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REPAIR OF INTERSTRAND DNA CROSSLINKS INDUCED BY OXIDATIVE STRESS AND ANTI-CANCER AGENTS

Abstract. Interstrand crosslinks (ICLs) occur when two complimentary strands of DNA are covalently linked together after exposure to crosslinking agents, therefore blocking the processes essential for cell survival such as DNA transcription, replication and recombination by preventing the strand separation and switching cell fate to apoptosis. Taking advantage of it, chemical agents such as cisplatin, mitomycin C and nitrogen mustards are widely used in chemotherapy against cancer and several hyperplasic diseases. However, cellular responses induced by ICLs and repair mechanisms counteracting their cytotoxic effect can lead to the appearance of acquired resistance in cancer cells thus limiting the efficiency of the treatment. In this review, we will discuss the main properties of several classes of ICL-forming agents and recent advances in our understanding of the mechanisms of ICL repair. Due to the recent developments on the repair mechanisms of various ICLs, our insight has broadened regarding the drug-specific formation and cellular processing of ICLs. Even though the main features of ICL repair remained the same, new players of repair machinery acting upon specific ICLs are being discovered. These new findings may furnish a basis to improve and adapt anticancer therapies by targeting DNA repair pathways in order to counteract the development of resistance to anti-cancer treatments.

Key words: DNA repair**,** oxidative stress, DNA crosslinks

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Оксидативті стресс пен антирактық агенттер әсерінен ДНҚ молекуласында пайда болған тізбекаралық айқасқан байланыстардың репарациясы

Аңдатпа. Химиялық агенттердің әсерінен екі комплементарлы ДНҚ тізбектері бір-бірімен, сутектік байланыс емес, коваленттi байланысқан жағдайда тізбекаралық айқасқан байланыстар (ICL) пайда болады. Сондықтанда ICL, транскрипция, репликация және ДНҚ рекомбинациясы сияқты клетканың тіршілігіне маңызды процестерді тежеп, клетканың апоптоз жолына түсуіне себеп болады. Осыған орай, цисплатин және митомицин С сияқты химиялық агенттер рак және гиперплазиялық ауруларына қарсы химиотерапия саласында кеңінен қолданылады. Алайда, ICL-ің цитотоксикалық әсеріне қарсы тұратын клеткаішілік процестер, сонымен қатар ДНҚ репарациясының механизмдері химиотерапиялық өңдеулердің емдік әсерін шектеп отыр. Осы мақалада, біздер ICL түзетін бірнеше заттардың қасиеттерін, сонымен қатар түзілген ICL-дің репрациялану механизмі туралы соңғы деректерді талқылаймыз. Соңғы кездері жарияланған деректер, ICL-дің пайда болу механизмдері мен клетканың ICL-ге қатысты жауабы туралы түсінігімізді кеңейтті. ICL зақымдалулардың репарациялану сипаттамалары еш өзгеріссіз болғанымен, ICL-дің түрлеріне қатысты жаңа репарациялану механизмдері айқындалып жатыр. Осы, ДНҚ молекуласындағы ICL түрлеріне қатысты репарациясының механизмі туралы жаңа деректер, ісікке қатысты терапияда ICL түзуші агенттерді пайдаланудың емдік әсерін арттыруға негіз бола алады деп ойлаймыз.

Түйін сөздер: ДНҚ репарациясы, оксидативті стресс, ДНҚ тізбекаралық байланысы.

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Репарация межцепочечных сшивок в ДНК, индуцированных оксидативным стрессом и антираковыми агентами

Аннотация. Межцепочечные перекрестные связи (ICL) возникают, когда две комплементарные цепи ДНК ковалентно связаны друг с другом после воздействия сшивающих агентов, поэтому они блокируют процессы, важные для выживания клеток, такие как транскрипция, репликация и рекомбинация ДНК, предотвращая разделение цепи и переключая судьбу клетки на апоптоз. В связи с этим, химические агенты, такие как цисплатин и митомицин С, широко используются в химиотерапии против рака и некоторых гиперплазических заболеваний. Однако клеточные ответы, индуцируемые ICL, и механизмы репарации, противодействующие их цитотоксическому эффекту, могут приводить к появлению приобретенной резистентности в раковых клетках, что ограничивает эффективность лечения. В этом обзоре мы обсудим основные свойства нескольких классов веществ, формирующих ICL, а также последние достижения в понимании механизмов репарации ICL. В связи с недавними результатами исследования механизмов репарации различных ICL, наше понимание расширилось в отношении специфического образования ICL и клеточной обработки ICL. Несмотря на то, что основные характеристики репарации ICL остались прежними, обнаруживаются новые механизмы репарации, действующие на конкретные ICL. Эти новые результаты могут послужить основой для улучшения и адаптации противоопухолевой терапии на основе знания механизмов репарации ДНК с целью противодействия развитию устойчивости к противораковому лечению.

