DNA repair inhibition to treat cancer and other serious illnesses

The stability of an organism's genome is essential for healthy functioning and survival. The information content and integrity of the genome are so important that a range of maintenance and repair systems have evolved.

By Professor Stephen P. Jackson and Cheryl L. Bishop

These systems, or molecular pathways, include those that sense or detect damage in the genome, those that signal the presence of genome perturbations to other pathways such as those involved in cell growth and division, and the systems that carry out the eventual repair of the DNA damage. So fundamental are these pathways to life that they must have evolved very early on. Consistent with this idea, the available evidence indicates that they are highly conserved across species, from yeast to man.

If the genome is damaged, this can lead to the generation of genetic mutations that can have dire consequences for the organism. In particular, errors in two types of genes can lead to cancer. These 'cancer predisposition' genes can be considered either as gatekeepers or caretakers of the cell. Generally, gatekeepers are those genes whose products serve to keep cells cycling or replicating in a controlled manner. They serve to ensure that cell growth and division only occur where needed in the organism and that inappropriate and sustained cell growth and division – as occurs in cancer – do not take place. By contrast, caretaker genes are those that encode proteins whose function is to detect damage to the genome and then make sure that this damage is repaired. Defects in caretaker genes thereby result in the accumulation of aberrations in the genetic code. When these aberrations occur in other caretaker genes or in gatekeeper genes, this can result is a cascade of ensuing genetic mutations, genomic instability and, ultimately, uncontrolled cell growth or cancer.

DNA damage repair

DNA damage repair systems are examples of caretaker systems and their sheer number indicates just how many types of genomic insults mammals and other organisms must be equipped to overcome. It has been estimated that in one day each cell in the human body sustains around 10,000 lesions; corresponding to a total of approximately 100,000,000,000,000,000 or 10^{17} lesions per person per day. **Figure 1**

