Cisplatin Damage: Are DNA Repair Proteins Saviors or Traitors to the Cell?

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Dedicated to Prof. Dr. Wolfgang Beck, Emeritus at the Department Chemistry and Biochemistry of the Ludwig Maximilian University of Munich, as a small token of appreciation of his life-work, for his material and mental support, and for his continuously inspiring presence.

Introduction

In spite of its successful use for over 30 years as an anticancer agent, there is almost no scientific article dealing with cisplatin (*cis*-diamminedichloroplatinum) that does not introduce its research subject with the words "… but its mechanism of action is not fully understood…". When we were students (some 25 years ago) and first heard of cisplatin, everything seemed so clear. Cisplatin tightly binds DNA, bends it locally at the sites of the lesions, and causes cellular demise by impeding DNA replication; at that time—we have to admit—we did not dwell very long on the question of whether it may be the steric hindrance or the structural distortion, or both, that brings about the inhibition of DNA replication. Since then, there has been much discussion in the literature of whether or not it is the progression of DNA replication that, when halted by cisplatin adducts, results in cell death. Although there is undoubtedly a strong effect on DNA replication, the latter may not be the reason but rather (one of) the consequence(s) of the lethally hit cells. Consider, for example, that cells proficient in DNA repair are able to survive despite inhibition of the DNA replication, whereas DNA-repair-deficient cells die at cisplatin concentrations that do not inhibit DNA replication.[1] Thus, inhibition of DNA synthesis correlates with the concentration of drug but not with the different sensitivities of the cell lines. In other words, cell death does not correlate with inhibition of DNA synthesis. In fact, cisplatin lesions are not necessarily an insuperable obstacle for DNA replication, as several polymerases are capable of synthesizing past damaged DNA sites (translesion synthesis; Figure 1).[2–5]

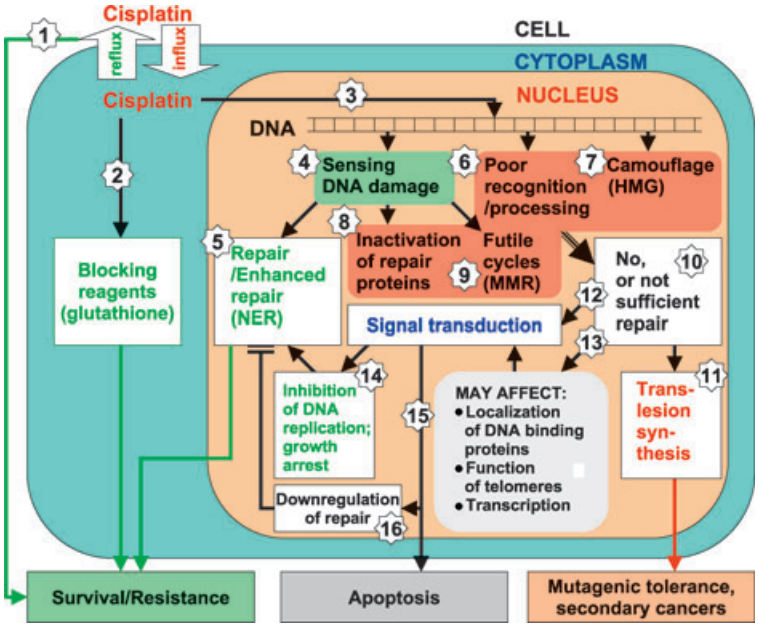

Figure 1. Cisplatin's fate, the levels of its action, and the possible biological outcomes (simplified). The potential degree of cytotoxicity of cisplatin is reduced by its reflux rate (1) and its detoxification by agents containing thiol groups (2). Cisplatin (in which the chloride ligands were replaced by water) reacts with the DNA upon entering the nucleus (3). DNA damage may be recognized by different sensors (4) and may be repaired (5), thereby enabling *survival* of the cell. Moreover, enhanced or altered repair may lead to *resistance* against cisplatin. Inefficient recognition and/or processing of the "naked" (6) or the masked (7) adduct, or inactivation of the repair/processing enzymes (8) or unsuccessful trials of repair (9), in the case of fine recognition, all lead to repair failure (10). Unrepaired lesions may be bypassed by DNA polymerases (11) that may incorporate inappropriate nucleotides opposite a cisplatin adduct, ultimately resulting in *manifestation of mutations* and *cell transformation*. In general, poor repair will lead, either directly by components of the repair system themselves (12) or indirectly by interference with cellular processes (13) through signal transduction within the nucleus and in the cytoplasm, to two possible outcomes: Inhibition of DNA replication and growth arrest (14) to give the cell another chance to repair the damage, after which the cell resumes proliferation or, alternatively, when there is excess DNA damage, to *programed cell death* (15). In the case where the cell decides to die, further energy-consuming repair is actively suppressed (16), in order to preserve the cell's energy for the apoptotic program.

Levels and Potential Causes of Cisplatin Cytotoxicity and Antitumor Activity

Nevertheless, due to genetic evidence, DNA is the commonly accepted biological target of cisplatin. Thus, several alternatives (or a combination thereof) to the stopping of DNA replication were suggested as the causes for cell decay, all of which centered around DNA (see Figure 1). For example, titration of essential DNA-binding proteins away from their natural sites of

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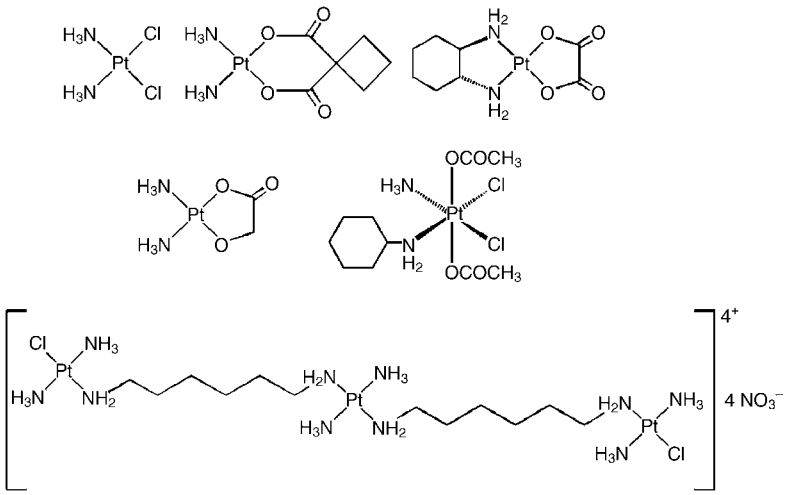
action by DNA sites bearing adducts of cisplatin,[6–12] futile cycles of the mismatch repair (MMR) system,[13] interference with telomere replication and/or function,[14] and general[15] or specific inhibition of the transcription of particular genes[16,17] have all been suggested.

The above enumeration addresses different levels and parameters through which cisplatin may operate, for example, the kind and persistence of DNA adducts, the structural distortion of the DNA, susceptibility (or lack thereof) to DNA-repair mechanisms, interference with synthetic processes of DNA (that is, DNA replication or transcription) or lesion bypass, and specific DNA loci. However, there are additional levels of cisplatin action as well, all of which can vary and affect the cytotoxic potential. These include uptake by the cell and reflux of the drug,[18–20] its *effective* concentration in the cell (dependent on the concentration and potency of cytoplasmic blocking reagents), its reactivity with DNA *under nuclear conditions*, and the availability of signal-transduction components of the cell-death machinery, that is, of apoptosis.[5] However, no theory revealing *the* reason for the cytotoxicity of cisplatin has prevailed so far, presumably because *all* of these parameters may contribute to a variable extent and at different levels.



