2 Role of Nonhomologous End-Joining and Recombinational DNA Repair in Resistance to Nitrogen Mustard and DNA Crosslinking Agents

Lawrence C. Panasci, MD, Zhi-Yuan Xu, MD, and Raquel Aloyz, PhD

CONTENTS

From: *Cancer Drug Discovery and Development: DNA Repair in Cancer Therapy*
Edited by: L. C. Panasci and M. A. Alaoui-Jamali © Humana Press Inc., Totowa, NJ

1. RESISTANCE TO THE NITROGEN MUSTARDS

The nitrogen mustards are an important group of alkylating agents with activity against several human tumors *(1–4)*. Many nitrogen mustard analogs are transported by carrier-mediated systems into cells and alkylate DNA, RNA, and proteins *(5–7)*. Alkylation of DNA and, more specifically, the formation of DNA interstrand crosslinks have been considered to be responsible for their cytotoxicity *(8–10)*. Resistance to the nitrogen mustards in murine and human tumor cells has been reported to be secondary to (1) alterations in the transport of these agents *(11)*, (2) alterations in the kinetics of the DNA crosslinks formed by these agents *(9,10,12)*, (3) cytoplasmic metabolism of the chloroethyl alkylating moiety to the inactive hydroxyethyl derivative *(13)* via glutathione (GSH)/glutathione-*S*-transferase (GST) *(14–16)*, (4) overexpression of metallothionein, which confers resistance to cis-platinum and cross-resistance to melphalan *(17)*, (5) changes in resistance to apoptosis *(18)*, and (6) altered DNA repair activity (*see* Fig. 1) *(19)*. There have been previous reports of alterations in the kinetics of DNA interstrand crosslink formation and removal associated with resistance to the nitrogen mustards *(9,10,12)*, although others have found no differences in the ability of sensitive or resistant cells to remove nitrogen mustard-induced crosslinks *(20,21)*. This review will concentrate on the involvement of DNA repair in nitrogen mustard drug resistance and cross-resistance to cisplatin. We will discuss results obtained in clinical samples and human cancer cell lines.

2. DNA CROSSLINKS VIS-À-VIS NITROGEN MUSTARD DRUG RESISTANCE

Nitrogen mustard-induced alkylation of DNA results predominantly in the development of purine–drug complexes *(22)*. The nitrogen mustards, including chlorambucil and melphalan, may also form intrastrand and/or interstrand crosslinks at N-7 guanines *(23)*. These interstrand crosslinks are considered to be important in the cytotoxicity of these drugs *(8–10)*. There are technical problems involved in quantitating nitrogen mustard-induced interstrand crosslinks. Nitrogen mustards produce thermolabile glycosylic bonds (N[7]-guanine adducts), which yield apurinic sites and which, in turn, can cause strand breaks and/or breaks of crosslinks (reviewed in ref. *24*). Strand breaks can interfere with molecular size-based assays. The ethidium bromide fluorescence assay has the advantage that strand breaks are less likely to influence the quantification of crosslinks *(25)*. A widely utilized assay to determine DNA crosslinks is the alkaline elution assay *(26–28)*. This technique involves molecular size differences. However, the strand breaks induced by nitrogen mustards may complicate interpretation of repair of interstrand crosslinks when utilizing this assay. More recently, the comet assay has been utilized to quantitate interstrand crosslinks, but there are similar problems with the alkaline assay, which may be less with a neutral assay *(29,30)*.

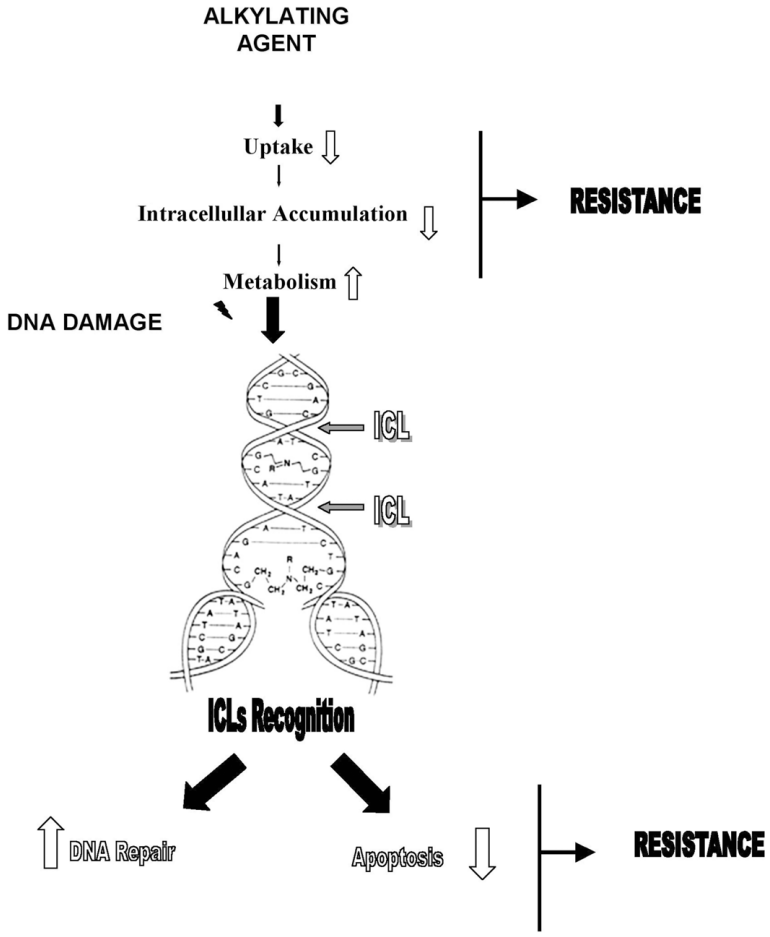

Fig. 1. Before DNA interstrand crosslink (ICL) are induced, decreased uptake (↓), decreased intracellular accumulation, or increased metabolism (↑) of the DNA-damaging agent may account for alkylating agent resistance. Downstream DNA damage recognition, alteration in the DNA repair, and/or apoptotic signaling pathways resulting in increased DNA repair (↑) and/or decreased apoptosis (↓) can mediate alkylating agent drug resistance.

3. NITROGEN MUSTARD DRUG RESISTANCE IN CHRONIC LYMPHOCYTIC LEUKEMIA

It is difficult to study clinical samples because of the heterogeneity of most tumor samples and the difficulty in obtaining serial samples from the same patient.

A model of drug resistance with direct relevance to clinical practice is a malignancy with easy access to a homogeneous population of malignant cells,

which represents the clinical status of the patients. Chronic lymphocytic leukemia is characterized by the proliferation and accumulation of B-lymphocytes that appear to be mature but are biologically immature. In some patients, chronic lymphocytic leukemia has an indolent course and does not require treatment for many years. When treatment is necessary, single-agent chemotherapy with a nitrogen mustard, usually chlorambucil, is the standard initial therapy, although fludarabine, a new exciting agent, may be incorporated in front-line treatment. At least 60–80% of patients respond to nitrogen mustard therapy, often for years, but eventually all patients become resistant to these agents *(31)*. Furthermore, many patients with chronic lymphocytic leukemia respond well to low-dose chlorambucil treatment, indicating that this disease is initially often very sensitive (hypersensitive) to these anticancer agents, to a greater extent than virtually all epithelial malignancies. A homogeneous monocellular population of malignant B-lymphocytes is easily obtained from chronic lymphocytic leukemia patients, thus providing a relatively unique opportunity to study clinically derived cells. We and others have previously demonstrated that there is a strong correlation between in vitro cytotoxicity of chlorambucil (measured by the microtiter [MTT] assay) and in vivo response in chronic lymphocytic leukemia patients *(32–34)*. Therefore, chronic lymphocytic leukemia is an excellent malignancy for in vitro studies, which should have direct clinical applicability.

