DNA damage repair and tolerance: a role in chemotherapeutic drug resistance

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ABSTRACT

A significant barrier to effective cancer therapy is the development of resistance to the drugs utilised. Standard chemotherapeutic regimens typically contain genotoxic agents, designed to damage DNA of existing tumour cells as well as prevent the synthesis of new DNA during proliferation. DNA damage in normal cells can be repaired efficiently or tolerated to preserve cellular and organ functionality. The mechanisms of DNA repair and tolerance are distinct for different types of lesion, but can be predicted if the mechanism of interaction of the drug with the DNA is known. There is now evidence in solid tumours to suggest that increased repair or tolerance of DNA lesions may contribute to the ability of the cancer cell to survive in high genotoxic stress environments afforded by the therapy. This review will explore the current understanding of drug resistance mechanisms to chemotherapy, but will focus on the new evidence for tolerance and repair, including some new data from the authors' laboratory on the haematological malignancy multiple myeloma. The review will focus particularly on the role of the 'specialised polymerases' which have flexible active sites capable of accommodating DNA lesions, allowing replication past the lesion by translesion synthesis and tolerance of the damage, which ultimately results in a phenotype of drug resistance.

KEY WORDS: Antineoplastic agents.
DNA damage.
DNA repair.
Drug resistance.

Introduction

Drug resistance is a physiological process that decreases the efficacy of a drug such that it is no longer effective enough to cure a disease, to eliminate the symptoms or prevent progression of a disease. Drug resistance is a major obstacle in the treatment of some diseases, especially as it can result from several different mechanisms, which complicates the decisions for alternative further therapies.

Drug resistance in cancer cells is a likely event, as reduction of the sensitivity to chemotherapy is one of the malignant cells' characteristics (cited in[1]). In some malignancies, such as multiple myeloma (MM), drug resistance has been reported in almost all patients despite an objective response to an initial dose of drug.[2–4]

Several mechanisms have been suggested to be involved in drug resistance in cancer, including increased efflux and decreased influx of drug, changes in targets and metabolic profile.[5,6] A key feature of many chemotherapeutic drugs is the creation of lesions in the DNA with the aim of inducing apoptosis through excessive damage.[7,8] In order to produce a drug-resistant phenotype with respect to chemotherapy-induced DNA lesions, it would require either increased removal (repair) or tolerance of the damage. It should be mentioned that DNA repair/lesion tolerance may be considered a potential drug resistance mechanism exclusive to genotoxic agents such as nitrogen mustards, nitrosoureas or topoisomerase inhibitors, and not a relevant mechanism for other drugs that are non-genotoxic.

To date, most research has been performed in animal models or solid tumours associating lesion tolerance with genotoxic drug resistance;[9–12] however, there is little evidence in the haematological malignancy setting despite malignancies such as MM demonstrating resistance in almost every patient.[13] Multiple myeloma represents a good example of drug resistance in a haematological setting, and the gold standard therapy has been melphalan,[14,15] suggesting a role for repair or tolerance in this cancer. The fact that survival is now improving for MM patients on non-genotoxic therapies[16] supports the concept of repair or tolerance.

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Recognised mechanisms of drug resistance

To date, a number of mechanisms of drug resistance have been described and investigated by different research studies and may apply to both genotoxic and non-genotoxic drugs.

Reduced cellular drug accumulation

This may arise due to a reduction in the drug uptake into the cell, or increase in efflux of the drug from the cell,[17,18] such that the drug never reaches its target. Here, some of the transporter proteins such as p-glycoprotein (p-gp) or multidrug resistance protein 1 (MDR1), MDR-associated protein (MRP), breast cancer resistance protein (BCRP) and lung resistance-related protein (LRP) play a major role in actively removing the drug from the cells.[19–22] CD98 is another membrane glycoprotein molecule known to be a transporter of some amino acids such as valine and tryptophan; it also transports L-phenylalanine, and, as melphalan is a derivative of L-phenylalanine, so down-

regulation of CD98 can result in a decrease of melphalan incorporation into cells, potentially resulting in a resistant phenotype for chemotherapeutic protocols utilising melphalan.[23]

Alteration in detoxification systems inside the cells

Drugs may be inactivated by sulphur-containing molecules such as glutathione, which facilitate detoxification of the compound.[24] These biochemical changes have been seen in human tumour cells resistant to alkylating agents, demonstrated by cytoplasmic metabolism of the active chloroethyl alkylating moiety of the nitrogen mustards to the inactive hydroxyethyl derivative via glutathione-S-transferase,[24] preventing covalent binding and crosslink formation of the drug with DNA – the key role of this group of drugs.[25,26] Elevated levels of some proteins such as glutathione can impair the function of a drug before it reaches its pharmacological target, and this has been suggested as a possible mechanism of drug resistance to melphalan and cisplatin.[24,27,28]

