Damage-recognition proteins as a potential indicator of DNA-damage-mediated sensitivity or resistance of human cells to ultraviolet radiation

Chuck C.-K. CHAO

Tumor Biology Laboratory, Departments of Biochemistry and Medicine, Chang Gung Medical College, Taoyuan, Taiwan 33332, Republic of China

We have previously identified damage-recognition proteins that bind to cisplatin[*cis*-diamminedichloroplatinum(II), a DNA cross-linking agent]- or u.v.-modified DNA in HeLa cells [Chao, Huang, Huang & Lin-Chao (1991) *Mol. Cell. Biol.* 11, 2075–2080; Chao, Huang, Lee & Lin-Chao (1991) *Biochem. J.* 277, 875–878]. In the present study we compared damage-recognition proteins in cells expressing different sensitivities to DNA damage. An increase in damage-recognition proteins and an enhancement of plasmid re-activation were detected in HeLa cells resistant to cisplatin and u.v. However, repair-defective cells derived from xeroderma-pigmentosum (a rare skin disease) patients did not express less cisplatin damage-recognition proteins than repair-competent cells, suggesting that damage-recognition-protein expression may not be related to DNA repair. By contrast, cells resistant to DNA damage consistently expressed high levels of u.v.-modified-DNA damage-recognition proteins. The results support the notion that u.v. damage-recognition proteins are different from those that bind to cisplatin. These findings also suggest that the damage-recognition proteins identified could be used as potential indicators of the sensitivity or resistance of cells to u.v.

INTRODUCTION

The DNA cross-linking agent *cis*-diamminedichloroplatinum(II), or cisplatin, is widely used for the treatment of a variety of tumours. Frequently, however, tumour cells become resistant to it, especially after long-term exposure to the drug, thereby limiting its use in cancer treatment. These clinically selected resistant cells can be mimicked in cell cultures by stepwise exposure of cells to increasing concentration of cisplatin [1–5]. Indirect evidence has suggested that cisplatin resistance (CPR) can be due to enhanced cellular ability in eliminating cisplatin–DNA adducts, including intra- and inter-strand cross-links [6–14]. This is supported by the observation that re-activation of genes on transfected plasmid DNA is enhanced in CPR cells [15–17]. The repair of cisplatin-modified DNA (CMD) involves a variety of enzymes and accessory proteins that presumably must have access to the damaged DNA for effective repair to occur [18,19]. It is reasonable to think that one or more of these factors can be identified through DNA-binding activity. In fact, it has been documented that nuclear extracts isolated from mammalian and yeast mutants in DNA repair fail to interact with damaged DNA [20,21]. Thus, for clinical application, the DNA-damage-mediated sensitivity and resistance of cancer cells to cisplatin may potentially be monitored by the use of a DNA-binding assay.

To test this possibility, we have applied gel-mobility-shift assay to detect CMD as well as u.v.-modified DNA (UVM) recognition proteins from HeLa cells [22,23]. In the present study, we further analysed the damage-recognition proteins (DRPs) identified in human cells expressing different sensitivities to DNA damage. The results indicate that there is overexpression of DRPs and an increase in DNA-repair capacity (reflected in plasmid re-activation) in resistant HeLa cells. However, repair-defective XP cells did not express less ‘cisplatin’ DRPs than

repair-competent cells. Our findings also suggest that DRPs could be used as potential indicators of the sensitivity or resistance of human cells to u.v. The unreliability of cisplatin DRPs as indicators of cellular sensitivity to the drug was discussed.

EXPERIMENTAL

Cell lines and determination of cytotoxicity

HeLa CPR and parental cells [15] were maintained in monolayer culture in Dulbecco-modified Eagle’s medium (DMEM) (GIBCO, Gaithersburg, MD, U.S.A.) containing 10 % (v/v) foetal-bovine serum (FBS), 100 µg of streptomycin/ml and 100 units of penicillin/ml, and incubated at 37 °C in a humidified atmosphere of 5 % (v/v) CO₂ in air. Control cell lines VA13 [WI-13 subline, American Type Culture Collection (A.T.C.C.)] and XP fibroblasts (xeroderma-pigmentosum complementation group A and group E, A.T.C.C.) were maintained according to the supplier’s instructions.

Cytotoxicity was assayed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric method [24]. The fold resistance of cells to cisplatin or u.v. is defined as the ratio of ID₅₀ (the cisplatin or u.v. dose resulting in 50 % survival) of the tested cells to that of the parental cells.