Our laboratory, utilizing the ethidium bromide fluorescence assay, originally reported that DNA interstrand crosslink formation at 4 h after melphalan incubation (a time-point believed to be associated with maximal crosslink formation) was decreased in malignant B-lymphocytes from resistant chronic lymphocytic leukemia patients *(35)*. However, when we examined crosslink formation and removal at 0, 4, and 24 h after a 35-min melphalan incubation, there was evidence of a greater amount of crosslinks at time 0 in malignant B-lymphocytes from resistant chronic lymphocytic leukemia patients as compared to those from untreated chronic lymphocytic leukemia patients. Moreover, the untreated patients' lymphocytes developed a greater amount of crosslinks at 4 h without evidence of removal at 24 h, whereas there was evidence of progressive removal of DNA crosslinks at 4 and 24 h in lymphocytes from resistant chronic lymphocytic leukemia patients. This suggests that enhanced DNA repair is implicated in this process *(36)*. In another study, a patient with advanced chronic lymphocytic leukemia was treated with iv cyclophosphamide and DNA interstrand crosslinks in the lymphocytes were measured by the alkaline elution technique. Maximal DNA interstrand crosslink formation occurred 12 h after injection. However, the level of crosslinks was just above the sensitivity of the assay at 12 and 24 h after drug administration *(37)*. Also, utilizing the alkaline elution technique, Johnston et al. examined DNA crosslink formation in chronic lymphocytic leukemia lymphocytes at 6 h after an in vitro incubation with chlorambucil. They found that the lymphocytes from two resistant chronic lymphocytic leukemia patients had

as many DNA crosslinks as the lymphocytes from patients sensitive to chlorambucil *(38)*.

4. DNA CROSSLINKING AGENT DRUG RESISTANCE IN EPITHELIAL CELL LINES

As concerns epithelial cancer cell lines, DNA repair has been implicated in DNA crosslinking agent drug resistance (enhanced repair of DNA interstrand crosslinks in some investigations) *(39–44)*, whereas in other investigations, drug resistance appears to develop independent of altered DNA repair *(45–47)*. As previously stated, the assays utilized to quantitate interstrand DNA crosslinks have technical problems that may render difficult the interpretation of "repair" of DNA interstrand crosslinks *(25–28)*. Thus, some of the investigations in which DNA repair is not implicated may be the result of these technical problems or may represent alternative mechanisms of drug resistance as initially discussed.

5. DNA REPAIR OF NITROGEN MUSTARD DNA CROSSLINKS IN CANCER CELLS

The mechanism of removal of DNA interstrand crosslinks in mammalian cells is poorly understood. There are several different DNA repair systems that could be involved in the repair of nitrogen mustard-induced DNA interstrand crosslinks, including base excision repair, nucleotide excision repair, and recombinational repair (*see* Fig. 2). The mammalian base excision repair enzyme, alkyl-*N*-purine DNA glycosylase (3-methyladenine-DNA-glycosylase), can excise damaged guanine bases from DNA treated with chlorambucil *(48)*. We measured 3-methyladenine-DNA-glycosylase activity in chronic lymphocytic leukemia extracts and found a significantly higher activity (approx 1.7-fold) in lymphocytes from resistant chronic lymphocytic leukemia patients as compared to those from untreated chronic lymphocytic leukemia patients. Because this activity may vary with cell proliferation, it was corrected for differences in DNA synthesis utilizing (^{3}H)thymidine incorporation (there were differences in DNA synthesis between the two groups even though the vast majority of malignant B-lymphocytes are nonproliferative) and this resulted in no significant difference in enzyme activity between the two groups *(49)*. Moreover, overexpression of the human alkyl-*N*-purine DNA glycosylase in CHO cells did not result in nitrogen mustard resistance, suggesting that alkyl-*N*-purine DNA glycosylase was not a rate-limiting enzyme in nitrogen mustard drug resistance *(50)*. Furthermore, mouse embryonic stem cells bearing null mutations in this enzyme are not hypersensitive to the nitrogen mustards *(51)*.

Possible insights into mechanism(s) of interstrand crosslink repair are gained by examining nitrogen mustard hypersensitivity in DNA repair mutants.

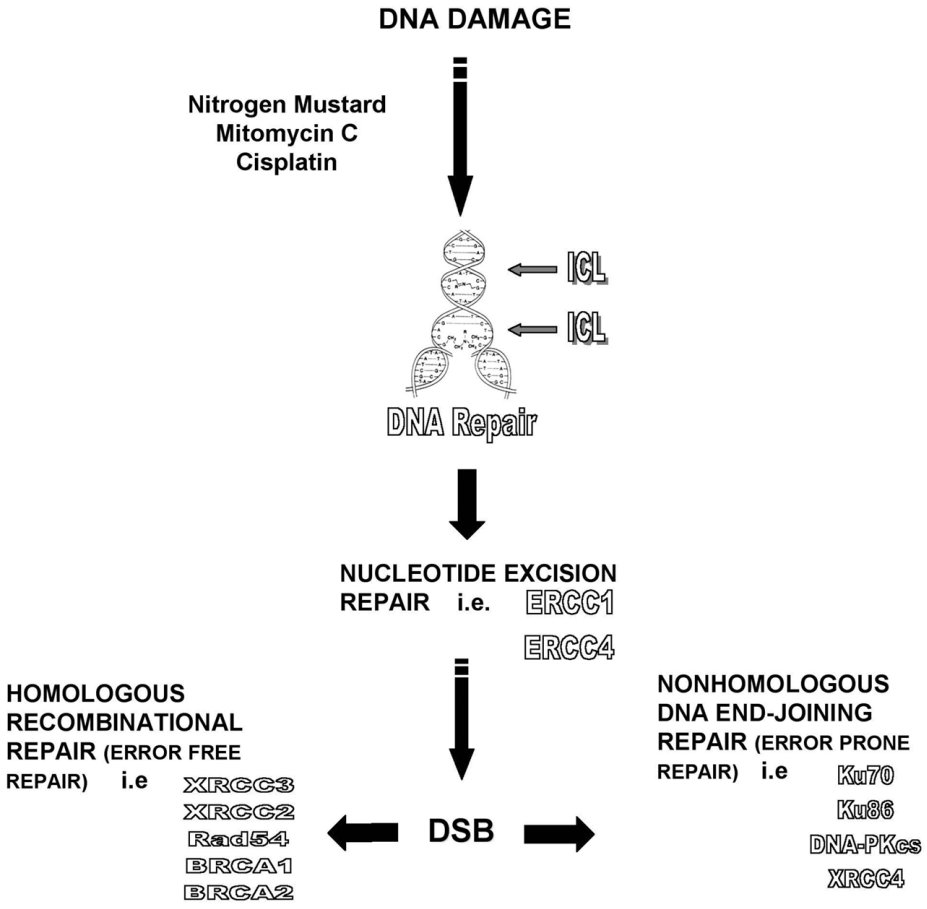

Fig. 2. The involvement of DNA repair proteins in DNA interstrand crosslinks (ICL) repair has been inferred by the fact that cells bearing mutations in such proteins are hypersensitive to ICL-inducing agents (nitrogen mustards, mitomycin c, and cisplatin). After DNA damage recognition, ICLs are unhooked by the nucleotide excision repair complex ERCC1/ERCC4, which could result in double-strand breaks (DSBs). The resulting DSBs would be repaired by homologous recombinational repair or nonhomologous DNA end-joining repair. The ICL-inducing agent's hypersensitive cell lines are defective in one of the following gene products: XRCC2, XRCC3, Rad54, BRCA1, BRCA2, Ku70, Ku86, DNA-PKcs, or XRCC4.

Significant DNA crosslinking agent hypersensitivity (varying from moderate to severe) is found in several DNA repair mutants, including ERCC-1, ERCC-4 (XPF), Xrcc-2, Xrcc-3, Rad54, Ku70, Ku86, and DNA-PK_{cs} *(52–56)*.

This analysis, along with information gained from studying crosslink removal in both bacteria and *Saccharomyces cerevisiae* and the possibility

that DNA double-strand breaks are repaired in a similar fashion to interstrand crosslinks, has resulted in the proposed model in mammalian cells that nucleotide excision repair, via the ERCC-1/ERCC-4 endonuclease, results in an incision 5' to the interstrand crosslink and that recombinational repair is involved in further processing of the lesion (reviewed in *57* and *58*). As concerns repair of double-strand breaks, nonhomologous DNA end-joining uses no, or very limited, sequence homology to rejoin ends directly, whereas homologous recombination requires extensive regions of DNA homology. Homologous recombinational repair would be necessary for error-free repair of interstrand crosslinks, whereas an illegitimate or nonhomologous DNA end-joining mechanism of repair could result in deletional repair of interstrand crosslinks. It is also conceivable that all three types of repair (nucleotide excision, homologous recombinational repair, and nonhomologous DNA end-joining) are implicated simultaneously or depending on the phase of the cell cycle, in the processing of interstrand crosslinks. The various genes implicated in nonhomologous DNA end-joining include the components of DNA-PK, Xrcc-4, and ATM, although ATM may be involved in homologous recombinational repair (*see* Fig. 3) *(58)*. Nonhomologous DNA end-joining is a major mechanism of double-strand breaks (DSB) repair in mammalian cells (reviewed in refs. *59* and *60*). Homologous recombinational repair in human cells implicates the HsRad51 family of proteins, including HsRad51, HsRad52, Rad51B, Rad51C, Rad51D, HsRad54, Xrcc-2, and Xrcc-3. Rad51 binding to DNA requires the precedent binding of Rad52. In addition, other Rad51 protein members are involved in the assembly of the Rad51 complex (*see* Fig. 3). Interactions of Rad51 with BRCA2, c-Abl kinase, and p53 have also been detected (reviewed in refs. *58* and *61*).