Regulatory proteins

Modification in the expression of oncogenes such as *c-foc*, *c-myc*, *H-ras*, *c-jun*, *c-abl*, as well as tumour suppressor genes such as *p53*, can alter the sensitivity of the cells to a drug.[18,29] Although the exact mechanism of action of these proteins is not yet fully understood, higher expression of *c-foc*, *c-myc* and *c-jun* has been found in cell lines resistant to cisplatin, as well as in some patients who failed to respond to cisplatin.[28]

Mutation of *ras* is one of the most common oncogenic mutations in several malignancies, including MM.[30] H-Ras also appears to be involved in the modulation of the specialised polymerase, polymerase beta (Polβ) expression (discussed below),[31] and it is suggested that this can be achieved through phosphorylation or dephosphorylation of proteins that bind to a critical promoter sequence of the *Polβ* gene.[32] Resistance to chemotherapy was also shown in a *ras*-transfected MM cell line.[33,34] These cell lines demonstrate progressive interleukin (IL)-6-independent growth and resistance to chemotherapeutic drugs.[34] Ras protein was also found in tumours resistant to cisplatin, such as tumours of the colon.[18,28]

The *c-abl* gene encodes a tyrosine kinase that can be activated in response to cisplatin; also, greater resistance in c-abl-deficient cells suggests a role for this gene in cisplatin-induced apoptosis.[18] The mechanisms by which DNA damage activates *c-abl* have not been fully clarified, but it involves the DNA-dependent protein kinase and ataxia telangiectasia mutated (ATM) gene product.[35]

Inhibition of apoptosis

Resistance to apoptosis can be induced by over-expression of anti-apoptotic proteins such as B-cell leukaemia protein 2 (Bcl-2), Bcl-XL, A1/Bfl1 and mutation of p53 protein.[4] The tumour suppressor gene *p53* plays a role in cell cycle control by promoting cycle arrest, and inducing either DNA repair or apoptosis.[36–38] Mutation of *p53* correlates with the cytotoxicity of therapeutic tumour necrosis factor-α (TNFα), demonstrating resistance to TNFα in sarcoma cell lines; *p53* may also be involved in resistance to melphalan.[39] Abnormalities of p53 are also found in malignant cells resistant to other drugs such as cisplatin.[28]

Alteration in DNA repair capacity or tolerance of damaged DNA as a proposed mechanism of resistance

DNA is a target molecule for many chemotherapeutic agents.[40] Removal of the DNA lesions caused by chemotherapy reduces the damage to the cell, and this can lead to the cell's survival. While increased DNA repair has been described as a mechanism of drug resistance,[41] DNA damage tolerance has been considered a potential mechanism of drug resistance, where the replication fork can continue to replicate DNA strands, bypassing DNA lesions rather than repairing them (Fig 1).[42] It is believed that specialised DNA polymerases play a key role in this process of tolerance, and evidence for their involvement is discussed below.[43]

Although there is no clear consensus on which of these mechanisms is most relevant in the clinical setting, the significance of DNA damage formation and repair or tolerance of damaged DNA in clinical resistance to a drug is evident.[44,45] Also, in many studies that have been performed using cisplatin, melphalan or other alkylating agents, enhanced DNA repair mechanisms are proposed as a major mechanism of drug resistance.[46–48]

Mammalian cells are continuously exposed to a wide range of endogenous and exogenous genotoxic factors such as free radicals, ultraviolet (UV) light, environmental pollutants, ionising radiation, as well as antineoplastic agents in cancer patients, which interact with DNA and lead to DNA damage.[49] In normal, healthy cells several mechanisms are involved in repairing damaged DNA and maintaining the genome stability. However, inaccurate DNA repair can result in mutagenic events, whereas tolerance of DNA damage can result in survival of the cell with DNA lesions, which can also lead to mutagenesis or tumourigenesis.[50] Both scenarios result in overall genome instability that favours progression of the tumour; not only because the cell can cope with the drug administered, but also because specialised polymerases have low fidelity of copying DNA and thus increase mutagenic events, promoting further instability.[45] DNA repair processes relevant to the removal of chemotherapeutic agents are briefly described below.

