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The Role of DNA Repair Processes in Determining Response to Cancer Therapy

Gillian Ross and Robert Brown

INTRODUCTION

THE MOLECULAR basis of the radiosensitivity and chemosensitivity of human cells both normal and malignant, remains one of the most important areas of research in cancer biology.

The field of mammalian DNA repair has undergone major developments over the last decade as a result of progress in somatic cell genetics and molecular biology. The aim of this paper is to review the contribution made by these areas to our current understanding of the role of DNA repair processes in determining the response of human cells to cancer therapy.

Variations in DNA repair proficiency in an individual may determine susceptibility to environmental mutagens, carcinogens, or cancer therapy. Evidence for the importance of DNA repair pathways comes most directly from the rare, inherited human disorders, in which defects in DNA repair mechanisms are associated with hypersensitivity to radiation and DNA damaging drugs. These disorders are pleiotropic in phenotype, but one feature is an associated cancer-proneness. See Table 1.

Although these syndromes are themselves rare in the general population, heterozygote carriers have been estimated at frequencies of 0.1–10%. It is conceivable that heterozygous individuals may also have some impairment of DNA repair, and indeed relatives of ataxia telangectasia homozygotes have an increased incidence of some common solid tumours [1]. Thus a defect in DNA repair processes may be involved in susceptibility to carcinogenesis and such individuals may be more prevalent in the cancer treatment population than the population generally.

The complexity of DNA repair processes in mammalian cells is well exemplified by our current level of knowledge of the ultraviolet radiation–DNA repair process. A large number of different cell lines have been established exhibiting hypersensitivity to ultraviolet radiation *in vitro*, both from rodents, and patients with xeroderma pigmentosum [2, 3]. Using cell fusion techniques, it is possible to demonstrate that such lines represent different genetic defects leading to expression of the ultraviolet hypersensitive phenotype, i.e. they belong to different 'genetic complementation' groups. One interpretation of this would be that a large number of genes are involved in repair of ultraviolet induced DNA damage.

DNA REPAIR PATHWAYS

DNA repair may be defined as those cellular responses associated with the restoration of normal nucleotide sequence and stereochemistry of DNA. Much of our knowledge of these processes has been obtained in yeast, bacteria, and only more laterly, rodents and humans [4, 5]. However, the major DNA repair processes appear to have been generally conserved through evolution, having served to counteract a wide spectrum of DNA damage, including ultraviolet light, heat-induced hydrolysis, ionising radiation, and environmental chemicals.

The excision repair pathway is responsible for the removal of many types of DNA lesion, including ultraviolet-induced cyclobutane pyrimidine dimers, bulky chemical adducts, alkylation, and base damage [6]. Its relevance to the processing of ionising radiation damage is uncertain, though both base damage, strand cross-links, and DNA–protein links are recognised in the spectrum of post-irradiation lesions [7]. See Fig. 1.

Genetic recombination involves the exchange of DNA sequence between DNA strands and has been proposed as a mechanism of repair of both DNA cytotoxic-induced adducts and strand breaks. The process is observed to occur during DNA repair in yeast, and can be demonstrated to occur in mammalian *in vitro* systems [8]. Non-homologous recombination is involved in both chromosome translocation, and gene amplifi-

Table 1. Human DNA repair deficiency syndromes

Syndrome	Phenotype	Hypersensitivity
Ataxia telangectasia	Cerebellar dysfunction Skin telangectasia Increased incidence of epithelial and lymphoid tumours	Ionising radiation Bleomycin
Bloom's	Photosensitivity, Immuno-deficiency. Growth retarded	Ultraviolet radiation Bifunctional alkylators
Cockayne's	Photosensitivity Dwarfism, ataxia Neural deafness Retinitis pigmentosum Progeria-like	Ultraviolet radiation
Xeroderma pigmentosum	Neurological defects Telangectasis Melanoma, squamous carcinoma Basal cell carcinoma	Ultraviolet radiation Bifunctional alkylators
Fanconi's anaemia	Hypoplastic anaemia, Skeletal defects Hypogonadism Thrombocytopenia Leukaemia	Bifunctional alkylators