Scheme 1. Chemical formulas of cisplatin, of some successful cisplatin analogues (carboplatin, oxaliplatin, nedaplatin), and of two promising derivatives in advanced clinical studies (satraplatin, BBR3464; from left to top to bottom).

In addition, besides the causes directly concerning *cytotoxicity*, there are other not conclusively answered questions regarding the biological action of cisplatin; for example, why is the cytotoxicity of cisplatin up to several orders of magnitude stronger against malignant cells than against nontransformed cells?[21] And furthermore, why is the antineoplastic effect of cisplatin restricted to certain tumor types (for example, testicular cancer)? Maybe the distinct targeted cells/organs differ in one or more of the parameters mentioned above (or in other characteristics not yet detected), thereby rendering them differentially susceptible to cisplatin. These issues pose additional levels of complexity in the cisplatin story. Hence, research into these unresolved but intriguing aspects is still feverishly ongoing.

Improving Parental Cisplatin

Obviously, matters have become more knotty than they seemed to be some decades ago. Nevertheless, despite our general ignorance with regard to the precise molecular mechanisms of cisplatin, significant progress has been made concerning important structural parameters that may influence, for example, the potency, organospecificity, and side effects of cisplatin. The main DNA adduct of cisplatin, the 1,2-deoxy(guanosine–phosphate–guanosine) (1,2-d(GpG)) intrastrand cross-link, that brings about local DNA untwisting and strong bending was suggested to be the principal cause of cytotoxicity.[22,23] Intense efforts led to the development of structural "rules" and to noteworthy, clinically relevant cisplatin derivatives, including carboplatin, oxaliplatin, and nedaplatin[24,25] (see examples in Scheme 1). However, these "rules", which emerged by trial and error, are rather empirical and several exceptions to them have been reported, such as unexpected active derivatives forming solely monofunctional adducts[26] and the activation of the *trans* geometry of diamminedichloroplatinum.[27] Therefore, there is still a strong need to try and comprehend the underlying molecular mechanisms of cisplatin action, particularly if one wishes to develop novel, better derivatives on a rational basis.

Central Lessons from Cisplatin Research and Application

Out of the seemingly confusing information about the responsible mechanisms of cisplatin cytotoxicity, two clear points emerged as a minimum common basis from the study of cisplatin and of other active, as well as inactive, platinum complexes. Firstly, in order to be cytotoxic, platinum complexes have to react with *and persist on* DNA; it is not (solely) the high reactivity of a compound that renders it cytotoxic because fast repair of DNA adducts leads to a compound's inactivation. Secondly, accumulating evidence suggests that persisting adducts of cisplatin (and of other effective cisplatin analogues or derivatives) may operate by an *active* mechanism to kill the cell, rather than by mere passive pathways, that is, impediment of or interference with synthetic processes of DNA leading to cell muddle. In other words, an active DNA adduct must elicit *signal transduction* from the damaged DNA to the cell-killing machinery.[28,29]

These upshots led us to appreciate that the most crucial features for a DNA adduct to be cytotoxic are, first, the ability to efficiently escape the DNA repair system(s) and, second, the ability to activate sensors that may translate the DNA damage into a cell-death signal. Recently, two noteworthy papers appeared[30,31] addressing these two key items. These reports confirmed old players and pathways of the cellular responses to cisplatin and revealed unexpected new ones.

Identification of Cellular Proteins Interacting with Cisplatin DNA Adducts

In the past, Lippard and co-workers had identified a high-mobility group (HMG) domain protein that is able to interact with DNA at the site of the major cisplatin adduct, the 1,2-d(GpG) intrastrand cross-link. HMG-domain proteins are nonhistone architectural constituents of chromatin that interact with DNA curvature and thus may be attracted by the profound DNA bend caused by this adduct. They do not interact with the cisplatin adduct directly. The "genetic screen" applied at that stage, however, cannot identify any "natural" proteins able to interact with cisplatin-modified DNA, for example, only after posttranslational modifications. To reveal the true events at the site of modification, it is reasonable therefore to search for proteins in their native form, that is, the form in which they exist inside the cell. Hence, in a recent communication published in the *Journal of the American Chemical Society*, Lippard and coworkers applied photoaffinity labeling in the isolation and identification of *native nuclear proteins* from HeLa cells that could interact with the 1,2-d(GpG) cross-link.[30] Affinity labeling is a classic biochemical method used, for instance, to identify important residues at the active site of an enzyme. In this method, a reactive substrate analogue is administered to an enzyme. Due to its *affinity* to the active site, this analogue binds to it and reacts covalently with a residue within the active site, thus stably *labeling* and marking it; this facilitates its identification by subsequent methods. Photoaffinity labeling is a variant of the method, in which the substrate analogue only becomes reactive after irradiation. In their work, Zhang et al. succeeded in labeling (and later identifying) proteins interacting with a 1,2-d(GpG) cisplatin adduct. To this end, these investigators tethered a *derivatized* cisplatin bearing a photoreactive benzophenone moiety through a $(CH_2)_6$ linker to the single central d(GpG) of a double-stranded oligodeoxyribonucleotide (25 mer; "oligo"). This oligo was equipped with two other useful modifications, radioactive end labels and/or a biotin group at one of the 3′ ends.

To check for a) a cisplatin-authentic structural distortion and b) the capacity of benzophenone to capture, upon irradiation, proteins intimately associated with the 1,2-d(GpG) lesion, Zhang et al. first incubated the modified oligo with HMG-box protein 1 (HMGB1) or HMG-domain derivatives, that is, polypeptides known to interact with the genuine 1,2-d(GpG) cisplatin adduct. After irradiation at 365 nm, the expected covalently joined protein–DNA complexes could be detected by autoradiography as mobility-shifted bands in a sodium dodecylsulfate (SDS) PAGE gel, a result attesting to the validity of the method. In an analogous manner, the oligo was incubated with HeLa total nuclear extract and irradiated; several resulting covalent protein–DNA complexes held together by the cisplatin–spacer–benzophenone chain were separated and visualized by autoradiography in an SDS PAGE gel.

To identify the bound proteins, the steps were repeated on a large scale and protein–DNA complexes were enriched by a procedure involving streptavidin-coated magnetic beads that largely removes noncovalently bound proteins. DNA-cross-linked proteins were then separated by SDS PAGE and identified by one or more of three methods: a) by mass spectrometry after transfer out of the gel matrix, b) by Western blotting and detection with selected antibodies, or c) by the demonstration that, after photo-cross-linking, the protein species from the HeLa extract comigrated with individual purified proteins in an SDS PAGE gel.

Involvement of HMG-Box Proteins and PARP in Cisplatin Action

Among other, as yet unidentified proteins, HMGB1, HMGB2, and poly(adenosine diphosphate–ribose)polymerase-1 (PARP-1) were found to have been cross-linked to the cisplatin lesion. In addition, in the particular case of PARP-1 the identity of this protein could also be unequivocally confirmed by its specific enzymatic reaction: PARP-1 catalyzes the transfer of several adenosine diphosphate–ribose (ADP–ribose) molecules from the nicotinamide adenine dinucleotide (oxidized form; NAD^+) onto itself whenever it encounters DNA discontinuities or other DNA damage.[32] Indeed, addition of NAD^+ prior to photo-cross-linking to the HeLa extract (or to pure PARP-1 as a positive control) yielded more slowly migrating (larger) cross-linked species in an SDS PAGE experiment, a result indicative of the autopoly(ADP-ribosyl)ation of PARP-1.