Base excision repair

Base excision repair (BER) is known as a major pathway for the repair of base damage and is responsible for repairing one or a small number of bases with smaller modifications such as alkylating and oxidative lesions caused by drugs such as nitrosoureas.[51,52] This process can be performed in either short-patch BER (a single DNA base) or long-patch BER (two to six DNA bases) which involve different proteins in the respective repair mechanisms. Five proteins including UDG, HAP1, Polβ, XRCC1 and DNA ligase I or III are needed for short-patch BER, while in long-patch BER DNase IV is also necessary.[53] It is a tightly balanced system for repair of alkylated bases such as N^7-methylguanine and N^3-methyladenine[54] and it is believed that *N*-alkylation, which represents the majority of alkyl DNA damage, is removed from DNA by the BER system.[55] Although BER can play a role in drug resistance by removing drug-induced DNA lesions from malignant cells, the balanced and proficient BER protein expression and BER capacity is

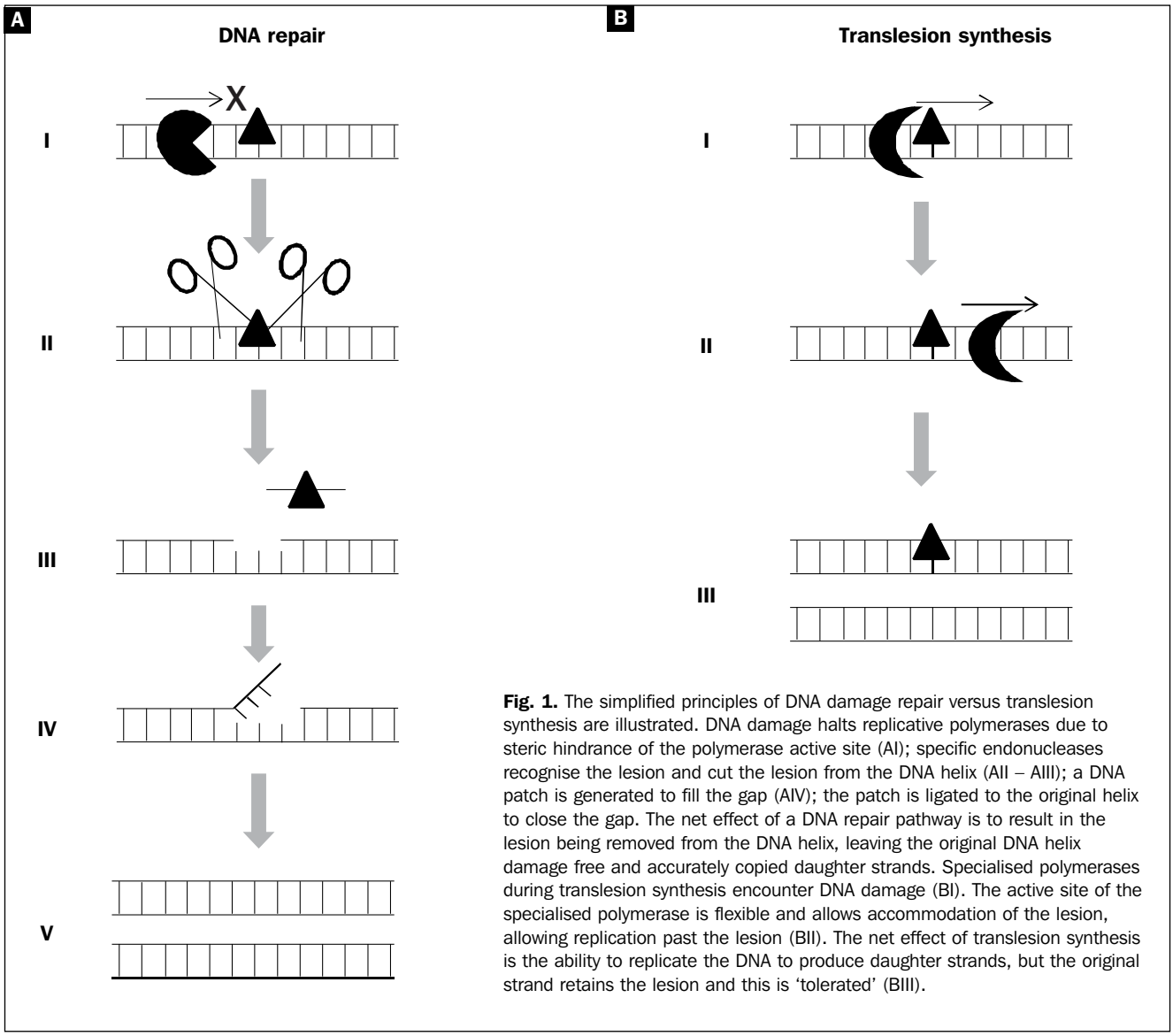

Fig. 1. The simplified principles of DNA damage repair versus translesion synthesis are illustrated. DNA damage halts replicative polymerases due to steric hindrance of the polymerase active site (AI); specific endonucleases recognise the lesion and cut the lesion from the DNA helix (AII – AIII); a DNA patch is generated to fill the gap (AIV); the patch is ligated to the original helix to close the gap. The net effect of a DNA repair pathway is to result in the lesion being removed from the DNA helix, leaving the original DNA helix damage free and accurately copied daughter strands. Specialised polymerases during translesion synthesis encounter DNA damage (BI). The active site of the specialised polymerase is flexible and allows accommodation of the lesion, allowing replication past the lesion (BII). The net effect of translesion synthesis is the ability to replicate the DNA to produce daughter strands, but the original strand retains the lesion and this is 'tolerated' (BIII).