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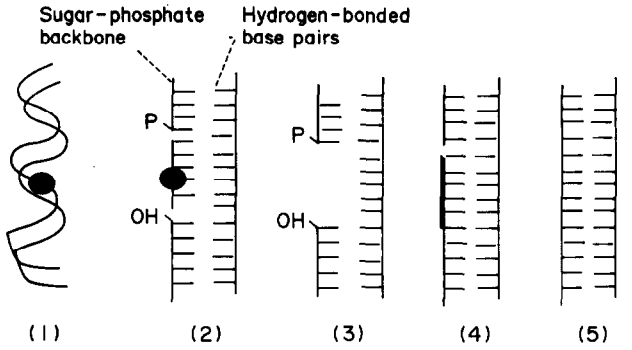

Fig. 1. Excision repair pathway. (1) DNA molecule with base damage or helix distortion. (2) Damage recognition, incision by DNA endonucleases. (3) Excision of damaged base sequence by endonucleases. (4) Polymerisation using undamaged DNA strand as template - polymerases. (5) Religation of strands to restore normal molecule - DNA ligases.

cation, and thus may play a vital role in the activation of oncogenes, and the evolution of drug resistance [9].

It is clear that DNA repair is not uniform throughout the genome. Only a small fraction of the genome is actively transcribed, with active genes packaged in altered nucleosome structures which are less condensed [10]. It is likely that the accessibility of particular genomic regions to repair enzymes depends upon the local topology of the DNA/chromatin and an early step in repair might control this access. Studies in both rodent and human xeroderma pigmentosum cells have demonstrated preferential repair of damage in transcriptionally active DNA [11]. Extracts from cells obtained from xeroderma pigmentosum patients have been shown to be capable of initiating the removal of pyrimidine dimers from purified DNA, but not from their own chromatin, demonstrating that their enzymes are adequate to perform repair with appropriate access to DNA [12].

GENE REGULATION FOLLOWING DNA DAMAGE

Given the presence of an 'SOS response' to cellular damage and an adaptive pathway for the repair of alkylation damage in bacteria, it is tempting to postulate that more complex mammalian cells might have similar, or even more efficient responses to genotoxic insults like radiation or cytotoxic drugs [2, 13, 14].

The complexity of the mammalian cellular response to DNA damage is becoming clearer, with more sophisticated analysis [15]. The expression of a number of genes is altered following DNA damage. Radiation can induce several classes of so-called 'early-response' genes, including *fos*, *jun*, and the *Egr*-1 families, which themselves code for transcription factors participating in the further regulation of cell responses [16]. It is possible that some of these genes might be involved in regulating cell cycle progression, and in particular, radiation-induced inhibition of DNA synthesis, to permit repair to occur [17].

Activation of genes which perturb normal signal transduction may result in resistance to radiation or cytotoxic drugs, and radiation may itself activate cellular oncogenes [18].

MOLECULAR AND BIOCHEMICAL APPROACHES TO HUMAN DNA REPAIR

Considerable efforts are being made in many laboratories to identify the altered genes induced in inherited human syndromes with DNA repair defects. Much of our current knowledge on genes involved in mammalian DNA repair has been obtained in rodent cells, for a variety of technical reasons. The genetic complementation approach relies on the isolation of hypersensitive mutants of a 'wild type' cell line, identified by their abnormal sensitivity to the DNA damage, e.g. ionising radiation. DNA is then transfected from normal cells, in an attempt to restore or 'complement' the defect in the deficient cells [19].

An alternative approach is to attempt to define and complement the defect at the biochemical level, e.g. by adding proteins in a cell-free assay, or microinjecting protein into a cell and observing restored function in a given repair system [6]. See Fig. 2.

This ability to analyse DNA repair processes in a stepwise, biochemical fashion, using cell-free systems, is becoming increasingly important, and has the further adantages of allowing us to work directly with human cells, either normal or malignant [20].

Modern recombinant DNA technology now enables us to use plasmid DNA molecules, of known sequence, or specially synthesised oligonucleotides, as the substrates for specific repair reactions [21]. The use of cell-free extracts and defined DNA substrates has recently yielded quantitative data on the ability of human cells to repair classes of DNA damage similar to those induced by ionising radiation or cytotoxic drugs [22].

The repair synthesis assay monitors the repair of DNA damage mediated by cell-free extracts of human cells, based on the incorporation of short patches of radiolabelled nucleotides into the substrate plasmid DNA molecule. Repair synthesis has been observed in this system as part of the biochemical processing of both ultraviolet radiation and cisplatin damage in human cell extracts [23]. See Fig. 3.