HMG-box proteins have been repeatedly suggested in the literature as an aid for DNA lesions to escape repair. DNA adducts of cisplatin, particularly the 1,2-d(GpG) adduct, may resist repair and persist on DNA[22,23,33,34] due to several reasons. These include: a) inactivation of repair proteins by cross-linking,[35] b) recognition but incapacity of repair proteins to cope with the lesion, for example, in futile cycles,[13] c) poor recognition and/or meager concomitant processing of particular lesions by repair proteins,[23] and d) "camouflage" of lesions by proteins that recognize, bind, and *protect* them from efficient repair. It is this last category to which the HMG-box proteins are supposed to belong. This scenario has been supported with a large body of biochemical and genetic evidence.[12,36–40] Thus, although this finding in the recent paper[30] is not novel but is actually expected, it strengthens the evidence by demonstrating again a direct association of "natural" HMG-box proteins with the major cisplatin lesion. However, this concept still does not address the question of whether HMG-box proteins bound to cisplatin lesions may also constitute a signal for cell death.

The second associated protein found, PARP-1, and poly(ADP-ribosyl)ation are involved in virtually all dynamic DNA processes, that is, replication, transcription, repair, and recombina-

tion.[32] With regard to repair, activation of PARP-1 has been previously associated with DNA lesions that can be handled by the base excision repair (BER) system. Although PARP-1 was thought until recently to have an absolute requirement for DNA strand breaks in order to become enzymatically active, more recent data[41] also implicated this protein in the repair of other types of lesions void of DNA breaks, that is, pyrimidine dimers, and known to be removed by a different repair system, that is, nucleotide excision repair (NER); NER is also responsible for the repair of cisplatin adducts. Thus, this finding of Zhang et al. is consistent with earlier results, in which cisplatin was reported to effect major poly(ADP-ribosyl)ation in the treated cells.[42] Moreover, sensitivity to cisplatin was shown to increase by treatment of the cells with PARP-1 inhibitors.[43] Hence, the report of the physical association of PARP-1 with the 1,2-d(GpG) cisplatin adduct may well be in line with a role of PARP-1 in the repair of this adduct.

PARP-1: Abolition or Boosting of Damage?

However, the interpretation of the PARP-1-related findings of Zhang et al. might be more complex. Association of PARP-1 with a DNA lesion is normally very short due to rapid autopoly(ADP-ribosyl)ation and repellence of PARP-1 from the DNA. Lagueux et al. found that the rate of catalysis (k_{cat}) of PARP-1 had already decreased by 25% *after 1 min of reaction*, probably due to a *decrease in the affinity* of PARP-1 for DNA.[44] Hence, it is striking that in the experimental set up of Zhang et al. autopoly(ADP-ribosyl)ated PARP-1 was *still sitting on the DNA* and was almost quantitatively captured by cross-linking, which followed *30 minutes* after incubation with NAD^+. One would assume that there would have been enough time for PARP-1 to at least partially dissociate from the DNA before cross-linking occurred. Several interpretations are possible. For instance, further operations at the 1,2-d(GpG) cisplatin adduct could have been stalled at the autopoly(ADP-ribosyl)ation step of PARP-1 (no dissociation) because of *insufficient modification*. It is known that the degree of poly(ADP-ribosyl)ation varies with regard to the particular DNA lesion. Accordingly, this state could also mirror a physiological situation, in which an undissociated PARP-1 would result in *protection*, not *repair* of the lesion (similar to the suggested action of HMG-box proteins). In fact, PARP-1 does not participate directly in the *repair* process, which begins only *after* heavily poly(ADP-ribosyl)ated PARP-1 has dissociated from the lesion.[32,45] In this context, PARP-1 has been suggested to shield damaged DNA sites, for example, strand breaks, from recombination[46,47] until downstream effectors have been recruited and/or activated. Incapacity of PARP-1 to dissociate would impede the next steps of repair, and further processing of the 1,2-d(GpG) cisplatin adduct might thus get stuck at this level. Accordingly, shielding of the 1,2-d(GpG) adduct by PARP-1 with concomitant escape from repair would indeed be more consistent with the claims that this lesion is a bad substrate for NER[22,23] rather than the theory of PARP-1 marking (or preparing) this lesion for repair.

Moreover, PARP-1 may be able not only to interfere with the repair of particular lesions but also to operate itself as a trigger for a cytotoxic response. Overactivation of PARP-1, for example, due to heavy DNA damage, may bring about the demise of the cells by *necrosis* due to NAD^+ and adenosine triphosphate (ATP) depletion.[32] A *proapoptotic* signal may result from less profane causes, that is, through the p53 pathway. There is clear evidence supporting a role of PARP-1 in the activation of p53, either by directly modifying the functional properties of the latter or by recruiting the protein to damaged sites of DNA.[32] On the other hand, it is known that activated p53, triggered by cisplatin lesions, may lead to apoptosis, for example, by transcription of the proapoptotic Bax protein.[48] An apoptotic response of the cell after the interaction of components of a *repair* system with cisplatin lesions is well established for MMR proteins.[49–52]

The possible additional roles of PARP-1 supposed here, either interfering with repair or even actively promoting apoptosis after binding to the 1,2-d(GpG) adduct, are not at odds with other findings mentioned above that favor prosurvival effects instead. First, results from studies with PARP-1 chemical competitive inhibitors (as well as from similar approaches like negative dominant or nutritional inhibition) must be interpreted with great scrutiny. Inhibitors, especially at high concentrations, are usually not monospecific; characteristically, at least one of the recently discovered nonclassical PARPs is also sensitive to 3-aminobenzamide, which is often used in PARP-1 inhibition studies. Moreover, inhibitors competing with NAD^+ do not hinder PARP-1 from binding to DNA lesions but instead hold back its dissociation from the DNA, thus *prolonging survival of genotoxic lesions*. Hence, the cytotoxicity of these inhibitors may erroneously suggest a *requirement* of PARP-1 for repair, although it is just the repair enzymes that are prevented from having access to the DNA lesions. Nevertheless, the requirement of PARP-1 in repair, at least in BER, could be unequivocally demonstrated by knock-out mice.[53] However, the effect of cisplatin on these animals has not been checked yet. PARP-1 might well play a role in NER too, yet it might aid the removal of cisplatin adducts *other than the reluctant 1,2-d(GpG) adduct*, adducts which are more easily repaired.[22,23] Furthermore, it is clear that the precise action of PARP-1 may depend on several additional factors. For example, it was shown previously that, after treatment of cells with methylnitrosourea, PARP-1 played a role mainly in the repair of *nontranscribed* genes, presumably serving to expose DNA to (base excision) repair enzymes through chromatin remodeling.[54] In the report of Flohr et al.,[41] PARP-1 was claimed to be actively involved in repair of DNA lesions (set at very low, noncytotoxic levels) handled by NER, but *only when a functional Cockayne syndrome complementation group B (CSB) protein was present*. CSB, on the other hand, is known to be essential in *transcription-coupled* repair, which also removes cisplatin adducts.[55] Thus, PARP-1 may appear important for particular repair processes under certain conditions but not under others. In conclusion, the outcome of the PARP-1 action, that is, repair or no repair, may depend inter alia on the specific damaging agent, on the particular repair system with which PARP-1 cooperates, and on

the genetic background of the cell. This notion may shed some light on the burning question of the apparent differential susceptibility of cells to cisplatin (see above). For these reasons, the detected physical association of PARP-1 with the 1,2-d(GpG) cisplatin adduct[30] does not necessarily signify *repair* of this adduct through PARP-1 assistance. Be that as it may, it is to the credit of Zhang et al. that they obtained direct evidence for a probable involvement of PARP-1 in processing the main cisplatin lesion. Their finding will undoubtedly instigate further exciting research.