necessary for genome stability and protection from hyperplasia and tumour formation.[56]

Nucleotide excision repair

Nucleotide excision repair (NER) is an important DNA repair mechanism necessary for repairing bulky DNA lesions such as crosslinks from bifunctional alkylating agents such as the nitrogen mustards.[57–59] Several proteins are involved in NER, and they can be categorised either as damage recognition/excision proteins or helicase proteins.[60] In brief, deformity in the DNA helix is recognised by the NER protein XPC-RAD23B, which binds to the DNA strand. The oligonucleotide is then excised on both sides of the lesion on the DNA strand. A repair patch is synthesised and DNA ligases seal the patch to the DNA.[59] Nucleotide excision repair is also the main mechanism of DNA repair and resistance to platinating agents such as cisplatin.[18,58]

Mismatch repair

Mismatch repair (MMR) is an important post-replicational repair system that plays an important role in the correction of DNA polymerase errors, either by preventing error-prone bypass replication or by correcting the formed mismatches.[61,62] Mismatch repair has also recently been shown to be involved in repairing oxidative and methylated DNA damage.[61,63] On the other hand, defective MMR can increase the risk of mutation as a result of unrepaired DNA lesions/mismatches or bypassing the lesions by error-prone DNA polymerases.[62] It has been shown that deficiency in MMR can be associated with endometrial cancer, due to the inability to repair the hypermethylation on DNA.[63] Hypermethylation of unmethylated CpG islands in the promoter regions of cancer-related genes in normal cells can prevent transcription of these genes, leading to the cell becoming cancerous.[63] Involvement of MMR in direct repair of anticancer drug-induced DNA damage has been shown, which suggests a possible role for MMR in chemotherapy resistance when DNA is the target, such as for alkylating agents.[64] Mismatch repair may also play a role as a linkage between cellular DNA damage and initiation of apoptosis.[65]

Direct repair of DNA

Direct repair of the DNA in the case of O6-alkylguanine lesions is also suggested, which can be performed by

O6-alkylguanine-DNA-alkyltransferase (AGT) and O6-methylguanine-DNA-methyltransferase (MGMT), and is limited to this type of lesion.[66] This is mainly associated with temozolomide and other alkylating agents by removing the alkyl group from the O6 position of guanine.[67] The protecting effect of MGMT has been demonstrated in human xenograft models[68] and human cell lines.[69]

DNA damage tolerance

Mammals are suggested to have at least 15 different DNA polymerases that collectively are capable of replicating DNA and repairing or tolerating DNA damage, and are subgrouped into families, largely according to their functionality.[70] High fidelity in genomic replication is maintained in mammalian cells by the function of the replicative DNA polymerases (mostly B family) which ensure the genomic stability.[71] Most of these high-fidelity DNA polymerases are incapable of replicating damaged DNA as lesions act as a replicative block.[72,73] Lesion bypass is a crucial response to unrepaired DNA damage during replication in a high genotoxic stress environment, and is performed either in an error-free (i.e., does not introduce mutation opposite the lesion) or error-prone manner (i.e., is frequently accompanied by mutation).[74,75]

The accurate replication of normal DNA requires the action of error-free DNA polymerases. However, where DNA damage cannot be repaired, in order for the cell to survive the damage it must be tolerated and this is achieved by the translesion synthesis (TLS) pathway catalysed by so-called specialised DNA polymerases.[76] These specialised DNA polymerases often have larger active sites capable of accommodating bulky lesions, and are able to insert bases opposite the damaged nucleotides.[77] One hypothesis suggests that TLS is performed using two or more of these specialised DNA polymerases, one or two for insertion of nucleotides and another polymerase for extension.[78]

Specialised DNA polymerases

The specialised DNA polymerases (also named adaptive, mutagenic or error-prone) are a group of polymerases that attempt to promote genomic stability during times of genotoxic stress; however, they show decreased fidelity when copying normal undamaged DNA, which can result in mutation and promote cancer.[45,74] Several of these polymerases participate in lesion bypass.[76] Most of these enzymes belong to the Y family of DNA polymerases that can be found in a wide variety of organisms, ranging from bacteria such as *Escherichia coli* to eukaryotes including humans. Different research illustrates that all members of this family replicate native DNA with a high error rate, but also facilitate TLS, at least *in vitro*, so they help cells to tolerate damaged DNA.[71,79] While the major replicative DNA polymerases are unable to carry out replication across the lesions such as cisplatin adducts, specialised DNA polymerases show the capability to bypass them in damaged DNA.[62,73]