Ключевые слова: репарация ДНК, оксидативный стресс, межцепочечная сшивка ДНК.

Introduction

In mammalian genome around 10^5 endogenous DNA lesions on average are formed per cell on daily basis as a result of replication errors, oxygen free radicals produced during cellular respiration, exposure to mutagenic environmental factors and modifications by both endogenous and exogenous genotoxic compounds [1, 2]. It is believed that these DNA lesions lie at the origin of cell lethality, tissue degeneration, aging, and cancer due to their cytotoxicity and miscoding properties, which affects normal genetic information inheritance and translation. Luckily most of these lesions have small and non-bulky character and are easily fixed by different DNA repair mechanisms that appeared in living organisms during the evolution to recognize, excise, and accurately replace specific forms of genetic modifications. However, there are also bulky and extremely cytotoxic DNA lesions such as double-strand breaks (DSB) and inter-strand DNA crosslinks (ICLs), which are very difficult to repair and thus require a coordinated contribution of several distinct repair pathways [3].

Inter-strand DNA crosslinks (ICLs) are among the most toxic DNA damages that block transcription and replication by preventing strand separation. A single ICL is enough to kill repair-deficient bacteria and yeast cell, whereas around 20-40 ICLs can lead to the death of a repair-proficient mammalian cell. Due to their high cytotoxicity, ICL-inducing agents like cisplatin, mitomycin C and psoralen are commonly used drugs against hyperplasic diseases such as cancer, psoriasis, and vitiligo [2, 4]. Like most of other DNA lesions, once induced, ICLs lead to the activation of cellular signaling and repair mechanism, which are being associated with the development of acquired resistance of tumor cells to anti-cancer drugs [5]. Due to the elevated mutation rate and specific changes in the genome organization of cancer cells, the sensitivity to DNA crosslinking agents greatly varies among tumors, making the choice of optimal therapy complicated. In this review we will discuss the properties of different DNA crosslinking agents and ICLs that they induce, as well as the current insight on understanding of ICL repair mechanisms in cells.

Formation of ICLs

The idea of using DNA inter-strand crosslinking agents in chemotherapy originated from extremely toxic mustard gas which was created as a deadly chemical weapon during the First World War. In 1943, during the Second World War US Merchant Ship with 60 tons of sulphur mustard bombs was

bombed and led to the contamination of the nearby city and suburbs. As a result of autopsy it was revealed that this chemical specifically attacked the white blood cells of the victims. It was proposed that these chemicals might be used as a treatment of leukaemia and later, after the war, the first study on the 'Nitrogen Mustard Therapy' was published [6].

After almost seven decades of continuous studies, nowadays there are different chemical DNA crosslinking drugs such as nitrogen mustards, platinum compounds, psoralens and mitomycin C widely used in the treatment of different solid tumors and leukaemia. Although these drugs have been used against tumor cells with remarkable efficiency since their anti-cancer property have been first discovered, the fact that they induce cell death by the ICLs formation was discovered much later on. As it appeared these agents form covalent bonds between neighboring nucleotides on one or two strands of DNA, inducing intra- or inter-strand crosslinks and only the latter ones, which represent only a small fraction of the total DNA adducts, are thought to be the main determinant of the cytotoxicity of these drugs [4, 7].

