Review

Multiple Roles of the ERCC1-XPF Endonuclease in DNA Repair and Resistance to Anticancer Drugs

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Abstract. *In this review, we focus on the discrepant roles of the DNA repair complex ERCC1/XPF in the prevention of cancer and in the resistance of cancer to chemotherapy. ERCC1/XPF is essential for nucleotide excision repair (NER) incising DNA 5' to the lesion. NER deficiency results in the skin cancer-prone inherited disease xeroderma pigmentosum (XP). The ERCC1/XPF complex is also involved in recombination, double strand break (DSB) and interstrand crosslink (ICL) repair cutting DNA overhangs around a lesion. In telomere maintenance ERCC1/XPF degrades 3' G-rich overhangs. In some types of cancer, high levels of ERCC1/XPF mRNA and protein correlate with poor overall survival and resistance to platinum-based chemotherapeutic treatments. Therefore, the ERCC1/XPF complex makes an attractive target for prediction of outcome for treatment in cancer patients as well as a novel drug target.*

The ERCC1/XPF Heterodimer

In 1984, Westerveld and colleagues (1) reported the successful cloning of human *ERCC1*. Since then, extensive genetic characterization of the protein and its partner XPF has been achieved. *ERCC1* is believed to have evolved from gene duplication of *XPF* where subfunctionalisation lead to the move from a homodimer of XPF proteins in lower eukaryotes to a heterodimeric complex in higher organisms (2, 3). The structure of the human ERCC1/XPF complex reveals that only XPF contains the nuclease domain of the complex, but it does require ERCC1 for subsequent nuclease activity (4). The shorter ERCC1 protein reveals a central domain which resembles the nuclease domain of XPF structurally, but lacks an active site. Instead, the groove contains basic and aromatic residues for ssDNA binding (5).

The central domain of ERCC1 binds with maximal affinity to single-stranded overhangs 15 nucleotides or longer with a preference for 5' overhangs (6). The interaction of XPF with ERCC1 has been confirmed to lie in their C-terminal regions, where both carry double helix-hairpin-helix $(HhH)_2$ domains (4). However, only the $(HhH)_2$ domain of ERCC1 has been found to have DNA binding properties and is thus required to ensure correct positioning of the XPF endonuclease upon DNA damage and interaction with other NER proteins such as XPA (3, 4, 7). XPA binds through a conserved GGGF motif which undergoes a disorder-to-order transition when in contact with the central domain of ERCC1 (5). A recent paper established that XPA binding to ERCC1 is only important in NER, but if disrupted, does not affect ICL repair or DSB repair (5). As shown, specific functions of the heterodimer can be distinguished by site-directed mutagenesis *in vitro* and *in vivo* and helps reveal more about the nuclease function of the complex. In fibroblasts isolated from human XP-F patients, only low levels of ERCC1 have been detected with small amounts of the heterodimer being apparent overall (8). The same is true for XPF protein in mammalian cells where only small amounts of the protein have been detected in the absence of ERCC1 (2). These studies suggest that XPF and ERCC1 are unstable in the absence of each partner in mammalian cells (2). In contrast, in a variety of cancer cell lines XPF protein levels were reduced when ERCC1 was targeted by RNAi, with *XPF* mRNA being stable, but reduced XPF levels did not result in a reduction of ERCC1 protein (9). *In vivo* studies might help clarify the stability of ERCC1 and XPF protein in the absence of each other.

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Comparison of mouse and human *ERCC1* genes have shown a high similarity and the promoter region have revealed a homologous sequence 50-90 bp upstream of the transcriptional start (10). The *N*-terminal of the mouse and human ERCC1 protein seems to be less conserved and a functional protein arises even when the *N*-terminus is deleted. Moreover, the *C*-terminus of the human and mouse proteins resemble each other (10). The similarity between human and mouse ERCC1 offers the convenient opportunity to use mice with *Ercc1* deficiencies as a model for human defects.

