinum treatments [26, 27]. This latter role of ERCC1 as a biomarker is important because it can guide clinicians

in their therapeutic decision-making and select the best treatment approach for a particular group of patients.
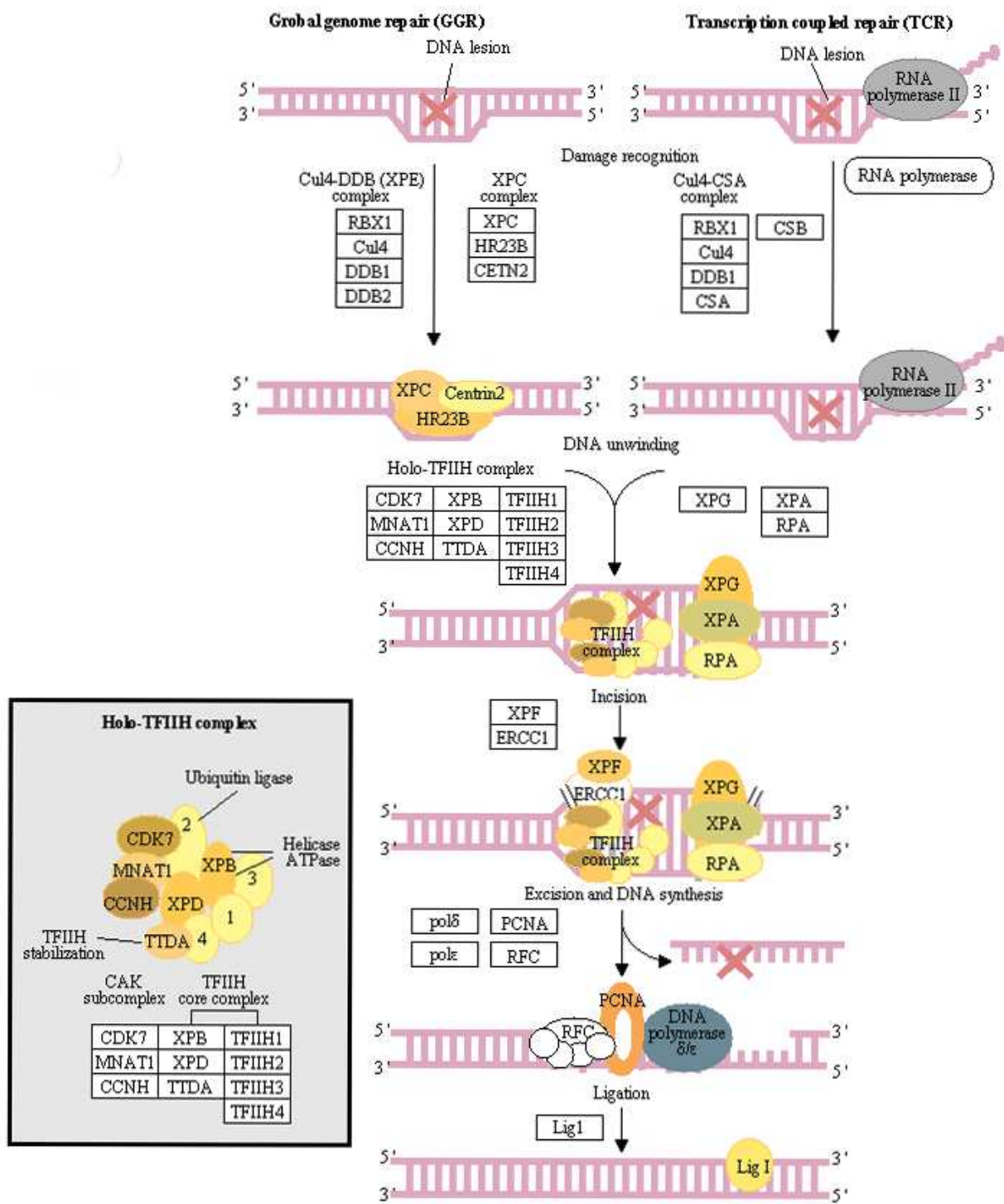
Figure 1. Steps of the nucleotide excision repair pathway. See text for details (adopted from the KEGG database [18]).

## 4. The ERCC1-XPA interaction is essential for a functional NER pathway

Regardless of the type of NER that is initiated, the XPA protein is equally essential to complete both pathways [28]. It plays a vital role in DNA lesion recognition and in the attraction of many other NER repair proteins. For example, prior to the incision step in NER, the ERCC1-XPF endonuclease is recruited to the damaged DNA site through a secondary interaction between ERCC1 and XPA [29, 30, 31]. Therefore, this protein-protein interaction is necessary for a functional NER mechanism. The NMR crystal structure was resolved by Tsodikov's group [13] and the critical residue-residue interactions were determined [4] through our binding energy predictions (see Figure 2). A 14-residue peptide from XPA that includes three essential consecutive glycines (residues 72–74) is buried within a hydrophobic cleft within the central domain of ERCC1. This peptide has two critical characteristics. First, it is necessary and sufficient for binding to ERCC1. Second, and more importantly, it can compete with the full-length XPA protein in binding to ERCC1 and disrupting NER *in vitro*.

