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Targeting DNA repair proteins for cancer treatment

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Introduction

With our growing understanding of the pathways involved in cell proliferation and signalling, targeted therapies for the treatment of cancer are entering the clinical arena. New and emerging targets are proteins that participate in DNA repair processes. Inhibition of proteins in these pathways can sensitize cells to DNA damaging agents, such as chemotherapeutics and ionizing radiation, the main tools currently available to improve the outcome of patients with advanced cancers. The efficacy of these treatment methods is directly related to the ability of the agent to specifically induce cytotoxic damage in tumor cells rather than in normal cells. This specific targeting is accomplished through molecular and cellular features of the cancer cell, such as its higher proliferative rate, which may be directly attacked using inhibitors of the cell cycle. Besides compounds that block cell division at the level of mitotic spindle formation (e.g. vinca alkaloids and taxanes), and growth signal inhibitors, which operate through hormonal manipulation by means of therapeutic antibodies or drugs, a growing number of cell cycle inhibitors encompass DNA damaging agents. Genomic damage may cause cell cycle arrest either directly or indirectly as a consequence of abortive DNA replication during the S-phase of the cell cycle. Notably, the ability of cancer cells to efficiently recognize and remove cytotoxic DNA damage plays a key role in therapeutic resistance, thus profoundly affecting therapeutic efficacy [1–3]. Moreover, since some DNA repair pathways are impaired or inactivated in some types of cancers, a detailed comprehension and strategic manipulation of DNA repair mechanisms could improve the efficacy of DNA damage-based anticancer therapies.

DNA repair pathways (such as BER, NER, TLS, HR, NHEJ, etc) (Fig. 1) can enable tumor cells to survive DNA damage that is induced by common cancer therapy; therefore, inhibitors of specific DNA repair pathways might prove efficacious when used in combination with DNA-damaging chemotherapeutic drugs (Table 1). In addition, alterations in DNA repair pathways that arise during tumour development can make some cancer cells reliant on a reduced set of DNA repair pathways for survival. Indeed, DNA repair pathways are highly interconnected and collaborative, and while a particular DNA lesion can be processed by multiple repair pathways, a single repair process is capable of repairing multiple DNA lesions.

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Moreover, DNA enzymology, epigenetic regulation and transcriptional regulatory mechanisms need to be highly interconnected and strictly coordinated to guarantee proper relief from the consequences of DNA damage. Therefore, knowledge of the fine-tuning mechanisms able to specifically control the activity of DNA repair enzymes is of the utmost importance both for cancer diagnosis and cancer treatment.

The present multi author review consists of nine contributions that cover some of the most important aspects of biochemical and functional regulation of key DNA repair enzymes, as well as the current state-of-art strategies to target these proteins in future cancer treatment paradigms. The first paper, by ML Hegde *et al.*, provides a thorough functional and structural overview of the Base Excision Repair (BER) pathway. BER is endowed with the responsibility of repairing small not-distorting DNA lesions, such as oxidized or aberrant bases and single strand breaks (SSBs), which are produced during cell metabolism or as a consequence of treatment with chemotherapeutic agents (e.g. bleomycin, temozolomide, etc.) [4,5]. Since these lesions are often mutagenic and have etiological linkage to sporadic cancers, the BER pathway is both important in the pathogenesis and treatment of cancer. The contribution by ML Hegde *et al.* focuses also on a novel structural aspect of the main enzymes in BER, i.e. the DNA glycosylases and apurinic/apyrimidinic (AP) endonuclease, where the disordered segments of these proteins operate as sites of molecular regulation of their activities.

A key enzyme in BER is the AP Endonuclease (APE1), which acts as the predominant AP site incision enzyme in mammalian cells. APE1 is a multifunctional ubiquitous and essential protein playing a role both in the pathogenesis of cancer and in resistance to DNA-interactive drugs, such as monofunctional alkylators and antimetabolites. For these reasons, APE1 is acquiring more and more interest as a novel and promising candidate target for anticancer treatment. APE1, which is altered in more aggressive tumors, is a pleiotropic protein playing a critical role not only in DNA repair but also regulating apoptosis, cell proliferation and an adaptive cell response to oxidative stress [6]. These observations, by the way, would be compatible with the caveat that in principle APE1 cannot be fully and stably knocked down, being essential for survival of cancer as well as normal cells, and therefore implying that specific inhibition of its activities only in cancer cells would be required for designing new efficient candidate drugs [7–10]. Thus, understanding the fine tuning mechanisms responsible for its functional regulation and its alteration in cancer cells is of the utmost importance in developing effective inhibitors. The review by G. Tell *et al.*, in this issue, describes the molecular basis for the multifunctional roles of this protein and is followed by an updated description of the post-translational modifications known to have a regulatory impact in redirecting APE1 toward a specific function (C. Busso *et al.*, this issue). The fourth contribution, by D. Wilson III and A. Simeonov, describes the current state of efforts to design potent and selective inhibitors against APE1 DNA-repair functions.