Further Protein Candidates Interacting with Cisplatin Lesions

What other proteins might have been cross-linked to the 1,2-d(GpG) target? Besides the described species, other proteins are known to interact in vitro and/or in vivo with cisplatin-damaged DNA, for example, histone H1,[11] and with the 1,2-d(GpG) lesion in particular, for example, transcription factor UBF[9,10] and the TATA binding protein (TBP).[6,7] HMGB1, HMGB2, and PARP-1 are abundant proteins; for example, one million molecules of the latter are found in mammalian cell nuclei.[45] Although UBF and TBP are more rare species, they interact with the 1,2-d(GpG) cisplatin adduct with a much greater affinity than HMGB1: their dissociation constants at equilibrium amount to $\approx$60 pM[10] and 300 pM,[56] respectively, in contrast to the value of $\approx$100–600 nM observed for HMGB1.[39,57] Therefore, these known proteins may be found among the few remaining predominant cross-linked bands. In addition, other proteins interacting with structured DNA (for example, topoisomerases or cruciform binding proteins) may also have been captured by the method used and may be identified. In contrast, we do not expect proteins proposed to initiate the NER response by recognizing or binding single-strand DNA regions, for example, replication protein A (RPA),[58,59] to be among the identified species, since the 1,2-d(GpG) cisplatin adduct is known not to perturb the double-strandedness of DNA. On the other hand, recombinant human RPA was indeed shown to bind to a duplex DNA containing a single site-specific 1,2-d(GpG) cisplatin adduct with a 4–6-fold increased affinity, as compared to an undamaged control DNA with an identical sequence.[60] However, single-stranded DNA seems to be a *prerequisite* for high-affinity binding of RPA to duplex cisplatin-damaged DNA.[61,62]

Weaknesses of Affinity Chromatography for the Identification of Interacting Partners

What is the physiological relevance of the approach of Zhang et al.? In fact, by this, as well as by related methods,[62] only abundant and/or quite strongly binding proteins can be identified. Rare or rather weakly binding species might elude detection. In addition, since the native compartmentalization of the nucleus is abolished, the identified proteins only reflect a pool of possible, but not necessarily actual, interactions. Furthermore, due to the inclusion of the strong detergent SDS for the separation of the cross-linked complexes by PAGE, proteins associated with the latter by noncovalent bonds also evade detection. Thus, potentially valuable information about the ensuing steps and signaling pathways might get lost. Perhaps a modification of the approach, for example, by blue native electrophoresis,[63,64] would preserve this information. These contemplations and suggestions can still be implemented. Meanwhile, the method introduced by Zhang et al. may provide an orientation point at which to start when proteins interacting with cisplatin lesions are to be isolated.

Involvement of DNA-Dependent Protein Kinase in Cisplatin Action

The second paper that appeared in April 2004 in the *Proceedings of the National Academy of Sciences U.S.A.*[31] amazed the cisplatin community with an entirely novel concept of cisplatin action, which at the same time underscores the "active" mode of cell killing by this drug.

The DNA-dependent protein kinase (DNA-PK) complex had been previously shown to bind to cisplatin-damaged (linear) DNA in vitro by a method similar to that of Zhang et al.[65] DNA-PK is instrumental in transducing signals from damaged DNA to repair factors. In particular, DNA-PK is involved in reunification of DNA double-strand breaks (DSBs), caused, for example, by ionizing radiation, through a kind of illegitimate recombination, dubbed nonhomologous end joining (NHEJ); in addition, it seems to play a role in senescence, in the structure of chromosomal ends,[66] and in DNA replication and transcription, as well as in growth control by transducing signals to other cellular components (for example, of the cell cycle or the apoptotic machinery).[67] DNA-PK consists of the two DNA-binding Ku polypetides, Ku70 and Ku80, and the catalytic serine/threonine kinase subunit, DNA-PK$_{cs}$; the latter is a member of the phosphatidylinositol 3 (PI3) kinase related family, to which ataxia telangiectasia mutated (ATM) and ATM- and Rad3-related (ATR) proteins, two more central players in DNA-damage signaling, also belong. The DNA-PK activity that binds in vitro cisplatin-damaged DNA ("damaged-DNA recognition protein-1", DRP-1) was purified and shown to be the Ku subunits (Ku heterodimer).[65] In order to check the function of DNA-PK in cisplatin-damage processing in vivo, Jensen and Glazer examined the response of immortalized mouse or hamster cells deficient in components of the DNA-PK complex (Ku80 or DNA-PK$_{cs}$) to one hour of treatment with cisplatin.[31] Surprisingly, they obtained an unexpected phenotype of the mutant cells. At relatively low cell densities there was no difference in sensitivity to cisplatin between mutant and wild-type cells (or mutant cells complemented with the respective functional protein). This would signify that DNA-PK is irrelevant for cisplatin damage and that its isolation by affinity chromatography on cisplatin-damaged DNA[62] would have been fortuitous and/or without physiological relevance. However, when cells were grown at *30–60-fold higher density* than the populations behaving indifferently, Ku80$^{-/-}$ or DNA-PK$_{cs}^{-/-}$ cells were in fact more *resistant* to cisplatin than their wild-type counterparts. This suggested that under conditions of high-density growth, DNA-PK may be a factor that sensitizes wild-type cells towards

cisplatin, for example, by exerting a shielding function on cisplatin adducts against repair (like HMG-box proteins; see above) or by transmitting a death signal from cisplatin damage to the apoptotic machinery (like MMR proteins; see above), or both. With regard to the first possibility, Jensen and Glazer proved that the unexpected higher sensitivity of wild-type cells towards cisplatin was dependent on an unimpaired *kinase* activity of DNA-PK. Thus, mere shielding of cisplatin lesions by binding of DNA-PK to damaged DNA would not suffice as an explanation for the observed sensitivity of wild-type cells. Hence, DNA-PK must be able to *actively* trigger cell death.

Transmission of a Cisplatin-Originated Death Signal to Neighbors through Gap Junctions

So, why was the increased sensitivity towards cisplatin of wild-type cells relative to DNA-PK mutants only perceived at high cell density? Obviously, a sort of communication may have been required that was only possible between cells grown in close proximity and that failed between dispersed, single cells. Three modes of intercellular communication *over short distances* are known: a) the paracrine mode, for example, through soluble cytokines or neurotransmitters, b) ligand–receptor interactions between cell surfaces, for example, at the immunologic synapse; and c) through gap junctions (gap-junction-mediated intercellular communication, "GJIC"), which are tiny channels built between and by two cells that allow the exchange of small molecules, that is, ions, metabolites, or secondary messengers of signal transduction. Jensen and Glazer could demonstrate that, for the presumed intercellular communication, gap junctions were responsible. This was shown compellingly at several different levels, that is, at the gene level by mutants deficient in connexin43, a main component of gap junctions, at the posttranscriptional level by RNA interference against connexin43 mRNA, and at the gene-product level by chemical inhibitors of gap junctions or by forced connexin43 expression in cells that do not express it. All approaches confirmed that functional gap junctions were required for the observed sensitive phenotype of wild-type cells treated with cisplatin. In contrast, abolishment of gap junctions or of their function rendered wild-type cells at high density as insensitive to cisplatin as they were at low density or as the DNA-PK mutants. This signifies that gap junctions are responsible for the *whole* effect, that is, they are necessary and *sufficient* for the propagation of the "entire" death signal to neighbors and that additional pathways (through cytokines or receptors) play no or only a marginal role. The absence of paracrine signaling was also largely corroborated by a separate experiment.