NER: Global Genome and Transcription-Coupled Repair

The classical role of the ERCC1/XPF complex lies in its involvement in the nucleotide excision repair pathway where the complex incises DNA 5' to a lesion. Typical substrates for NER are UV-induced photoadducts such as cyclobutane pyrimidine dimers (CPDs) and (6-4) photoproducts (11), intrastrand crosslinks caused by endogenous and exogenous sources and bulky chemical adducts. NER is therefore an important pathway in the repair of intrastrand crosslinks and bulky adducts that are induced by chemotherapy. Dealing with many different kinds of DNA damage that occur in the form of bulky adducts, NER is one of the most flexible of all DNA repair mechanisms with similar principles in prokaryotes and eukaryotes (Figure 1A) (12). NER is carried out if DNA damage occurs in the cell with a preference given to transcribing genes. Transcription-coupled NER operates quickly to ensure that the transcription of genes can be carried out correctly (12). In contrast, global NER repairs lesions in the overall genome more slowly, preventing mutations from being passed on to the next generation and keeping the integrity of the genome stable (12). Initial damage recognition in global genome NER is carried out by the XPC/ HHR23B and XPE complex. The two subunits of XPE, DNA damage binding protein (DDB) 1 and 2, are central players in chromatin remodelling during NER with their strong affinity for UV- damaged DNA and their association with known factors involved in chromatin remodelling (13). In transcription coupled NER, RNA polymerase I or II stalled at a lesion constitutes the recognition step, with CSA and CSB ensuring removal and degradation of the polymerase by ubiquitination (11). So far, only a weak affinity of the DNA-binding proteins for their substrates in the pre-incision complex has been found, with XPB being thought to then lock those factors in place (11). XPB is part of the TFHII complex with the helicase subunit XPD being crucial for unwinding of the DNA around the lesion in both subpathways of NER (11). XPA and RPA appear to have a role in damage verification and are also necessary to load and position the ERCC1/XPF complex correctly onto the damaged DNA in order to start the incision process (14).

Gillet and Schärer (13) proposed a model in which XPG initially only helps to keep the unwound DNA stable. After ERCC1/XPF binding and 5' incision at the site of the lesion, a conformational change then results in the activation of the XPG catalytic activity and subsequent incision of the DNA 3' of the lesion. In addition, the creation of a free 3'-hydroxyl end after ERCC1/XPF incision is thought to immediately initiate repair (2, 14). Excision of the 24-32 nucleotide oligomer containing the lesion is followed by gap-filling carried out by RPA, RFC, PCNA and DNA polymerase δ and ε. DNA ligase I completes the final repair step in TC-NER with GG-NER using XRCC1 and DNA ligase III (Figure 1 Panel A) (12). The re-assembly of chromatin after GG-NER is thought to be mediated by the histone chaperone CAF-1 which seems to be recruited by PCNA after the repair is completed.

ERCC1/XPF in Recombination, Double-Strand Break and Interstrand Crosslink Repair

Several pathways evolved to deal with more toxic lesions such as interstrand cross links and DSB in humans. One of these pathways, homologous recombination (HR), has been extensively studied in *Saccharomyces cerevisiae* and is regarded as being an error-free process. A template, usually a sister chromatid, is needed to carry out the repair event (15). Therefore, HR is only functional in late S/G_2 phase. Human and murine fibroblasts, but not murine ES cells, are hypersensitive to IR as determined by survival assays, with γH2AX foci, a marker for DSB, persisting in ERCC1/XPF deficient cells, indicating a cell type specific role for the heterodimer to protect against DSB (16). *In vitro* evidence suggests that Ku86 and ERCC1/XPF act together to mediate DSB repair in murine fibroblasts (16). However, mammalian cells tend to make more extensive use of non homologous end-joining (NHEJ), the second pathway dealing with DSB (15, 17). This mechanism is not as accurate but has an advantage for eukaryotes as no extensive homology is required. This pathway is therefore able to operate in G_1. Variations of these pathways are single strand annealing (SSA) and microhomology-mediated end joining (MMEJ), where regions of microhomology are used to mediate recombinational events (16). SSA is usually used in mammals when HR or NHEJ pathways are impaired (16). MMEJ was recently confirmed to act in murine B- cells when XRCC4 and Ligase 4 are disturbed (18). The roles of the ERCC1/XPF complex in DSB and ICL repair are distinct from its role in NER as the XPA binding domain of ERCC1 is required for NER only (5).