Over-expression of specialised DNA polymerases enhances TLS, which helps malignant cells to cope with genotoxic effects of chemotherapeutic drugs, and increasing the mutation rate may provide cancer cells with selective benefits.[45] So far, specialised DNA polymerases have been discussed in the literature mainly with regard to their role in mutagenesis due to environmental chemicals[80–82] and less in the context of drug resistance. However, the most well-known specialised polymerases that have been shown to bypass lesions produced by chemotherapy are illustrated in Table 1. It is of note that polymerase iota (Polι) has only been shown to recognise crosslinks from UV therapy, but to date does not appear to recognise lesions from any other chemotherapy, and so will not be discussed in detail below. Similarly, the majority of work on specialised polymerases and chemotherapy has focused on cisplatin and its analogues (see Table 1).

Polβ

Polβ is the smallest DNA polymerase with a molecular mass of 39 kDa.[11] It is a housekeeping enzyme that is expressed at a low level throughout the cell cycle[93] and is inducible by some genotoxic agents such as *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine-(MNNG), methyl methanesulphonate (MMS) and *N*-acetoxy-2-acetylaminofluorene (AAAF).[94] The main characteristics of Polβ that discriminate it from other DNA polymerases include its small size, lack of associated proof-reading activity, high infidelity in replicating DNA *in vitro*, ability to perform TLS, and poor ability to discriminate nucleotides at the level of binding.[1,95] Polβ has a 3′→5′ and 5′→3′ exonuclease activity and the crystal structure of Polβ by X-ray shows a U-like cleft that suggests the ability of Polβ to accommodate DNA.[96] Polβ is unusual in that it is capable of performing both BER and TLS.[97]

Polβ plays an essential role in maintaining DNA strand integrity[98] as well as its role in DNA repair, which has been suggested by the study of mammalian cells with DNA polymerase inhibitors such as pamoic acid, trans-communic acid (CA), mahureone (MH) and masticadienonic acid (MA).[11,99] *In vitro* studies illustrate the ability of Polβ to bypass DNA lesions produced by crosslinking agents such as cisplatin and alkylating agents.[100,101]

Hypersensitivity to the cytotoxic effect of these agents has also been shown in cells deficient in Polβ, which is believed to be due to loss or decrease of BER.[102] It is known as an error-prone DNA polymerase involved in BER[100] and plays a major role in protein-protein interactions among the various BER proteins.[1] Purified Polβ from calf thymus is able to bypass platinum d(GpG) adducts placed at the N-7 position of the two adjacent guanine bases of codon 13 of the human *H-Ras* gene.[103]

Increasing evidence illustrates the elevated expression of Polβ in drug-resistant cells; high expression of Polβ was seen in a large percentage of cancer cell lines and human tumours including oesophageal, ovarian, colorectal, glioma, colon adenocarcinoma, pancreatic, prostate, kidney, stomach, lung and breast cancer.[45,56,100] Also, it has been shown that down-regulation of Polβ by small interfering RNA (siRNA) can resensitise cancer cells to cisplatin.[45] Similarly, *in vitro* tests illustrated the reduced drug sensitivity in the presence of purified Polβ when melphalan, cisplatin or mechlorethamine were used as anticancer drugs.[1]

Polκ

Polymerase kappa (Polκ) is one of the translesion polymerases and is believed to act as an extender in TLS.[75,104]

Table 1. Chemotherapeutic drugs recognised by specialised polymerases during replication.

Agent	Polβ	Polη	Polι	Polη	Polζ	References
Cisplatin and analogues	+	+			+	1, 10, 83, 84, 86, 87, 91
Melphalan	+					1
Mechlorethamine	+					1
Gemcitabine		+				10
Cytarabine		+				10
Mitomycin C				+		85, 89
Temozolomide						88
Tamoxifen	+	+		+		90
UV therapy			+			92

Chemotherapeutic agents listed are used to treat sarcomas, small cell lung cancer, non-small cell lung cancer, ovarian cancer, lymphoma, germ cell tumours, testicular cancer, multiple myeloma, malignant melanoma, prostate, pancreatic, bladder and breast cancers, acute myeloid leukaemia, acute lymphocytic leukaemia, oesophageal and anal cancer, astrocytoma. UV therapy is the only recognised lesions for Polι and may be used in the treatment of conditions such as eczema and psoriasis.