In order to gain insight into possible mechanisms of DNA crosslink removal in nitrogen mustard-resistant chronic lymphocytic leukemia, Bramson et al. analysed in vitro cross-resistance in chronic lymphocytic leukemia *(32)*. Chlorambucil-resistant chronic lymphocytic leukemia lymphocytes were completely cross-resistant to melphalan and mitomycin c, partially cross-resistant to cis-platinum, and not cross-resistant to ultraviolet (UV) light or methylmethane sulfonate. Because UV radiation damage is repaired by nucleotide excision repair and methylmethane sulfonate is repaired by base excision repair, it appears that these repair systems are not upregulated in nitrogen mustard drug resistance in chronic lymphocytic leukemia *(32)*. Also, ERCC-1 protein levels were not increased in nitrogen mustard drug-resistant chronic lymphocytic leukemia lymphocytes *(62)*. Nucleotide excision repair activity was very low in most chronic lymphocytic leukemia lymphocytes, including the majority of those obtained from previously treated chronic lymphocytic leukemia patients *(63)*.

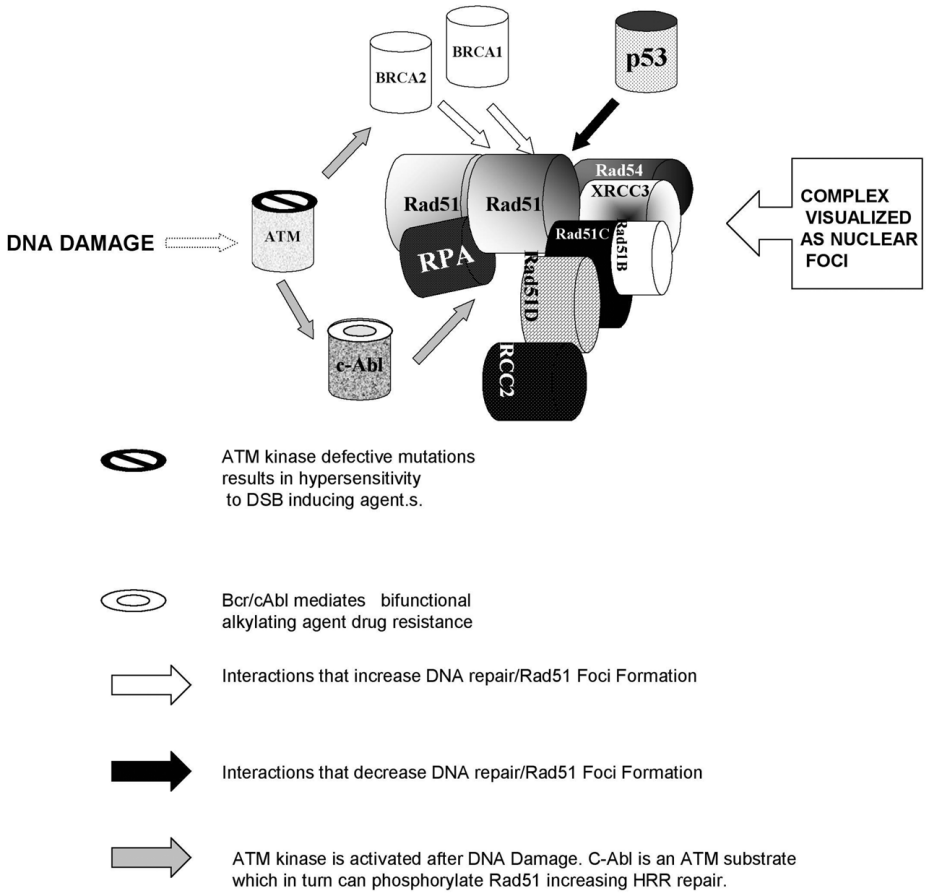

Fig. 3. The modulation of the homologous recombinational repair process can affect DNA damaging agent sensitivity. Homologous recombinational repair is mediated by multienzyme complexes, which includes the Rad51 paralogs (Rad51, Rad51B, Rad51C, Rad51 D, Xrcc2, Xrcc3 and Rad54). In response to DNA damage, the DNA repair complexes relocalize into the nucleus in discrete foci and can be visualized immunocytochemically using Rad51 antibodies. Other proteins known to interact and associate with these core complex include RPA, BRCA1, BRCA2, c-abl, and P53. Upon DNA damage, the serine–threonine kinase, ATM, is activated. Targets downstream of ATM include the BRCA1, BRCA2 and the c-abl tyrosine kinase proteins that are known to functionally interact with Rad51. BRAC 1/BRAC2—Rad51 interaction results in increased DNA repair and Rad51 foci induced by DNA damage. Constitutively active c-abl kinase (BCR/CABL) results in resistance to bifunctional alkylating agents resistance and induced Rad51 foci. The tumor suppresser p53 interacts with Rad51 decreasing homologous recombinational repair and Rad51 foci formation.

6. NONHOMOLOGOUS DNA END-JOINING

Because ionizing radiation results in DSBs that are largely repaired by nonhomologous DNA end-joining *(59)* and because DSBs are probably repaired in a similar fashion to interstrand crosslinks, cross-resistance studies between chlorambucil and ionizing radiation may be informative. Indeed, it appears that there is evidence of cross-resistance between ionizing radiation and chlorambucil in chronic lymphocytic leukemia lymphocytes *(64)*.

DNA-dependent protein kinase, a nuclear serine–threonine kinase, is a protein complex including a catalytic subunit of 460 kDa, DNA-dependent protein kinase$_{cs}$, and a DNA-binding subunit, the Ku autoantigen (a dimer of the Ku70 and Ku86 proteins). Ku binds to DSBs and other discontinuities in the DNA and recruits DNA-dependent protein kinase$_{cs}$ to the damaged site *(59,60)*. The active DNA-dependent protein kinase complex can then phosphorylate many DNA-bound proteins in the vicinity *(65)*. Because mutations in DNA-dependent protein kinase result in X-ray and alkylating agent sensitivity *(53,54)* and because X-ray resistance develops in parallel with chlorambucil resistance in chronic lymphocytic leukemia *(64)*, determination of DNA-dependent protein kinase activity in chronic lymphocytic leukemia should be informative. In a preliminary report with a small sample of chronic lymphocytic leukemia patients, an increase in DNA-dependent protein kinase activity was found in resistant samples *(66)*. In collaboration with Muller and Salles, our laboratory examined DNA-dependent protein kinase activity in a group of 34 patients (18 patients resistant to chlorambucil both in vitro and in vivo). There was an excellent linear correlation between DNA-dependent protein kinase activity and in vitro chlorambucil cytotoxicity ($r = 0.875$, $p = 0.0001$) *(67)*. The increased DNA-dependent protein kinase activity was independent of other clinical and biological factors. The regulation of DNA-dependent protein kinase activity was associated with increased DNA-binding activity of its regulatory subunit, Ku, and increased Ku protein levels. Interestingly, most untreated chronic lymphocytic leukemia patients have very low levels of DNA-dependent protein kinase activity, suggesting that, initially, resistance in chronic lymphocytic leukemia may be simply a state in which tumor cells lose an abnormal sensitivity to alkylating agents. In approx 25% of the samples from untreated chronic lymphocytic leukemia patients, a variant (truncated) form of the Ku86 protein was associated with very low DNA-dependent protein kinase activity and hypersensitivity to chlorambucil *(67)*. Wortmannin, a nonspecific inhibitor of DNA-dependent protein kinase, which also inhibits other phosphatidylinositol 3-kinases, sensitized chronic lymphocytic leukemia lymphocytes to the effects of chlorambucil. Moreover, there was a significant correlation between the synergistic sensitization and fold decrease in DNA-dependent protein kinase activity, but because wortmannin also inhibits other phosphatidylinositol 3-kinases, these results must be interpreted with caution