Adair and co-workers (19) reported that ERCC1was required for removal of long non-homologous tails from 3'-OH ends of invading strands during targeted homologous recombination in Chinese hamster ovary cells. Wood's group

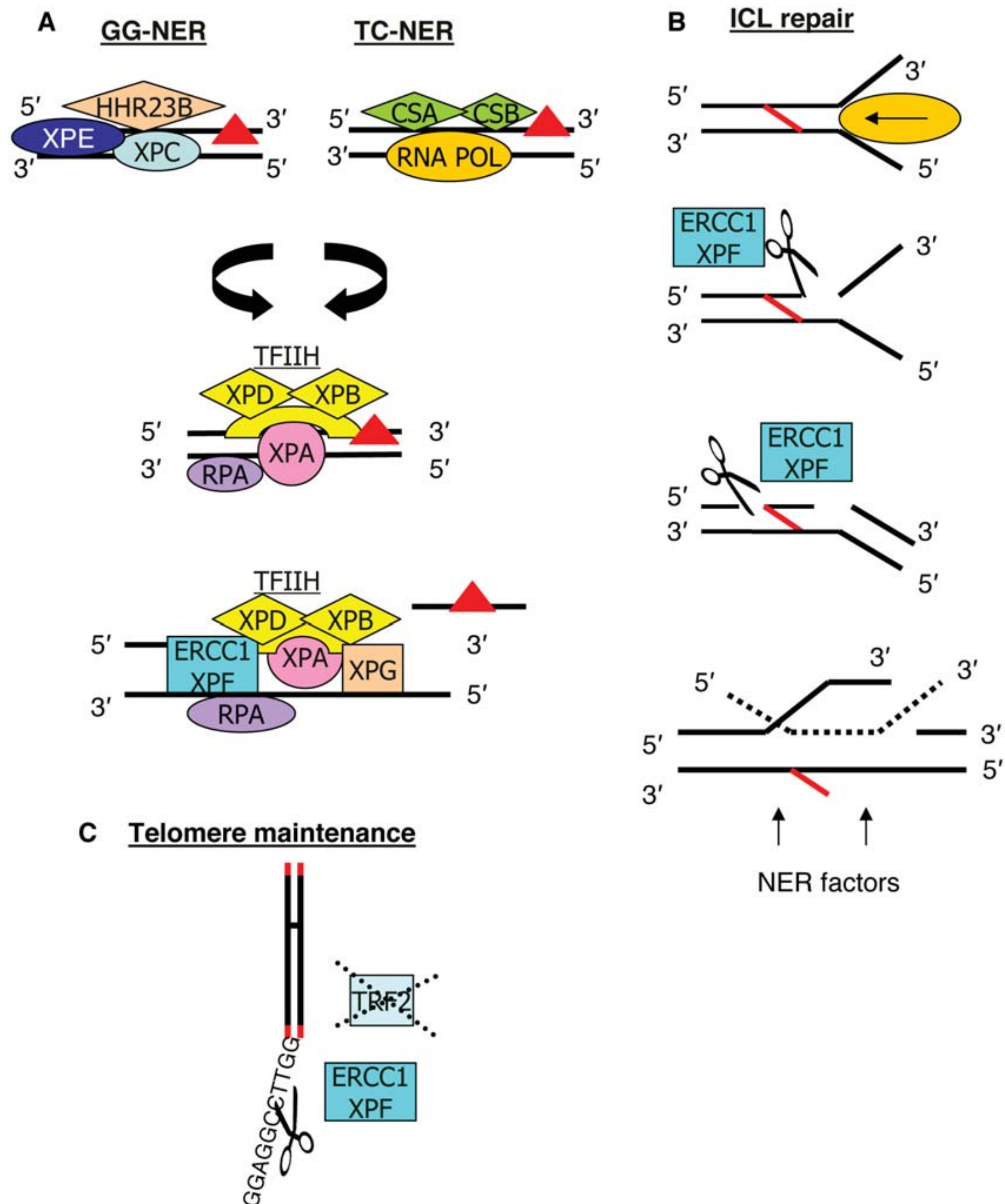

Figure 1. *Role of ERCC1. A: Global genome (GG) and transcription-coupled (TC) nucleotide excision repair (NER) in vitro. In GG-NER DNA damage recognition occurs upon binding of XPC in complex with HHR23B. XPE with its subunits DDB1 and DDB2 helps in the recognition process. In TC- NER, stalled RNA polymerase II (RNA pol) in complex with CSA and CSB recognises DNA damage. In both pathways, transcription initiation factor IIH (TFIIH), consisting of ten subunits including XPB and XPD, and RPA protein are recruited to the complex to unwind the DNA around the lesion. XPA, RPA and XPG then help to form a pre-incision complex around the lesion. Upon recruitment of ERCC1/XPF complex to the damaged site, 5' incision leads to a conformational change in XPG, catalysing the 3' incision around the lesion. Immediate repair around the excision site might be initiated by the 5' incision through ERCC1/XPF. RPA, RFC, PCNA and DNA polymerases δ and ε carry out the gap-filling process. DNA ligase I completes the repair process in TC-NER, with GG-NER utilising XRCC1 and DNA ligase III. DNA strands are symbolised by black lines. DNA lesions are marked as red triangles. B: Model of interstrand crosslink repair according to Kuraoka and colleagues (20) and Fisher and colleagues (21). The formation of a Y structure near the crosslink due to replication fork stalling or with the help of DNA helicase initiates a combination of repair events. ERCC1/XPF first cleaves at the 3' side of one arm of the crosslink and then makes an additional incision at the 5' side. The fork collapses and recombination and NER events can take place to complete the repair. The dotted line represents the invading, homologous DNA strand. C: ERCC1/XPF plays a role in telomere maintenance where it degrades 3' G-rich overhangs when TRF2 function is inhibited.*