Polκ in humans is encoded by the *DINB1* gene[82] and it is the only member of the Y family polymerases with homologues in prokaryotes and archea, including the *DinB* gene in *E. coli*.[105] Polκ has an inability to bypass cisplatin adducts[82] but is capable of recognising lesions formed by mitomycin C[85,89] and tamoxifen.[90] Polκ is capable of bypassing DNA lesions in both an error-free and error-prone TLS manner *in vitro*; however, with an extraordinary low fidelity and high error rate, it is likely to promote a mutator phenotype.[74,75]

High expression of Polκ is reported in adrenal glands and ovaries with the highest levels being found in the testis.[106] Up-regulation of *Polκ* can also be seen in lung cancer.[107] It seems that transcriptional regulation of Polκ depends on p53 and inactivation of p53 promotes Polκ over-expression.[108] Over-expression of Polκ affects the fidelity of DNA replication, which demonstrates the role of Polκ in the genetic instability of cancer cells, offering a selective growth advantage to malignant cells during cancer evolution.[109]

Polη

Polymerase eta (Polη) is encoded by the *XPV* gene in humans and mutations in the gene lead to the disease xeroderma pigmentosum variant, where patients are incapable of tolerating sunlight and are prone to skin cancer.[110] This disease demonstrates the importance of TLS in the development of clinical conditions. It plays a role both in error-free and error-prone lesion bypass which has been shown by *in vitro* studies.[111] However, in addition to UV lesion tolerance, Polη is the most efficient polymerase to bypass platinum adducts such as 1,2-d(GpG) adducts due to cisplatin or oxaliplatin therapy.[112]

Polζ

Polymerase zeta (Polζ) is introduced as one of the major polymerases able to bypass cisplatin lesions.[73] It is encoded by the *REV3* gene and analysis of *REV3*-null mutants revealed that a large number of mutations are associated with the activity of Polζ.[113] Polζ participates in TLS as an extender,[73] and reduced expression of the catalytic subunit increases the sensitivity and decreases the resistance of human fibroblasts to cisplatin.[73] Most lesions produced by cisplatin are intrastrand crosslinks and it appears that Polζ is responsible both for resistance and mutagenicity of cisplatin by its error-prone replication past cisplatin adducts.[73]

In addition, Polζ is known as the only mutase that is able to add more bases when the 3′ end of the daughter strand is not very well matched with the template strand, and seems to work with one of the other several mutases to complete bypassing DNA adducts. In the case of cisplatin, it seems that it works in this capacity with Polι[114] or Polη.[115]

Evidence of the involvement of TLS and specialised DNA polymerases in drug resistance

Expression of specialised DNA polymerases in various tumour samples including kidney, breast, prostate, uterus, ovary, colon, lung, cervix and stomach has been examined by Albertella *et al.*,[45] and it was shown that specialised DNA polymerases are over-expressed in 45% of the examined tumour tissues by two-fold or more. In particular, Polβ was over-expressed in one-third of all tumours studied both at the mRNA and protein level; also down-regulation of Polβ expression by siRNA reinstated sensitivity to cisplatin, suggesting the role of Polβ in the tolerance of cisplatin-induced DNA damage.[45]

A cytotoxicity study comparing SV40-transformed Polη-deficient (XP30RO) cells and the wild-type GM637 fibroblast cells illustrates the higher sensitivity of Polη-deficient cells to cytosine arabinoside and cisplatin (approximately three-fold), gemcitabine (approximately two-fold) and a combination of gemcitabine/cisplatin (approximately 10-fold). As all these drugs affect the DNA elongation process, which can lead to cell replication inability, modulation of sensitivity due to the expression of Polη can reveal the role of this polymerase in TLS.[10] The role of Polη is confirmed further by transfecting the wild-type cell with siRNA targeting exon 11 of the Polη gene (70–75% decrease in Polη compared with the original expression) resulted in a notable increase in sensitivity of wild-type cells to the drugs.[10]

Comparison between the human ovarian tumour cell line 2008 and its cisplatin-resistant derivative (2008/C13*5.25) demonstrated no elevated glutathione level, alteration in mismatch repair proteins or increase in nucleotide excision

repair in resistant cells, but an eight-fold higher expression and higher activity of Polβ was found, suggesting bypass replication as a predominant mechanism to explain cisplatin resistance in this cell line.[100]

Similar results have been achieved using calf thymus Polβ, which could efficiently bypass the d(GpG) cisplatin crosslink adduct in DNA.[103] Involvement of Polβ in TLS leading to cisplatin resistance was also shown by inhibiting Polβ, using trans-communic acid (CA), mahureone (MH) or masticadienonic acid (MA), which increased the sensitivity to cisplatin in 2008/C13*5.25 cells.[11] Hypersensitivity of Polβ-null cells has been shown by measuring their nucleosomal degradation of DNA in the presence of alkylating agents (MNNG, MMS and maphosphamide).[102] Transfection of these cells by a Polβ-expressing vector caused a reduction in apoptosis even when the Polβ was expressed at a low level (equal or lower than 20% of Polβ expression in wild-type cells).[102]