In a recent study, Barbara et al. [32] reported mutations in the central domain of ERCC1 that had a significant impact on NER activity *in vitro* and *in vivo*. These mutations occur at the XPA binding site within ERCC1, preventing the interaction between the two proteins. Due to these mutations, the ERCC1-XPF nuclease was not recruited to the damaged DNA sites after exposing cells to ultra violet (UV) radiation. Consequently, the last incision step that is performed by ERCC1-XPF was never completed leading to a dysfunctional NER mechanism in these cells and, hence, a hypersensitivity to UV radiation. These results are consistent with previous findings on the importance of XPA in NER, where no cellular function beyond NER has been observed for XPA [12]. Interestingly, these mutations did not affect the activity of ERCC1-XPF in other DNA repair pathways leading to two distinctive conclusions. First, the XPA-ERCC1 interaction is only necessary for NER but not for other DNA repair pathways in which ERCC1-XPF is important for their activity. Second, the involvement and recruitment of ERCC1-XPF to the different DNA repair pathways is coordinated through different and not overlapping protein-protein interactions mediated by ERCC1. Based on these findings, one can selectively disrupt the activity of ERCC1-XPF within these DNA repair pathways by inhibiting its interactions with the recruitment factors to the damaged sites. These observations, coupled with the available crystal structure of this interaction make ERCC1 and XPA an extremely attractive target for computationally assisted development of small molecule inhibitors targeted for use in combination therapies involving cisplatin.
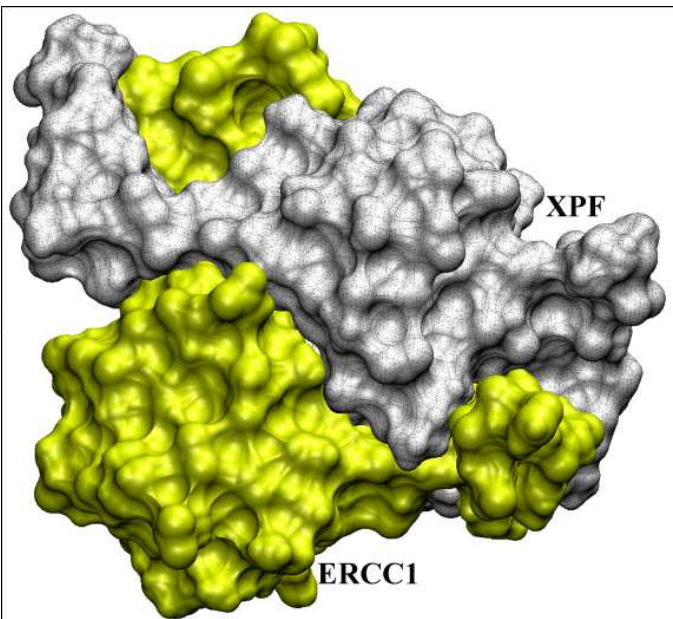
5. ERCC1 interacts massively with XPF

As shown in Figure 3, ERCC1 in engaged in a tight interaction with XPF in which almost every residue from XPF is either interacting or being affected by an interaction with ERCC1 residues. The main interaction sites are located within the C-terminal domains of the two proteins. The most tightly interacting regions in XPF include residues 828 to 835, 859 to 862, 878 to 882 and 892 to 905. These exhibit almost no flexibility in the bound structure, demonstrating a contri-

bution to binding with ERCC1. The two proteins form the heterodimer enzyme that is responsible for the cleavage of one side of the damaged nucleotides chain.
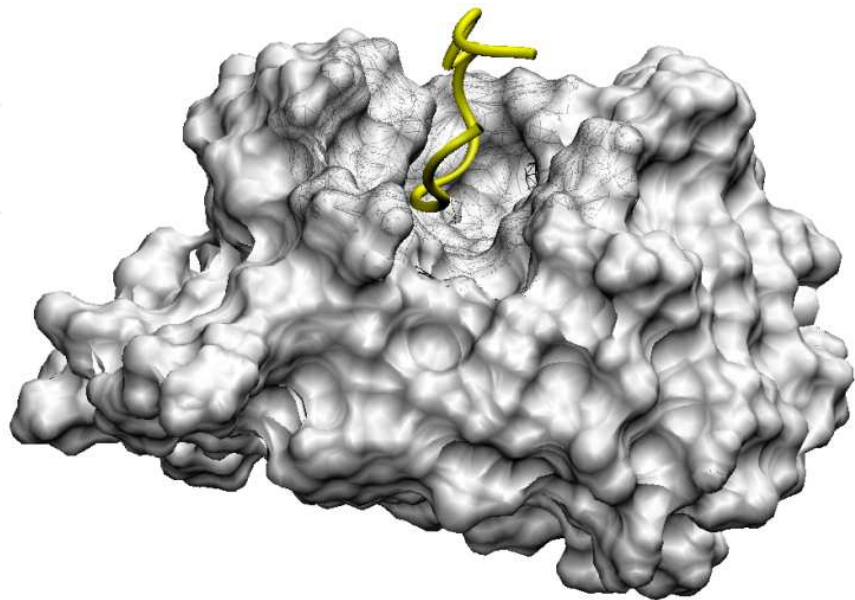
Figure 2. XPA-ERCC1 protein-protein interaction. The binding between ERCC1 (whight) and XPA (yellow) is predicted [4] to be primarily mediated by 5 residues from XPA peptide, namely; G72, G73, G74, F75 and I76. On the other hand, the contribution from the ERCC1 binding site is distributed among the following 10 residues: R106, Q107, G109, N110, P111, F140, L141, S142, Y145 and Y152.