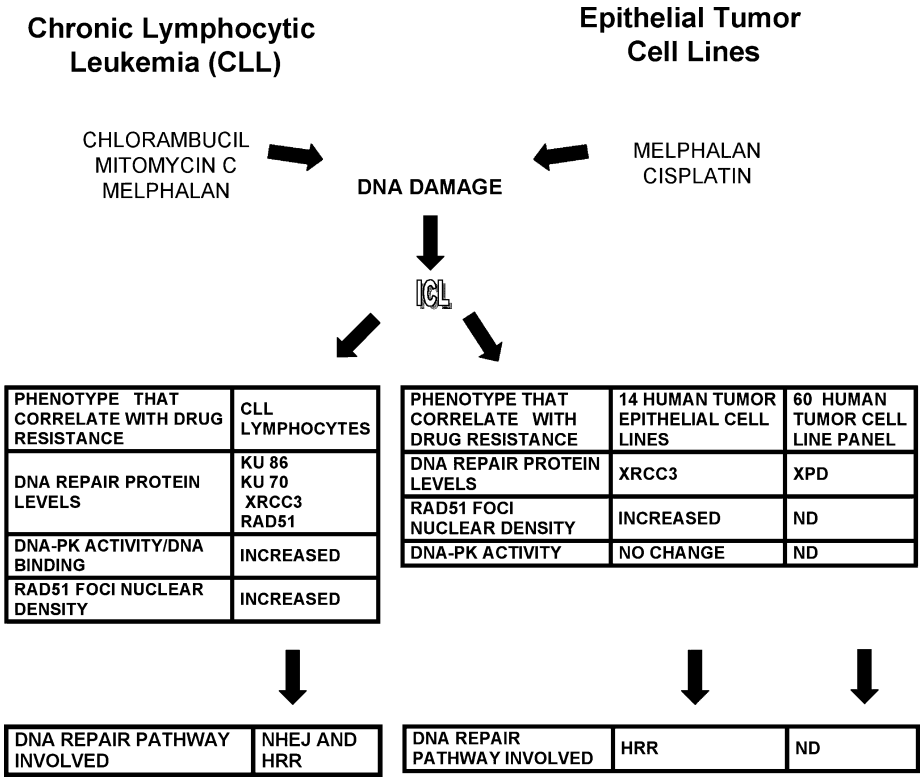

Fig. 4. The expression levels of DNA repair proteins essential for ICL repair (summarized in Fig. 2) can affect drug sensitivity. XRCC3 protein levels and DNA-damage-induced Rad51 foci correlates with chlorambucil drug resistance in lymphocytes from chronic lymphocytic leukemia (CLL) patients and with melphalan and cisplatin resistance in epithelial tumor cell lines, indicating that increased homologous recombinational repair (HRR) can be involved in drug resistance. Moreover, in CLL lymphocytes but not in epithelial cell lines, drug resistance can be mediated by increased non-homologous DNA end-joining (NHEJ) because Ku70 and Ku86 protein levels correlated with drug resistance and DNA-PK activity. Interestingly, the levels of the nucleotide excision repair protein XPD correlated with drug resistance in a human tumor cell line panel.

(68). In contrast, neither Ku protein levels nor DNA-dependent protein kinase activity correlated with melphalan resistance in epithelial tumor cell lines, suggesting that DNA-dependent protein-kinase-related DNA repair is not a rate-limiting step in epithelial cancers (*see* Fig. 4) *(69)*.

The immunohistochemical expression of Ku autoantigen and DNA-dependent protein kinase$_{cs}$ was examined in various human tissues. There was a large variation in expression depending on the specific tissue type *(70)*. This supports our

results that there is a variation in DNA-dependent protein kinase expression in human tissues.

Although it appears reasonable that increased DNA-dependent protein kinase activity is associated with increased repair of nitrogen mustard-induced interstrand crosslinks in chronic lymphocytic leukemia and thus, increased drug resistance, it is possible that other mechanisms are involved, including a role for DNA-dependent protein kinase with respect to apoptosis *(71,72)*.

DNA-dependent protein kinase$_{cs}$ is a member of the phosphatidylinositol (PI) 3-kinase superfamily. Other members include the gene mutated in ataxia telangiectasia (ATM) and the cell cycle checkpoint protein ATR *(60)*. Recently, loss of heterozygosity (LOH) or mutations of the ataxia telangiectasia gene and a decrease in ataxia telangiectasia protein levels have been found in approx 30–40% of B-chronic lymphocytic leukemia patients. These factors appear to be associated with a shorter survival, at least in younger patients *(73–76)*. The association of ataxia telangiectasia with nitrogen mustard drug resistance in cancer has not been investigated to date.

7. HOMOLOGOUS RECOMBINATIONAL REPAIR

The involvement of nucleotide excision repair and homologous recombinational repair in the repair of interstrand crosslinks is inferred from the fact that the mutant cell lines with the greatest sensitivity (10- to 100-fold) to alkylating agents that produce interstrand crosslinks are those that are deficient in or lacking Xrcc-2, Xrcc-3, ERCC1, and ERCC4/XPF *(52,56,58)*. The nucleotide excision repair complex (ERCC-1/ERCC-4) in mammalian cells makes dual incisions 22–28 bp apart, 5' to the interstrand crosslink on the same strand, *(77)*. This would then be followed by homologous recombinational repair. Alternatively, it is possible that strand invasion mediated by the Rad51 repairasome, including Xrcc-2 and Xrcc-3, occurs prior to ERCC1/XPF endonuclease-induced incision *(78)*.

Several human genes implicated in homologous recombinational repair have been characterized, including HsRad52, HsRad51, Rad51B, Rad51C, Rad51D, HsRad54, Xrcc-2, and Xrcc-3 (reviewed in refs. *58* and *61*). A recent model of interaction in yeast proposes that Rad52 interacts with RPA, followed by Rad52 association with Rad51. This leads to the assembly of Rad51 and associated proteins onto single-stranded DNA (ssDNA), which then initiate recombinational DSB repair (*see* Fig. 3) *(79)*. Xrcc-3 is necessary for the assembly of Rad51 foci and these proteins physically interact *(56,80)*. In fact, if all of the interactions described occur in one complex, then HsRad51, Xrcc-3, Rad51C, Rad51B, Rad51D, and Xrcc-2 are complexed together (reviewed in ref. *58*). Rad54 appears to be required after the association of the above-mentioned proteins, and Rad54 may assist Rad51 in interacting with damaged DNA *(81)*.

In view of the critical role of the Rad51 protein in homologous recombinational repair and its probable involvement in repair of interstrand crosslinks, our laboratory investigated HsRad51 foci formation after in vitro chlorambucil treatment of chronic lymphocytic leukemia lymphocytes. In vitro chlorambucil treatment induced HsRad51 expression as measured by increased immunopositive staining in all chronic lymphocytic leukemia samples. In the chlorambucil-resistant chronic lymphocytic leukemia lymphocytes, there was a linear correlation between induction of HsRad51 foci at 5.4 μ*M* chlorambucil and the in vitro LD_{50} concentration of chlorambucil *(82)*. Moreover, there was a significant correlation between Rad51 protein levels and, to a lesser extent, Xrcc-3 protein levels and chlorambucil cytotoxicity in chronic lymphocytic leukemia samples *(83)*. Thus, it appears that Rad51-directed homologous recombinational repair as evidenced by Rad51 foci, Rad51 protein levels, and Xrcc-3 protein levels is implicated in the development of nitrogen mustard drug resistance in chronic lymphocytic leukemia (*see* Fig. 4). Recent investigations of overexpression of fusion tyrosine kinases such as bcr/abl in myeloid cells results in DNA-crosslinking-agent drug resistance associated with increased homologous recombinational repair and Rad51 protein levels. Furthermore, overexpression of Rad51 results in DNA crosslinking agent drug resistance in these myeloid cells *(84,85)*.

