Drug resistance and DNA repair in leukaemia

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Abstract

Most cytotoxic agents exert their action via damage of DNA. Therefore, the repair of such lesions is of major importance for the sensitivity of malignant cells to chemotherapeutic agents. The underlying mechanisms of various DNA repair pathways have extensively been studied in yeast, bacteria and mammalian cells. Sensitive and drug resistant cancer cell lines have provided models for analysis of the contribution of DNA repair to chemosensitivity. However, the validity of results obtained by laboratory experiments with regard to the clinical situation is limited. In both acute and chronic leukaemias, the emergence of drug resistant cells is a major cause for treatment failure. Recently, assays have become available to measure cellular DNA repair capacity in clinical specimens at the single-cell level. Application of these assays to isolated lymphocytes from patients with chronic lymphatic leukaemia (CLL) revealed large interindividual differences in DNA repair rates. Accelerated O^6-ethylguanine elimination from DNA and faster processing of repair-induced single-strand breaks were found in CLL lymphocytes from patients nonresponsive to chemotherapy with alkylating agents compared to untreated or treated sensitive patients. Moreover, modulators of DNA repair with different target mechanisms were identified which also influence the sensitivity of cancer cells to alkylating agents. In this article, we review the current knowledge about the contribution of DNA repair to drug resistance in human leukaemia.

Abbreviations: AGT – O^6-alkylguanine-DNA alkyltransferase; AML – acute myeloid leukaemia; ALL – acute lymphatic leukaemia; AP – apurinic site; BER – base excision repair; CLL – chronic lymphatic leukaemia; CML – chronic myeloid leukaemia; EtNU – *N*-ethyl-*N*-nitrosourea; ICA – immunocytological analysis; LRP – lung resistance related protein; MDR – multiple drug resistance; MMR – mismatch repair; MRP – multiple drug resistance-related protein; NER – nucleotide excision repair; O^6-ethylguanine – O^6-EtGua; PARP – poly(ADP-ribose)polymerase; SCGE – single-cell gel electrophoresis; TCR – transcription-coupled repair.

Introduction

Drug resistance remains a major obstacle in the chemotherapy of acute and chronic leukaemias. After initial therapeutic responses, many patients develop recurrent disease which is often refractory to chemotherapy due to the emergence of drug resistant cell clones. Much attention has been paid to the elucidation of cellular mechanisms responsible for drug re-

sistance in experimental models. Cell lines have been established from patients before and after treatment, and resistant sublines were developed from parent cell lines by *in vitro* selection procedures. These models allowed the characterisation of cellular resistance mechanisms, although it is important to consider the limitations of laboratory experiments. Selection pressures which occur in deriving a cell line from primary malignant cells will inevitably change the original

phenotype. The validity of these models in relation to the clinical situation can, therefore, be questioned. However, information gained from such experiments may be used to assess the clinical relevance of molecular mechanisms of resistance in leukaemic cells from patients with haematological malignancies.

One line of research investigated the putative major site of drug action as the most likely target involved in drug resistance, i.e. the action of topoisomerase II inhibitors such as anthracyclines or etoposide (Valkov and Sullivan, 1997). However, nonspecific detoxifying mechanisms, such as transport-proteins, may also confer resistance to a variety of cytotoxic drugs. The prototype of drug efflux pumps that mediate the classical multiple drug resistance phenotype (MDR), P-glycoprotein, was first described by Juliano and Ling (1976). Expression of P-glycoprotein causes resistance to a broad range of structurally unrelated anticancer drugs, such as anthracyclines and vinca alkaloids, which are important agents in the treatment of leukaemias. In the early nineties, it has been shown that P-glycoprotein expression correlates with clinical drug resistance in acute myeloid leukaemias (AML)(Pirker et al., 1991). However, experimental and clinical studies indicate that further cellular mechanisms of resistance contribute to treatment failure in leukaemias. Recently, it was found that a second membrane transporter, the MDR-related protein (MRP), belonging to the same ATP-binding cassette (ABC) superfamily as P-glycoprotein, also confers resistance to a variety of cytotoxic agents (Cole et al., 1992). A further drug pump, the lung resistance protein (LRP), has been linked to the MDR phenotype (List, 1997). At present, only few studies have addressed the clinical significance of MRP or LRP expression for the development of drug resistance in leukaemia (Filipits et al., 1997). It is, however, clear that leukaemic cells possess a battery of different mechanisms of resistance which may operate synergistically in the clinical setting.

As most chemotherapeutic agents exert their action via DNA damage, the repair of drug induced DNA lesions remains another cellular defence mechanism whose clinical importance remains to be explored. The integrity of the genome is preserved by a complex network of repair processes designed to eliminate cytotoxic and mutagenic lesions or mismatched nucleotides from DNA. Generally, repair mechanisms in mammalian cells can be classified into four categories depending on the basic molecular reactions involved 1) direct repair 2) base excision repair (BER), 3) nucleotide excision repair (NER) and 4) mismatch repair (MMR) (Figure 1).