Figure 3. The ERCC1-XPF complex. The C-terminal domain of each protein interacts massively with its counterpart from the other protein forming the heterodimer endonuclease enzyme.
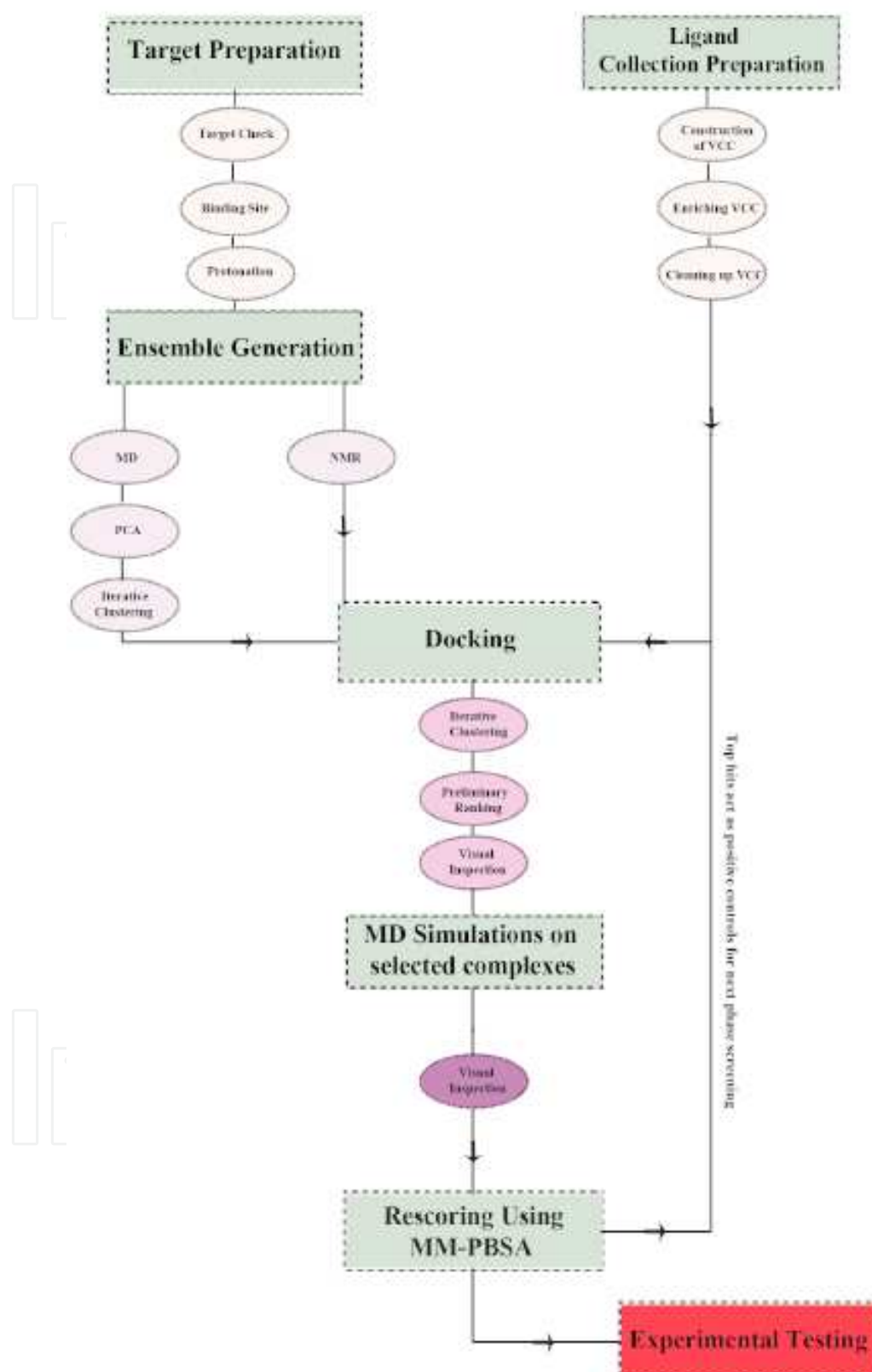
Figure 4. The implemented virtual screening protocol.

6. Earlier efforts to identify NER inhibitors

Although the NER pathway has been recognized as one of the most important factors that increase the resistance against platinum-based therapy, little work has been done so far on regulating its activity. Here, we wish to point to three major studies that identified inhibitors for the NER mechanism. First is the work done by Barret et al. [33] and their discovery of F11782. Second are the findings of Jiang and Yang [34] on the effects of the cell cycle checkpoint abrogator UCN-01 (7-hydroxystaurosporine) on NER. Finally, the work on the DNA damaging agent Et743 is a landmark result [35]. We briefly describe these outcomes below.