In order to determine if our results in chronic lymphocytic leukemia were applicable to other malignancies, epithelial cell lines were investigated. We determined Rad51 foci formation in the epithelial cell lines. There was a good correlation between the density of Rad51 foci formation induced by 5.5 μ*M* melphalan and melphalan drug resistance. Also, melphalan-induced Rad51 foci density correlated with cisplatin resistance *(69)*. Xrcc-3 may be a determining factor in Rad51-related recombinational repair and nitrogen mustard resistance in epithelial cell lines. There is a correlation between Xrcc-3 protein levels and melphalan cytotoxicity in the epithelial cell lines, suggesting that Xrcc-3 may be important in the induction of Rad51-mediated recombinational repair and drug resistance. Rad51 protein levels did not correlate with melphalan/cisplatin resistance in the 14 epithelial cell lines *(69)*. Additionally, overexpression of Rad51 in CHO cells produced minimal (1.5-fold to fold in synchronized cells in S-phase) resistance to cisplatin (*86*; M. DeFais, personal communication) suggesting that the role of Rad51 vis-à-vis DNA crosslinking agent drug resistance may be somewhat different in epithelial cells as compared to hematopoetic cells (myeloid and lymphocytic cells) (*see* Fig. 4). These data are consistent with the hypothesis that Rad51-mediated homologous recombinational repair is associated with DNA crosslinking agent drug resistance. These results suggest a novel mechanism of DNA crosslinking agent drug resistance with significant potential clinical implications.

8. OVEREXPRESSION OF Xrcc-3/XPD RESULTS IN DRUG RESISTANCE ASSOCIATED WITH ENHANCED Rad51-RELATED HOMOLOGOUS RECOMBINATIONAL REPAIR AND PROLONGED S-PHASE CHECKPOINT

Xrcc-3, a Rad51 paralog that binds to Rad51, was overexpressed in MCF-7 cells (a cell line with low Xrcc-3 protein levels and sensitive to cisplatin and melphalan). The Xrcc-3-transfected cells (Xrcc-3/MCF-7) have sixfold higher Xrcc-3 protein levels as compared to mock-transfected cells. The Xrcc-3/MCF-7 cells were twofold resistant to cisplatin/melphalan and fivefold resistant to mitomycin c utilizing the MTT assay. Initial results suggest that alkylating agent-treated Xrcc-3/MCF-7 cells demonstrate enhanced Rad51 foci density as compared to mock-transfected cells *(87)*.

Because nucleotide excision repair is also implicated in DNA crosslinking agent drug resistance, my laboratory in collaboration with the US National Cancer Institute determined the protein levels of XPA, XPD, XPB and ERCC-1 in their 60 cancer cell line panel utilized for drug screening and then correlated the protein levels with the cytotoxicity of 170 compounds screened in this panel. In this study, only XPD protein levels correlated significantly with alkylating agent drug resistance (*see* Fig. 4) *(88)*.

The XPD helicase is a component of the TFIIH transcription factor that participates in DNA unwinding to allow either gene transcription by RNA polymerase II and/or the removal of DNA lesions induced by a variety of genotoxic agents, including UV light and some anticancer drugs by nucleotide excision repair *(89)*. Our knowledge regarding the role of XPD in drug efficacy comes from correlations between loss of XPD function and changes in cell sensitivity to DNA damage. It has been reported that XPD mutations that impair nucleotide excision repair activity result in minimal or no DNA crosslinking agent hypersensitivity *(52,90)*.

In order to determine if XPD plays a role in DNA crosslinking agent drug resistance, my laboratory overexpressed XPD in the SK-MG-4 human glioma cell line. The XPD-overexpressing cell line (hereafter called XPD) was twofold to threefold resistant to cisplatin and melphalan but not to UV light as compared to mock-transfected cells (hereafter called PCD). As anticipated, there was no difference in nucleotide excision repair activity between XPD and PCD cells. Also, the basal doubling time and basal percentage of cells in the S-phase were similar. Following cisplatin treatment, XPD cells removed interstrand crosslinks faster than PCD cells. Consistent with these results, XPD overexpression increased homologous recombinational repair visualized as Rad51 foci density after DNA damage. Moreover, immunochemical and immunoprecipitation studies demonstrate that XPD and Rad51 interact constitutively and that this interaction is increased after cisplatin treatment. This is the first description of

functional crosstalk between a nucleotide excision repair protein XPD and Rad51-homologous recombinational repair resulting in DNA crosslinking agent drug resistance and accelerated removal of interstrand crosslinks *(91)*.

Overexpression of XPD did not alter the doubling time or the percentage of cells in the S-phase in the basal state. However, cisplatin treatment significantly increased the percentage of cells in the S-phase in cells overexpressing XPD. This suggests that overexpression of XPD results in a prolongation of the S-phase checkpoint, allowing more time for Rad51-related DNA repair *(91)*. Similar studies are in progress with the Xrcc-3-overexpressing cells. Because Rad51-related homologous recombinational repair probably occurs mainly in the S-phase *(58)*, Rad51-related homologous recombinational repair and the S-phase checkpoint process are intimately interrelated. Thus, it is difficult to determine if enhanced Rad51-related HRR or prolonged S-phase arrest is primarily responsible for the DNA crosslinking agent drug resistant phenotype of the XPD-overexpressing cell lines (*see* Fig. 5).

9. SUMMARY OF RESULTS WITH RECOMBINATIONAL GENES

The regulation of DNA-dependent protein kinase activity appears to be tightly associated with the development of chlorambucil drug resistance in chronic lymphocytic leukemia. In particular, low DNA-dependent protein kinase activity is associated with hypersensitivity to chlorambucil. Furthermore, increased levels of DNA-dependent protein kinase activity are associated with chlorambucil resistance in chronic lymphocytic leukemia. Moreover, chlorambucil-induced HsRad51 foci, Rad51 protein levels, and Xrcc-3 protein levels correlate with chlorambucil drug resistance. The increased HsRad51 foci formation after chlorambucil treatment in resistant chronic lymphocytic leukemia samples may represent an active DNA repair process involving other Rad-51-related proteins. A plausible hypothesis to explain these results is that low DNA-dependent protein kinase activity defines a hypersensitive state, whereas high DNA-dependent protein kinase activity along with increased homologous recombination, as determined by HsRad51 foci formation, Rad51 protein levels, and Xrcc-3 protein levels contribute to the resistant state in chronic lymphocytic leukemia (*see* Fig. 4).

Regarding epithelial cancers (as represented by epithelial cancer cell lines), it appears that nonhomologous end-joining (i.e., DNA-dependent protein kinase) does not correlate with melphalan cytotoxicity but that HsRad51-related homologous recombinational repair is implicated in the development of nitrogen mustard and cisplatin drug resistance (*see* Fig. 4). Furthermore, Xrcc-3 protein levels are implicated in this process, as demonstrated by the correlation of Xrcc-3 protein with melphalan drug resistance and the fact that overexpression of Xrcc-3 results in DNA crosslinking agent drug resistance. The complexity of this situation is illustrated by the fact that overexpression of a nucleotide excision repair

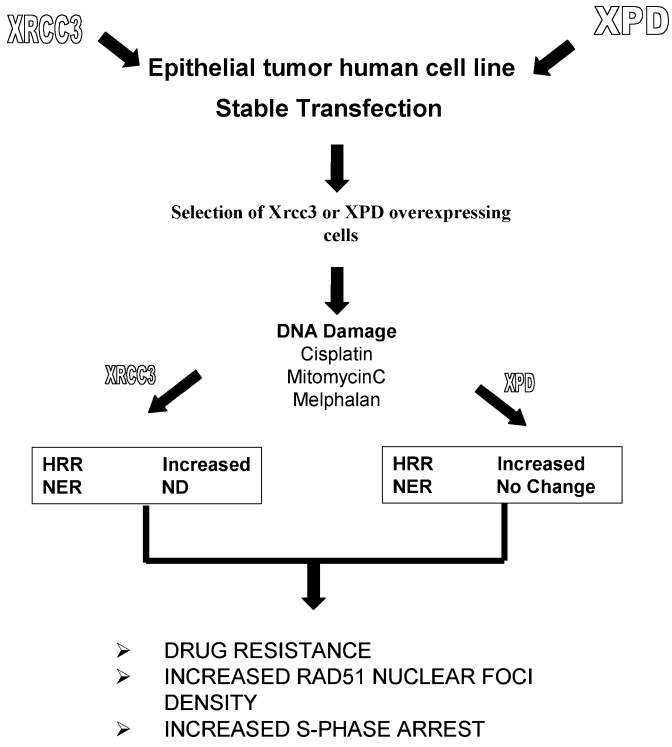

Fig. 5. To further assess the results summarized in Fig. 4, Xrcc3 or XPD open reading frames were stably transfected in human epithelial tumor cell lines. The cell lines were chosen from a cell line panel taking in account their relatively high sensitivity to alkylating agents and the low levels of the proteins to be overexpressed. Both cell lines XPD (XPD-overexpressing cells) and XRCC3 (Xrcc3-overexpressing cells) displayed similar phenotypes such as (1) resistance to alkylating agents (cisplatin, Mitomycin C, and melphalan), (2) increased HRR after DNA damage as assessed by Rad51 foci density, and (3) an increased percentage of cells in the S-phase after DNA damage.

protein, XPD, results in DNA crosslinking agent drug resistance via an interaction with Rad51-related homologous recombinational repair. The enhanced Rad51-related repair is also associated with cisplatin-induced S-phase arrest, suggesting that S-phase arrest may be a determining factor in enhanced Rad51-related repair. Because Rad51-related repair occurs largely in the S-phase, these two processes are intertwined and difficult to separate (*see* Fig. 5).