Classification of 97 colorectal cancer patients into two groups of high and low expression of Polβ (examining their Polβ mRNA by reverse transcription polymerase chain reaction [RT-PCR]) demonstrates the higher percentage of lymph node, liver and distant metastases in the high-expression group as well as their significantly poorer prognosis when analysing their five-year overall survival.[116] In the same study, suppression of Polβ in a CaR-1 (human colon cancer) cell line by siRNA resulted in increased sensitivity to cisplatin but not to oxaliplatin, which might be due to the different structure of these two drugs.[116]

Clonogenic assays using Polζ-deficient human fibroblast cells (6I cells) that express a high level of REV3L antisense mRNA show that they are hypersensitive to cisplatin by 1.4-fold compared to the parental 9N58 cells, which were genetically engineered to express Polζ proficiently.[73] DNA analysis both in Polζ-deficient and -proficient cell lines demonstrates relatively similar initial adduct formation (3.32±0.36 versus 3.49±0.30, respectively), and there is no significant difference in adduct removal between the two cell lines.[73] As NER is a major adduct removal mechanism in cisplatin, it is proposed that Polζ functionality plays a major role in resistance to cisplatin via TLS.[73]

Investigation of TLS as a mechanism of resistance in haematological malignancies

Multiple myeloma is the most common haematological malignancy, representing 1.5% of all cancers in the UK in 2009 (17th most common cancer)[117] compared to 3% for all the leukaemias collectively (10th most common cancer in the UK).[117] Almost without exception, patients eventually become resistant to their drugs.[2–4] As melphalan, a nitrogen mustard and crosslinking agent,[118] has been a gold standard of therapy for MM,[14,15] the authors wished to explore if there was any evidence of a role for specialised polymerases and TLS in drug resistance in MM.

Several MM-resistant cell lines were developed by gradually increasing exposure to melphalan utilising methods similar to those described in Bellamy *et al.*,[17] producing resistant lines for RPMI 8226, U266 and Jim3. Analysis of the resistant cell lines showed that U266 was the most resistant to melphalan, and RPMI 8226 the least resistant. All cell lines showed some limited cross-resistance to cisplatin, mechlorethamine and chlorambucil (data not shown), which might be expected for TLS, as the lesions produced by these drugs are similar in structure and therefore likely to be recognised by the same polymerase(s). Attention was focused on Polβ as a possible candidate for recognising melphalan crosslinks, as this has previously been described to recognise these lesions.[1] With the knowledge that Polβ is capable of removing crosslinking lesions by BER,[119] as well as tolerating lesions via TLS,[97] both pathways needed consideration. However, the primary mode of removal of melphalan crosslinks is by NER (and alkylative bases by BER), so demonstration of increased repair of crosslinks would not likely be due to Polβ expression, if increased repair was shown to be a factor.

Western blot analysis demonstrated expression of Polβ in all cell lines (both sensitive and resistant), but only the RPMI 8226-resistant cell line showed raised Polβ protein levels, despite being the least resistant cell line (data not shown). Analysis of DNA damage, lesion tolerance and repair was determined using the comet assay (reviewed in[120]), which was able to indicate clearly that neither excessive DNA repair nor TLS capability appear to play a role in the development of drug resistance in MM to melphalan, at least *in vitro* (Fig. 2). The data clearly show that over the 12 months it took to generate the resistant cell lines, baseline DNA damage levels both in sensitive and resistant cell lines were the same, and there was no evidence of any accumulation of either alkylative or crosslink damage over this time. At time zero, the DNA in the comet tail is retarded, demonstrating that all cell lines were equally capable of generating crosslink lesions. The comet tail gradually increased over the next 24–72 hours, showing that the crosslinks are released by cutting with repair enzymes (Fig. 1; DNA repair steps AII to AIII) and then finally resolved back to baseline levels at 96 hours, when repaired DNA was re-ligated.

The data clearly show no accumulation of damage, either long- or short-term, demonstrating no role for TLS in melphalan resistance in MM *in vitro*. Also, there is limited evidence for increased repair, where Jim3 and U266 showed statistical difference between sensitive and resistant cells at 0–48 hours and 48–96 hours, respectively ($P<0.05$). Interestingly, inhibition of Polβ both in sensitive and resistant cell lines with oleanolic acid alone showed statistically significant differences ($P<0.05$) in cell viability, with all resistant cell lines succumbing more readily (data in preparation). This suggests that Polβ may play a role in maintaining myeloma cells following genotoxic stress, but is unlikely to play a major role in MM during chemotherapeutic exposure.