(20) proposed a new model for the repair of interstrand crosslinks taking the incision properties of the ERCC1/XPF heterodimer into account. Their model suggests the formation of a Y structure near the damage, for example at a stalled replication fork, or by a DNA helicase. ERCC1/XPF then cleaves at the 3' side of one arm of the crosslink and then makes an additional incision at the 5' side. The fork collapses and recombination and NER events can take place to complete the repair (Figure 1 Panel B) (20). Another group used Y-shaped DNA in mammalian cells, mimicking a stalled fork structure, to investigate the role of ERCC1/XPF in ICL repair *in vitro* (21). Fisher and co-workers found that the heterodimer does indeed not only cut 5' of the psoralen lesion but also cuts 3' of the ICL, resulting in a DSB near the cross-linked site. Therefore, ERCC1/XPF appears to be involved in unhooking of ICLs and therefore initiating the repair process for this most toxic lesion in mammals (21).

Although some involvement of ERCC1 in recombination repair pathways cannot be denied, it seems not to be an essential factor. Melton and co-workers (22) used mitomycin C to induce interstrand crosslinks in *Ercc1*-deficient murine cells and only detected a moderate sensitivity, concluding that ERCC1 is not essential for homologous recombination and that an additional pathway may exist to deal with the damage. However, ERCC1/XPF endonuclease was found to be involved in completion of HR after induction of ICLs by Mitomycin C in mammalian cells where an impairment of the HR pathway was observed in *ERCC1*-deficient mammalian cells (23).

In *S. cerevisiae,* the XPF/ERCC1 homologues RAD1-RAD10 are involved in processing of non-homologous 3' tails during single strand annealing (24). Single-strand annealing is a pathway that evolved to repair ICLs which are located between direct tandem repeat sequences (25). It was confirmed that ERCC1/XPF plays a role in SSA in mammalian cells, but low frequencies of SSA in G_1-arrested mammalian cells with higher frequencies in G_2/S phase of the cell cycle were noted (26). Sarkar and co-workers (27) examined interstrand crosslink repair in *S. cerevisiae* further, suggesting that a combination of pathways might be involved in lesion repair in G_1 phase. Yeast cells are without homologous recombination substrate during the G_1 stage of the cell cycle and can therefore not rely on the classical recombination based pathway for repair of ICL (27). Instead, NER factors, such as ERCC1/XPF and XPG, incise DNA around the ICL without removal of the lesion. When the high fidelity DNA polymerase β stalls at the adducted oligo site, signalling to the RAD6 pathway occurs, leading to monoubiquitination of PCNA at K164. A polymerase switch to polymerase ζ, a B-family polymerase known to replicate through a variety of lesions then occurs (27). Translesion synthesis and re-initiation of replication downstream of the lesion follows. An interplay between the predominant NER pathway and the translesion bypass pathway helps to bypass ICL during G_1 of the cell cycle in yeast. Zhang and colleagues (28) confirmed a mixture of pathways being involved in ICL repair in mammals, where ICLs seem to stimulate HR only when the DSB occurs near the ICL site. They proposed a model in which fork collapse leads to ERCC1/XPF mediated ICL uncoupling, a possible translesion bypass step followed by fork restoration after HR and NER monoadduct removal or replication and another translesion bypass.

ERCC1/XPF in Telomere Maintenance

The ERCC1/XPF complex has also been implicated in telomere maintenance where it interacts with the telomere binding protein 2 (TRF2) (29). At telomeric ends, ERCC1/XPF is required for degrading 3' G-rich overhangs when TRF2 function is inhibited (Figure 1 Panel C) (29). In addition, overexpression of TRF2 in mouse keratinocytes led to *XPF*-dependent telomere loss, increased DNA damage, premature ageing and cancer (30). Wu and co-workers (31) added to the picture by showing that XPF protein harbouring mutations in its conserved nuclease domain was deficient in DNA repair, but could still function in TRF-mediated telomere shortening. Which XPF domain is responsible for this telomere interaction is still elusive.