Depending on a number of factors, including cellular uptake and metabolic activation, ICL agents have different cytotoxicities. Nitrogen mustards and their derivatives such as melphalan, chlorambucil, and cyclophosphamide induce both intra-strand and inter-strand crosslinks by reacting with guanines at 5'-GNC-3' cites. Nitrogen mustards act very rapidly inducing ICLs with 14° strand bending distortion within 20 minutes after treatment [8, 10]. Psoralens that are used to treat several skin diseases like psoriasis can be activated by UVA to induce thymine monoadducts and ICLs between thymines at 5'-TA-3' and 3'-AT-5' sequences in DNA. Psoralen-induced ICLs cause unwinding of 25° and minor local distortion of DNA's helical structure. Natural antitumor antibiotic mitomycin C forms adducts at the N-7 and N-2 of guanine, intra-strand cross-links, and ICLs between the N-2 of guanines at 5'-GC-3' and 3'-CG-5' sequences in the minor groove. Widely used anti-cancer drug cisplatin mainly reacts with guanines, forming 65% d(GpG) intra-strand cross-links, 25% d(ApG) intra-strand cross-links and 5–8% ICLs between the guanines in the sequence d(GpC) [8, 9]. Among all platinum compounds cisplatin is the one causing the largest DNA distortion by 45° bending and 79° unwinding. Carboplatin adducts are similar to cisplatin adducts, with 3–4% ICLs. Although the percentage of cisplatin-induced ICLs is low, they seem to cause major DNA distortions, whereas another platinum compound transplatin with up to 30% of ICL induce is less toxic, which could be due to several reasons such as different structure of the ICL allowing faster repair, slower conversion of transplatin monoadducts to ICLs, and a lack of the highly toxic d(GpG) intra-strand adducts [11-13].

Although most of the current interest in ICL-inducing agents appeared from their use in anti-cancer chemotherapy, endogenous crosslinking agents are considered to be behind the evolution of the cellular responses that these exogenous agents trigger. ICLs appear endogenously as a result of the reaction between DNA and by-products of lipid peroxidation, including acrolein and crotonaldehyde R, β-unsaturated aldehydes [14, 15], that are also found in food, tobacco and pesticides and their concentrations increase in the cells of subjects with a high-fat diet and alcoholism [16, 17]. As long as cellular survival is seriously threatened by the presence of such adducts, they might be considered as a driving evolutionary force for the development of the repair and signaling pathways of ICLs.

Therefore, ICLs can occur naturally and are something that cells must deal, even though they are rare in healthy cells. The existence of a rare genetic human disorder Fanconi anemia (FA), where patients are extremely sensitive to ICL-inducing agents, underscore the importance of being able to process ICLs in normal cells [18]. As a result, the ICL repair pathways are vital for healthy cells, but at the same time they are a cause of the resistance to ICL-inducing agents under therapeutic conditions.

Consequences of ICL formation

The processes necessary for normal functioning of cells such as replication and transcription requires separation of the two strands of a DNA double helix. ICL inducing agents create covalent bonds between two bases in opposite strands, which are more difficult to repair, compared to those on one DNA strand, called intra-strand DNA crosslinks [7]. The latter is assumed to be less toxic due to the presence of the universally conserved nucleotide excision repair pathway (NER) which can remove these lesions and also specific translesion synthesis DNA polymerases that may bypass these DNA adducts during the replication. Moreover, since only one strand of DNA is distorted in the case of intra-strand crosslinks, the opposite complimentary strand might be used as a template during the repair (mostly by NER pathway) and replication, which is not possible with ICLs that damage both strands [9]. As it was shown in the first studies of ICL repair

in *E. coli* by Cole [19], the interplay of multiple repair pathways such as nucleotide excision repair (NER) and homologous recombination (HR) is required to deal with ICLs. In eukaryotes the repair of ICLs is even more complex due to the presence of additional layers of the regulation such as chromatin and dependence of the repair pathway choice on the cell cycle. Therefore, as well as NER and HR, other DNA repair and signaling pathways such as FA, mismatch repair (MMR) and translesion synthesis (TLS) are involved in ICL repair [20].

In addition, the cellular processing of ICLs can generate DSBs during the replication due to the replication fork stall and collapse. The formation of DSBs leads to the number of processes such as cell-cycle arrest, HR pathway-mediated repair of the breaks, and also to apoptosis, in case of large number of unrepaired DNA lesions. At present, it is not clear whether apoptosis induced by UV-lights and ICLs, which induce different types of DNA lesions, occurs because of the replication induced DSBs [21, 22]. What is more, it is remains unclear how a cell decides to survive through the repair or dye via apoptosis. In this review, we will focus on two fields regarding the ICL-induced cellular response, ICL removal from DNA and its regulation by Fanconi anemia (FA) pathway.