Conclusions

Based on recent findings, specialised DNA polymerases appear to play a significant role in lesion bypass and DNA repair, especially when the normal high-fidelity DNA polymerases are overwhelmed by lesions. It is evident that DNA repair and DNA damage tolerance may play a role in drug resistance in the case of chemotherapy with DNA-damaging drugs, but there is little evidence to support this in MM *in vitro*. Certainly there is accumulating evidence that

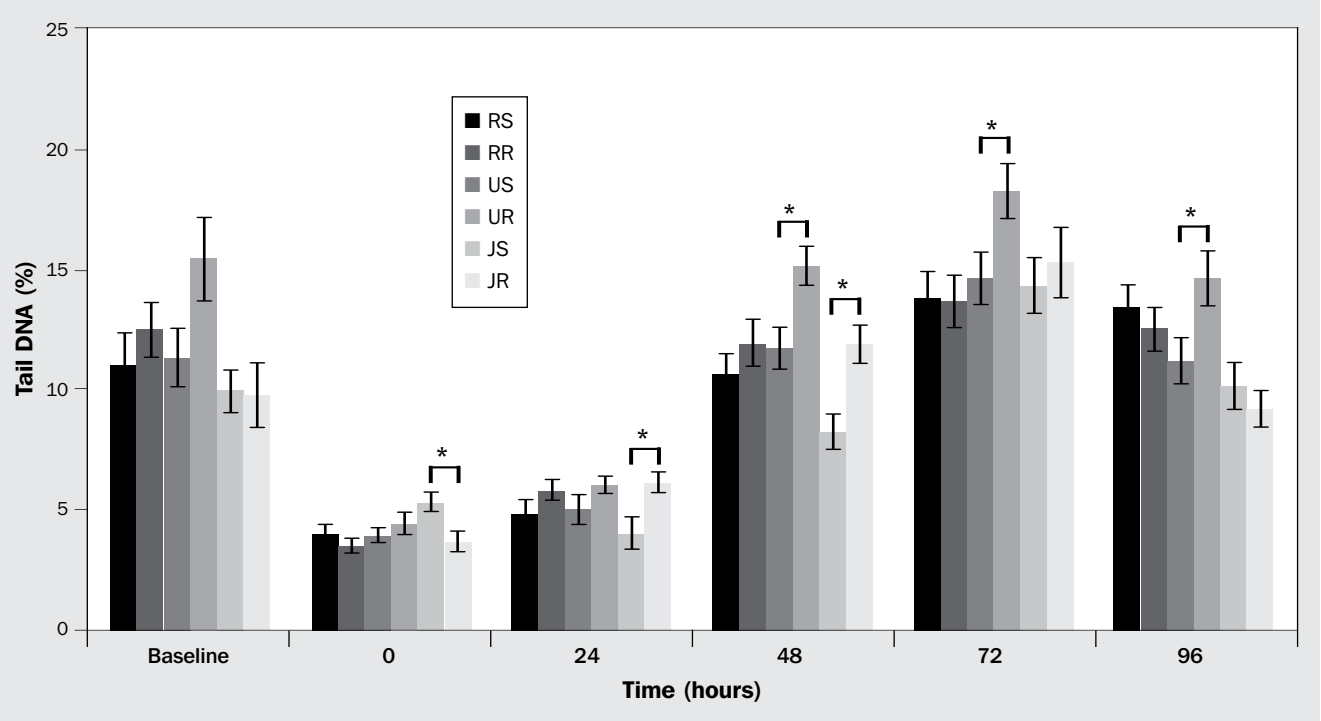

Fig. 2. Comet assay analysis represented as %DNA in the tail of sensitive and resistant multiple myeloma cells was performed at baseline, immediately after 1-h exposure to 32.8 μmol/L (clinically relevant dose) melphalan (0 h), and at each day post-exposure (24–96 h) in drug-free medium. Cells ($1x10^5$/mL) were combined with low melting agarose and transferred to a microscope slide. After lysis of the cells, electrophoresis was performed and DNA stained using SybrGreen. Comets were analysed using Comet Assay IV software (Perceptive Instruments) and represent the mean of three independent experiments. RS: RPMI 8226-sensitive; RR: RPMI 8226-resistant; US: U266-sensitive; UR: U266-resistant; JS: Jim3-sensitive; JR: Jim3-resistant (*$P<0.05$).

TLS can be suggested as a major mechanism of drug resistance for cisplatin, which has been most widely studied,[11,62,103,112,115] and therefore further research is required with the haematological malignancies and a range of drugs before these mechanisms can be discounted.

To date, specialised DNA polymerases are better defined according to the environmental chemical lesions that they evolved to recognise, as well as with respect to their mutagenesis potential. Less is known of their critical role in drug resistance, and in particular which DNA lesions produced by chemotherapy they might recognise. In addition, much of the research has investigated these polymerases in solid tumours, with limited information on their role in haematological malignancies.[13] Future study in this area may lead to a new generation of chemotherapeutic drugs that impair the function of specialised DNA polymerases or form lesions to block these polymerases;[121] however, such approaches are likely to compromise normal cells and thus will require full evaluation prior to use. □

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