ERCC1 and *XPF* Deficiencies in Humans

Xeroderma pigmentosum (XP) is associated with very high photosensitivity and 1000-fold increased risk for skin cancer, as well as 10-to-20 fold higher risk for internal cancer (32). Other characteristics in XP patients include ocular and neurological abnormalities with a rate of 40% and 30-40% of the cases being affected respectively (32). XP is a disease of defective NER. Seven complementation groups have been identified within the NER deficient group of XP patients, XPA to XPG.

Most patients presenting with XPF mutations show a mild phenotype with slight sun sensitivity, late onset of skin cancer and low ERCC1/XPF protein content (8). Neurological abnormalities are seldom observed, but can be severe in some cases (33). Several mutations were found in XPF patients, resulting in low levels of the protein and low levels of NER (33, 34). Recently, a patient with more severe *XPF* mutations presented showing profound crosslinking sensitivity and a severe progeroid syndrome (35). Recently, the first reported case of ERCC1 deficiency in humans was reported (36). The patient exhibited very severe clinical features consistent with cerebro-oculo-facio-skeletal syndrome, albeit with only a moderate hypersensitivity of the patient's cells to mitomycin C and UV. The maternal allele exhibited a point mutation leading to a premature stop codon at Q158, leaving a functional null. In contrast, the paternal allele contained a F231L mutation, leaving residual enzymatic activity of the ERCC1/XPFcomplex. The patient died aged 14 months (36).

Ercc1 and *Xpf* Deficiency in Mice

Ercc1 knockout mice were runted at birth and died before weaning due to liver failure displaying severe liver abnormalities (37, 38). Hepatocytes were found to contain nuclei with variable sizes and showed premature polyploidy and aneuploidy by three weeks of age (37). In addition, p53 levels were elevated in liver, kidney and brain as part of a stress response. DNA replication and normal binucleation events seem to be reduced as part of a protective role for p53 in *Ercc1*-deficient cells. It was suggested that p53 ensures that cells with DNA damage do not proceed through replication or binucleation to reduce the risk of fixation of mutations and formation of chromosomal aberrations (39). To investigate the cause of polyploidy in the liver further an adenovirus carrying a Cre recombinase was used in combination with *Ercc1* floxed primary hepatocytes (40). Increased levels of apoptosis were apparent in *Ercc1*-deficient cultures, both spontaneously and after UV irradiation and oxidative DNA damage. Simple *Ercc1*-deficient livers also showed increased apoptosis at the time of onset of polyploidy along with mitochondria with disrupted outer membranes. Lipid accumulation was observed in older *Ercc1*-deficient hepatocyte cultures and in young *Ercc1*-deficient livers, suggesting a reduced ability to repair oxidative DNA damage and a malfunction of oxidative pathways (40).

A mouse line with a truncated form of *Ercc1*, lacking the last seven amino acids, showed delayed onset of premature aging with an extended life span of six months (41). Mutation frequencies were assessed using a lacZ reporter gene in *Ercc1*-truncated livers and were found to be two-fold increased when compared to control littermates at 28 weeks of age. Interestingly, only liver was affected by the truncated form of *Ercc1* with no increase in mutations found in other organs such as the kidney. In addition, livers of three week old complete *Ercc1* knockout mice showed no increased genome instability and mutation frequency when compared to control littermates (41). These findings suggest that mutation frequencies in *Ercc1*-deficient livers arise from weaning onwards, thereafter leading to large genome rearrangements. These large rearrangements could impact normal patterns of gene expression in liver and lead to age-related cellular degradation and liver failure.

Prasher and co-workers (42) investigated the haematopoietic system of *Ercc1* knockout mice and found the basal haematopoiesis and the reserve capacity of the system reduced in those mice. This functional decline of the haematopoietic system is normally found in humans aged 70 and older, where mild anaemia and reduced neutrophil production can be observed (42). No such reduction in haematopoiesis could be noted in *Xpa* mice which are only malfunctional in NER.

The original *Ercc1* knockout mice accurately modelled the patient with severe XPF mutations with increased apoptosis, up-regulation of anti-oxidant defences and reduced growth hormone/insulin-like growth factor 1 (IGF1) signalling being apparent (35). Aged mice, mice under calorie restriction or chronic genotoxic stress showed a similar phenotype (35). The authors concluded that unrepaired DNA damage leads to reallocation of resources from growth to somatic preservation and extension of life span, a process mediated by the IGF1/insulin pathway.

