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ORIGINAL ARTICLE

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Human colon cancer cells surviving high doses of cisplatin or oxaliplatin in vitro are not defective in DNA mismatch repair proteins

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Abstract *Purpose:* Alterations in the DNA mismatch repair (MMR) proteins have been associated with an increased resistance of many cancer cell lines to cisplatin. The aim of this work was to investigate whether defects in DNA MMR proteins are involved in the survival of human colorectal cancer cells in the presence of high concentrations of cisplatin and oxaliplatin, a diaminocyclohexane (DACH) platinum compound whose adducts are not recognized by the MMR system. *Methods:* Six unselected human colon cancer cell lines (HT29, HCT15, HCT116, Caco2, SW480 and SW620) were treated with a single 3-h exposure to cisplatin or oxaliplatin at suprapharmacological concentrations, ranging from 50 to 200 μg/ml. The microsatellite stability and the expression of MMR proteins in the parental cell lines and in the drug-selected subpopulations were studied. *Results:* Most cells underwent apoptosis in the days following the cisplatin or oxaliplatin treatment, but some colonies expanded 3 to 4 weeks after, suggesting the presence of innately resistant cells in the six parental cell lines. Microsatellite instability (MIN), which reflects genetic defects in the DNA MMR system, was detected only in the HCT116 parental cell line and its drug-selected counterparts, due to a known mutation in the *hMLH1* gene. No acquired MIN was observed in the other cisplatin-selected sublines derived from the HT29, HCT15, Caco2, SW480 or SW620 parental cells. In the same way, Western blot analysis showed that expression of the DNA MMR proteins hMLH1, hPMS1, hPMS2, hMSH2 and hMSH6 did not differ between the parental and the drug-surviving cells. *Conclusions:* These results indicate that high-level resistance of human colon cancer cells to high doses of cisplatin and oxaliplatin does not seem to be related to acquired defects in the DNA MMR proteins.

Keywords Cisplatin · Oxaliplatin · Drug resistance · DNA mismatch repair · Colon cancer

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Introduction

Cisplatin is a potent anticancer drug whose biological activity is a consequence of the formation of covalent adducts between the platinum compound and some bases in the DNA [1]. Cisplatin contributes to the cure of many testicular and some ovarian carcinomas. However, the efficiency of cisplatin is low in colorectal cancer (CRC), with fewer than 20% clinical responses when used alone or in combination [2, 3]. Oxaliplatin is a new diaminocyclohexane (DACH) platinum compound. This cisplatin analogue produces different types of DNA adducts which are not recognized by the mismatch repair (MMR) system [4]. Oxaliplatin is clinically active in metastatic CRC, with a response rate of about 50% when used with 5-fluorouracil and folinic acid [5]. Nevertheless, oxaliplatin is less active when used alone with a response rate of 18% in previously untreated patients [6]. Moreover, an acquired resistance to oxaliplatin-fluorouracil combinations always develops in patients with CRC after a few months of treatment.

Numerous mechanisms have been advocated to explain the acquired or inherent resistance of cancer cells to cisplatin and its derivatives (reviewed in reference 7). These include decreased accumulation of the drug,

increased cellular detoxification by glutathione or metallothioneins, increased removal of cisplatin DNA adducts and enhanced DNA repair, tolerance to platinum-DNA damage, and alterations in signal transduction pathways involved in apoptosis activation that occur in tumour cells following drug exposure.

Several recent studies have focused on the possible role of defects in the DNA MMR system in the resistance of cancer cells to cisplatin [8, 9, 10] or to alkylating agents [11, 12]. This DNA repair system recognizes and restores misincorporated bases which frequently occur during DNA replication [13]. In cells with mutated DNA MMR proteins, mono- to hexanucleotide repeat sequences of genomic DNA, termed microsatellites, are not accurately replicated and length alteration of microsatellites results in additional bands on DNA electrophoresis after PCR amplification. This phenomenon is termed replication error phenotype (RER+) or microsatellite instability (MSI or MIN). Mutations or deletion of DNA MMR genes probably play a role in the genesis of CRC by increasing DNA instability and favouring the mutation of oncogenes, such as *K-ras*, *APC* or *TGFbetaR2* [14, 15]. Mutations in the DNA MMR genes, namely *hMSH2*, *hMLH1*, *hPMS1*, *hPMS2* and the more recently described *hMSH6*/*GTBP*, are involved in the most common form of hereditary CRC, hereditary nonpolyposis CRC (HNPCC) [16] and are also acquired at an early stage of the cancer genesis in sporadic non-hereditary CRC [17].