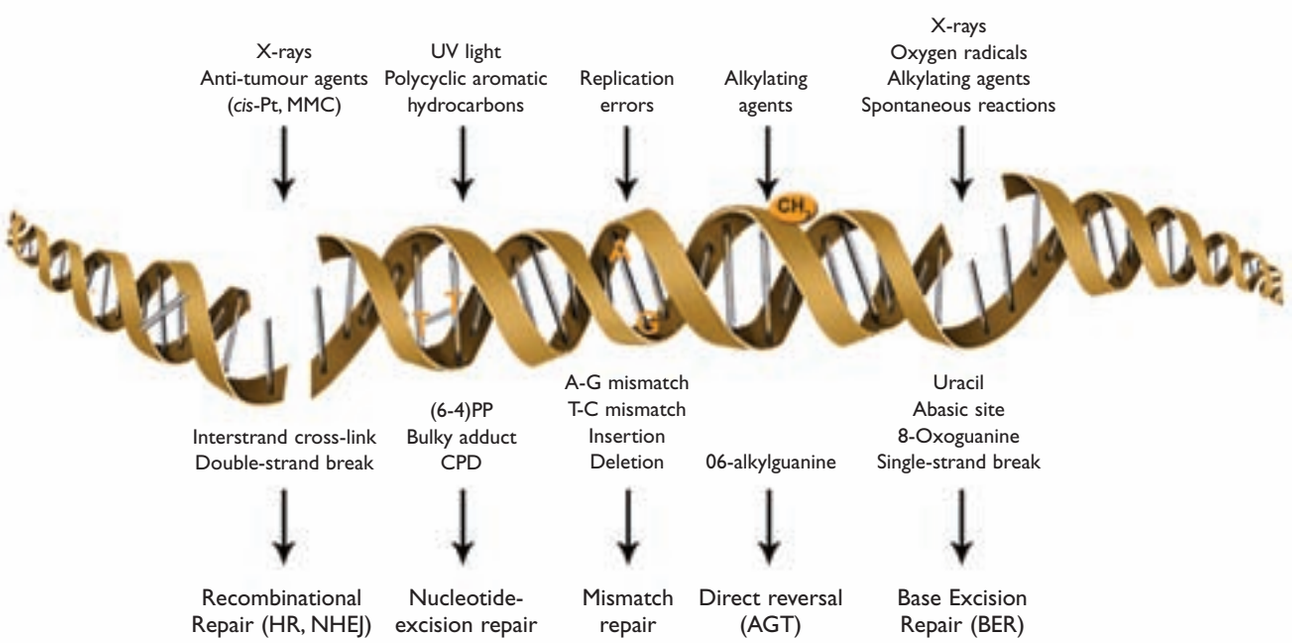

Figure 1 illustrates some of the different types of DNA damage that can occur, including DNA cross-links, double- or single-strand DNA breaks, base adducts and base mismatches. Importantly, although certain forms of DNA damage occur frequently within the cell, others such as DNA double-strand breaks (DSBs) do not occur frequently under normal circumstances but are instead generated in specific situations, such as when a person is treated with radiotherapy.

Evolution has provided our cells with various ways to overcome the myriad DNA lesions which are caused by both endogenous agents, such as reactive oxygen species generated by cellular metabolism, and exogenous damaging agents, such as X-rays, cigarette smoke and UV light. Because of the distinct nature of the various forms of DNA damage, a specific molecular repair pathway has evolved to fix each one. Each of these pathways is in turn made up of proteins and enzymes that may have overlapping and multiple functionality. The main DNA repair pathways currently known to science include nucleotide-excision repair (NER), base-excision repair (BER), DSB repair by both homologous recombination (HR) and non-homologous end joining (NHEJ), direct reversal of the damage and mismatch repair (MMR).

Failure of DNA damage detection and repair pathways due to mutations or in-born genetic defects can result in severe medical conditions and, in particular, to a heightened disposition to cancer. For example, the genomic instability syndromes Xeroderma pigmentosum, Cockayne's syndrome and trichothiodystrophy arise due to defects in the NER pathway. Another human genetic disorder, ataxia-telangiectasia (A-T), is caused by a mutation in the gene *ATM* (ataxia-telangiectasia mutated). A-T has an incidence of around 1 in 100,000 and is characterised by growth retardation, premature ageing and an increased likelihood of developing cancer. At the cellular level, A-T is characterised by a high degree of genome instability.

It is important to recognise that disorders such as those described above are generally familial; that is the genetic mutations are inherited and patients affected by these diseases are suffering from lifelong absence of various enzymes and proteins. Sustained dysfunction of these pathways, particularly during key stages of early development, is likely to have a much greater impact on health than would be caused by a temporary inactivation of such pathways at later stages in life. Consequently, if proteins such as ATM could be inactivated for shorter periods – for example to treat diseases as described below – this could provide significant therapeutic benefit without causing overt toxicity to the patient.

DNA repair inhibition

If a DNA damage detection or repair pathway is missing or is inhibited in a cell, then the cell will be much more easily killed by DNA damaging agents that cause the type of damage that is acted upon by such a pathway. Bearing this in mind, one can see how therapeutic inhibition of these pathways could be useful in clinical settings where the goal is to kill diseased cells. In the treatment of cancer, for example, the primary rationale for the administration of radiotherapy or chemotherapy is to cause cancer cell death. In the case of radiotherapy, cancer cell killing is caused primarily through the generation of DNA DSBs. Unfortunately, in many cases the DSB detection and repair pathways operating in cancer cells allow them to rectify the DNA damage and thereby survive, leading to ineffective therapy and cancer recurrence. If the clinician could couple radiotherapy with an inhibitor of DSB detection and/or repair – so that DSB repair does not take place in the cancer cells – then this would be expected to enhance the likelihood of effective cancer cell killing. Thus, specific, transient inhibition of DNA damage detection or repair could have utility in combating cancer.