In both the cellular and biochemical approaches, modification of the DNA damage can be studied by following structural changes in the DNA molecule or restoration of gene function coded within the gene in which the lesion was initially produced [20].

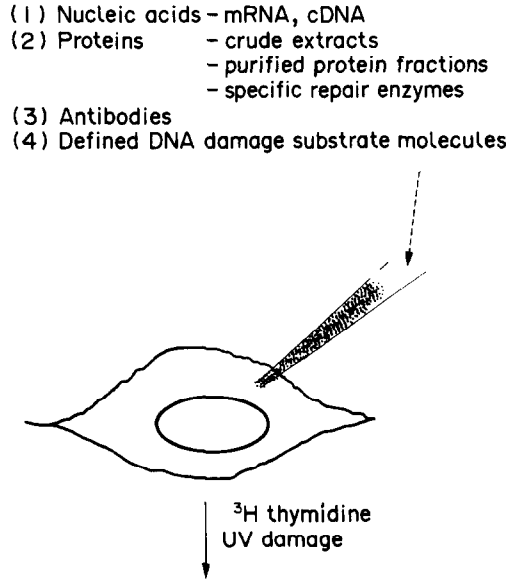

Fig. 2. *In vivo* cellular microinjection.

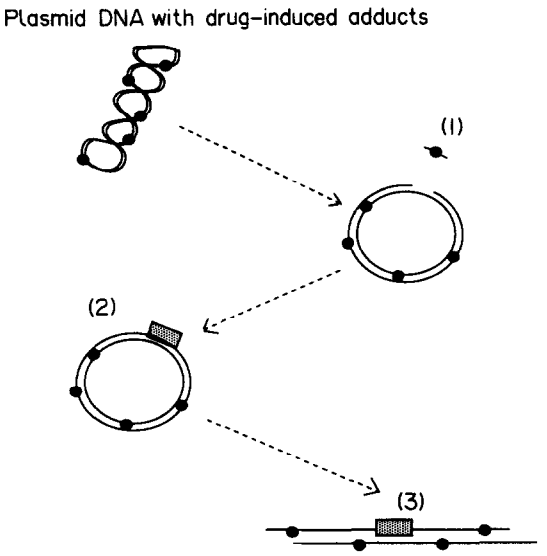

Fig. 3. (1) Cell extract-mediated incision at damage site. (2) Cell extract-mediated polymerisation and ligation, incorporates radiolabelled nucleotides at site of 'repair synthesis'. (3) Plasmid DNA linearised by enzyme, prior to gel electrophoresis and autoradiographic detection of repair patch.

ROLE OF DNA REPAIR PROCESSES IN CYTOTOXIC DRUG THERAPY

DNA and DNA metabolising enzymes are crucial targets for the action of many successful anticancer drugs. However, for many of these agents it is unclear which is the specific cytotoxic lesion that results in a selective effect against tumours. For a detailed review of drug-induced DNA damage see Ref. 24.

With an increased understanding of mechanisms of repair in mammalian cells, and especially with the availability of molecular assays for expression of repair proteins, it should become possible to analyse DNA repair enzymes in human tumour samples.

One of the first steps in a given repair pathway must be recognition of damaged DNA. Proteins have now been identified in human tumour cells which can bind to DNA damage produced by UV radiation, or cisplatin [25, 26]. These damage recognition proteins have been shown to be defective in certain xeroderma pigmentosum cell lines [27], and over-expressed in cisplatin-resistant cell lines with increased DNA repair proficiency, therefore may be involved in the initial steps of the excision repair pathway. However, their role in clinical chemoresistance to platinum compounds has yet to be explored.

DNA topoisomerases are enzymes which alter the structural conformation of DNA during processes which require strand cleavage, resynthesis, and then strand rejoining [28]. They have been suggested as targets for the activity of many clinically useful cytotoxic agents, including doxorubicin, etoposide, and mitoxantrone. With the development of assays of topoisomerase activity, antibodies to the proteins, and molecular probes for topoisomerase genes, their involvement in processing DNA damage should become clearer.

The biochemical pathways involved in the processing of alkylating drug damage have been studied extensively in both bacterial and human systems. Clinically useful compounds such as lomustine and carmustine, interact with DNA by alkylating the O^6 position of a guanine base, then bind to a second base forming a cross-link. The enzyme O^6 alkylguanine DNA alkyltransferase removes the adduct in a suicide reaction, transferring it to an acceptor cysteine residue. Cells which are proficient in the repair of alkylator damage at this site are termed MER+ or MEX+, and have significant increased levels of the enzyme. Increased levels of the enzyme have been strongly correlated with reduced alkylation-mediated mutagenicity, and cytotoxicity, though O^6 alkylguanine DNA damage does not correlate directly with cytotoxicity [29].