In recent years, strategies have become available to assess and modify the DNA repair capacity of leukaemic cells in patients: i) methods have been developed to measure either the expression or the activity of DNA repair pathways at the single-cell level in heterogeneous tumour cell populations, and ii) DNA repair modulators are being developed which influence specific steps of repair processes and thereby increase the cytotoxicity of alkylating agents. Moreover, genetherapeutic approaches utilising DNA repair proteins are being developed; e.g. the transfection of stem cells with genes encoding specific DNA repair proteins with the aim to reduce haematopoetic toxicity of alkylating agents (Wang et al., 1996). At present, these studies are mainly designed to improve the therapeutic index of chemotherapy in solid tumours, and will, thus, not be further reviewed here. The contributions of different DNA repair processes to drug resistance in vitro have been recently reviewed (Chaney and Sancar, 1996). In this article we shall try to summarise our knowledge about the contribution of DNA repair to clinical drug resistance in leukaemias.

DNA Alkylation Damage and the Corresponding Repair Pathways

In one-step DNA repair, alkyl groups covalently bound to the O^6-atom of guanine in DNA are eliminated in a single step by the repair protein O^6-alkylguanine-DNA alkyltransferase (AGT; EC 2.1.1.63; Pegg, 1990). This 22-kDa polypeptide restores the integrity of DNA by transfer of the alkyl group from guanine to one of its own cysteine residues in a suicidal reaction. This pathway eliminates very efficiently O^6-methylguanine from DNA. Larger residues attached to the O^6-atom of guanine, e.g., ethyl or butyl residues, can be removed in parallel by alternative mechanisms such as excision repair (Thomale et al., 1994; Engelbergs et al., 1998).

During excision repair, a damaged base or nucleotide is cut off from a DNA strand and replaced by a regular base using the complementary strand as a template (Sancar, 1994, 1995; Cunningham, 1997). In case of BER, the damaged base is released by a DNA glycosylase and then the abasic sugar moiety (apurinic/apyrimidinic (AP) site) is excised by an AP endonuclease. A polymerase fills the resulting gap and the nick is sealed by a ligase. Various glycosylases
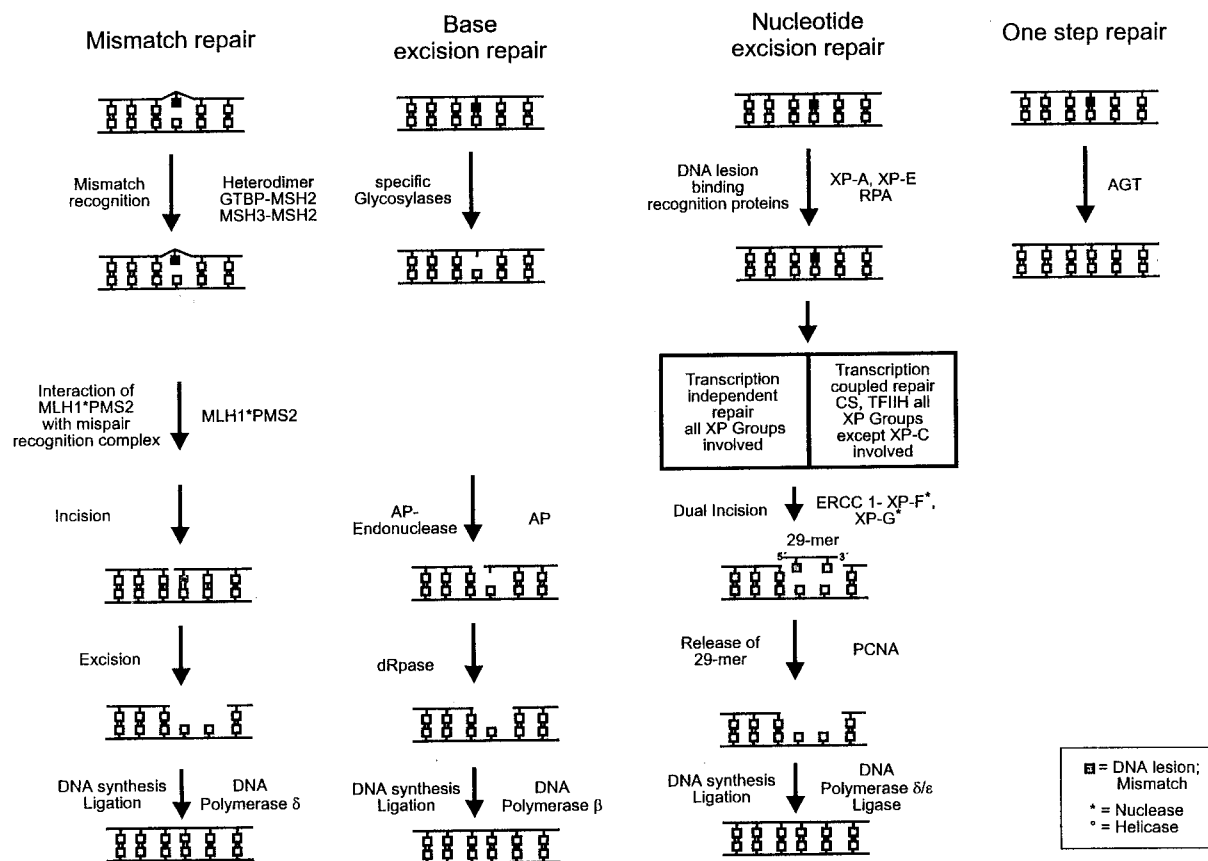
Figure 1. Pathways of DNA repair in mammalian cells.