Significantly, a growing body of research indicates that in many situations cancer cells are particularly vulnerable to inhibition of DNA repair processes. One reason for this may be that cancer cells tend to divide more frequently than normal cells, meaning that they have less time than normal cells to repair DNA damage before duplicating their genome or dividing. Another reason for cancer cells being very susceptible to DNA repair inhibition is that, by definition, they are genetically unstable, having lost the proper functioning of one or more gatekeeper or caretaker genes. By contrast, the non-cancerous cells in the body will generally have retained their full complement of such genes. Consequently, a cancer cell is typically highly reliant on the repair pathways that it still retains, meaning that inhibition of such pathways will have a greater effect on the cancer cell than on normal cells. As we learn more about cancer genetics, molecular diagnostics is expected to change the future of cancer treatment selection, and this is likely to be particularly so for treatments that involve the generation of DNA damage. As described below, DNA damage sensing and repair inhibition holds promise in the treatment of cancer and several other life-threatening diseases whose pathology is linked to the generation of DNA damage.

The DNA repair enzyme AGT and resistance to DNA alkylating drugs

Alkylating agents (also known as methylating agents) such as temozolomide, dacarbazine and carmustine are used to treat many solid tumours, and their cytotoxicity is mediated principally through methylation of the O^6 position of guanine, one of the building blocks of DNA. These agents are, however, most effective in only a small range of cancers which include glioma and melanoma.

It is hypothesised that the relatively limited response of many tumours to these alkylating agents is due to the high expression of the DNA repair enzyme O^6-alkylguanine-DNA alkyltransferase (AGT) in some types of cancer. AGT repairs the damage caused by methylating agents through direct reversal of the damage: AGT binds directly and irreversibly with the methyl group that has been added to the guanine residue and thus removes it. Furthermore, *in vivo* and *in vitro* studies indicate that the higher the level of AGT present in a cell, the less effective DNA methylating agents tend to become. Therefore, a temporary depletion of AGT through inhibition of the enzyme during treatment with these alkylating agents may result in improved efficacy of the cytotoxic methylating drug. Moreover, efficacy in high AGT expressing tumour types that were previously unresponsive to the actions of these alkylating agents may also be achieved. These added anti-tumour effects are also expected to increase the therapeutic index of methylating agents. Currently, there are two AGT-depleting DNA repair inhibitors in clinical trials: O6BG (Alkylade®, Phase III, Access Oncology) and Patrin™ (Phase II, KuDOS Pharmaceuticals).

The potential for DNA-PK and ATM inhibition in cancer therapy

Both DNA-PK and ATM respond to DSBs and have multiple functions within the cell. DNA-PK is a key player in the DSB repair process of NHEJ, and in addition functions in the modulation of chromatin structure and telomere maintenance. Although aspects of the NHEJ repair process remain a subject of study, DNA-PK is known to sense DNA DSB damage and then activate and recruit repair factors to these lesions through a number of phosphorylation events. By contrast, ATM is not required for NHEJ but is involved in several additional cellular processes that are triggered by DNA DSBs. These include the modulation of repair activities and the activation of signalling pathways that control the cell cycle. As for DNA-PK, ATM exerts its influences through phosphorylating a range of target proteins. Importantly, loss of DNA-PK or ATM leads to elevated sensitivity towards ionising radiation and certain chemotherapeutic drugs that generate DNA DSBs.