By comparing the number of cell survivors at a given cisplatin concentration, for example, 10 $\mu g\,mL^{-1}$, Jensen and Glaser determined that about 60% of GJIC-negative cells (resistant against intercellular killing) were killed, whereas this amount increased to about 95% with GJIC-positive cells (susceptible to intercellular cell killing). Thus, a substantial portion of wild-type cell demise (about one third) was supposed to be due to intercellular cell killing, which makes this phenomenon an important factor of cisplatin action.

Theoretically, at least two possibilities exist to explain the increased sensitivity of wild-type cells caused by gap junctions. Contact of the two hemichannels ("connexons"), each one on an adjacent cell, may elicit a mutual potentiation of the death signal generated *within* the same cell by an (unknown) outside-in signaling mechanism or it may result in an inside-out transmission of the death signal generated in each cell to its neighbor. Jensen and Glazer demonstrated inside-out directionality of a death signal released by a sensitive cell to its neighbor by showing that wild-type cells (sensitive) mixed with Ku80$^{-/-}$ mutants (more resistant) and, grown at a cisplatin concentration of 5 $\mu g\,mL^{-1}$, could sensitize the mutants to an extent that was dependent on the ratio of sensitive to resistant cells. In order to receive the death signal from a sensitive cell, the gap junctions of the recipient were required to be intact (Figure 2).

Significance of the Results of Jensen and Glazer

In general, signal transduction through gap junctions is well documented.[68–72] Moreover, transmission of a *growth-inhibition* signal or a *death* signal from dying cells to unsuspecting

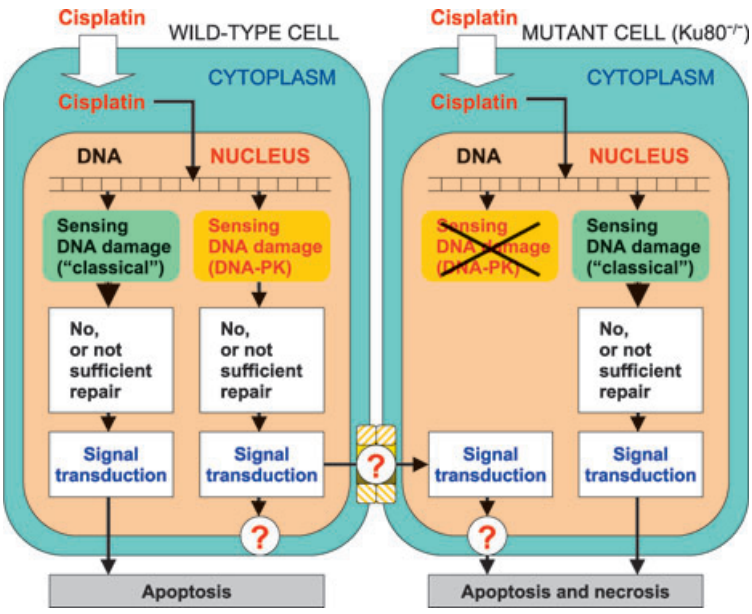


Figure 2. Simplified scheme and open issues (denoted by question marks) of the novel mechanism of cisplatin action suggested by Jensen and Glazer. A condensed "classical" cytotoxic pathway is depicted for lucidity. A signal in a wild-type cell generated by DNA-PK recognition of a cisplatin–DNA lesion is transmitted to a neighboring cell (a Ku80$^{-/-}$ cell is shown here) through a gap junction (drawn here in section), thereby eliciting a death signal in the latter cell. The signal-transmitting molecule, the signal-transduction pathway, and the reason for the (implicit) "self-immunity" of the assassin cell are not known. (See the text for further explanations.)

neighbors through gap junctions has also been described in the literature,[73–75] including the well-known "bystander" phenomenon. The latter is the production both in vitro and in vivo of transmissible, cell-to-cell effects, for example, genotoxic damage, between hit and nonhit cancer cells individually exposed to ionizing irradiation[76–85] or to chemotherapeutic agents.[86] So, what then is the new dimension in the study of Jensen and Glazer? It is the fact that decay of cells due to intercellular communication and collateral injury was also shown for cisplatin. Jensen and Glazer firmly established that gap junctions mediate a considerable portion of cell killing by cisplatin and that this commences at the DNA level by DNA-PK signaling. This also provides an explanation for the earlier observation that cisplatin potentiated the effects of forced expression of connexin26 and vice versa,[75] a fact suggesting that cisplatin may synergize with gap junctions to promote cell death.

The results of Jensen and Glazer are of twofold benefit. First, they add to our understanding of the differential susceptibility of cells to cisplatin; for instance, tumor cells (growing at high density in tissues) that are or become resistant to cisplatin may do so, at least in part, by losing their gap junctions. In fact, some cancerous *tissues* have been found to downregulate expression of connexin genes,[72,87] a process which may render these tissues resistant to the cisplatin regimen. Second, this knowledge may inspire adjuvant cancer therapy on a more rational basis, that is, by (pharmacological or gene-therapy-aided) reexpression in cancerous tissue not only of the tumor-suppressing connexin genes as suggested[72,75,87] but also of DNA-PK in combination with cisplatin treatment.

What Might Be the Signal Mediator?

What might be the, so far unknown, signal entity that traverses gap junctions of cisplatin-treated cells? Only molecules of ≤ 1 kDa are allowed to pass the tiny opening. In other reports of GJIC, Ca^{2+} ions,[74] cyclic adenosine monophosphate (cAMP),[68] and mediators of oxidative stress[80] have been discussed. However, Ca^{2+} ions may be precluded as possible messengers in the work of Jensen and Glazer, since the GJIC inhibitor oleamide, which selectively restricts gap junction permeability to Ca^{2+} ions,[74] was able to reduce cell death of wild-type cells grown at high density down to that of wild-type cells grown at low density. Moreover, "stretched" *macromolecules* may also come into consideration. Consider that, in an *unfolded state*, proteins can get through the mitochondrial membrane through translocases. However, *newly* synthesized proteins are less likely to mediate this intercellular communication, since the transmission of the death signal occurred rapidly (within 30–60 minutes). In addition, double-stranded RNA, for example, small interfering RNAs (siRNAs) that are known to overcome cell barriers, could probably slide longitudinally through gap junctions rather easily. In fact, a putative siRNA mediator could also be a direct consequence of *Ku80-dependent transcription*, since Ku80 is known or thought to participate in additional cellular processes besides the repair of DNA, for example, gene expression.[88]

Possible Physiological Importance of Killing "Innocent" Cells

What is the point of killing neighbors, that is, the bystander effect? First of all, with regard to X-ray radiation, it was demonstrated that the bystander effect may, in fact, be detrimental for micromass cultures of limb bud cells irradiated by a high, challenging X-ray dose; nevertheless, at a low, conditioning (radioadaptive) dose the bystander effect was important for the induction of a *protective* response for cell proliferation and differentiation.[77] However, a "chemoadaptive" effect on the treated cells at low concentrations of cisplatin was not reported by Jensen and Glazer. One hypothesis for the role of bystander effects in biological systems is that they are protective because they terminate division in cells with collateral or possibly preexisting DNA damage that is not properly repaired.[89]