10. FUTURE PERSPECTIVES

The mechanism of low DNA-dependent protein kinase activity in sensitive chronic lymphocytic leukemia lymphocytes involves decreased Ku protein levels

and a variant form of Ku86, as compared to resistant samples. The regulation of this process needs to be further investigated at both the translational and transcriptional levels. Inhibition of DNA-dependent protein kinase activity by small molecules in combination with nitrogen mustard chemotherapy may improve the therapeutic index of the latter compounds in chronic lymphocytic leukemia. As concerns homologous recombinational repair, the implication of other Rad51 paralogs and accessory proteins needs to be examined. Increased expression of bcr/abl in myeloid cells results in an associated increase in expression of Rad51 and Rad51 paralogs along with DNA crosslinking agent drug resistance *(84)*. Some of these proteins may be markers of drug resistance in clinical specimens. A model of repair of DSBs has been proposed in which either Ku or HsRad52 binds DSBs, thus directing entry into nonhomologous DNA end-joining or homologous recombinational repair, respectively *(92)*. The relationship between Ku and HsRad52 with respect to repair of DNA crosslinks should also be examined. Experiments designed to alter the expression of genes involved in nonhomologous DNA end-joining and/or homologous recombinational repair vis-à-vis DNA crosslinking agent drug resistance should help to clarify their respective roles in this process. Again, inhibition of homologous recombinational repair (e.g., Xrcc-3 or Rad51 inhibition) may result in sensitization of epithelial tumors and chronic lymphocytic leukemia lymphocytes that are resistant to cisplatin and the nitrogen mustards.

XPD and Xrcc-3 overexpression results in DNA crosslinking agent drug resistance associated with enhanced Rad51 foci density *(87,91)*. In addition, cisplatin-treated XPD-overexpressing cells are arrested in the S-phase *(91)*. Thus, it is not clear if DNA-crosslinking-agent drug resistance is a result of enhanced Rad51-related homologous recombinational repair or S-phase arrest or both. Because recombinational repair and S-phase arrest are intimately intertwined, the use of dominant-negative constructs and mutant cell lines should help to determine if S-phase arrest is essential for the development of DNA crosslinking agent drug resistance associated with XPD overexpression. There are a number of defective cell lines with mutated genes for ATM, CHK2, NBS, and MRE11. These genes are involved in the S-phase checkpoint as outlined in the chapter and these mutant cell lines are defective in S-phase arrest *(93–95)*. Furthermore, dominant-negative constructs of ATM, ATR, NBS, CHK1, and CHK2 can be utilized to determine the effect of loss of function on the XPD/Xrcc-3-DNA crosslinking agent phenotype *(96–99)*. These studies should help to clarify the role of S-phase arrest in Rad-51-related DNA crosslinking agent drug resistance.

REFERENCES

1. Ochoa M Jr. Alkylating agents in clinical chemotherapy. *Ann NY Acad Sci* 1969;163:921–930.
2. Bergsagel DE, Griffith KM, Haut A, et al. The treatment of plasma cell myeloma. *Adv Cancer Res* 1967;10:311–359.

3. Fisher B, Carbone P, Economou SG, et al. L-Phenylalanine mustard (L-Pam) in the management of primary breast cancer. *N Engl J Med* 1975;292:117–122.
4. Young RC, Chabner BA, Hubbard SP, et al. Advanced ovarian adenocarcinoma. *N Engl J Med* 1978;299:1261–1266.
5. Goldenberg GJ, Land HB, Cormack DV. Mechanism of cyclophosphamide transport by L5178Y lymphoblasts *in vitro*. *Cancer Res* 1974;34:3274–3282.
6. Tew KD, Taylor DM. Studies with cyclophosphamide labelled with phosphorus-32:nucleic acid alkylation and its effect on DNA synthesis in rat tumor and normal tissues. *J Natl Cancer Inst* 1977;58:1413–1419.
7. Hoes I, Lemiere F, Van Dongen W, et al. Analysis of melphalan adducts of 2'-deoxynucleotides in calf thymus DNA hydrolysates by capillary high-pressure liquid chromatography-electrospray tandem mass spectroscopy. *J Chromatogr B Biochem Sci Appl* 1999;736:43–59.
8. Ross WE, Ewig RA, Kohn KW. Differences between melphalan and nitrogen mustard in the formation and removal of DNA crosslinks. *Cancer Res* 1978;38:1502–1506.
9. Zwelling L, Michaels S, Schwartz H, et al. DNA crosslinking as an indicator of sensitivity and resistance of L1210 leukemia cells to *cis*-diaminedichloroplatinum (II) and L-phenylalanine mustard. *Cancer Res* 1981;41:640–649.
10. Parsons PG, Carter FB, Morrison L, et al. Mechanism of melphalan resistance developed *in vitro* in human melanoma cells. *Cancer Res* 1981;41:1525–1534.
11. Moscow JA, Swanson CA, Cowan KH. Decreased melphalan accumulation in a human breast cancer cell line selected for resistance to melphalan. *Br J Cancer* 1993;68:32–37.
12. Parsons PC. Dependence on treatment time of melphalan resistance and DNA crosslinking in human melanoma cell lines. *Cancer Res* 1984;44:2773–2778.
13. Suzukake K, Vistica BP, Vistica DT. Dechlorination of L-phenylalanine mustard by sensitive and resistance tumor cells and its relationship to intracellular glutathione content. *Biochem Pharmacol* 1983;32:165–167.
14. Green JA, Vistica DT, Young RC, et al. Potentiation of melphalan cytotoxicity in human ovarian cancer cell lines by glutathione depletion. *Cancer Res* 1984;44:5427–5431.
15. Kramer RA, Greene K, Ahmad S, et al. Chemosensitization of L-phenylalanine mustard by the thiol-modulating agent buthionine sulfoximine. *Cancer Res* 1987;47:1593–1597.
16. Morgan AS, Ciaccio PJ, Tew KD, et al. Isozyme specific glutathione S-transferase inhibitors potentiate drug sensitivity in cultured human tumor cell lines. *Cancer Chemother Pharmacol* 1996;37:363–370.
17. Kelley SL, Basu A, Teicher BA, et al. Overexpression of metallothionein confers resistance to anticancer drugs. *Science* 1998;241:1813–1815.
18. Reed JC. Bcl-2 family proteins: regulators of apoptosis and chemoresistance in hematologic malignancies. *Semin Hematol* 1997;34:9–19.
19. Tan KB, Mattern MR, Boyce RA, et al. Elevated topoisomerase II activity and altered chromatin in nitrogen mustard-resistant human cells. *NCI Monogr* 1987;4:95–98.
20. Dean SW, Johnson AB, Tew KD. A comparative analysis of drug-induced DNA effects in a nitrogen mustard resistant cell line expressing sensitivity in nitrosoureas. *Biochem Pharmacol* 1986;35:1171–1176.
21. Robson CN, Lewis AD, Wolf CR, et al. Reduced levels of drug-induced DNA cross linking in nitrogen mustard-resistant Chinese hamster ovary cells expressing elevated glutathione-*S*-transferase activity. *Cancer Res* 1987;47:6022–6027.
22. Bank BB, Kanganis D, Liebes LF, et al. Chlorambucil pharmacokinetics and DNA binding in chronic lymphocytic leukemia lymphocytes. *Cancer Res* 1989;49:554–559.
23. Lawley PD, Brookes PJ. Interstrand crosslinking of DNA by difunctional alkylating agents. *J Mol Biol* 1967;25:143–160.
24. Ojwang JO, Grueneberg DA, Loechler EL. Synthesis of a duplex oligonucleotide containing a nitrogen mustard interstrand DNA-DNA crosslink. *Cancer Res* 1989;49:6529–6537.