A skin-specific *Ercc1*-deficient mouse line, carrying two loxP sites flanking exon 3 and 5 of the *Ercc1* gene, was created (43). Activation of Cre recombinase, which was under the control of the skin-specific keratin 5 gene promoter, drove the recombination of the loxP sites, resulting in the excision of exon 3-5 of the *Ercc1* gene. When UVB was used as a DNA damaging agent, *Ercc1* flox/null mice showed a high incidence of skin tumours 8-17 weeks after exposure to UVB with control littermates under the same protocol not showing any carcinogenic changes in the skin (43).

Xpf-deficient mice were created by Tian and colleagues (44). Unsurprisingly, *Xpf*-deficient mice showed the same phenotype as *Ercc1*-deficient mice. Animals were runted, died before weaning and displayed an increase in polyploid hepatocyte nuclei.

ERCC1/XPF in Cancer and in Resistance to Chemotherapy

ERCC1 has been implicated in resistance to platinum based chemotherapy in a variety of cancer types and there is currently considerable interest in using *ERCC1* status as a predictive marker for chemotherapeutic outcome. Non-small cell lung cancer is one of the more extensively studied cancers for platinum-based chemoresistance. High *ERCC1* mRNA and protein levels seem to correlate with poor outcome (45, 46). Patients with non-small cell lung tumours with low expression of ERCC1 protein profited from adjuvant chemotherapy in terms of survival when compared to a control group and it was proposed that ERCC1 levels could serve as a determinant of successful adjuvant chemotherapeutic treatment (46). In addition, in patients with metastatic non-small cell lung cancer high ERCC1 expression was correlated with resistance to chemotherapy (47). However, longer overall survival was noted in patients who did not receive chemotherapy and displayed high amounts of ERCC1 protein in the tumours (46), demonstrating that expression levels of ERCC1 need to be seen in a context-specific manner. In patients with advanced squamous cell carcinoma of the head and neck, the risk of cancer death was higher in patients with high ERCC1 protein expression when treated with concurrent chemo- and radiotherapy, again making low ERCC1 expression a possible predictive marker for better response to treatment (48). When samples from patients with advanced gastric cancers were examined for *ERCC1* mRNA and protein expression, better response to chemotherapy and longer overall survival was

noted with low ERCC1 expression (49, 50). The same was true in patients with advanced metastatic bladder cancer and oesophageal cancer where low mRNA and protein levels of ERCC1 could predict longer overall survival after chemotherapy (51, 52). These studies demonstrate that ERCC1 could be useful as a predictive marker for a large variety of cancer types and is not just specific to one or two cancer types.

Several groups found that enhanced expression of BRCA1 or ERCC1 in ovarian cancer patients and cell lines correlated with resistance to platinum-based chemotherapy and poor prognosis in patients (53, 54). Moreover, high levels of both *BRCA1* and *ERCC1* mRNA were related to each other in patients with advanced tumour stages and correlated with diminished overall survival (53). One could hypothesise that both proteins are somehow involved in DSB repair pathways that complement each other. Pancreatic cancer patients show a poor five-year survival rate and, so far, one of the best studied markers for efficiency of the standard chemotherapeutic treatment with gemcitabine is the regulatory subunit of ribonucleotide reductase (RRM1) (55). Performing a tissue microarray, Akita and co-workers (55) found that the best prognosis for disease-free and overall survival in patients with primary pancreatic adenocarcinoma was achieved when protein levels of RRM1 and ERCC1 were both high in the primary tumour prior to surgery, probably due to intact DNA repair pathways being able to contain the disease by preventing the accumulation of additional mutations needed for metastasis. However, once patients presented with recurrent disease, a benefit of gemcitabine treatment was noted when RRM1 protein levels were low (55). This demonstrates that using several proteins as markers for disease outcome before and after chemotherapy can make predictions more reliable.