have been identified with different substrate specifities. The glycocylases involved in BER have a narrow substrate range; they are in close contact with the lesion during cleavage of the N-glycosyl bond. This includes endogenous DNA damage, e.g. induced by oxygen stress, and exogenous damage, e.g. caused by alkylating agents.

Helix-distorting DNA damage as induced by 'bulky' alkylating agents is predominantly repaired by the NER (Lindahl *et al.*, 1997). This repair system hydrolyses two phosphodiester bonds, one on either side of the lesion, to release an oligonucleotide carrying the damage. The cleavage properties of the nucleases suggest strongly an opened DNA structure as repair intermediate. Opening of a sequence of at least 25 base-pairs appears to occur before the dual incision by 5' and 3' specific endonucleases. The excised oligonucleotide is released from the helix, and the resulting gap is then filled in and ligated to complete the repair reaction. The incision pattern is rather precise and, depending on the size of the lesion, the patch is removed

as a 27 to 29 nt oligomer in eukaryotic cells. Bulky lesions, however, are not the only substrates for NER. Smaller lesions, such as O^6-alkylguanines, which do not distort the helix to a greater extent, are also substrates for this repair pathway. In mammalian cells, 16 polypeptides including seven xeroderma pigmentosum (XP)-related proteins, the trimeric replication protein A (RPA) human single-stranded DNA binding protein (HSSB), excision repair cross complementing (ERCC1)-containing complex, and the multisubunit general transcription factor TFIIH are required for damage recognition and dual incisions. NER may work either by sequential assembly of factors, or by the action of a preformed 'repairosome', or by an interaction of intermediate subassemblies. Indicating the coregulation of different DNA repair proteins, similar expression levels of three genes involved in NER (ERCC1, ERCC2, ERCC6) have been found within one individal (Dabholkar *et al.*, 1993).

Regarding NER, an important point is the preference of one of its subpathways for transcribed regions

of the genome (so-called transcription-coupled repair, TCR). In TCR, the transcribed strand of an active gene is repaired more rapidly than the nontranscribed strand. Some authors have suggested that TCR is a better indicator of the cellular response to geno- and cytotoxic agents than the slower overall repair representative of the nontranscribed sequences of genomic DNA (Petersen *et al.*, 1996).

DNA interstrand crosslinks constitute a significant fraction of the DNA lesions induced, e.g., by bi-functional alkylating agents and are considered to be the major cytotoxic damage caused by these drugs. No pathway has been clearly defined yet for the repair of interstrand crosslinks; however, it is assumed that these lesions are eliminated from DNA by the combined action of excision repair and recombination. Preliminary experimental data suggest that BER also contributes to the processing of DNA interstrand cross-links (unpublished results).

Poly(ADP-ribose)polymerase (PARP; EC 2.4.2.30) is a 116 kDa nuclear enzyme which catalyses the formation of long homopolymers of ADP-ribose from NAD^+ in response to DNA strand breaks (Sancar, 1995). Poly(ADP-ribose) synthesis leads to structural alterations in chromatin, resulting from the electrostatic repulsion from DNA of the negatively charged PARP and modified histones, thus allowing the access of repair enzymes to damaged DNA. A further mechanism involves the binding of unmodified PARP to free DNA ends, thus preventing further processing. PARP is released after automodification allowing gap filling and ligation to occur.

In recent years much attention has been paid to the investigation of mismatch repair (Modrich, 1994; Karran and Hampson, 1996; Kolodner, 1995). Mismatch repair proteins correct polymerase errors during DNA replication. Mutations in human homologues of the bacterial genes MutS and MutL, which play key roles in mismatch recognition and repair, play a crucial role in hereditary nonpolyposis colorectal carcinoma and are implicated in some sporadic colorectal cancers and secondary leukaemias (Kolodner, 1995).

DNA Repair and Cytotoxic Drug Resistance: Experimental Data

Different alkylating anticancer drugs are contained in protocols for the treatment of haematological malignancies including high- and low-grade lymphomas, chronic lymphatic leukaemias (CLL), chronic myeloid leukaemias (CML) and acute lymphatic leukaemias (ALL). Cisplatin, which also binds covalently to DNA forming intra- and interstrand crosslinks, is not as efficient as conventional alkylating agents in the therapy of haematological malignancies. In acute myeloid leukaemias (AML), alkylating agents are usually not contained in first-line chemotherapeutic regimens due to their low therapeutic efficacy.