6.1. F11782

Using the 3D (DNA Damaged Detection) assay first proposed by Wood *et al.* [36] and later modified by Salles's team [37], Barret et al. [33] screened for NER inhibitors and identified F11782. The compound was already known as an inhibitor of both the topoisomerases II and I [38]. Moreover, F11782 did not show any activity toward other enzymes such as DNase I or T4 polynucleotide kinase, indicating that the compound targets one of the proteins that are involved in NER. Further investigations on F11782 limited its NER inhibitory activity to one of the earlier steps of the pathway, specifically either the helicase or the incision steps, with more preference given to the incision step [33].

6.2. UCN-01

Jiang and Yang [34] analyzed the effects of UCN-01, which is a well-known protein kinase C inhibitor and cell cycle checkpoint abrogator [39], on the NER pathway. These findings showed that UCN-01 inhibited the repair of cisplatin-induced DNA damage both *in vitro* and *in vivo* and indicated that UCN-01 has a dramatic inhibitory effect on the interaction of NER proteins. The drug enhanced the activity of cisplatin only in NER-proficient cells, but not in the deficient ones. However, no direct binding of UCN-01 to any of these proteins has been reported and it has been speculated that the observed inhibitory activity may result from UCN-01-mediated regulation of the signaling pathway that involves post-translational modifications of repair proteins. Although Jiang and Yang [34] attributed the loss of NER activity to an attenuation in the ERCC1-XPA protein-protein interaction, their careful and detailed binding analysis of the compound to the two proteins revealed that UCN-01 did not interact directly with either of them. However, in this work we used UCN-01 as a positive control, assuming it can bind to the XPA binding site within ERCC1, particularly because the drug can fit within the binding pocket despite its limited interactions with the protein.

6.3. Ecteinascidin 743

A final compound that has been shown [35] to interfere with NER is Ecteinascidin 743 (Et743]. At the time of writing this article, Et743 is in phase II/III clinical development and its main mode of action is as a DNA damaging agent. The drug seems to specifically obstruct the TCR-NER sub-pathway, however, it does not act as an inhibitor of any of the proteins that are

involved in the NER mechanism. A model proposed by Gregory et al. [35] suggests that the DNA adducts formed by Et743 are more efficient than those of cisplatin in dealing with NER. These authors suggest that the Et743-guanine adducts trap the TCR-NER pathway at the incision or ligation steps, preventing the pathway from being completed.

7. Recent attempts to discover novel NER inhibitors

As mentioned above, most of the earlier NER inhibitors listed above were not discovered to be potent or specific NER inhibitors. In other words, they were found mainly by chance to partially inhibit the NER pathway. Given the impact of regulating the NER pathway on improving many of the chemotherapeutic drug cocktails currently in clinical use, it is very important to directly target elements of NER pathway itself. Following this path, our group has been focusing on this problem in hope of implementing a novel strategy that would reverse resistance and potentiate the efficacy of cisplatin and other similar chemotherapeutic agents. The foremost endeavor is to specifically and separately target the two protein-protein interactions described above, namely the XPA-ERCC1 [4, 40] and XPF-ERCC1 [41] interactions. These efforts have already resulted in two successful examples where inhibitors identified by us via virtual screening were able to sensitize cells to ultra violet radiation (UV) and potentiate the efficacy of cisplatin in cancer cells. Here, we briefly describe the methods used and their outcomes. The studies described below primarily utilized computational tools to develop inhibitors that disturb these interactions. This was then followed by experimental validation of the predicted effects of these inhibitors on cancer cells.

7.1. The method

In the following studies, virtual screening identified small molecules that bind to and fit within the binding site within the interacting proteins in order to disturb its binding to the other protein in the complex. The virtual screening (VS) protocol that was used is shown in Figure 4. It is an improved version of the relaxed complex scheme (RCS) technique reported by McCammon and his team [42]. In the original RCS approach, all-atom MD simulations (e.g., 2-5 ns simulation) are applied to explore the conformational space of the target, while docking is subsequently used for the fast screening of drug libraries against an ensemble of receptor conformations. This ensemble is extracted at predetermined time intervals (e.g., every 10 ps) from the simulation, resulting in hundreds of thousands of protein conformations. Each conformation is then used as a target for an independent docking experiment.

The RCS methodology has been successfully applied to a number of cases. An excellent example is that of an HIV inhibitor, raltegravir which became the first FDA approved drug targeting HIV integrase [43, 44]. Other successful examples include the identification of novel inhibitors of the acetylcholine binding protein [45], RNA-editing ligase 1 [46], the influenza protein neuraminidase [47] and *Trypanosoma brucei* uridine diphosphate galactose 4'-epimerase [48]. These applications employed alternative ways to solve two main problems with the method, namely, reducing the number of extracted target conformations and deciding on how

to select the final set of hits after carrying out the screening process. For the first problem, a number of studies suggested extracting the structures at larger intervals of the MD simulation, e.g. every 5ns or so [45], condensing the structural ensemble generated from MD simulations using QR factorization [46], or clustering the MD trajectory using root-mean-square-deviation (RMSD) conformational clustering [47, 48]. On the other hand, to rank the screened compounds and suggest a final set of top hits, some studies used only docking predictions [45-47], while others suggested using a more accurate scoring method (e.g. MM/PBSA (Molecular Mechanics/ Poisson Boltzmann Surface Area)) to refine the final selected hits [42]. All of these approaches, similar to the work presented here, were aimed at keeping the balance between significantly reducing the number of target structures and retaining their capacity to describe the conformational space of the target. Figure 4 describes the approach that was used to put together and improve the RCS to target the strong protein-protein interactions described above.