Cisplatin and doxorubicin resistance have been reported to be associated with the absence of hMLH1 and greatly reduced levels of hPMS2 [15, 18] or hMSH2 [9] in ovarian tumour cells. In melanoma cells exhibiting resistance to cisplatin, etoposide and vindesine, the nuclear content of each DNA MMR protein hMLH1, hMSH2, hMSH6 is reduced by 30–70% [19]. Two cisplatin-resistant sublines of the ovarian adenocarcinoma cell line A2780 (A2780/CP70 and A2780/MCP-1) are defective in hMLH1 expression and are more resistant to cisplatin than the parental MMR-proficient cells [20]. Loss of hMLH1 expression has also been shown to be a high-frequency event following in vivo exposure of human ovarian cancer cells to cisplatin. hMLH1-deficient lines appear to have lost the ability to engage G1 and G2 cell cycle arrest after cisplatin damage [10]. It has been hypothesized that futile cycles of synthesis, past the cisplatin-DNA adducts, followed by removal of newly synthesized DNA strands by an active MMR system, may lead to cell death [21]. Thus, loss of MMR could result in drug resistance, either directly by impairing the ability of the cell to detect DNA damage and activate apoptosis, or indirectly by increasing the rate of mutations throughout the genome [11]. Defects in hMLH1 and hMSH6 proteins could also result in increased cisplatin resistance through an increased replicative bypass of cisplatin adducts [8].

However, in most studies that have shown an association between a deficiency in MMR proteins after cisplatin exposure and cisplatin resistance, non-digestive tract cancer cells have been used. No information is currently available on resistance to high doses of platinum drugs in CRC or on the MMR status in colon cancer cells after cisplatin or oxaliplatin treatment. We found that clonogenic colon cancer cells grew after a single 3-h exposure of common unselected human colon cancer cell lines (HT29, HCT15, HCT116, Caco2, SW480 and SW620) to cisplatin at suprapharmacological concentrations. To investigate whether defects in DNA MMR proteins are involved in the survival of these human CRC cells in the presence of cisplatin, we studied the microsatellite stability and the expression of MMR proteins in the six unselected human colon cancer cell lines and in the cisplatin-selected sublines. We also sought to determine whether MIN and the expression of DNA MMR proteins are altered in cells surviving oxaliplatin whose adducts are not recognized by the MMR system.

Materials and methods

Cell lines, cell culture and drug treatment

The HT29, HCT15, HCT116, Caco2, SW480 and SW620 human colon cancer cell lines were obtained from the American Tissue Culture Collection (ATCC, Rockville, Md.). Cells were routinely grown in monolayers at 37°C in DMEM (4.5 g/l glucose) supplemented with 10% fetal bovine serum (GIBCO, Life Technologies, Cergy-Pontoise, France) and 1% L-glutamine. Cell lines were subcultured after detachment with trypsin/EDTA.

For drug sensitivity assays, subconfluent cells were exposed for 3 h to cisplatin or oxaliplatin at concentrations ranging from 50 to 200 μg/ml. The cells were then washed and incubated in drug-free complete medium, with medium renewal twice a week. The survival of resistant cells after cisplatin treatment was evaluated 10 days after drug treatment. Surviving cells were fixed by a 5-min exposure to pure ethanol, stained for 5 min with crystal violet dye (1% in a mixture of water/ethanol, 90/10) and rinsed with abundant tap water. Dye was eluted with acetic acid (33% in distilled water) to measure the optical density (OD) at 540 nm and determine the percentage of surviving cells.

For clonogenic assays, subconfluent cells were exposed for 3 h to cisplatin or oxaliplatin at concentrations ranging from 50 to 200 μg/ml. The cells were then washed and incubated in drug-free complete medium, with medium renewal twice a week. The survival of resistant cells after cisplatin treatment was evaluated 3 to 4 weeks after drug treatment. To count colonies, cells were fixed by a 5-min exposure to pure ethanol, stained for 5 min with crystal violet dye (1% in a mixture of water/ethanol, 90/10) and rinsed with abundant tap water. The maximal concentration of drug at which colonies appeared 3 to 4 weeks after cisplatin treatment was referred to as the maximal tolerated concentration (MTC). Subsequent characterization of the resistant subpopulations was performed on global resistant populations obtained from a mixture of drug-selected colonies as well as individual colonies expanded to obtain monoclonal populations.