Several studies have examined DNA repair mechanisms in cell lines sensitive or resistant to various types of alkylating agents and to cisplatin. These experimental models have provided insights into the relationship between DNA repair and chemoresistance (Chaney and Sancar, 1996). However, it is important to recognise the limitations of *in vitro* models with regard to the complexity of the DNA repair network and its multiple functional links to downstream processes initiating cell death (apoptosis). An increased repair rate of a given DNA adduct does not specify the particular repair pathway involved. Furthermore, the observation that distinct repair proteins are present at higher levels in resistant cell lines does not necessarily prove that the efficiency of a multiprotein repair pathway is enhanced. Reduced expression or loss of specific DNA repair proteins, such as 3-methyladenine DNA glycosylase (Engelward *et al.*, 1996), the ERCC1 protein (Cappelli *et al.*, 1995), DNA topoisomerase II alpha (Eder *et al.*, 1995), or the damage recognition protein XP-A (Cleaver *et al.*, 1995), increase cellular sensitivity to DNA-damaging drugs. However, diminished rather than increased, resistance to alkylating agents has been observed in hamster cells overexpressing 3-methyladenine DNA glycosylase, which catalyses an early step in BER (Ibeanu *et al.*, 1992). It is likely that imbalanced expression of constituent proteins of a given repair pathway may lead to accumulation of repair intermediates such as single-strand breaks, which may contribute significantly to the cytotoxicity of alkylators in addition to the primary DNA alkylation products.

In mammalian cell lines, elevated AGT levels confer increased cellular resistance to the cytotoxic effects of methylating or chloroethylating agents and, conversely, depletion of cellular AGT activity by the inhibitor O^6-benzylguanine sensitises mammalian cells to these drugs (Pieper *et al.*, 1991; Müller *et al.*, 1993). Cross resistance studies in AGT proficient and deficient cell lines have revealed that increased expression of this protein provides protection against the cytotoxic effect of nitrosoureas but not to bulky alkylating agents such as mafosfamide or chlorambucil (Preuss

et al., 1996). Recent studies with cell lines and mice either deficient in AGT alone or carrying a double-knockout for AGT and MSH2 (MMR) indicate that the persistence of O^6-alkylguanines *per se* is not the major trigger of cytotoxicity. Rather the futile attempt of the MMR system to repair the mismatched base pair by excision and resynthesis of the opposite strand lead to genomic instability and cell death (Modrich, 1994).

The repair capacity for damage of pRSV-CAT plasmid was significantly elevated in a human breast cancer cell line resistant to L-phenylalanine mustard; however, no differences were observed between the sensitive parent line and resistant subline regarding the mRNA expression of the NER genes ERCC1, XPD (ERCC2), XPB (ERCC3) and polymerase beta (Yen *et al.*, 1995).

The initial level of cisplatin-DNA binding – as measured by quantitative immunocytochemistry using an antiserum against cisplatin modified DNA – correlated to sensitivity to cisplatin in several different mammalian cell lines (Terheggen *et al.*, 1990). Correspondingly, repair of cisplatin-interstrand crosslinks was enhanced in cisplatin-resistant vs. -sensitive cell lines (Johnson *et al.*, 1994). However, no correlation between the cytotoxicity of cisplatin and the formation or removal of cisplatin-adducts or the overall platination level of DNA was observed in immunocytoma cell lines (Vendrik *et al.*, 1997).

Regarding TCR, enhanced interstrand crosslink repair in transcribed genes was found in cisplatin-resistant cell lines compared to sensitive parent lines, whereas no differences were observed between the overall genomic repair activity of both cell lines (Petersen *et al.*, 1996).

Treatment of human ovarian carcinoma cell lines with cisplatin *in vitro* can induce mutations that functionally alter DNA mismatch repair proteins, and – paradoxically at first glance – this loss of MMR activity results in increased resistance to cisplatin in these cells (Aebi *et al.*, 1996). In colorectal carcinoma cell lines defective in MMR, the increased resistance to methylating agents overrides the dependence of resistance on AGT and its inhibition by O^6-benzylguanine. However, AGT mediates resistance to chloroethylating agents in these cells (Liu *et al.*, 1996).

Resistance of cells to 4-hydroperoxy-cyclophosphamide appear to be mediated by multiple mechanisms, including elevated levels of aldehyde dehydrogenases and decreased induction and tolerance to interstrand crosslink formation (Andersson *et al.*, 1994). In general, resistance of cells to alkylating agents

and platinum compounds appears to be multifactorial not only with regard to the involvement of different DNA repair pathways but also concerning detoxifying mechanisms unrelated to repair.