Cisplatin and u.v. modification of plasmid DNA

For cisplatin modification, the pRSVcat or f103 DNA (see below) at a concentration of 100 µg/ml (300 µM-nucleotide phosphate) was treated with cisplatin in 3 mM-NaCl and 1 mM-sodium phosphate, pH 7.4, at 37 °C for 20 h as described by Ushay *et al.* [25]. The cisplatin concentration varied between 0.3 and 30 µM. The molar ratio of cisplatin to nucleotide phosphate at the onset of incubation, *r_i*, varied between 0.001 and 0.10. Under these conditions, the molar ratio of bound cisplatin to nucleotide phosphate, *r_b*, reaches a steady-state level of ~ 0.8 *r_i*.

Abbreviations used: cisplatin, *cis*-diamminedichloroplatinum(II); CMD, cisplatin-modified DNA; CPR, cisplatin resistance; DRP, damage-recognition protein; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; UVM, u.v.-modified DNA; DMEM, Dulbecco-modified Eagle’s medium; FBS, foetal-bovine serum; ID₅₀, dose resulting in 50 % survival; CAT, chloramphenicol acetyltransferase; XP, xeroderma pigmentosum; XPA or XPE, XP group A or E cells.

Fig. 2. Identification of CMD DRP

(a) Competition of CMD-binding activity with indicated amount of unlabelled CMD (UCMD). A typical reaction mixture included 2 μ g of HeLa nuclear extracts (N.E.), 2 μ g of poly(dI·dC) and 0.3 ng of cisplatin-treated f103 probe (r_f = 0.08). Control lanes are: lane 1, without nuclear extract; lane 2, untreated f103 probe. (b) Dose response of CMD-binding activity. Nuclear extract was incubated with f103 probe carrying the indicated amount of cisplatin modification (r_f). The control lane (lane 1) contained untreated f103 probe; f, unbound free DNA probe; arrowhead, protein-bound DNA probe.

Fig. 3. Identification of UVMD DRP

(a) Competition of UVMD binding activity with indicated amount of unlabelled UVMD (UUVMD). A typical reaction mixture included 2 μ g of HeLa nuclear extracts (N.E.), 2 μ g of poly(dI·dC) and 0.3 ng of 2 kJ/m² u.v.-irradiated f130 probe. Control lanes are: lane 1, without nuclear extract; lane 2, unirradiated f130 probe. (b) Dose response of UVMD-binding activity. Nuclear extract was incubated with u.v.-modified f130 probe carrying the indicated fluence of u.v. The control lane (lane 1) contained unirradiated f130 probe. Symbols are the same as those in Fig. 2.

The calculated fold increase is indicated in parentheses. Relative to HeLa cells, CPR and revertant cells show a 3.4- and 1.2-fold increase in re-activation of cisplatin-damaged plasmid, and a 2- and 1.15-fold increase in u.v. damage. In contrast, XPA cells showed a diminished ability to re-activate transfected plasmid modified by both damaging agents.

Identification of DRPs

A CMD DRP was identified in HeLa nuclear extracts by using the gel-mobility-shift assay. To verify that the observed DRP is specific for CMD, increasing amounts of unlabelled competitor were included in the binding reaction. As shown in Fig. 2(a), increasing the amount of CMD competitor effectively lowered the CMD DRP (lanes 3–6; indicated by the arrowhead). For example, 10-fold competitor (3 ng) competed for 70 % (15–20 % variation in three determinations) of the binding activity (compare lanes 3 and 5). This was supported by the dose-response studies. As shown in Fig. 2(b), CMD DRP is nearly proportional to cisplatin damage detected. For example, the DRP was readily detected in CMD with an r_f greater than 0.01 (lanes 5–7). The data indicate that the detected DRP is specific for CMD.

A UVMD DRP was also identified. The specificity of UVMD-

Fig. 4. Overexpression of DRPs in resistant cells

(a) CMD-binding activity. (b) UVMD-binding activity. A typical binding-reaction mixture included 2 μ g of nuclear extracts of parental (HeLa), resistant (CPR), revertant (HeLa-rev), VA13, XP group E (XPE) or group A (XPA) cells. Symbols are the same as those in Fig. 3.

binding activity was studied as follows. The u.v.-modified f130 competitor significantly competed for the UVMD binding (see lanes 4–6 of Fig. 3a). For example, more than 50 % of the binding activity was abolished by 3 ng (i.e. 10-fold that of the UVMD probe) of the competitor (compare lanes 3 and 5). The dose response of UVMD DRP was also determined. As shown in Fig. 3(b), the level of UVMD DRP increases with increasing amount of u.v. damage (lanes 2–4). However, the binding activity did not further increase with DNA damage induced by 5 kJ/m² of u.v. irradiation, compared with 3 kJ/m², suggesting that the UVMD-binding activity saturates at higher levels of u.v. damage. These results support the notion that the identified UVMD DRP is specific for UVMD. In addition, it was also found that the identified CMD- and UVMD-binding factors were sensitive to proteinase K, but not to RNAase (results not shown), suggesting that these DRPs are proteins and that their activity is not dependent on RNA molecules.