ICL removal in G0/G1 cells

The vast majority of studies indicate that the repair of ICLs occurs mainly via replication-dependent mechanism [20]. However, the removal of ICLs in quiescent G0/G1 phase cells may proceeds by different mechanism. The induction of strong replication fork blockage by DNA crosslinking agents is the main reason of their high cytotoxicity of . Cells lacking the replication-coupled ICL repair exhibit high sensitivity to DNA crosslinking agents. However, in the case of quiescent (G0/G1 phases) cells the removal of ICLs is also essential because of the transcription stall, but the repair mechanisms and the main players are different from that of replication-dependent ICL repair. In all eukaryotes from yeast to human cells replication-independent repair of ICLs in G0/G1 phases occurs through two-step incision of ICL by NER machinery. First, ICL-containing region in DNA is recognized and cut by NER enzymes [23, 24]. In case of transcription blockage by ICL, incision complex is loaded by the two specific factors of transcription-coupled NER, which are CSA and CSB but in non-transcribed regions by XPC-HHR23B complex *via* global-genome NER (GG-NER). In this step, the incision complex consists of XPA-RPA, TFIIH, XPF-ERCC1 and XPG. Once the dual bracketed incisions are done, the gap generated only on one side of DNA duplex is bypassed by a TLS polymerase such as DNA polymerase κ, DNA polymerase ζ, or REV1 [23, 25-27]. The short oligonucleotide covalently-bound to a nucleotide on another strand goes through the second round of incision with different composition of enzymatic complex. And finally, the second gap is filled in by Pol δ and PCNA.

The repair of ICLs in S phase

The ICL induced stall of the replication machinery by the blockage of strand separation and DNA polymerase activity is a major cause of ICL cytotoxicity , thus it is assumed that the replication fork stall triggers the initiation of ICL repair. The removal of ICLs during DNA replication is a very complex process that requires involvement of several repair pathways and involvement of various enzymes acting in different cellular processes. Several studies suggest that cell exposure to ICL-inducing agents leads to the generation of double-strand breaks (DSB) in S phase of cell cycle, which in turn are repaired by HR but not by the classical non-homologous end joining (NHEJ) pathway [23]. Therefore ICL-induced DSBs were associated with the replication fork, although the details of the replication-dependent ICL repair remained unknown until very recently. It was shown that in *S. cerevisiae*, in proliferating as well as in quiescent cells, ICLs are recognized and excised by NER machinery, thus making NER-deficient yeast mutants extremely sensitive to ICL-inducing agents [27-30]. However, in case of mammalian cells, hypersensitivity to crosslinking agents was demonstrated only for the cells deficient for XPF and ERCC1 which are components of a hetero-dimeric endonuclease XPF/ERCC1, that is able to recognize and cleave single-stranded branched DNA structures with high specificity [27-30]. In addition, several classes of proteins, such as, structure-specific endonuclease MUS81-EME1, homologue of XPF-ERCC1, TLS polymerases Polζ and Rev1, and HR proteins: Rad54, XRCC2 and XRCC3 were found to be involved in ICL-repair based on the sensitivity of mutant cell lines lacking these proteins to ICLs [20]. MUS81-EME1 specifically binds to double-stranded branched DNA structures, Holliday junctions and 3'-flaps, and as well as XPF-ERCC1 is found to be implicated in ICL-induced DSB generation in replicating cells [31]. At present it is not clear, whether, the formation of DSB is a

consequence of the ICL repair, this should be further investigated [9, 32]. Recently many nucleases have been shown to be involved in the ICL incision and without any doubt the accumulation of knowledge coming from various studies have shed light on our understanding of ICL repair. Here, we summarize the most recent model of the ICL repair in replicating cells. Dual bracketed incisions of an ICL during DNA replication result in the generation of DSB in one of newly synthesized sister chromatid, which is then repaired by HR pathway. The studies of the roles of *S. cerevisiae* RAD51, RAD52, RAD54, RAD59 and MRE11 genes showed that they are important for the ICL repair, since the mutant strains deficient for any of these genes exhibited hypersensitivity to DNA crosslinking agents. Interestingly, although YKU70 mutants (deficient for NHEJ) did not demonstrate increased sensitivity to ICLs as compared to control, YKU70/RAD52 double mutants exhibited the same sensitivity profile as RAD52 mutant alone. As it was mentioned above, high DSBs accumulation and DSB repair failures in HR-deficient cells suggest that the NHEJ pathway is not involved in the ICL-induced DSBs repair [10]. In addition, the mammalian cells with mutations in RAD51 paralogues, RAD54, RAD54B and BRCA2 demonstrated increased sensitivity to ICL-causing agents, compared to wildtype, which is not the case for the HNEJ-deficient mutants [33]. It was also hypothesized that HR is responsible for the restart of stalled replication forks after the repair of ICL-induced DSB. In vertebrates, the Fanconi anemia (FA) system plays major role in the replication-coupled ICL repair. Indeed, the FA patients with this cancer-prone inherited disorder, are extremely sensitive to DNA crosslinking agents. In fact, there are at least twenty genes in which mutations are associated with the FA phenotype and although their biological role is not well understood yet, they are known to control HR in the replication-dependent ICL repair.