BER, by virtue of its handling of extremely toxic pathway intermediates, is coupled to cell death and recombination mechanisms, both of which are activated should BER stall or fail. Our understanding of these back-up mechanisms in BER and various linkages to other processes, such as replication, is in its earliest stage, and our understanding of the components of the BER sub-pathways themselves is still limited [11–12]. Because of this lack of information, we are unable to make accurate predictions on clinical therapeutic approaches to either enhance or interfere with BER. Similarly, we are not able to make accurate predictions on the biological effects of environmental stressors that trigger BER. A deeper understanding of BER will eventually allow us to conduct more meaningful clinical interventions and also use BER capability in risk analyses and prevention of toxicant-induced disease. In the fifth review, S. Wilson *et al.* will cover historical and recent information on BER and DNA polymerase β and discuss approaches currently underway toward use of small molecule inhibitors to manipulate BER and potentiate cytotoxicity. The following article by F. Mégnin-

Chanet *et al.* is an overview of the PARP family of enzymes as essential players in repairing SSBs. Further discussion covers the emerging evidence that PARPs are ideal candidate targets for single agent therapies acting through the principle of *synthetic lethality* with homologous recombination-deficient (e.g. *BRCA*) tumors [3,13,14].

Alkylating agents-induced DNA damage, besides being repaired via BER, is also acted upon by O⁶-Methylguanine-DNA Methyltransferase (MGMT), a suicide protein that specifically removes methyl (or alkyl) groups from O⁶ position of guanine or O⁴ thymine. Since these adducts are formed as a consequence of a number of common alkylator drugs, MGMT levels represent a key mechanism of anticancer drug resistance, particularly in malignant gliomas and melanomas [15,16]. The contribution by B. Kaina and M. Christmann focuses on MGMT inhibitors as promising tools for cancer therapy.

One of the most important class of drugs used in cancer chemotherapy forms DNA interstrand crosslinks (ICLs), which are extremely cytotoxic lesions in that they block replication and transcription in eukaryotic cells [17]. However, to date, relatively little information is available on the molecular mechanisms responsible for their removal within cells in part due to the limited availability of well-defined substrates. A. Guainazzi and O.D. Schärer describe, in the eight contribution, a survey of the current understanding of the biological responses triggered by ICLs, including Translesion Synthesis (TLS) bypass, Homologous Recombination (HR) and Nucleotide Excision Repair (NER) pathways [18,19]. The review focuses particularly on the use of synthetic adducts that have facilitated recent studies and on how these findings may be translated into the design of new therapeutic agents for cancer chemotherapy.

Double strand breaks (DSBs) are considered one of the most harmful and lethal forms of DNA damage and are also a main cause of genomic instability associated with the onset of cancer [20]. DSBs, which may result as the consequence of many chemotherapeutic agents that induced damage that blocks DNA replication, are repaired through Homology-directed Repair (HR) or Non Homologous End joining (NHEJ) [21]. The last Review by F.V. Rassool and A.E. Tomkinson focuses on the molecular aspects of DSB repair, their alterations in cancer and on the future directions for cancer therapies that target these pathways.

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Figure 1. Schematic overview of DNA-repair systems and emerging anticancer targets

Repairing toxic DNA lesions induced during most common cancer treatments relies on proper DNA-repair response, which has the ability to dictate the cellular outcome, e.g. cell death or cell survival.

Triggering of a specific repair pathway (DR, BER, NER, MMR, HR and NHEJ pathways) depends on the nature of the DNA damage. The main components and the signaling pathways of each repair response is indicated. Yellow boxes highlight emerging anticancer targets which are discussed in this issue. The availability as anticancer targets of other components of these pathways requires further testing. Abbreviations: DR, Direct Reversal; BER, Base Excision Repair; NER, Nucleotide Excision Repair; GGR, Global Genome Repair; TCR, Transcription Coupled Repair; MMR, Mismatch Repair; HR, Homologous Recombination; MRN, Mre11–Rad50–Nbs1 complex; NHEJ, Non-Homologous End Joining; O⁶MeG, O⁶-methylguanine.

Table 1

List of the known repair pathways involved in repairing toxic DNA lesions induced by most common cancer treatments

Cancer treatment	Toxic lesion	Major repair pathways involved
<u>Radiomimetics and radiotherapy</u> <ul style="list-style-type: none"> • Ionizing radiation • Bleomycin 	Single-strand breaks Double-strand breaks Base damage	NHEJ, BER, HR
<u>Monofunctional alkylators</u> <ul style="list-style-type: none"> • Temozolomide • Nitrosourea compounds • alkylsulphonates 	Base damage, bulky adducts, replication lesions	DR, BER, HR, NER
<u>Bifunctional alkylators</u> <ul style="list-style-type: none"> • Cisplatin • Mitomycin C • Nitrogen mustards • Psoralen 	Double-strand breaks, DNA crosslinks, replication lesions, bulky adducts	HR, NER
<u>Antimetabolites</u> <ul style="list-style-type: none"> • 5-Fluorouracil • Thiopurines • Folate analogues 	Base damage, replication lesions	BER, ?
<u>Topoisomerase inhibitors</u> <ul style="list-style-type: none"> • Camptothecins • Etoposide 	Single-strand breaks, double-strand breaks, replication lesions	HR, NHEJ
<u>Replication inhibitors</u> <ul style="list-style-type: none"> • Aphidicolin • Hydroxyurea 	Double-strand breaks, Replication lesions	HR, NHEJ