25. de Jong S, Zijlstra JG, Timmer-Bosscha H, et al. Detection of DNA crosslinks in tumor cells with the ethidium bromide fluorescence assay. *Int J Cancer* 1986;37:557–561.
26. Kohn KW. DNA filter elution: a window on DNA damage in mammalian cells. *BioEssays* 1996;18:505–513.
27. Kohn KW. Principles and Practice of DNA filter elution. *Pharmacol Ther* 1991;49:55–77.
28. O'Connor PM, Kohn KW. Comparative pharmacokinetics of DNA lesion formation and removal following treatment of L1210 cells with nitrogen mustards. *Cancer Commun* 1990;2:387–394.
29. Merk O, Speit G. Detection of crosslinks with the comet assay in relationship to genotoxicity and cytotoxicity. *Environ Mol Mutagen* 1999;33:167–172.
30. Merk O, Reiser K, Speit G. Analysis of chromate-induced DNA-protein crosslinks with the comet assay. *Mutat Res* 2000;471:71–80.
31. Foon KA, Rai KR, Gale RP. Chronic lymphocytic leukemia: new insights into biology and therapy. *Ann Int Med* 1990;113:525–539.
32. Bramson J, McQuillan A, Aubin R, et al. Nitrogen mustard drug resistant B-cell chronic lymphocytic leukemia as an *in vivo* model for crosslinking agent resistance. *Mutat Res* 1995;336:269–278.
33. Hanson JA, Bentley DP, Bean EA, et al. *In vitro* chemosensitivity testing in chronic lymphocytic leukaemia patients. *Leuk Res* 1991;15:565–569.
34. Silber R, Degar B, Costin D, et al. Chemosensitivity of lymphocytes from patients with B-cell chronic lymphocytic leukemia to chlorambucil, fladarabine, and camptothecin analogs. *Blood* 1994;84:3440–3446.
35. Panasci L, Henderson D, Skalski V, et al. Transport, metabolism, and DNA interaction of melphalan in lymphocytes from patients with chronic lymphocytic leukemia. *Cancer Res* 1988;48:1972–1976.
36. Torres-Garcia SJ, Cousineau L, Caplan S, et al. Correlation of resistance to nitrogen mustards in chronic lymphocytic leukemia with enhanced removal of melphalan-induced DNA crosslinks. *Biochem Pharmacol* 1989;38:3122–3123.
37. DeNeve W, Valeriote F, Edelstein M, et al. *In vivo* DNA crosslinking by cyclophosphamide: comparison of human lymphatic leukemia cells with mouse L1210 leukemia and normal bone marrow cells. *Cancer Res* 1989;49:3452–3456.
38. Johnston JB, Israels LG, Goldenberg GJ, et al. Glutathione S-transferase activity, sulfhydryl group and glutathione levels and DNA crosslinking activity with chlorambucil in chronic lymphocytic leukemia. *J Natl Cancer Inst* 1990;82:776–779.
39. Batist G, Torres-Garcia S, Demuys JM, et al. Enhanced DNA crosslink removal: the apparent mechanism of resistance in a clinically relevant melphalan-resistant human breast cancer cell line. *Mol Pharmacol* 1989;36:224–230.
40. Bedford P, Fox BW. Repair of DNA interstrand crosslinks after busulphan. A possible mode of resistance. *Cancer Chemother Pharmacol* 1982;8:3–7.
41. Hill BT, Shellard SA, Hosking LK, et al. Enhanced DNA repair and tolerance of DNA damage associated with resistance to *cis*-diammine-dichloroplatinum (II) after *in vitro* exposure of a human teratoma cell line to fractionated X-irradiation. *Int J Radiat Oncol Biol Phys* 1990;19:756–83.
42. Johnson SW, Swiggard PA, Handel LM, et al. Relationship between platinum–DNA adduct formation and removal and cisplatin cytotoxicity in cisplatin-sensitive and -resistant human ovarian cancer cells. *Cancer Res* 1994;54:5911–5916.
43. Ali-Osman F, Rairkar A, Young P. Formation and repair of 1,3-bis-(2-chloroethyl)-1-nitrosourea and cisplatin induced total genomic DNA interstrand crosslinks in human glioma cells. *Cancer Biochem Biophys* 1995;14:231–241.
44. Dong Q, Bullock N, Ali-Osman F, et al. Repair analysis of 4-hydroperoxycyclophosphamide-induced DNA interstrand crosslinking in the c-myc gene in 4-hydroperoxy-

cyclophosphamide-sensitive and -resistant medulloblastoma cell lines. *Cancer Chemother Pharmacol* 1996;37:242–246.
45. Rawlings CJ, Roberts JJ. Walker rat carcinoma cells are exceptionally sensitive to *cis*-diamminedichloroplatinum(II) (cisplatin) and other difunctional agents but not defective in the removal of platinum-DNA adducts. *Mutat Res* 1986;166:157–168.
46. Petersen LN, Mamentaq EL, Stevnsner T, et al. Increased gene specific repair of cisplatin induced interstrand crosslinks in cisplatin resistant cell lines, and studies on carrier ligand specificity. *Carcinogenesis* 1996;17:2597–2602.
47. Roy G, Horon JK, Roy R, et al. Acquired alkylating drug resistance of a human ovarian carcinoma cell line is unaffected by altered levels of pro- and anti-apoptotic proteins. *Oncogene* 2000;19:141–150.
48. Mattes WB, Lee CS, Laval J, et al. Excision of DNA adducts of nitrogen mustards by bacterial and mammalian 3-methyladenine-DNA glycosylases. *Carcinogenesis* 1996;17:643–648.
49. Geleziunas R, McQuillan A, Malapetsa A, et al. Increased DNA synthesis and repair-enzyme expression in lymphocytes from patients with chronic lymphocytic leukemia resistant to nitrogen mustards. *J Natl Cancer Inst* 1991;83:557–564.
50. Bramson J, O'Connor T, Panasci LC. Effect of alkyl-*N*-purine DNA glycosylase overexpression on cellular resistance to bifunctional alkylating agents. *Biochem Pharmacol* 1995;50:39–44.
51. Allan JM, Engelward BP, Dreslin AJ, et al. Mammalian 3-methyladenine DNA glycosylase protects against the toxicity and clastogenicity of certain chemotherapeutic DNA crosslinking agents. *Cancer Res* 1998;58:3965–3973.
52. Hoy CA, Thompson LH, Mooney CL, et al. Defective DNA crosslink removal in Chinese hamster cell mutants hypersensitive to bifunctional alkylating agents. *Cancer Res* 1985;45:1737–1743.
53. Caldecott K, Jeggo P. Cross-sensitivity of gamma-ray-sensitive hamster mutants to crosslinking agents. *Mutat Res* 1991;255:111–121.
54. Tanaka T, Yamagami T, Oka Y, et al. The scid mutation in mice causes defects in the repair system for both double strand DNA breaks and DNA crosslinks. *Mutat Res* 1993;288:277–280.
55. Essers J, Hendriks RW, Swagemakers SMU, et al. Disruption of mouse RAD54 reduces ionizing radiation resistance and homologous recombination. *Cell* 1997;89:195–204.
56. Liu N, Lamerdin JE, Tebbs RS, et al. Xrcc-2 and Xrcc-3, new human Rad51-family members, promote chromosome stability and protect against DNA crosslinks and other damages. *Mol Cell* 1998;1:783–793.
57. Thompson LH. Evidence that mammalian cells possess homologous recombinational repair pathways. *Mutat Res* 1996;363:77–88.
58. Thompson LH, Schild D. Homologous recombinational repair of DNA ensures mammalian chromosome stability. *Mutat Res* 2001;477:131–153.
59. Anderson CW, Lees-Miller SP. The nuclear serine/threonine protein kinase DNA-dependent protein kinase. *Crit Rev Eukaryot Gene Expr* 1992;2:283–314.
60. Jeggo PA. DNA-dependent protein kinase at the cross-roads of biochemistry and genetics. *Mutat Res* 1997;384:1–14.
61. Shinohara A, Ogawa T. Rad51/RecA protein families and the associated proteins in eukaryotes. *Mutat Res* 1999;435:13–21.
62. Bramson J, McQuillan A, Panasci LC. DNA repair enzyme expression in chronic lymphocytic leukemia vis-à-vis nitrogen mustard drug resistance. *Cancer Lett* 1995;90:139–148.
63. Barret J-M, Calsou P, Laurent G, Salles B. DNA repair activity in protein extracts of fresh human malignant lymphoid cells. *Mol Pharmacol* 1996;49:766–771.
64. Bentley P, Salter R, Blackmore J, et al. The sensitivity of chronic lymphocytic leukaemia lymphocytes to irradiation *in vitro*. *Leuk Res* 1995;19:985–988.
65. Lees-Miller SP, Chen YR, Anderson CW. Human cells contain a DNA-activated protein-kinase that phosphorylates simian virus 40T antigen, mouse p53 and the human Ku autoantigen. *Mol Cell Biol* 1990;10:6472–6481.