Human tumours have been found to generally express the MER+ phenotype, and evidence correlating enhanced alkylation repair, in human tumours, and 'clinical' resistance to alkylators is relatively sparse [30, 31].

Assays measuring the kinetics of removal of DNA lesions from human tumour DNA *in vivo* are not yet available. However, the development of antibodies to specific types of DNA damage may make this possible. For instance, antibodies to lesions induced by cisplatin have been developed, and have been used to monitor the removal of such damage from human cells.

Several pieces of evidence would suggest a role for enhanced DNA repair in cisplatin resistant cells. Increased repair of intrastrand crosslinks has been observed in a resistant human ovarian tumour line, as measured by an increase in unscheduled DNA synthesis [32]. Similarly, cisplatin sensitive testicular tumour cells appear to be less proficient in the removal of DNA aducts than resistant bladder tumour cells [33]. Further indirect support for the notion that DNA repair contributes to the cellular outcome following cisplatin treatment comes from the use of aphidicolin. This drug acts as a DNA polymerase inhibitor, and can modulate the response to cisplatin in resistant cell lines.

It is a general observation that much of the data on DNA repair proficiency in human cells has been derived using cell lines selected for drug resistance in a step-wise fashion. Much more work is needed using systems permitting the quantification of relevant repair processes directly from biopsy material.

DNA REPAIR FOLLOWING IONISING RADIATION DAMAGE

The biochemical pathways involved in the repair of ionising radiation-induced DNA damage are much less clear than those responsible for the repair of ultraviolet damage, making evaluation of the possible role of repair proficiency in determining response to radiation difficult.

Although there is a body of evidence associating proficiency of repair of the DNA double strand break (dsb) with cell lethality, it is clear that this represents a mechanistic oversimplification [34–36].

On the basis of neutral elution studies in bulk DNA, mammalian cells 'repair' the majority of dsb structurally. However, the accuracy or fidelity of strand break rejoining has been questioned in the case of ataxia telangectasia [22, 37].

Studies attempting to correlate cellular radiosensitivity with either variations in initial induction of dsb or rate of rejoining have reached differing conclusions. Schwartz *et al.* [38] found that radioresistant human tumour lines repaired dsb more rapidly than sensitive lines, in addition to incurring a lower level of initial damage. Similarly, McMillan *et al.* [39] have demonstrated that human tumours of differing radiosensitivity incur varying amounts of initial damage for a given dose of radiation, but that the level of correlation between induced damage and radiosensitivity was not simple, with differences in cell survival between lines having comparable levels of initial damage.

Differing classes of DNA double strand break can be created

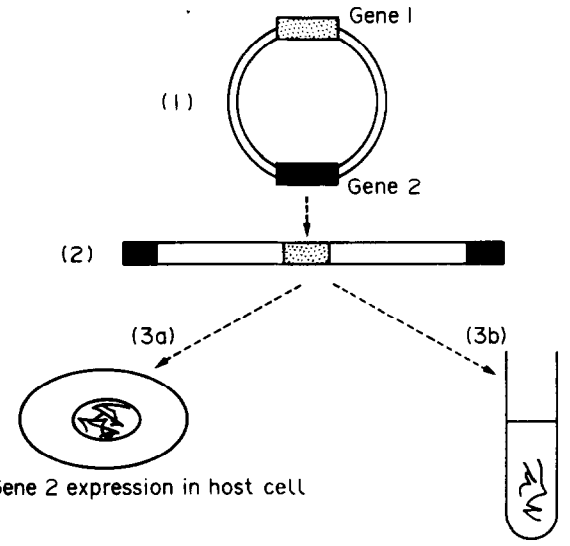

Fig. 4. (1) Plasmid DNA molecule with 2 'marker' genes. (2) Restriction digest to yield linear DNA, with modelled double strand break in gene 2. (3a) Transfection of damaged DNA into cell of interest. (3b) Damaged plasmid DNA mixed with cell extract of interest. Experimental endpoints: reformation of circular DNA molecule, or expression of gene 2, either in host cell, or after bacterial transformation.

using restriction enzymes, similar to those produced by ionising radiation in genomic DNA. The fate of such lesions have been assessed in two ways. Using *in vitro* techniques, rejoining of such DNA breaks can be mediated by proteins contained within extracts produced from the particular cell of interest. Using this system a defect has been proposed in the accuracy of DNA dsb rejoining in cell extracts prepared from ataxia telangectasia [22]. Similarly, such modelled dsb can be used to study repair in the cellular environment, following transfection of the damaged DNA into rodent or human cells [20]. See Fig. 4.