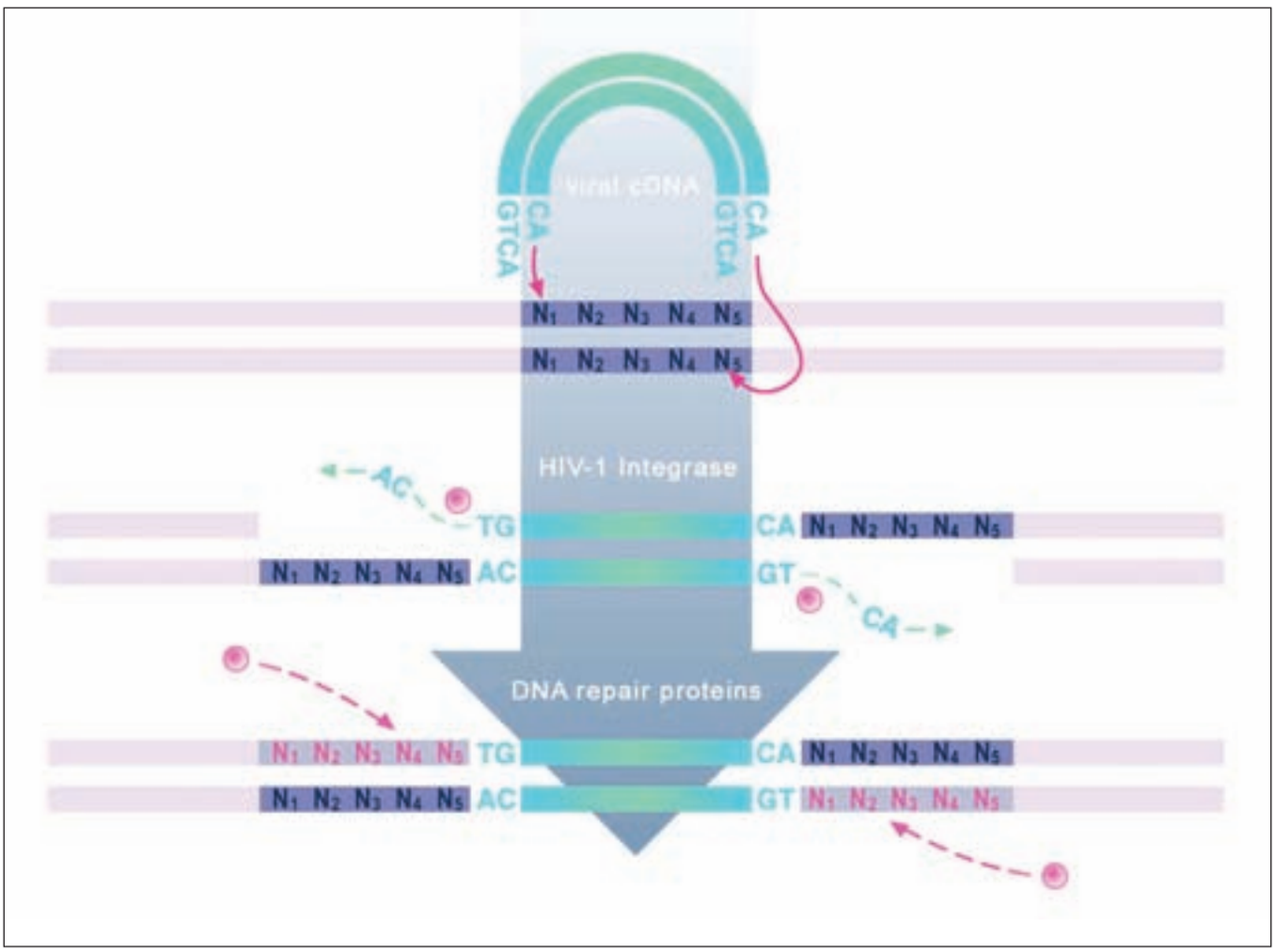

Figure 2

Consequently, both DNA-PK and ATM make promising targets for the development of inhibitors that could be used to enhance the efficacy of cancer radiotherapy and chemotherapy.

DNA repair and viral integration

Retroviruses such as HIV infect cells by incorporating their own genome into that of the host using a combination of host cell proteins and proteins that the virus itself encodes. This then programmes the host cell to produce new viruses and they can then go on to infect other cells, or other individuals. Thus, integration is an important step in the retrovirus life cycle, without which the virus would be unable to replicate.