66. Muller C, Salles B. Regulation of DNA dependent protein kinase activity in leukemic cells. *Oncogene* 1997;15:2343–2348.
67. Muller C, Christodoulopoulos G, Salles B, et al. DNA-dependent protein kinase activity correlates with clinical and *in vitro* sensitivity of chronic lymphocytic leukemia lymphocytes to nitrogen mustards. *Blood* 1998;92:2213–2219.
68. Christodoulopoulos G, Muller C, Salles B, et al. Potentiation of chlorambucil cytotoxicity in B-cell chronic lymphocytic leukemia by inhibition of DNA-dependent protein kinase activity using Wortmannin. *Cancer Res* 1998;58:1789–1792.
69. Wang ZM, Chen ZP, Xu ZY, et al. Xrcc-3 protein expression and induction of Rad51 foci correlate with melphalan resistance in human tumor cell lines. *J Natl Cancer Inst* 2001;93:1473–1478.
70. Moll U, Lau R, Sypes MA, et al. DNA-dependent protein kinase, the DNA-activated protein kinase is differentially expressed in normal and malignant human tissues. *Oncogene* 1999;18:3114–3126.
71. Jackson SP. DNA-dependent protein kinase. *Int J Biochem Cell Biol* 1997;29:935–938.
72. Song Q, Lees-Miller SP, Kumar S, et al. DNA-dependent protein kinase catalytic subunit: a target for an ICE-like protease in apoptosis. *EMBO* 1996;15:3238–3246.
73. Starostik P, Manshouri T, O'Brien S, et al. Deficiency of the ATM protein expression defines an aggressive subgroup of B-cell chronic lymphocytic leukemia. *Cancer Res* 1998;58:4552–4557.
74. Bullrich F, Rasio D, Kitada S, et al. ATM mutations in B-cell chronic lymphocytic leukemia. *Cancer Res* 1999;59:24–27.
75. Stankovic T, Weber P, Stewart G, et al. Inactivation of ataxia telangiectasia mutated gene in B-cell chronic lymphocytic leukaemia. *Lancet* 1999;353:26–29.
76. Schaffner C, Stilgenbauer S, Rappold GA, et al. Somatic ATM mutations indicate a pathogenic role of ATM in B-cell chronic lymphocytic leukemia. *Blood* 1999;94:748–753.
77. Bessho T, Mu D, Sancar A. Initiation of DNA interstrand crosslink repair in humans: the nucleotide excision repair system makes dual incisions 5' to the crosslinked base and removes a 22 to 28-nucleotide-long damage-free strand. *Mol Cell Biol* 1997;17:6822–6830.
78. De Silva IU, McHugh PJ, Clingen PH, et al. Defining the roles of nucleotide excision repair and recombination in the repair of DNA interstrand crosslinks in mammalian cells. *Mol Cell Biol* 2000;20:7980–7990.
79. Hays SL, Firmenich AA, Massey P, et al. Studies of the interaction between Rad52 protein and the yeast single-stranded DNA binding protein RPA. *Mol Cell Biol* 1998;18:4400–4406.
80. Bishop DK, Ear U, Bhattacharyya A, et al. Xrcc-3 is required for assembly of Rad51 complexes *in vivo*. *J Biol Chem* 1998;273:21482–21488.
81. Tan TL, Essers J, Citterio E, et al. Mouse Rad54 affects DNA conformation and DNA-damaged induced Rad51 foci formation. *Curr Biol* 1999;9:325–328.
82. Christodoulopoulos G, Malapetsa A, Schipper H, et al. Chlormabucil induction of HsRad51 in B-cell chronic lymphocytic leukemia. *Clin Cancer Res* 1999;5:2178–2184.
83. Bello VE, Aloyz RS, Christodoulopoulos G, et al. Homologous recombinational repair *vis-à-vis* chlorambucil resistance in chronic lymphocytic leukemia. *Biochem Pharmacol* 2002;63:1585–1588.
84. Slupianek A, Schmutte C, Tombine G, et al. BCR/ABL regulates mammalian RecA homologs, resulting in drug resistance. *Mol Cell* 2001;8:795–806.
85. Slupianek A, Hoser G, Majsterek I, et al. Fusion tyrosine kinases induce drug resistance by stimulation of homology-dependent recombination repair, prolongation of G(2)/M phase, and protection from apoptosis. *Mol Cell Biol* 2002;22:4189–4201.
86. Vispe S, Cazaux C, Lesca C, et al. Overexpression of Rad51 protein stimulates homologous recombination and increases resistance of mammalian cells to ionizing radiation. *Nucleic Acids Res*. 1998;26:2859–2864.
87. Xu R, Aloyz R, Panasci LC. Xrcc-3 overexpression results in melphalan/cisplatin drug resistance. *Proc Am Assoc Cancer Res* 2002;43:424.

88. Xu Z, Chen Z-P, Malapetsa A, et al. DNA repair protein levels vis-à-vis anticancer drug resistance in the human tumor cell lines of the National Cancer Institute drug screening program. *Anticancer Drugs* 2002;13:511–519.
89. Egly JM. The 14th Datta Lecture. TFIIH: from transcription to clinic. *FEBS Lett* 2001;498:124–128.
90. Damia G, Imperatori L, Stefanini M, et al. Sensitivity of CHO mutant cell lines with specific defects in nucleotide excision repair to different anti-cancer agents. *Intl J Cancer* 1996;66:779–783.
91. Aloyz R, Xu Z-Y, Bello V, et al. Regulation of cisplatin resistance and homologous recombination repair by the TFIIH subunit XPD. *Cancer Res.* 2002;62:5457–5462.
92. Van Dyck E, Stasiak AZ, Stasiak A, et al. Binding of double-strand breaks in DNA by human Rad52 protein. *Nature* 1999;398:728–731.
93. Walworth NC. Cell-cycle checkpoint kinases: checking in on the cell cycle. *Curr Opin Cell Biol* 2000;12:697–704.
94. Falck J, Mailand N, Syljuasen RG, et al. The ATM-Chk2-Cdc25A checkpoint pathway guards against radioresistant DNA synthesis. *Nature* 2001;410:842–847.
95. Abraham RT. Cell cycle checkpoint signaling through the ATM and ATR kinases. *Genes Dev* 2001;15:2177–2196.
96. Cliby WA, Roberts CJ, Cimprich KA, et al. Overexpression of a kinase-inactive ATR protein causes sensitivity to DNA-damaging agents and defects in cell cycle checkpoints. *EMBO J* 1998;17:159–169.
97. Falck J, Petrini JH, Williams BR, et al. The DNA damage-dependent intra-S phase checkpoint is regulated by parallel pathways. *Nature Genet* 2002;30:290–294.
98. Lim DS, Kim ST, Xu B, Maser RS, et al. ATM phorphorylates p95/nbs1 in an S-phase checkpoint pathway. *Nature* 2000;404:613–617.
99. Guo N, Faller DV, Vaziri C. Carcinogen-induced S-phase arrest is Chk1 mediated and caffeine sensitive. *Cell Growth Differ* 2002;13:77–86.

http://www.springer.com/978-1-58829-298-8

DNA Repair in Cancer Therapy
Panasci, L.C.; Alaoui-Jamali, M.A. (Eds.)
2004, XI, 363 p. 50 illus., Hardcover
ISBN: 978-1-58829-298-8
A product of Humana Press