Interindividual Variation in the Activity of DNA Repair Pathways

'No one supposes that all the individuals of the same species are cast in the very same mould. These individual differences are highly important for us...' (Charles Darwin, The Origin of Species, 1859). Substantial interindividual differences in the expression or activity of DNA repair proteins in humans have been described. Person-to-person differences were found in the activity or expression of specific DNA repair proteins such as AGT (Waldstein *et al.*, 1982; Gerson *et al.*, 1985). Variation in the expression of AGT was also observed between different human normal tissues, e.g. being high in liver and very low in bone marrow myeloid precursors (Gerson *et al.*, 1986). Furthermore, the levels of AGT activity in different tissues and cell types vary greatly between species (Gerson *et al.*, 1986). The degree of variability has not so far been studied systematically for other DNA repair pathways. However, a wide range in the expression-levels of genes involved in NER (ERCC1, ERCC2 and ERCC6) was observed in bone marrow specimens from cancer patients (Dabholkar *et al.*, 1993).

Nature may allow variability in the function of an important cellular defence system such as DNA repair to promote genetic diversity within species. A 'side-effect' of this phenomenon is, however, increased risk for cancer in individuals having low repair capacity for mutagenic lesions (Goth and Rajewsky, 1974). E.g., lack of AGT in histologically normal brain adjacent to primary human tumours has been proposed as a predisposing factor for human brain cancer (Silber *et al.*, 1996).

Clinical Significance of DNA Repair as a Mechanism of Resistance to Chemotherapy in Leukaemia

It is not yet known whether this wide interindividual range in DNA repair phenotypes translates into clinical responsiveness to treatment with DNA-reactive anti-cancer drugs. Regarding solid tumours, this lack of knowledge is partly due to the current absence the of

methods sufficiently sensitive to measure DNA repair kinetics in individual cells of heterogeneous biopsy samples. On the other hand, haematological malignancies are particularly suited to study the development of resistance due to the easy accessibility of malignant cells, which can be enriched from peripheral blood. The resulting cell suspensions usually contain relatively few nonmalignant cells. However, even those contaminations can lead to false results in the case of very sensitive assays such as polymerase chain reaction. Regarding P-glycoprotein expression as a mechanism of resistance, a consensus conference recently recommended single-cell assays to be applied to clinical specimens (Beck *et al.*, 1996). As alkylating agents play a major role in first-line chemotherapy regimens, CLL represents an excellent model for studying the clinical significance of DNA repair as a determinant of drug resistance. At the time of diagnosis of CLL, intensive treatment aiming at cure remains the rare exception. If necessary, oral chemotherapy with the alkylating DNA crosslinker chlorambucil is generally used to alleviate clinical symptoms and to reduce peripheral lymphocyte counts. During the chronic course of the disease, a proportion of CLL patients gradually becomes resistant to this regimen. In extracts from lymphocytes of CLL patients nonresponsive to chlorambucil, an increased expression of the NER protein ERCC1 and elevated activity of 3-methyladenine-DNA glycosylase (BER), were observed (Geleziunas *et al.*, 1991). Elimination kinetics of crosslinks suggested that lymphocytes from patients with resistant CLL compared to untreated patients have an enhanced capacity to repair these lesions (Torres-Garcia *et al.*, 1989). Furthermore, the amount of melphalan-induced interstrand crosslinks in DNA from lymphocytes from resistant CLL patients was found to be significantly lower compared to untreated patients (Panasci *et al.*, 1988). Neither a transport defect nor altered intracellular melphalan levels appeared to contribute to this effect. NER activity (incision and repair synthesis) has been measured in extracts from CLL lymphocytes. It was noted that NER activity was elevated in CLL lymphocytes from pretreated compared to untreated patients (Barret *et al.*, 1996). Interestingly, NER activity was measurable in isolated normal lymphocytes only when these were stimulated to proliferate (Barret *et al.*, 1995).

Other studies, however, did not find a relation between the repair of crosslinks or the expression of various DNA repair genes and treatment outcome in leukaemia patients (Joncourt *et al.*, 1993; Bramson *et al.*, 1995). No difference in the repair of chlorambucil-induced DNA crosslinks in CLL lymphocytes was observed between patients either sensitive or resistant to treatment with this alkylating agent (Begleiter *et al.*, 1991). The clinical significance of DNA repair as a major determinant of drug resistance thus remained unclear in these cases.