Still Open Questions

In conclusion, the report of Jensen and Glazer indeed fuels intriguing questions, provokes several speculations, and paves the way to further, exciting research. However, this study also raises some issues. Firstly, Jensen and Glazer obtained clearly different behavior in wild-type cells to that in mutant cells at rather high cisplatin concentrations (from 3.33 μM [1 μg mL^{-1}] to 66.7 μM [20 μg mL^{-1}]); at concentrations of <3.33 μM, the difference was very small to nonexistent. The IC_{50} value for cisplatin in most sensitive cells in culture is around 1 μM, depending on the cell line and the assay used. Since the pharmacologically relevant concentration (for example, that found in tumor cells in animals treated with a therapeutic dose of cisplatin) is about 5 μM,[90] it may be questioned whether the effect seen by Jensen and Glazer at these high cisplatin concentrations would be reflected in vivo to a significant extent. Secondly, Jensen and Glazer mention that the effect they observed was not noticed before, partially due to neglect of the respective cell densities or to a possible lack of gap junctions in the tested cells. Yet a different outcome to that found by Jensen and Glazer has indeed been described upon DNA-PK inactivation, that is, DNA-PK mutants were markedly *sensitized* to cisplatin (three- to fourfold) when compared with their respective parental cell line.[91] In the latter work, exponentially growing cells at very low density were exposed to cisplatin for one hour (conditions equivalent to those of Jensen and Glazer). By contrast, Jensen and Glazer found *no difference* in the viability of mutant cells versus wild-type cells at low density. Thirdly, for the observed effect of DNA-PK signaling in vivo upon cisplatin damage to DNA, Jensen and Glazer claim that the kinase function of DNA-PK must be active. However, in the past, other authors[29,92] obtained evidence by in vitro assays that DNA adducts of cisplatin *inhibited* the DNA-PK kinase activity, the alleged prerequisite for the cell's sensitization. *Inhibition* of the DNA-PK phosphorylation activity is also in line with the fact that cisplatin sensitizes tumor cells to ionizing radiation, probably through ensuing *defects in double-strand break repair*.[62] It is difficult to reconcile the arising *resistance* found by Jensen and Glazer with such reports demonstrating *sensitivity* of cells

defective in DNA-PK. Fourthly and most importantly, it is not at all clear what kind of pathway is supposed to generate and convey the death signal. Jensen and Glazer determined that wild-type cells and those mutant in Ku80 or GJIC grown at high density were *equally sensitive* to DNA damage induced by agents other than cisplatin, while Ku80$^{-/-}$ cells retained their known *extreme sensitivity* against ionizing radiation, a result indicating that the pathway might be damage specific. Moreover, Jensen and Glazer mentioned that cells deficient in MMR, which are also known to show moderate resistance to cisplatin,[51,52] did *not* display an apparent density dependence of the resistant phenotype analogous to that of the DNA-PK mutants. Therefore, the mechanism bestowing resistance through DNA-PK inactivation must be fundamentally different to that of MMR deficiency, or, inversely, the mechanism conferring sensitivity through functional DNA-PK must be fundamentally different to the signal transduction through MMR. In fact, the putative new mechanism might be much more peculiar than thought at first glance. It is quite striking that the wild-type cells supposed to generate and transmit a death signal through DNA-PK had *the same phenotype* as the corresponding DNA-PK mutants at low density, since this implies that *the former must have been immune against their own death signaling* (see Figure 2). However, all major known or presumed signal cascades that are triggered by DNA damage also regularly lead to repair or cell death *within the cell, in which they occur*.[93] Therefore, even at low densities one would expect the wild-type cells to be slightly more sensitive than the mutants in the work of Jensen and Glazer. Yet the death signal supposed to be produced by DNA-PK seems to have an *exclusive* effect on neighboring cells and *no impact on the cells that produce it*. Jensen and Glazer demonstrated that wild-type cells in the presence of cisplatin could kill the (more resistant) *Ku80 mutants* at high cell density. It would be interesting to also check whether wild-type cells treated with cisplatin would be able to kill naive (that is, untreated) *wild-type cells* or if the latter would in fact be resistant to GJIC killing.

Possible Interrelations of PARP and DNA-PK in Cisplatin Action

Overall, Jensen and Glazer showed that the generated death signal required intact DNA-PK and had to be transmitted through gap junctions. The events in between remain obscure. Jensen and Glazer reported cell death of sensitive (wild-type) cells to have been apoptotic as well as necrotic, a fact indicating that the signal transduction may involve several pathways, for instance, interaction of DNA-PK with other sensors of DNA damage. In this context, it is reasonable to speculate whether *PARP-1* could be implicated in the process, since PARP-1 is known to effect cell necrosis upon the heavy DNA damage[32] that is to be expected at the high cisplatin concentrations used by Jensen and Glazer. Is PARP-1 able to recruit and perhaps regulate DNA-PK? PARP-1 and DNA-PK were actually found to colocalize at the matrix attachment regions (MARs) of chromatin[94] and, moreover, to copurify by affinity chromatography on "base-unpairing regions" of MARs.[95] Interestingly, Ku autoantigen can form a molecular complex with PARP-1 in the absence of DNA that also suggests a possible *functional* interaction between PARP-1 and DNA-PK.[95,96] DNA-PK$_{cs}$ as well as Ku70 contain poly(ADP-ribose)-binding motifs that could allow PARP-1 to target DNA-PK through poly(ADP-ribose) and regulate some of its domain functions.[97] In fact, DNA-PK was reported to be stimulated in vitro by PARP-mediated protein ADP-ribosylation.[98] On the other hand, DNA-PK was found to suppress PARP-1 activity in vitro, probably through direct binding,[96] in line with the in vivo result that PARP-1 *deficiency* can rescue the site-specific recombination events at the gene loci of the light- and heavy-chain immunoglobulins (V(D)J recombination) in SCID (DNA-PK$_{cs}^{-}$) mice.[47] Finally, PARP has recently been suggested to also provide an (alternative) route for *DSB repair* that complements the DNA-PK/XRCC4/ligase IV dependent NHEJ.[99] The latter may not be surprising in light of the fact that the affinity of PARP to blunt ends in vitro has been determined to be about *tenfold higher* than that of DNA-PK.[100] In conclusion, these findings strongly suggest not only an interaction but possibly also a *mutual coordination of activity* of these enzymes in vivo in response to DNA damage. Therefore, it would be interesting to check whether PARP$^{-/-}$ cells also display a higher resistance *towards cisplatin* than wild-type cells *at high density*.

These last remarks round off the comments on the two considered papers, perhaps anticipating an unexpected connection of the research subjects. It is obvious that a lot more research is required to clarify all the matters arising and to resolve the apparent contradictions. At the same time, it is equally clear that cisplatin, thirty-five years after the discovery of its antitumor activity in various tumor systems by Rosenberg, continues to be a source of utmost scientific excitement.