Apart from the incision-dependent ICL repair, which leads to the collapse of the replication fork and generation of DSBs, the alternative incision-independent ICL repair *via* unhooking of the crosslinked DNA bases by NEIL3 DNA glycosylase from Fpg/Nei family has been described in recent studies. In two different studies [34-37], enzymatic activity of NEIL3 DNA glycosylase upon psoralen-induced ICLs was demonstrated using biochemical in vitro reconstitution system. Walters and colleagues using cell-free *Xenopus* egg extracts have shown efficient unhooking of the ICL via hydrolysis of the N-glycosydic bond between the adducted base and deoxyribose sugar by NEIL3, and creating an AP site on one DNA strand and psoralen-thymine monoadduct on the other complementary DNA strand. It has been suggested that a basic site is then removed by the AP endonuclease or AP lyase activity, while monoadduct might be removed via base excision repair (BER) pathway [36]. On the other hand, Saparbaev's laboratory has demonstrated that NEIL1 and NEIL3 DNA glycosylases are able to resolve psoralen-crosslinked DNA fragments in three- and four-stranded DNA structures [34-35, 37]. Although, these experiments have been performed in vitro, the recent studies revealed that NEIL3 is overexpressed in various tumors that characterized by high resistance to ICL-inducing agents such as cisplatin, nitrogen mustards and mitomycin C. These observations imply the importance of this gene in the resolving of CLs in the incision-independent repair pathways without generation of highly toxic DSB [38-41].

FA pathway-coordinated removal of ICLs

FA is a rare genetic disease resulting in aplastic anemia, bone marrow failure, congenital abnormalities, and high predisposition to develop hematological (typically acute myelogenous leukemia) and squamous cell cancers. After exhaustive genetic and functional complementation studies, 22 genes were found so far to be mutated in FA patients, making this disorder genetically highly heterogeneous [7, 42]. What is more, FA gene products play roles in the ICL response and repair of associated DNA damage and they found to be involved in double-strand break (DSB) repair, mismatch repair (MMR), and nucleotide excision repair (NER) pathways. Mutations in FANC genes lead to high sensitivity to DNA crosslinking agents and genetic instability [18]. Products of nine FA genes (FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL, FANCM, FANCT) form FA core complex, whereas at least five of them are associated proteins (FAAP100, FAAP24, HES1, MHF1 and MHF2). In addition, there are two FA core complex proteins that have catalytic activities – FANCM and FANCL. The former, FANCM, is a DNA translocase, that interacts with MHF1-2 and FAAP24 [42-45], this complex is a first sensor to be recruited to DNA damage sites and it promotes remodeling of the stalled replication forks. It has been suggested that FANCM plays a role in loading of the FA core complex to the stalled replication fork structures, but if FA core complex is absent, it activates checkpoint signaling [18]. Two other

proteins, UHRF1 and UHRF2, recently, have been found to also act as ICL sensors, and to be recruited to the lesion site within seconds to promote activation of the FA pathway [46]. The FANCL is an ubiquitin ligase required for the monoubiquitination of the FANCD2-FANCI proteins, a second component of the FA pathway [20]. Monoubiquitination of this complex is essential for DNA repair, because it leads to the co-localization of this heterodimer with several DNA repair factors, such as FANCD1 (also known as BRCA2) in chromatin, which is, on the other hand, necessary to induce resistance to ICL-forming agents. Using *Xenopus* cell-free extracts, the role of FANCD2-FANCI proteins in cisplatin-induced ICL repair has been demonstrated by in vitro reconstitution assay [47]. As it appeared, the absence of FANCD2/I complex or mutations which makes its ubiquitination impossible, result in the blockage of the ICL unhooking and subsequent translesion synthesis bypass. It was suggested that mono-ubiquitinated FANCD2-FANCI complex is one of the key factors in the recruitment of structure-specific endonucleases and translesion synthesis DNA polymerases to the ICL sites. In addition, it was suggested that the gene products of FANCD1 (BRCA2), FANCJ (BACH1 or BRIP1 helicase) and FANCN (PALB2) are necessary for the efficient repair of the ICL-induced DSBs by HR machinery [18].