Our implementation follows the same guidelines as in the RCS method. We first use MD simulations and generate large enough trajectories that can progress through the phase space of the binding site. The length of the MD simulations (usually on the order of 100 ns) is determined by applying metrics that employ principal component analysis (PCA). Once the trajectory reaches an adequate sampling of target conformations, clustering analysis extracts representative structures that describe the dominant dynamics of the binding site. The extracted structures are then used as rigid targets to screen the whole library of compounds and suggest models for the most preferred ligand-protein complexes, hence, utilizing the "conformational sampling" model. These bound structures are then solvated and used to run all-atom MD simulations to relax the two molecules and generate new trajectories that represent their "induced fit" models. The MM-PBSA method finally ranks the newly generated structures and suggests a set of top hits for experimental testing.

7.2. XPA-ERCC1 inhibitors

Our earliest challenge was to directly disturb the interaction between the ERCC1 and XPA proteins. Two subsequent screening experiments were used. The initial study screened two compound databases for inhibitors of the ERCC1-XPA interaction and constructed a pharmacophore model demonstrating the crucial features necessary for their inhibition. The databases used included the National Cancer Institute Diversity Set (NCIDS) and DrugBank compounds.

The NCIDS is a collection of approximately 2,000 compounds that are structurally representative as scaffolds of a wide range of molecules, representing almost 140,000 compounds that are available for testing at the NCI. A number of its ligands contain rare earth elements and cannot be properly parameterized for docking experiments, leaving us with 1,883 compounds that can be actually used. This work exploits a cleaned 3D version of the NCIDS formatted for use in AutoDock and it was prepared by the AutoDock Scripps team. What makes the NCIDS so valuable and extensively screened by many groups (even in HTS) is that its individual molecules have distinctive structures and are the cluster representatives of their parent families. Once screened and a number of its molecules rank high in the hit list, one can return back and screen the whole family of the representative structure, instead of screening the actual NCI set of compounds. On the other hand, the DrugBank database is not only a set of molecules

representing FDA-approved drugs, but it also represents a unique bioinformatics and chem-informatics resource. It relates each drug to its target(s). It includes details about the different pathways, structural information and chemical characteristics of these targets and the way they take part in inducing a particular disease. This information is stored in a freely available website that is linked to other databases (KEGG, PubChem, ChEBI, PDB, Swiss-Prot and Gen-Bank) and a range of structure displaying applets. The DrugBank collection includes ~4,800 drug structures including >1,350 FDA-approved small molecule drugs, 123 FDA-approved biotech (protein/peptide) drugs, 71 nutraceuticals and >3,243 experimental drugs. Once a hit is identified from this library, it simply represents a drug. This means many barriers of pre-clinical and clinical tests can be readily overcome and the molecule can be tested directly for its novel biological activity. Moreover, a hit from this collection may explain a mysterious side effect that would not be discovered before its identification as a regulator for the examined target.

This initial study utilized a minimized model of the XPA binding site within ERCC1 to employ flexible residue docking as implemented in AutoDock 4.0. This was then followed by RCS docking, where MD simulations and RMSD conformational clustering were used to generate a set of forty-four representative conformations of the binding site within ERCC1. AutoDock was then used to screen against a set of seven target conformations, composed of the six most dominant cluster-representative structures along with an equilibrated folded conformation for the binding site produced by employing principal component analysis on the ERCC1 trajectory. Top hits were rescored by docking them to the whole set of cluster-representative structures and ranked by their weighted average binding energy. The non-redundant hits from these screens were then used to identify a dynamic binding-site pharmacophore that target the ERCC1-XPA interaction. The pharmacophore model was then compared to docking results for the weak inhibitor of NER, UCN-01 (7-hydroxystaurosporine). A number of selected hits from this study are shown in Figure 5.
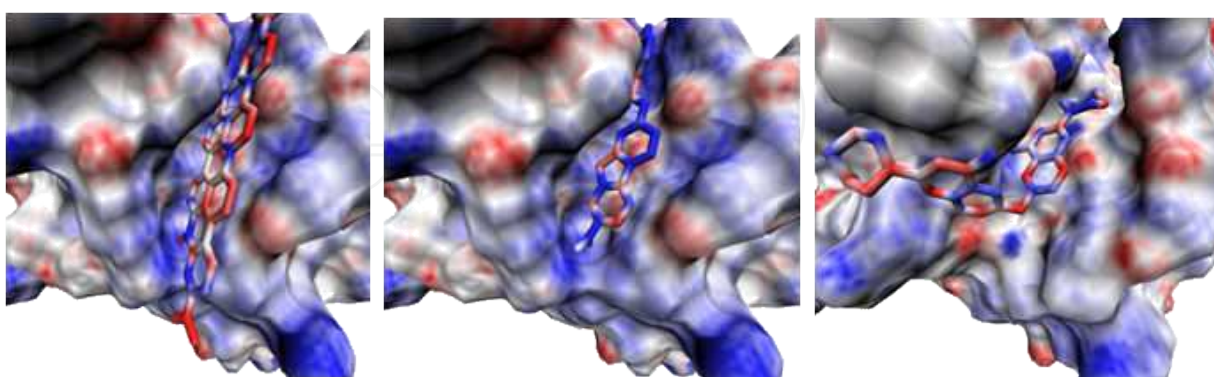

Figure 5. Three selected hits within their preferred binding site conformations. Adopted from [4].