The application of modern molecular and biochemical techniques to the study of ionising radiation DNA damage and repair in human tumours is a relatively new development; it is hoped that such approaches will yield valuable insights into the molecular basis of variations in intrinsic cellular radiosensitivity.

CONCLUSIONS

Over the last decade increasingly sophisticated techniques in somatic cell genetics, molecular biology and biochemistry have made significant contributions to our knowledge of DNA repair processes in human cells. However, there are still major gaps in our understanding of how these pathways might influence normal tissue sensitivity to drugs or radiation, or contribute to the observed heterogeneity of tumour response to therapy.

It is vital that we continue to refine assays which permit quantification of repair of likely DNA lesions at functionally relevant levels of genomic organisation. The observation of genomic variability in repair proficiency should lend impetus to the development of techniques permitting evaluation of repair within functional subunits of the genome and ideally at the level of specific genes of interest.

It is hoped that technical advances in molecular biology will continue to identify genes involved in human DNA repair, and characterise the function of their protein products, in addition to defining more specific molecular targets for therapy.

Application of this knowledge might permit more selective 'targetting' of drugs and radiation at the level of the gene, or facilitate specific repair inhibition.

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Understanding Anticancer Drug Resistance: Opportunities for Modulation and Impact on New Drug Design

Ian R. Judson

INTRODUCTION

RESISTANCE TO cytotoxic drugs is an important cause of treatment failure. The causes are complex and may be determined by a combination of the tumour characteristics, such as the proportion of resting phase cells, adequacy of blood supply, etc, and specific cellular mechanisms, as in the multidrug resistance (MDR) phenotype associated with enhanced expression of the *mdr*-1 gene. Tumours with particular sensitivity to cytotoxic drugs are clearly the exception. Not only do such tumours tend to have a higher growth fraction but they may also be deficient in DNA repair mechanisms or certain natural cellular defences. These include P-glycoprotein and glutathione *S*-transferases, which are known to be present in certain normal tissues and are often expressed in the corresponding tumours [1]. Enhanced expression of such mechanisms may follow treatment with a variety of cytotoxic drugs [2, 3]. Furthermore, resistance mechanisms may be genetically linked such that exposure to one class of agent triggers increased expression of a group of proteins conferring resistance to several drug classes [4].

Resistance to cytotoxic agents can be raised in tumour cell lines *in vitro* by continuous exposure to increasing drug concentrations. Doubts have been expressed regarding the relevance of such models to clinical drug resistance. However, the progressive development of resistance has been demonstrated in patients, together with evidence of appropriate biochemical changes in the tumour [2, 3]. Also, human tumour cell lines appear subsequently to retain similar patterns of response to those of the original tumour [5]. The important question is whether a better understanding of the mechanisms of anticancer drug resistance can lead to improvements in antitumour therapy?

MECHANISMS OF RESISTANCE

Increased Drug Efflux

The resistance mechanism which has generated the most interest is the multidrug resistance (MDR) associated with overexpression of a 170 kD membrane glycoprotein, known as P-glycoprotein [6]. A family of closely related *mdr* genes, has been identified one of which, *mdr*1, codes for P-glycoprotein [7]. This is an ATP-dependent membrane efflux pump with a very wide substrate specificity and its tissue distribution in the gut, kidney, liver and other organs implies a natural mechanism for the elimination of toxic molecules. A disparate group of antitumour drugs derived from natural products, e.g. vincristine, etoposide, doxorubicin, actinomycin D, are susceptible to this efflux process. Clinical drug resistance does appear to correlate with increased expression which is thought to result in reduced intracellular accumulation of these agents. A striking example was reported in children with soft tissue sarcoma in whom P-glycoprotein expression was associated with disease recurrence and poor survival in contrast with the excellent prognosis in the P-glycoprotein negative group [8].

If P-glycoprotein expression is induced by exposure to chemotherapy and this correlates with the degree of resistance [9], this

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