As described above, the FA pathway mainly operates during replication of DNA, in S-phase of cell cycle, cooperating with the structure-specific DNA endonucleases, TLS DNA polymerases and HR proteins which repairs the ICL-induced DSBs. This replication-dependent ICL repair pathway taken place in proliferating cells is considered to be the main system that counteracts the genotoxic effects of ICLs. However in slowly dividing and non-dividing at all highly differentiated cells such as neurons, the replication-independent repair of ICLs such as TC-NER can be used to preserve genetic integrity. Currently, the mechanism of FA pathway outside of S-phase is poorly understood and requires further investigations [48].

ICL repair inhibitors

As a result of the accumulation of knowledge coming from various studies, the FA pathway has become increasingly complex; thus the understanding of detailed molecular mechanisms of its functioning can be used to detect the susceptibility of tumor cells to DNA crosslinking anti-cancer agents. Therefore this information can be essential for predicting the success of treatment as well as the inhibition of FA pathway may sensitize tumor cells to the treatment with DNA crosslinking agents.

As it was demonstrated previously, efficient repair of ICLs leads to the development of acquired resistance of tumor cells to DNA crosslinking agents [49, 50] and the inhibition of key processes in the ICL repair pathways is one of the main aims of anti-cancer therapy. Recently, it has been shown, that even without using DNA damaging agents, inhibition of single-strand DNA break (SSB) repair protein PARP1 leads to the synthetic lethality of the BRCA2 (FANC1)-deficient cells [51], which implies the promising future for the development of new anti-cancer therapy by targeting the DNA repair proteins.

The ICL repair is a complex process, which involves various proteins from different DNA repair pathways, thus in order to successfully inhibit the whole process, is sufficient to identify the key players and target them. Due to high sensitivity of FA-patients to DNA crosslinking agents, this pathway attracts a lot of attention from researchers as a valuable target for DNA repair inhibition. So far, the disturbance of FANCC and FANCG, which are FA core complex proteins, resulted in the increased sensitivity of adenocarcinoma cells to DNA crosslinking agents in mice studies [52]. Various studies have identified the key factors essential for normal functioning of the FA pathway one of them the most important step of FA is the mono-ubiquitination of FANCD2/I complex, which is now being the most studied target for DNA repair inhibition [49, 50]. So far several products, including curcumin and its derivatives were identified as inhibitors of the FA pathway [53, 54]. Very recently, it was also found that phosphorylation of FANCD2 suppresses the FA pathway by reducing the DNA binding activity of FANCD2-FANCI complex [47]. The novel mechanism that controls the load of FANCD2/FANCI complex to DNA, allowing to switch the FA pathway on and off. This seems to be very attractive target to downregulate the FA pathway which may lead to the increased sensitivity of tumor cells to DNA crosslinking agents. However deeper understanding of this mechanism is necessary to be able to apply it in clinic. So far several other studies with promising results were done with much attention to FANCD2/FANCI complex and its monoubiquitination by FA core proteins [55, 56]. Further development of the inhibitors of FA pathway requires understanding of the whole picture of FA pathway and its mechanisms to specifically downregulate this repair system.

Conclusion

ICLs are highly cytotoxic DNA lesions which damage both DNA strands in the duplex and require efficient cooperation of many different DNA repair and signaling pathways to be removed. After decades of investigations, our picture of the mechanisms of ICL repair which involves the network of interactions between signaling and repair proteins induced by ICLs still remains incomplete. So far, the studies of ICL repair face the difficulties due to rare character of these lesions in the non-treated healthy cells, however now thanks to the development of biochemical methods to synthesize oligonucleotides and plasmids containing site-specific ICLs as well as to use of cell-free extracts, researchers are able to progress in the understanding of the molecular mechanisms of the ICL repair and beyond, which undoubtedly would help the understanding the development of anti-cancer therapy resistance. The knowledge accumulating from different studies around the world would allow us to improve and adapt the anti-cancer therapies by using the combination of DNA crosslinking agents and the specific inhibitors targeting the essential DNA repair proteins in order to increase the efficiency of treatment of cancer patients.

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