Upon entering a cell, the virus first makes a complementary DNA (cDNA) copy of the RNA that it carries, using the viral enzyme reverse transcriptase. It then uses integrase, another viral enzyme, to co-ordinate the joining of two ends of this cDNA into the host DNA. The virus relies on host DNA repair enzymes, however, to join the remaining two ends (**Figure 2**). Because DSBs are key intermediates in the retroviral lifecycle, it makes intuitive sense that DNA damage detection and repair enzymes will influence the integration process. Consistent with this notion, ATM and DNA-PK are needed for the efficient integration of retroviral DNA into the genome of a host cell. This makes them attractive targets for developing new anti-retroviral drugs.

Drugs targeting host enzymes such as DNA-PK and ATM might have certain advantages over existing anti-retroviral agents. Current anti-retroviral therapies target viral enzymes, such as reverse transcriptase, and thus put considerable pressure on the virus to mutate in order to render its proteins immune from such drugs. These mutations thus result in resistance to anti-retroviral therapy and lack of efficacy in patients. HIV drug resistance is a growing problem that clinicians are combating by giving combination and often very toxic

drug regimens. Significantly, a drug target encoded by the host cell, such as DNA-PK or ATM, would not be subject to such mutational pressure. Consequently, retroviruses would not easily be able to develop resistance to drugs targeting these enzymes. More work, however, is needed before we can fully assess the anti-retroviral potential of DNA repair inhibition.

PARP and ischaemic diseases

Poly(ADP-ribose) polymerase-1 (PARP-1) is another DNA repair enzyme that could be inhibited to treat various diseases. Although work is ongoing to reveal the precise functions of other members of the PARP family, work on PARP-1 has clearly established that it detects and signals the presence of DNA single- and double-strand breaks. Upon detecting such damage, PARP–1 binds to the site of injury and catalyses the conversion of NAD+ into nicotinamide and ADP-ribose, producing highly charged branched chains of poly-ADP ribose on PARP-1 itself and on a series of other proteins. These activities of PARP-1 are thought to aid recruitment of other factors that help to repair the DNA damage. Notably, loss of PARP-1 is known to sensitise cells to a range of DNA damaging agents, including a variety of anti-cancer chemotherapeutic agents. Inhibitors of PARP-1 therefore have considerable potential to improve the efficacy of cancer therapies.

In addition, PARP inhibitors have potential in other therapeutic areas. Notably, if cells undergo high levels of DNA damage through oxidative stress, such as that present following myocardial infarction or stroke, PARP-1 activity is dramatically induced. Indeed, under such conditions, through its catalytic activity, PARP-1 severely depletes the cellular energy reserves and results in cell death due to necrosis and irreversible tissue damage. Under these circumstances then, PARP-1 activation actually contributes to the ensuing pathology. Consequently, one might anticipate that temporary PARP-1 inhibition in these situations would at least partially alleviate the cell killing. Consistent with this idea, work in preclinical models indicates that administering a PARP inhibitor during or shortly after an ischaemia-reperfusion event such as myocardial infarction, decreases the infarct size and overall damage to the organ.

Conclusions and future directions

Over the past few years, we have witnessed dramatic advances in our understanding of how cells detect and repair DNA damage, and how defects in these events can have pathological consequences. Indeed, it is now clear that underlying deficiencies in DNA damage detection and repair are associated with the majority, if not all, cases of human cancer. Although DNA repair pathways generally operate for the good of the organism, a significant conceptual development has been the realisation that temporary inhibition of these pathways has major therapeutic potential. This is particularly so in the oncology arena, where inhibition of specific DNA damage signalling or repair pathways might dramatically extend the efficacy of existing radiotherapy and chemotherapy regimes. There is also the exciting possibility that inhibitors of DNA repair and related pathways will have utility in other therapeutic areas, such as in the treatment of retroviral infections and ischaemic diseases. Given the breakneck speed of advances in the field of DNA damage detection and repair, and because of the increasing level of interest in this area by the pharmaceutical sector, the next few years promise to be very exciting indeed. DDW

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Further reading

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