One well-studied polymorphism in *ERCC1* codon 118 in exon 4 leads to a C:T substitution without change in the polypeptide sequence (N118N) (56). Yu and colleagues (57) suggested that this silent *ERCC1* polymorphism could lead to diminished mRNA and protein levels in ovarian cancer cell lines with functional consequences for removal of cisplatin-induced DNA damage when a T allele was present. It was also proposed that the codon N118N transition leads to differential *ERCC1* mRNA levels which might influence repair capacity in non-small cell lung cancer (58). Moreover, patients with epithelial ovarian cancer who received platinum-based adjuvant chemotherapy without placitaxel treatment, displaying a C:C genotype at codon N118N and high *ERCC1* mRNA expression were at greater risk of disease progression (59). However, Park and co-workers (60) reported that patients with metastatic colorectal cancer carrying the minor C allele at codon N118N expressed lower levels of *ERCC1* mRNA and showed better response to cisplatin-based treatment. In prostate cancer patients, two distinct polymorphisms in the *ERCC1* gene led to reduced mRNA expression (61). However, the clinical significance of these findings was not tested.

Predicted survival for cisplatin treated non-small cell lung cancer patients decreases when one *ERCC1* allele contains the T variant at codon N118N. The same polymorphism is also associated with decreased overall survival and time to progression of colorectal cancer in patients receiving chemotherapy (62). Higher expression of *ERCC1* mRNA was detected in lesions of patients with colorectal adenomas and carcinomas when compared to normal tissue (63), no association of polymorphisms at codon N118N and K259T of the *ERCC1* gene could be made with increased risk of colorectal cancer or response to oxaliplatin treatment (64, 65). When a cohort of patients with inoperable pancreatic cancer, or after relapse, were examined for ERCC1 codon N118N polymorphisms after treatment with the new drug S-1 and two modulators alone or in combination with cisplatin, better progression-free and overall survival was found in patients carrying the T variant compared to those homozygous for the C variant when combining both drugs (66). However, SNP analysis of 481 patients with pancreatic cancer and 621 controls did not reveal a significant genotype difference between haplotypes at N118N in risk of pancreatic cancer (67). A positive correlation with melanoma incidence could be found for other gene polymorphisms in *ERCC1* and *XPF*, where a G:A polymorphism at position 19007 in exon 4 of *ERCC1* and the T:C polymorphism at position 30028 in exon 11 of *XPF* were overrepresented in melanoma patients (68). From these studies, it is clear that polymorphisms in the *ERCC1* gene can influence cancer outcome, but a consistent picture for the role of particular alleles needs further clarification.

Relationship between *ERCC1* Expression and Anticancer Drugs

So far, it has been extensively demonstrated that *ERCC1* mRNA and protein levels can influence response to chemotherapy. However, little is known about the regulatory mechanisms involved in this resistance. Human ovarian cancer cell lines and specimens were examined for the presence of a 42 bp splicing variant of *ERCC1* (69). The 5'-UTR region of *ERCC1* mRNA includes an untranslated region in exon 1. The loss of the 42 bp sequence from the untranslated region was associated with a rise in *ERCC1* mRNA levels in ovarian cancer specimens, indicating a negative transcriptional regulatory effect of this region on *ERCC1* expression. However, when a variety of human cancer and non-cancer cell lines were examined the 42 bp splice variant was found ubiquitously and, in this study, the 42 bp splice variant was not cancer related (70). Chen and co-workers (71) demonstrated for the first time, that hypermethylation 5.4kb upstream of the *ERCC1* promoter region was correlated with cisplatin sensitivity in glioma cell lines and human glioma samples. In addition, methylation status of the CpG island was correlated with *ERCC1* mRNA

and protein levels (71). It is likely that other chromatin modifications may play a role in the regulation of ERCC1 in normal cells and in response to drug resistance.

Cisplatin treatment in ovarian cancer cell lines led to an increase in *ERCC1* mRNA expression (72). This *ERCC1* mRNA up-regulation was preceded by an increase in *c-fos* and *c-jun* mRNA expression along with c-Jun protein phosphorylation, and an increase in *in vitro* nuclear extract binding activity to an AP-1-like site in the *ERCC1* promoter region (72). Yan and colleagues (73) examined *ERCC1*- related cisplatin resistance in more detail. The upstream promoter region around 410 base pairs upstream of the normal *ERCC1* initiation site contains a variety of transcription factor motifs and seems to be responsible for resistance to cisplatin. Upon exposure to cisplatin, the transcription repressor MZF1 was down-regulated followed by *ERCC1* up-regulation. Similarly, MZF1 overexpression led to lower levels of ERCC1 upon cisplatin administration, indicating a regulatory function for MZF1 on *ERCC1* expression (73). When ERCC1/XPF was down-regulated by RNAi in non-small cell lung cancer, ovarian and breast cancer cell lines, cisplatin cytotoxicity and efficacy was increased considerably (9). Both inter- and intrastrand crosslink repair was reduced upon down-regulation of ERCC1/XPF protein levels and DSB, as measured *via* γH2AX foci, persisted *in vitro* (9). These findings make down-regulation of the heterodimer an attractive approach to enhance cisplatin-based chemotherapy in a variety of cancers.