In our own studies, a monoclonal-antibody based immunocytological assay (ICA) has been used as a functional test for the accumulation and repair of a specific alkylation product, O^6-ethylguanine (O^6-EtGua), in the nuclear DNA of individual leukaemic cells (Seiler *et al.*, 1993; Thomale *et al.*, 1994). After pulse-exposure using the model alkylating agent *N*-ethyl-*N*-nitrosourea *in vitro*, O^6-EtGua was stained using a monoclonal antibody and elimination kinetics for this DNA alkylation product was determined (Figure 2). Thereby, a wide range of different O^6-EtGua repair phenotypes was observed in specimens derived from individual leukaemia patients. Inhibition of AGT by O^6-benzylguanine revealed that O^6-EtGua is removed from DNA by both AGT and excision repair (Thomale *et al.*, 1994). Regarding clinical drug resistance, CLL lymphocytes of patients nonresponsive to alkylating agents displayed higher rates of O^6-EtGua elimination in comparison to responsive patients (Müller *et al.*, 1994). Additionally, a single-cell gel electrophoresis (SCGE, 'comet' assay) was applied to study the time course of formation and persistence of repair-induced intermediates such as abasic sites and single-strand breaks (Olive *et al.*, 1991; Buschfort *et al.*, 1997). Using this assay, CLL lymphocytes from nonresponsive patients exhibited faster processing of secondary repair intermediates compared to untreated or sensitive patients (Müller *et al.*, 1997). Both observations underline the clinical significance of DNA repair as a determinant of resistance to alkylating agents in leukaemic cells. Future studies must elucidate whether enhanced DNA repair in resistant CLL lymphocytes occurs in response to systemic treatment or is the result of the selective survival and outgrowth of repair-competent, resistant cells from the malignant cell population. Interestingly, the treatment of lung cancer patients with chlorambucil failed to induce *in vitro* resistance in normal lymphocytes, possibly indicating a lack of (short-term) induction of DNA repair proteins in these cells (Bentley *et al.*, 1996).

The ICA assay measures the combined overall efficiency of all mechanisms which eliminate O^6-EtGua from DNA including AGT and excision repair. Later
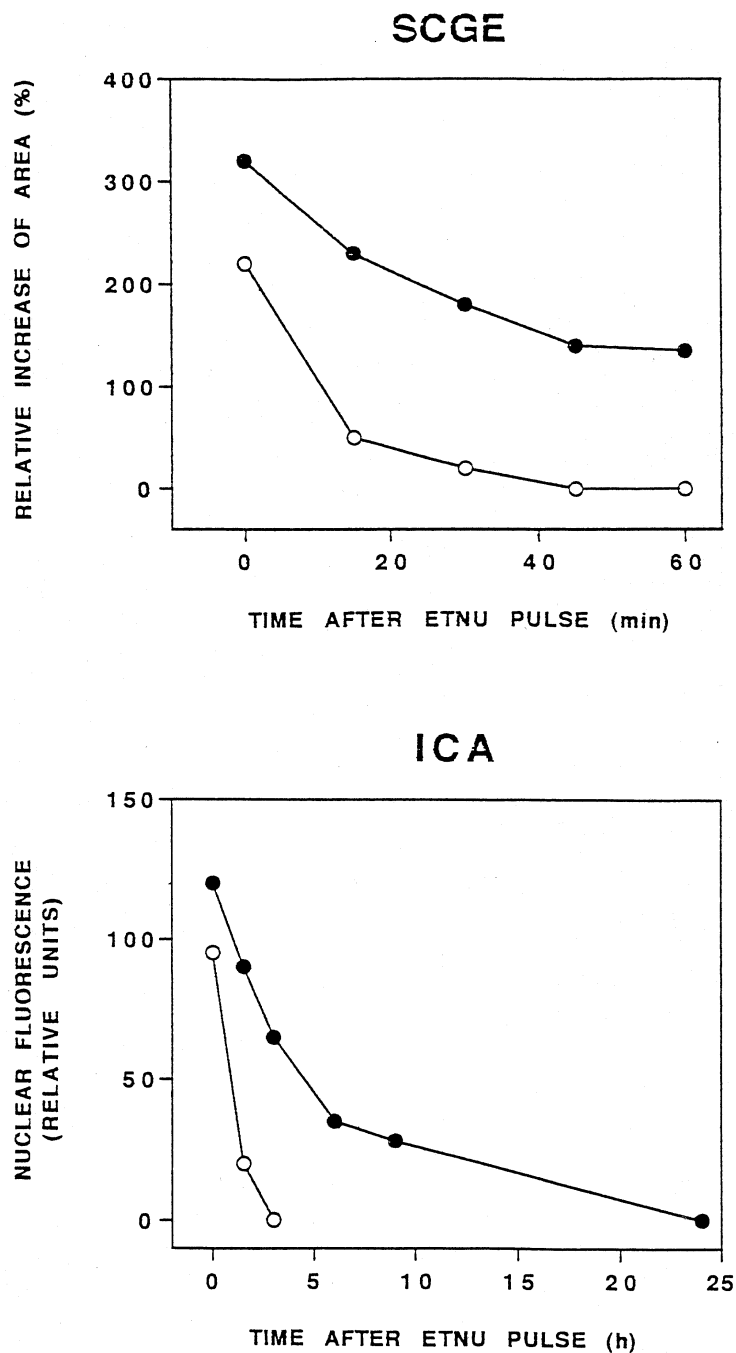
SCGE