Chemicals

Cisplatin [CDDP, *cis*-diamminedichloroplatinum(II)] and oxaliplatin [*trans*-1-diaminocyclohexane (DACH) platinum, L-OHP] were obtained in their commercial forms Cisplatyl and Eloxatine from Roger Bellon Laboratories (Neuilly, France) and Sanofi Winthrop (Gentilly, France), respectively. Both drugs were diluted in culture medium immediately before use.

Immunoblotting

Cells were rinsed with phosphate-buffered saline (NaCl 8 g/l containing KCl 0.2 g/l, KH_2PO_4 0.2 g/l, $Na_2HPO_4{\cdot}7H_2O$ 2.16 g/l) and lysed for 30 min on ice in 150 m*M* NaCl containing 1% NP-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulphate, 5 m*M* EDTA, 50 m*M* Tris-HCl (pH 8), 1 m*M* aprotinin and 0.1 m*M* phenylmethylsulfonyl fluoride. Protein concentrations were determined by the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, Calif.). Equal amounts of total cellular proteins (20 µg) were separated in a 7% SDS-polyacrylamide gel and electrophoretically transferred onto a polyvinylidene difluoride membrane (Bio-Rad Laboratories). Actin was labelled using a monoclonal antibody (mAb) (Innogenex, San Ramon, Calif.), hMLH1 using the G168-15 mAb (Pharmingen, San Diego, Calif.), hMSH2 with the G219-1129 mAb (Pharmingen), hMSH6 using the 44 mAb (Transduction Laboratories, Lexington, Ky.), hPMS2 using the 37 mAb (Transduction Laboratories) and a polyclonal antiserum raised in rabbits against an internal peptide of hPMS1 (kindly provided by Prof. J. Jiricny, Institute of Medical Radiobiology, University of Zurich, Switzerland) as first antibodies. A peroxidase-conjugated anti-mouse antibody (Jackson Immunoresearch Laboratories, West Grove, Pa.) and an enhanced chemiluminescence (ECL) kit (Amersham Pharmacia Biotech, Little Chalfont, UK) were used for the visualization. Analysis of protein labelling was performed using ImageQuant software (Molecular Dynamics, Cambridge, Mass.).

Microsatellite instability

DNA was extracted according to the method of Sambrook et al. [22] and stored at 4°C in 10 m*M* Tris-HCl containing 1 m*M* EDTA buffer.

PCR

The characteristics of the nine microsatellites studied are shown in Table 1. PCR reactions were performed in a 25-µl volume containing 50 ng genomic DNA, 200 m*M* dNTPs (Quantum Bioprobe, Illkirch, France), 1.5–2.0 m*M* magnesium chloride, 0.75 U BioTaq DNA polymerase (Quantum Bioprobe) and 0.32 µ*M* of each primer. The following labelled primers were used: Bat26-HEX, D8S256-HEX, D10S197-6-FAM, D11S2179-FAM, TH01-HEX, AR-TET, D17S855-TET, D3S1514-FAM and TP53-TET (Applied Biosystems, Courtaboeuf, France). Amplifications were performed in a PCR Express Hybrid thermocycler. PCR conditions were: initial denaturation of 5 min at 94°C followed by 30 cycles of 30 s at 94°C, 30 s at the appropriate annealing temperature (55–65°C), 30 s at 72°C and a final extension at 72°C for 5 min. The PCR products in 5-µl aliquots were analysed on 2% agarose gel after ethidium bromide staining. Equal densities, calculated with a Gel Doc 1000 UV system (Bio-Rad), of parental and drug-selected cell DNA, were used for electrophoretic analysis.