Comparing the methodology that was used here to the workflow discussed in the above, one can make three observations. First, the virtual screening methodology depended mainly on

docking scoring to rank the compounds. Second, the clustering analysis that was used to extract dominant conformations of the target was not iterative, it used a cut off RMSD value that is commonly employed in the literature. Finally, no post-docking refinements were performed on the final set of compounds. These shortcomings were properly adjusted in the subsequent study [49].
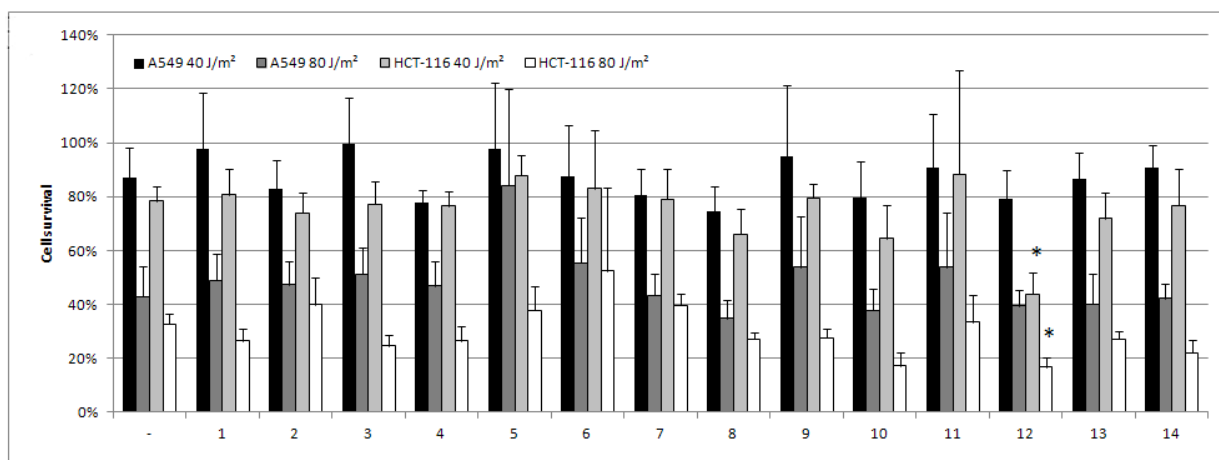
Figure 6. Sensitivity of cancer cells to UVC irradiation alone or in combination with potential inhibitors of the interaction between ERCC1 and XPA. IC50 values (J/m²). Compound 12 showed promising effects on cancer cells and was henceforth termed NERI01.

The new study used the CN chemical library for virtual screening. The CN chemical library (~50,000 compounds) is a repository of all synthetic, natural compounds and natural extracts in the existing French public laboratories. The whole database is divided into two main categories. The first part includes information about all synthetic products, while the second contains the natural compounds and extracts. In this work, we used the whole CN database in our screening. In contrast to the previously mentioned databases, compounds in this library are represented by 2D SDF structures with no hydrogen atoms attached. This required a number of cleaning and preparation steps before using them in VS simulations.

The second ERCC1-XPA study exactly followed the screening protocol described above. The hit rate of the new study was higher than that of the one described here, indicating the importance of utilizing more accurate scoring, performing iterative clustering and refining the docked structures using MD simulations. A promising hit, shown in Figure 6 as compound 12, was discovered and validated on a UV radiation sensitivity cell-based assay [40]. The validated hit was termed NER inhibitor 01 (NERI01) has been shown to be effective in sensitizing colon cancer cells to UV radiation, which induces the same type of damage as cisplatin and its lesions are removed by ENR.

7.3. XPF-ERCC1 inhibitors

The final study focused on the more challenging problem of interfering with the ERCC1-XPF interaction. As shown in Figure 4, the two proteins have a very close interaction with each

other. A comparison of atomic fluctuations (as revealed by the corresponding B-factor values) between the unbound-XPF and the bound-XPF structures is shown in Figure 7. Almost all XPF residues are rigid in the bound case compared to the free structure. This indicates a massive interaction between ERCC1 and XPF in which almost every residue from XPF is either inter‐ acting or being affected by an interaction with ERCC1 residues. The most flexible regions in XPF include residues 828 to 835, 859 to 862, 878 to 882 and 892 to 905. These have almost no flexibility in the bound structure, demonstrating a contribution to binding with ERCC1.