Moreover, the naturally occurring tyrosine kinase inhibitor emodin has been shown to exhibit anticancer effects in a variety of cancers including non-small cell lung cancer (74). High levels of ERCC1 and RAD51 increase the resistance to DSB-inducing DNA damaging agents. However, emodin is known to regulate ERCC1 and RAD51 levels in order to act as a cytotoxin. In non-small cell lung cancer cells, down-regulation of ERCC1 and RAD51 protein levels by emodin was mediated through enhancement of mRNA and protein instability, and enhanced proteolysis by the 26S proteasome (74). In addition, the activation of the MKK1/2-ERK1/2 signalling pathway was shown to be the upstream signal for maintaining the expression of ERCC1 and RAD51 proteins and mRNA in non-small cell lung cancer cell (74). Emodin could therefore be seen as a new anticancer drug on its own or prior to the administration of other chemotherapeutic agents, such as platinum based agents, in order to sensitise cells to chemotherapy. Overall, several upstream factors seem to play a role in the regulation of *ERCC1* mRNA in relation to cisplatin with context specificity being a likely explanation for the diversity of transcription factors involved.

ERCC1 as a Target for Therapy

It is becoming increasingly clear that ERCC1 makes an attractive therapeutic target. Most conventional anti-cancer drugs work by inducing DNA damage, mostly the very toxic DSB or crosslinks, but also bulky adducts. However, many tumours seem to develop resistance to chemotherapy by up-regulating DNA repair pathways that can deal with these toxic lesions (45-51). ERCC1 is one of the proteins with increased expression in many different tumours but as the protein itself does not have an endonuclease domain it will be difficult to regulate its activity (75). Because we are now able to link specific domains of the ERCC1 protein to specific functions in DNA repair, small molecules targeting a specific site on ERCC1, or its partner XPF, could be developed. In addition, the crystal structure of parts of the proteins are now available (3-7). For example, the central domain of ERCC1 which makes contact with XPA and which lends ERCC1 its specificity for NER, could be targeted to inhibit NER function in cancer cells, thereby inhibiting intrastrand crosslink repair and the repair of bulky DNA lesions. Indeed, a synthetic XPA peptide, consisting of amino acids 67 to 80, was found to bind to ERCC1 and to subsequently inhibit NER in mammalian cells (7). A search for a suitable small molecule to inhibit the interaction site of ERCC1 with XPA is already underway (76). In addition, UCN-01, a synthetic derivate of staurosporine, has been found to potentiate cisplatin response by interfering with the interaction of XPA and ERCC1 in mammalian cells (77). Once repair capacity is impaired, tumours should be sensitised to *e.g.* platinum-based chemotherapy. Of note, the most toxic lesions induced by cisplatin, namely interstrand crosslinks, are not dealt with by the NER function of ERCC1/XPF, but through their involvement in HR, SSA and related pathways. As the formation of the heterodimer is crucial, it would be most beneficial to disrupt the actual binding site between ERCC1 and XPF which lies in the *C*-terminal region and carries a double helix-hairpin-helix domain in each of the heterodimeric proteins. As an alternative, the endonuclease activity of the heterodimer, which lies in the central domain of XPF, could be targeted by an inhibitor.

Conclusion

The heterodimer ERCC1/XPF is involved in multiple DNA repair pathways and telomere maintenance, having therefore an important role in genome maintenance. Because of its many roles the phenotype seen in mice and humans is complex, making it hard to elucidate the importance of each function in isolation. More studies designed to relate specific domains or even just amino acid sequences of the complex to certain functions might help to explain the complexity further. Not much is known of the regulation of ERCC1/XPF at a protein and chromatin level. Heterochromatic marks and protein modifications specific to a certain scenario (*e.g.* UV-induced DNA damage) might give important hints on the regulation of the heterodimer. It is becoming increasingly clear that, although ERCC1/XPF is essential for genome

maintenance, high *ERCC1/XPF* mRNA and protein levels in cancer patients can be counterproductive in a chemotherapy setting, often leading to chemoresistance and poor outcome. This is not only true for a specific type of cancer, but seems to be a general pattern in a variety of cancer types, making the ERCC1/XPF heterodimer a potentially interesting target not only for predictive outcome of treatment in patients, but also for drug therapy.

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