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[1] C. M. Sorenson, A. Eastman, *Cancer Res.* **1988**, *48*, 6703.
[2] Z. Wang, *Mol. Interventions* **2001**, *1*, 269.
[3] M. Garcia-Diaz, J. F. Ruiz, R. Juarez, G. Terrados, L. Blanco, *Sci. World J.* **2003**, *3*, 422.
[4] Y. Zhang, F. Yuan, H. Xin, X. Wu, D. K. Rajpal, D. Yang, Z. Wang, *Nucleic Acids Res.* **2000**, *28*, 4147.
[5] M. Kartalou, J. M. Essigmann, *Mutat. Res.* **2001**, *478*, 23.
[6] F. Coin, P. Frit, B. Viollet, B. Salles, J. M. Egly, *Mol. Cell. Biol.* **1998**, *18*, 3907.
[7] P. Vichi, F. Coin, J. P. Renaud, W. Vermeulen, J. H. Hoeijmakers, D. Moras, J. M. Egly, *EMBO J.* **1997**, *16*, 7444.
[8] J. Kasparkova, V. Brabec, *Biochemistry* **1995**, *34*, 12379.
[9] P. Jordan, M. Carmo-Fonseca, *Nucleic Acids Res.* **1998**, *26*, 2831.

[10] D. K. Treiber, X. Zhai, H. M. Jantzen, J. M. Essigmann, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 5672.
[11] J. Yaneva, S. H. Leuba, K. van Holde, J. Zlatanova, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 13448.
[12] D. B. Zamble, S. J. Lippard in *Cisplatin—Chemistry and Biochemistry of a Leading Anticancer Drug* (Ed.: B. Lippert), Wiley-VCH, Weinheim, **1999**, p. 73.
[13] J. A. Mello, S. Acharya, R. Fishel, J. M. Essigmann, *Chem. Biol.* **1996**, *3*, 579, and references therein.
[14] T. Ishibashi, S. J. Lippard, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 4219.
[15] J. S. Mymryk, E. Zaniewski, T. K. Archer, *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 2076.
[16] G. L. Evans, J. D. Gralla, *Biochem. Biophys. Res. Commun.* **1992**, *184*, 1.
[17] G. L. Evans, J. D. Gralla, *Biochem. Pharmacol.* **1992**, *44*, 107.
[18] S. Ishida, J. Lee, D. J. Thiele, I. Herskowitz, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 14298.
[19] A. K. Holzer, K. Katano, L. W. Klomp, S. B. Howell, *Clin. Cancer Res.* **2004**, *10*, 6744.
[20] R. Safaei, A. K. Holzer, K. Katano, G. Samimi, S. B. Howell, *J. Inorg. Biochem.* **2004**, *98*, 1607.
[21] A. G. Quiroga, J. M. Perez, E. I. Montero, J. R. Masaguer, C. Alonso, C. Navarro-Ranninger, *J. Inorg. Biochem.* **1998**, *70*, 117.
[22] D. E. Szymkowski, K. Yarema, J. M. Essigmann, S. J. Lippard, R. D. Wood, *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 10772.
[23] J. G. Moggs, D. E. Szymkowski, M. Yamada, P. Karran, R. D. Wood, *Nucleic Acids Res.* **1997**, *25*, 480.
[24] I. Judson, L. R. Kelland, *Drugs* **2000**, *59* (Suppl. 4), 29.
[25] M. J. Piccart, H. Lamb, J. B. Vermorken, *Ann. Oncol.* **2001**, *12*, 1195.
[26] L. S. Hollis, W. I. Sundquist, J. N. Burstyn, W. J. Heiger-Bernays, S. F. Bellon, K. J. Ahmed, A. R. Amundsen, E. W. Stern, S. J. Lippard, *Cancer Res.* **1991**, *51*, 1866.
[27] N. Farrell, L. R. Kelland, J. D. Roberts, M. van Beusichem, *Cancer Res.* **1992**, *52*, 5065.
[28] A. Eastman in *Cisplatin—Chemistry and Biochemistry of a Leading Anticancer Drug* (Ed.: B. Lippert), Wiley-VCH, Weinheim, **1999**, p. 111.
[29] M. Kartalou, J. M. Essigmann, *Mutat. Res.* **2001**, *478*, 1.
[30] C. X. Zhang, P. V. Chang, S. J. Lippard, *J. Am. Chem. Soc.* **2004**, *126*, 6536.
[31] R. Jensen, P. M. Glazer, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 6134.
[32] D. D'Amours, S. Desnoyers, I. D'Silva, G. G. Poirier, *Biochem. J.* **1999**, *342*, 249.
[33] R. B. Ciccarelli, M. J. Solomon, A. Varshavsky, S. J. Lippard, *Biochemistry* **1985**, *24*, 7533.
[34] W. J. Heiger-Bernays, J. M. Essigmann, S. J. Lippard, *Biochemistry* **1990**, *29*, 8461.
[35] B. Lambert, J.-L. Jestin, P. Brehin, C. Oleykowski, A. T. Yeung, P. Mailliet, C. Pretot, J.-B. Le Pecq, A. Jacquemin-Sablon, J.-C. Chottard, *J. Biol. Chem.* **1995**, *270*, 21251.
[36] S. J. Brown, P. J. Kellett, S. J. Lippard, *Science* **1993**, *261*, 603.
[37] J. C. Huang, D. B. Zamble, J. T. Reardon, S. J. Lippard, A. Sancar, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 10394.
[38] M. M. McA'Nulty, S. J. Lippard, *Mutat. Res.* **1996**, *362*, 75.
[39] P. M. Pil, S. J. Lippard, *Science* **1992**, *256*, 234.
[40] D. B. Zamble, D. Mu, J. T. Reardon, A. Sancar, S. J. Lippard, *Biochemistry* **1996**, *35*, 10004.
[41] C. Flohr, A. Bürkle, J. P. Radicella, B. Epe, *Nucleic Acids Res.* **2003**, *31*, 5332.
[42] A. Bürkle, G. Chen, J. H. Kupper, K. Grube, W. J. Zeller, *Carcinogenesis* **1993**, *14*, 559.
[43] S. J. Miknyoczki, S. Jones-Bolin, S. Pritchard, K. Hunter, H. Zhao, W. Wan, M. Ator, R. Bihovsky, R. Hudkins, S. Chatterjee, A. Klein-Szanto, C. Dionne, B. Ruggeri, *Mol. Cancer Ther.* **2003**, *2*, 371.
[44] J. Lagueux, L. Menard, B. Candas, G. Brochu, F. Potvin, A. Verreault, P. F. Cook, G. G. Poirier, *Biochim. Biophys. Acta* **1995**, *1264*, 201.
[45] T. Lindahl, M. S. Satoh, G. G. Poirier, A. Klungland, *Trends Biochem. Sci.* **1995**, *20*, 405.
[46] T. Lindahl, M. S. Satoh, G. Dianov, *Philos. Trans. R. Soc. London Ser. B* **1995**, *347*, 57.
[47] C. Morrison, G. C. Smith, L. Stingl, S. P. Jackson, E. F. Wagner, Z. Q. Wang, *Nat. Genet.* **1997**, *17*, 479.
[48] T. Miyashita, J. C. Reed, *Cell* **1995**, *80*, 293.
[49] A. Nehme, R. Baskaran, S. Aebi, D. Fink, S. Nebel, B. Cenni, J. Y. Wang, S. B. Howell, R. D. Christen, *Cancer Res.* **1997**, *57*, 3253.
[50] J. G. Gong, A. Costanzo, H. Q. Yang, G. Melino, W. J. Kaelin, M. Levrero, J. Y. Wang, *Nature* **1999**, *399*, 806.
[51] D. Fink, S. Nebel, S. Aebi, H. Zheng, B. Cenni, A. Nehme, R. D. Christen, S. B. Howell, *Cancer Res.* **1996**, *56*, 4881.
[52] J. T. Drummond, A. Anthoney, R. Brown, P. Modrich, *J. Biol. Chem.* **1996**, *271*, 19645.
[53] J. M. de Murcia, C. Niedergang, C. Trucco, M. Ricoul, B. Dutrillaux, M. Mark, F. J. Oliver, M. Masson, A. Dierich, M. LeMeur, C. Walztinger, P. Chambon, G. de Murcia, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 7303.
[54] L. S. Ray, S. Chatterjee, N. A. Berger, V. I. Grishko, S. P. LeDoux, G. L. Wilson, *Mutat. Res.* **1996**, *363*, 105.
[55] K. B. Lee, D. Wang, S. J. Lippard, P. A. Sharp, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 4239.
[56] Y. Jung, Y. Mikata, S. J. Lippard, *J. Biol. Chem.* **2001**, *276*, 43589.
[57] Y. Jung, S. J. Lippard, *Biochemistry* **2003**, *42*, 2664.
[58] A. Sancar, *Annu. Rev. Biochem.* **1996**, *65*, 43.
[59] R. D. Wood, *Biochimie* **1999**, *81*, 39.
[60] S. M. Patrick, J. J. Turchi, *Biochemistry* **1998**, *37*, 8808.
[61] S. M. Patrick, J. J. Turchi, *J. Biol. Chem.* **1999**, *274*, 14972.
[62] J. J. Turchi, K. M. Henkels, I. L. Hermanson, S. M. Patrick, *J. Inorg. Biochem.* **1999**, *77*, 83.
[63] M. M. Camacho-Carvajal, B. Wollscheid, R. Aebersold, V. Steimle, W. W. Schamel, *Mol. Cell. Proteomics* **2004**, *3*, 176.
[64] H. Schagger, W. A. Cramer, G. von Jagow, *Anal. Biochem.* **1994**, *217*, 220.
[65] J. J. Turchi, K. Henkels, *J. Biol. Chem.* **1996**, *271*, 13861.
[66] C. Featherstone, S. P. Jackson, *Mutat. Res.* **1999**, *434*, 3.
[67] A. Nussenzweig, C. Chen, V. da Costa Soares, M. Sanchez, K. Sokol, M. C. Nussenzweig, G. C. Li, *Nature* **1996**, *382*, 551.
[68] A. S. Burnside, P. Collas, *Eur. J. Cell Biol.* **2002**, *81*, 585.
[69] A. Ito, N. Morita, D. Miura, Y. Koma, T. R. Kataoka, H. Yamasaki, Y. Kitamura, Y. Kita, H. Nojima, *Carcinogenesis* **2004**, *25*, 2015.
[70] W. H. Evans, P. E. Martin, *Bioessays* **2002**, *24*, 876.
[71] A. M. Simon, D. A. Goodenough, *Trends Cell. Biol.* **1998**, *8*, 477.
[72] K. K. Hirschi, C. E. Xu, T. Tsukamoto, R. Sager, *Cell Growth Differ.* **1996**, *7*, 861.
[73] P. P. Mehta, J. S. Bertram, W. R. Loewenstein, *Cell* **1986**, *44*, 187.
[74] V. A. Krutovskikh, C. Piccoli, H. Yamasaki, H. Yamasaki, *Oncogene* **2002**, *21*, 1989.
[75] M. Tanaka, H. B. Grossman, *Hum. Gene Ther.* **2001**, *12*, 2225.
[76] H. Zhou, G. Randers-Pehrson, M. Suzuki, C. A. Waldren, T. K. Hei, *Radiat. Prot. Dosim.* **2002**, *99*, 227.
[77] B. Wang, H. Ohyama, Y. Shang, K. Fujita, K. Tanaka, T. Nakajima, S. Aizawa, O. Yukawa, I. Hayata, *Radiat. Res.* **2004**, *161*, 9.
[78] E. I. Azzam, S. M. de Toledo, J. B. Little, *Cancer Res.* **2003**, *63*, 7128.
[79] E. I. Azzam, S. M. de Toledo, J. B. Little, *Oncogene* **2003**, *22*, 7050.
[80] J. B. Little, E. I. Azzam, S. M. de Toledo, H. Nagasawa, *Radiat. Prot. Dosim.* **2002**, *99*, 159.
[81] E. I. Azzam, S. M. de Toledo, J. B. Little, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 473.
[82] E. I. Azzam, S. M. de Toledo, A. J. Waker, J. B. Little, *Cancer Res.* **2000**, *60*, 2623.
[83] W. F. Morgan, *Mil. Med.* **2002**, *167*, 44.
[84] C. Mothersill, C. B. Seymour, *Nat. Rev. Cancer* **2004**, *4*, 158.
[85] K. M. Prise, O. V. Belyakov, M. Folkard, B. D. Michael, *Int. J. Radiat. Biol.* **1998**, *74*, 793.
[86] M. Tanaka, G. C. Fraizer, J. De La Cerda, R. J. Cristiano, M. Liebert, H. B. Grossman, *Gene Ther.* **2001**, *8*, 139.
[87] D. W. Laird, P. Fistouris, G. Batist, L. Alpert, H. T. Huynh, G. D. Carystinos, M. A. Alaoui-Jamali, *Cancer Res.* **1999**, *59*, 4104.
[88] E. D. Arrington, M. C. Caldwell, T. S. Kumaravel, A. Lohani, A. Joshi, M. K. Evans, H. T. Chen, A. Nussenzweig, N. J. Holbrook, M. Gorospe, *Free Radical Biol. Med.* **2000**, *29*, 1166.
[89] C. Mothersill, R. J. Seymour, C. B. Seymour, *Radiat. Res.* **2004**, *161*, 256.
[90] B. Rosenberg in *Cisplatin—Chemistry and Biochemistry of a Leading Anticancer Drug* (Ed.: B. Lippert), Wiley-VCH, Weinheim, **1999**, p. 3.
[91] C. Muller, P. Calsou, P. Frit, C. Cayrol, T. Carter, B. Salles, *Nucleic Acids Res.* **1998**, *26*, 1382.
[92] J. J. Turchi, S. M. Patrick, K. M. Henkels, *Biochemistry* **1997**, *36*, 7586.