Overexpression of DRPs in resistant cells

To understand further whether the development of CPR may be reflected by the level of DRPs in cells, the DRPs identified above were measured in cells featuring different levels of sensitivity to DNA damage and DNA repair. As shown in Fig. 4(a), HeLa-CPR cells exhibited a CMD-binding activity (indicated with arrowheads) 2-fold that of the parental HeLa cells (compare lanes 3 and 4). HeLa-rev cells showed slightly reduced binding activity compared with HeLa-CPR cells (compare lanes 4 and 5). In addition, VA13 and XP cells (lanes 6–8) expressed a similar level of CMD-binding activity. The data suggest that the DNA-binding activity in these cells is only weakly correlated, if at all, with the CPR phenotype.

The differential expression of UVMD DRP in these cells is much more dramatic. Under optimized binding conditions, two UVMD-binding activities were identified (indicated with arrowheads in Fig. 4b). Both UVMD-binding factors were overexpressed by 4–5-fold in HeLa-CPR cells as compared with HeLa parental cells (compare lanes 3 and 4). The enhanced UVMD-binding activity of HeLa-rev cells diminished to a level

comparable with that of HeLa cells (compare lanes 3–5). VA13 cells also exhibited high levels of UVMD-binding activity, i.e. ~80% of that shown by HeLa-CPR cells. Interestingly, UVMD-binding activity was not detected in XP group E (XPE) cells (lane 7). XPA cells consistently showed less than 10% of the UVMD binding of VA13 cells (compare lanes 6 and 8). These UVMD-binding factors are probably the same as the XPE factor identified by Chu and colleagues [20]. However, it is not clear whether the two UVMD DRPs are the same, because one cannot exclude the possibility that the slower-migrating band is the dimeric form of the UVMD DRP.

DISCUSSION

In the present study we have shown that HeLa-CPR cells expressed increasing binding activities for both CMD and UVMD compared with HeLa parental cells. Repair-defective XP cells did not express (XPE), or expressed a reduced level (XPA) of, UVMD-binding factors compared with repair-competent VA or HeLa cells. Therefore the CPR and u.v. resistance of CPR cells is associated with the overexpression of DRPs in these cells. It should be noted that the DRPs identified are specific for cisplatin or u.v. damage, because cross-competition was not observed, and undamaged DNA did not compete for DRPs [22–23]. These results suggest that the identified UVMD-binding factors may be potentially useful as indicators of the sensitivity or resistance of cancer cells to DNA-damaging agents. However, XP cells also expressed a significant level of CMD DRP, indicating that the sensitivity or resistance of cells to cisplatin does not necessarily correlate with recognition of DNA damage.

Lippard and his colleagues have recently reported that the apparent molecular mass of a CMD-binding factor identified in human cells by gel-mobility-shift assay is 91 kDa [36]. We have also identified a CMD DRP with a molecular mass of about 95 kDa using Southwestern-blot hybridization [23]. However, there was no overexpression of CMD DRP in their resistant HeLa cells [36]. Therefore the level of CMD DRP in resistant cells is not necessarily correlated with the sensitivity of cells to cisplatin. In addition, it is not clear what the connection is between the binding factors detected by gel-mobility-shift assay and those found by the modified Southwestern-blotting procedure.

Repair of UVMD and CMD involves a nucleotide excision–repair pathway in mammalian cells [37]. The overexpression of DRPs which recognize both types of DNA damage in our CPR cells is not surprising. However, it is hard to imagine that XPA cells do not express a reduced level of CMD DRP. One possibility is that the constitutive level of DRPs does not reflect the cellular ability in DNA repair, because other proteins and enzymes also participate in this process. Alternatively, it is possible that the CMD DRP has nothing to do with DNA repair. In addition, it is known that, unlike the repair of u.v. damage (pyrimidine dimer), cisplatin cross-links are not repaired in a strand-specific manner in rodent cells [38], supporting the notion that repair of CMD is not exactly the same as that of UVMD. Furthermore, the inducible expression of DRPs [23] may be involved in the CPR phenotype. The damage-inducible response has been well demonstrated in a bacterial system [39]. A similar inducible response may exist in mammalian cells [18]. A u.v.-inducible UVMD binding activity has also been observed in HeLa cells (C. C.-K. Chao, unpublished work) and monkey cells [40]. Indirect evidence has suggested an inducible repair activity in an increasing spectrum of cells, including excision–repair in human cells [41] and photorepair in frog [42] and yeast [43] cells. However, questions about the inconsistency of CMD DRP in CPR and XP cells remain unanswered. Further studies are

needed in order to answer a few among many other questions; for example, are the two separately identified binding factors for UVMD and CMD related? What role do the identified damaged-DNA-binding factors play in the CPR phenotype? Answering these questions will facilitate an understanding of the development of CPR phenotype.

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