The enthalpic contribution, as calculated by the MM-PBSA analysis, to the binding energy between the two proteins is exceptionally large (-123 kcal/mol). While the solvation energy contributed passively to the interaction (298 kcal/mol), compensation from the electrostatic and van der Waals interactions dominated the overall interaction (-238 kcal/mol and -184 kcal/ mol, respectively). From these analyses we showed that ERCC1-residues shared ~50% of the total energy with PHE293 being the residue that contributes the most to the ERCC1-XPF in‐ teraction (-11 kcal/mol). On the XPF side, PHE894 has been found to contribute -7.7 kcal/mol to the binding energy. With the exception of ASP839 from XPF which disfavored the interaction by ~1 kcal/mol, the indicated residues favored the binding between ERCC1 and XPF. This allowed us to identify a binding site on the XPF surface that was used to identify putative inhibitors of this protein-protein interaction.
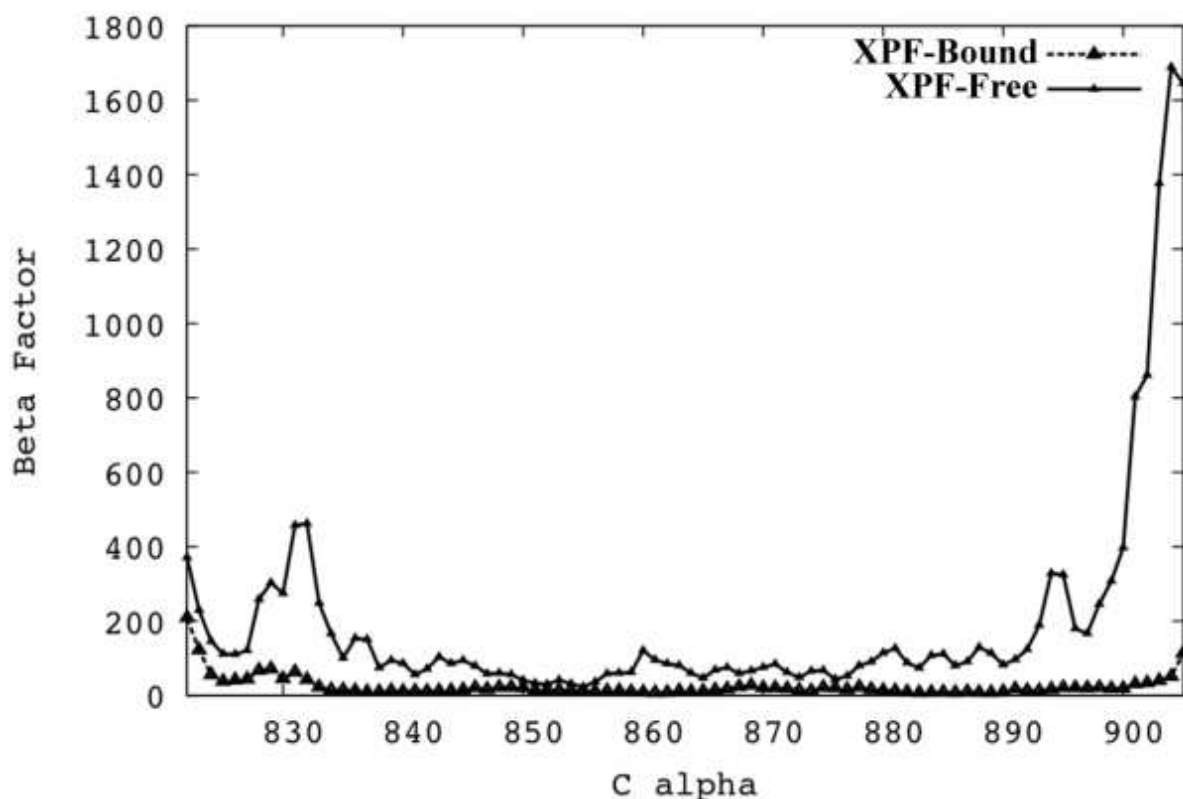
Figure 7. Flexibility of the XPF residues. Atomic fluctuations for the free and bound XPF proteins are shown here. Binding of ERCC1 to XPF considerably stabilized the protein, indicating a wide range of protein-protein interaction.