[93] C. Bernstein, H. Bernstein, C. M. Payne, H. Garewal, *Mutat. Res.* **2002**, *511*, 145.
[94] F. Tramontano, S. Di Meglio, P. Quesada, *J. Cell. Biochem.* **2004**.
[95] S. Galande, T. Kohwi-Shigematsu, *Crit. Rev. Eukaryotic Gene Expression* **2000**, *10*, 63.
[96] Y. Ariumi, M. Masutani, T. D. Copeland, T. Mimori, T. Sugimura, K. Shimotohno, K. Ueda, M. Hatanaka, M. Noda, *Oncogene* **1999**, *18*, 4616.
[97] J. M. Pleschke, H. E. Kleczkowska, M. Strohm, F. R. Althaus, *J. Biol. Chem.* **2000**, *275*, 40974.
[98] T. Ruscetti, B. E. Lehnert, J. Halbrook, H. Le Trong, M. F. Hoekstra, D. J. Chen, S. R. Peterson, *J. Biol. Chem.* **1998**, *273*, 14461.
[99] M. Audebert, B. Salles, P. Calsou, *J. Biol. Chem.* **2004**.
[100] I. D'Silva, J. D. Pelletier, J. Lagueux, D. D'Amours, M. A. Chaudhry, M. Weinfeld, S. P. Lees-Miller, G. G. Poirier, *Biochim. Biophys. Acta* **1999**, *1430*, 119.

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