ICA

Figure 2. Kinetics of comet formation and disappearance (SCGE, 'comet assay', top) and of the elimination of O^6-ethylguanine from nuclear DNA (ICA, bottom) in two specimens of CLL lymphocytes after 20 min exposure to EtNU. Curves were obtained from two CLL patients who were either sensitive (closed symbols) or resistant (open symbols) to treatment with alkylating agents. In case of SCGE, the area of stained nuclear DNA is given as the increase relative to untreated control cells. Regarding ICA, mean values of relative nuclear fluorescence signals of 100 cells/time point are plotted. The $t_{50\%}$ repair values – reflecting either the time required to reduce the initial amount of single-strand breaks by 50% (SCGE) or to repair 50% of induced O^6-EtGua initially formed in DNA (ICA) – were determined graphically.
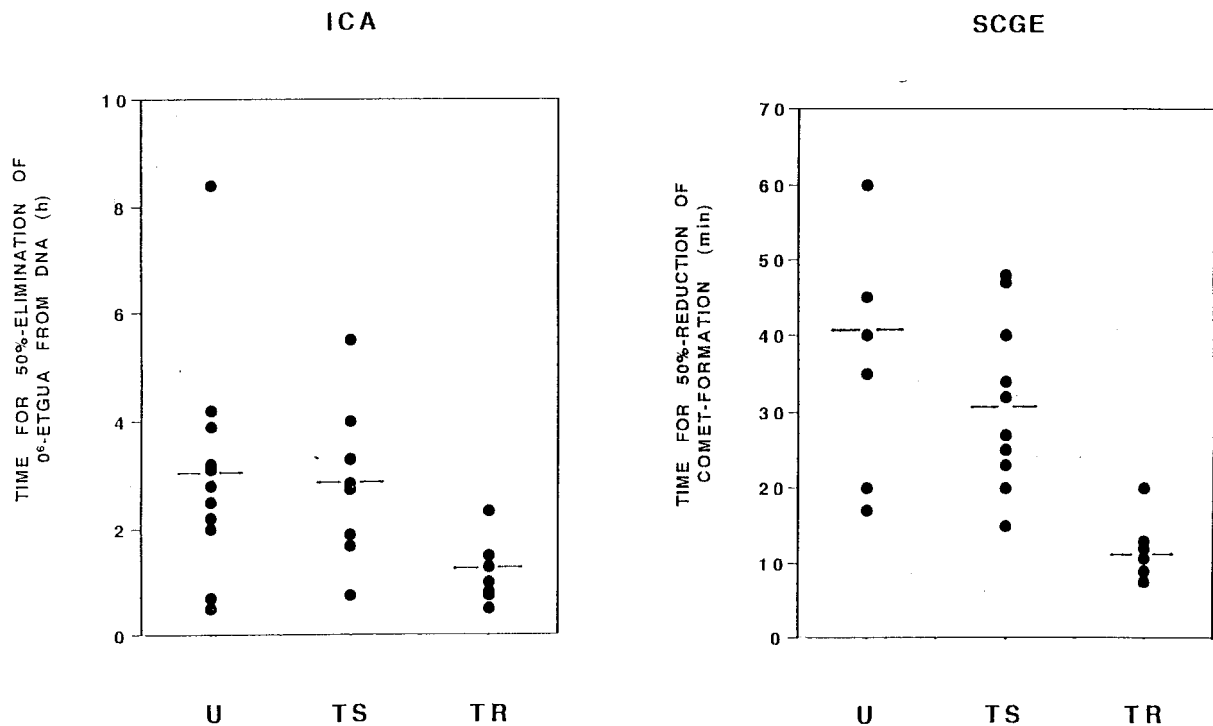
ICA   SCGE

Figure 3. Repair of O^6-EtGua (ICA) and single-strand breaks (SCGE) in the nuclear DNA of CLL lymphocytes in relation to treatment outcome. The t$_{50\%}$ repair values (see Figure 2) were determined in specimens obtained from CLL patients who were either untreated (U), sensitive (TS) or resistant (TR) to treatment with alkylating agents.

stages of damage processing, such as gap filling and rejoining of DNA strands, are monitored by the comet assay (SCGE). Furthermore, the relative repair rates determined by these functional assays covering different areas of the DNA repair network were correlated (Müller *et al.*, 1997). This correlation suggests at least partial coordination of different rate-limiting repair steps. It remains to be determined whether this reflects the predominant action of one particular step measured by both assays, or rather coregulation of key components of distinct repair pathways. However, it is clear that both functional assays could potentially be employed to predict clinical resistance in patients with leukaemia.