The screening methodology adopted the VS protocol shown in Figure 4 and used to screen the CN chemical library, NCI diversity set and DrugBank compounds for inhibitors of this inter-action. A number of promising hits were experimentally validated and were very effective in disrupting the NER pathway and potentiating cisplatin efficacy. The most promising com-pounds with binding modes are shown in Figure 8.
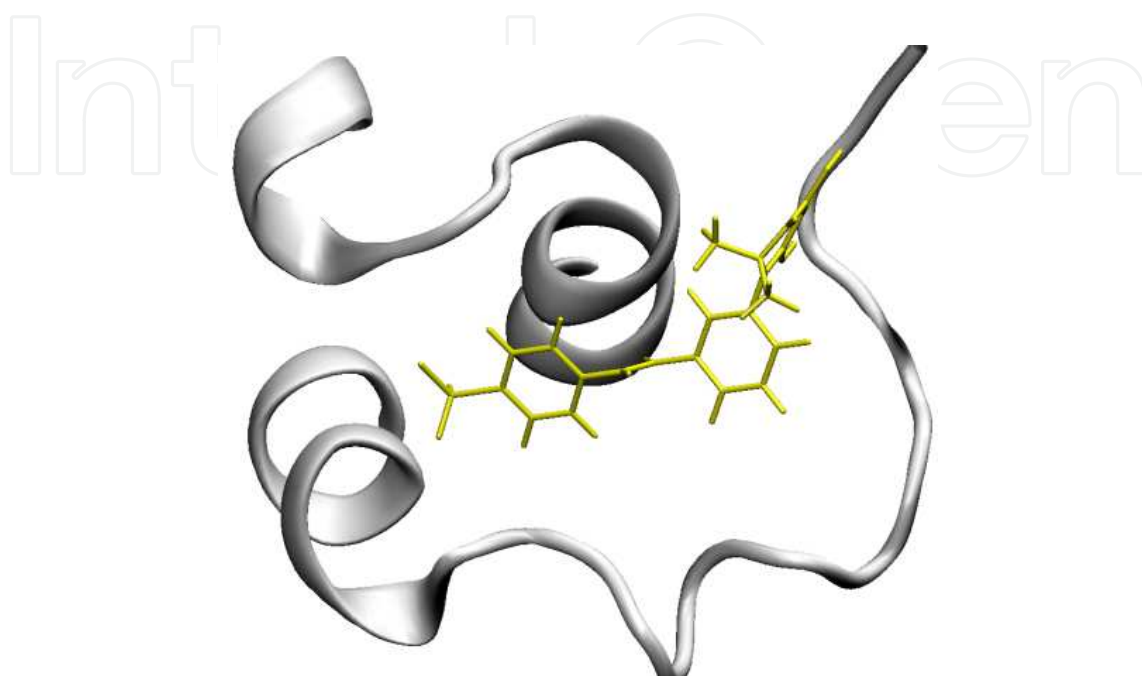
Figure 8. Binding mode of most promising XPF-ERCC1 inhibitor.

8. Conclusions

DNA damaging agents induce lesions into the genome aiming at preventing cancer cells from proliferating and invading the surrounding tissue. However, DNA repair pathways remove the induced damage and, hence, increase resistance to an otherwise efficacious treatment [1]. This approach has validated DNA repair enzymes as new molecular targets in the context of the battle against cancer. Nucleotide excision repair (NER) is a major DNA repair mechanism that removes mainly DNA lesions that distort the DNA helix or form bulky injuries to the genome. Among the most affected drugs with NER activity are platinum compounds such as cisplatin, the backbone for many treatments of solid tumors including testicular, bladder, ovarian, head and neck, cervical, lung and colorectal cancer. It has been demonstrated that NER is the major DNA repair mechanism that removes cisplatin-induced DNA damage, and that resistance to platinum-based therapy correlates with high expression of ERCC1, a major element of the NER machinery. Therefore, one way to improve such drugs and reduce their acquired resistance is by developing inhibitors that would regulate the NER machinery.

This chapter reviewed the state-of-the-art efforts that were made to identify inhibitors of the NER pathway. We discussed the various methods that were used toward this aim and illus-

trated the mode of action of the identified inhibitors. The earlier efforts were not focused on NER as a target. However, the first identified NER-inhibitors were discovered unintentionally. These efforts include the examples of finding the three drugs F11782 [33], UCN-01 (7-hydroxystaurosporine) [34] and Et743 as weak inhibitors of the NER activity. Recent studies exploited the fact that ERCC1 and its associated proteins XPA and XPF have a considerable correlation between resistance to cisplatin and their over-expression in cancer cells [19]. The latter studies were aimed at discovering specific inhibitors that target these interactions in the hope of disturbing their binding and hence reducing the NER activity. In this regard, we described the development of two different classes of NER inhibitors. The first class, represented by the lead compound NERI01 target the ERCC1-XPA interaction, while the second class represented by NERI02 targets the ERCC1-XPF interaction. Future directions of this research include the development of derivative structures for the identified hits and their optimization for improved drug-like properties and higher specificity to target their representative protein interactions. While great efforts have been done both *in silico* and *in vitro* to identify and validate novel inhibitors for the two mentioned NER targets, no *in vivo* studies have been performed on them yet. This is mainly due to the fact that the two proteins have been very recently recognized as druggable targets and no one in the past thought of regulating NER as a way to improve cancer therapy. However, we think that the studies presented here offer a proof-of-concept that inhibiting the interaction of ERCC1 with either XPA or XPF has a considerable impact on the NER mechanism and, therefore, enhances the efficacy of chemotherapeutic treatments that are associated with acquired resistance due to over expression of the NER elements. We hope that this chapter will be found of value to the researchers and clinicians interested in developing new drug candidates that can improve the efficacy of and reduce resistance against platinum treatments and other DNA damaging agents as a way to arrest tumor progression.

Acknowledgements

Author details

K. Barakat[1,2] and J. Tuszynski[3,4]

1 Department of Physics, University of Alberta, Canada

2 Department of Engineering Mathematics and Physics, Fayoum University, Fayoum, Egypt

3 Department of Physics, University of Alberta, Canada

4 Department of Oncology, University of Alberta, Canada

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