Indirect evidence for the importance of DNA repair as a mechanism of resistance was obtained by assessing the profiles of *in vitro* chemosensitivity of CLL lymphocytes to a variety of alkylating agents. Lymphocytes of CLL patients displayed cross-resistance between bifunctional bulky alkylating agents such as chlorambucil and melphalan, but not methyl methane sulfonate and UV light, indicating that neither BER nor NER, but rather crosslink repair is rate-limiting in resistance (Bramson *et al.*, 1995). Cross-resistance

has also been observed between mono- and bifunctional alkylating agents in CLL lymphocytes suggesting common repair mechanisms for cytotoxic lesions (Müller *et al.*, 1997). Increased *in vitro* chemoresistance to alkylating agents was observed in CLL lymphocytes exhibiting a 'fast' DNA repair phenotype. This underlines the importance of DNA repair as a mechanism of cellular resistance to alkylating agents in human leukaemic cells.

Compared to CLL, much less is known about the contribution of DNA repair to drug resistance in CML, ALL or AML. Increased levels of AGT were observed in ALL and AML blasts compared to normal bone marrow, but no relationship was found to the treatment outcome of these patients (Joncourt *et al.*, 1993). In CML cells, AGT activity was relatively low, possibly reflecting the sensitivity of these cells to nitrosoureas. We investigated the repair of O^6-EtGua in the DNA of isolated blasts derived from AML patients using the ICA assay (Müller *et al.*, 1994). No correlation was found between DNA repair time and treatment outcome in AML patients. This is probably due to the fact that MDR-related drugs but not alkylating agents were contained in the treatment regimens for these patients.

Future Perspectives: Strategies for Modulating Repair Activity

Several agents are known that interfere with specific steps of DNA repair pathways. These compounds also modulate the sensitivity of cancer cells to DNA-reactive chemotherapeutic agents. Enhancement of the cytotoxicity of alkylating agents by methylxanthines such as caffeine or pentoxifylline has been known for more than 25 years (Walker and Reid, 1971). The underlying mechanisms are probably the inhibition of excision repair proteins or cell cycle effects. Differential sensitisation to alkylating agents was observed in CLL lymphocytes and AML blasts, e.g. AML blasts were more sensitive to mafosfamide than CLL lymphocytes following pretreatment with pentoxifylline. However, sensitisation by pentoxifylline was not observed to other alkylators such as 1,3-bis(2-chloroethyl)-1-nitrosourea and dacarbazine in either cell type (Müller *et al.*, 1993). The AGT inhibitor O^6-benzylguanine sensitised CLL lymphocytes but not AML blasts to the DNA-methylating agent dacarbazine. Interestingly, a chemosensitising effect of both repair modulators was found in subgroups but not in the total population of leukaemia patients. Future studies should, therefore, relate the observed sensitising effect of O^6-benzylguanine and pentoxifylline to a specific DNA repair mechanism.

A different target for the modulation of cellular chemosensitivity to alkylating agents is PARP. PARP inhibitors were first developed by Purnell and Whish (1980), one of them being 3-amino-benzamide. PARP has been identified as a damage recognition protein in CLL lymphocytes exposed to melphalan *in vitro* (Bramson *et al.*, 1993). Application of PARP inhibitors retards the rejoining of DNA strand breaks and potentiates the cytoxicity of DNA-damaging agents such as temozolomide or radiotherapy in cell lines (Boulton *et al.*, 1995; Griffin *et al.*, 1995). Benzamide also increases the fraction of apoptotic human leukaemic cells after treatment with the methylating agent temozolomide (Tentori *et al.*, 1997).

Aphidicolin, a tetracyclic diterpenoid antibiotic obtained from Cephalosporium aphidicola, has been shown to inhibit DNA repair by adhering to nucleotide binding sites on DNA polymerases alpha and delta, and thereby to prevent long-patch excision repair of platinum-induced DNA lesions (Beketic-Orescovic and Osmak, 1995). Aphidicolin overcomes platinum resistance in fresh biopsy samples or ascites cells from ovarian cancer patients (Sargent *et al.*, 1996).

Fludarabine, a purine analogue, is currently evaluated for the treatment of CLL and other low-grade lymphomas. Fludarabine was shown to inhibit NER of cisplatin-DNA adducts at the steps of incision and repair synthesis (Li *et al.*, 1997). In cell lines, the combination of fludarabine with cisplatin resulted in synergistic cytotoxicity accompanied by a reduced removal of interstrand crosslinks (Yang *et al.*, 1995).

To summarise, a battery of repair modulators with different targets in the DNA repair network is known which also influence chemosensitivity in leukaemic cells. Differential patterns of sensitisation were observed in leukaemic cells from individual patients, depending on the target molecule of the repair modulator, the alkylating agent and the target cell type. These modulation approaches, however, face problems because of the likelihood that multiple resistance mechanisms – besides DNA repair – operate in the clinical setting. Strategies to circumvent drug resistance in the clinic should, therefore, also address this question.

In conclusion, the vast amount of information in the fields of DNA repair and other mechanisms underlying drug resistance must be merged and ultimately exploited to improve the treatment of leukaemias. Monitoring DNA repair in leukaemia patients using functional assays such as ICA and SCGE provides a means to facilitate the rational design of chemotherapeutic regimens.

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