Microsatellite analysis

Dilutions of parental and drug-selected cell DNA were prepared in distilled water and 1 µl of the dilution was added to 1 µl GS500-TAMRA size standard and 18 µl loading buffer Template Suppression Reagent (Applied Biosystems). Reaction products were denatured for 5 min at 95°C and the fragments separated under denaturing conditions using an ABI Prism 310 automated fluorescent sequencer. The data collected were further analysed with Genescan and Genotyper software (Applied Biosystems). Peak heights were compared for parental and drug-selected cell lines. MIN was scored when novel alleles were observed. Allele ratios were calculated for informative loci using peak height for each parental and drug-selected counterpart as follows: S2:S1/R2:R1, where S2 and R2 are the height values of the larger length allele product peak for the parental and drug-selected cells, respectively, and S1 and R1 are the height values of the shorter length allele product peak for the parental and drug-selected cells, respectively. All ambiguous samples were analysed at least three times under different PCR conditions and on different electrophoretic runs.

Results

Isolation and characterization of cisplatin- and oxaliplatin-resistant cells

Six unselected human colon cancer cell lines were exposed for 3 h to suprapharmacological concentrations of cisplatin and oxaliplatin ranging from 50 to 200 µg/ml. The cells were then washed and incubated in drug-free complete medium for 3 to 4 weeks. In the days following drug exposure, most cells displayed classical apoptotic figures with progressive cell detachment. After

Table 1. Characterization of the microsatellites analysed in this study

Locus	Nucleotide repeat	Location	Primer sequence	Annealing temperature (°C)	Size (bp)
BAT 26	A	2p16	TGACTACTTTTGACTTCAGCC AACCATTCAACATTTTTAACCC	55	80–100
D8S256	CA	8q24.13	GTTCAAGGGCTCAGGGTTCT CTTCCACCTTTAGCCAAGGA	55	210–232
D10S197	CA	10p11.2	ACCACTGCACTTCAGGTGAC GTGATACTGTCCTCAGGTCTCC	55	161–173
D11S2179	2n	11q23	TAGGCAATACAGCAAGACCCTG GCACTGGAATACGATTCTAGCAC	60	130–144
TH01	TCAT	11p15.5	GTGGGCTGAAAAGCTCCCGATTAT ATTCAAAGGGTATCTGGGCTCTGG	65	179–203
AR	CAG	Xq13	TGGGGAGAACCATCCTCACC TCCAGAATCTGTTCCAGAGC	65	202–250
D17S855	CA	17q21	GGATGGCCTTTTAGAAAGTGG ACACAGACTTGTCCTACTGCC	55	143–155
D3S1514	4n	3p21–14.2	GGCAACAGAGCAAGATGC CCAGCCAGCAGAATTATGA	55	200–280
TP53	CA	17p13.1	ACTGCCACTCCTTGCCCCATTC AGGGATACTATTCAGCCCGAGGTG	65	103–135

2 weeks, virtually all cells seemed to have been killed by the drug treatment. However, a few colonies of adherent cells reappeared from all the treated cell lines, as illustrated for the HT29 cell line (Fig. 1), indicating that some cells did resist the drug exposure.

The maximal concentration of drug at which colonies appeared 3 to 4 weeks after cisplatin treatment (the MTC) was lower for Caco2 (MTC 50 μg/ml) than for HT29, HCT15, HCT116, SW480 and SW620 cells (MTC 200 μg/ml). The MTC of oxaliplatin was equal for the six cell lines (MTC 200 μg/ml).

To obtain sublines following drug exposure of the six cell lines, the resistant colonies were trypsinized and expanded. The cisplatin-resistant subpopulations were designated Caco2-CP50, and HT29-, HCT15-, HCT116-, SW480- and SW620-CP200; the oxaliplatin-resistant subpopulations were designated -OX200.

Drug sensitivity assays were performed on the HT29 and HT29-CP200 cells to estimate the resistance level of each population. A 2.2-fold increase in survival was observed in the HT29-CP200 subpopulation after cisplatin treatment (IC_{50} 110 μg/ml) compared to the HT29 parental cells (IC_{50} 50 μg/ml; Fig. 2). HT29-CP200 cells were also exposed repeatedly to cisplatin in order to estimate the percentage of surviving cells after a second, third or a fourth treatment. The number of surviving colonies increased after each cycle of cisplatin treatment (Fig. 3).

Analysis of microsatellite instability in the parental and drug-selected cells

In order to determine whether alterations in the DNA MMR system were involved in the tolerance of cisplatin and oxaliplatin cytotoxicity, we analysed MIN in parental cells and resistant subpopulations (Table 2). The parental HCT116 cell line is known to have two MIN loci [21, 23]. It extended to three additional loci after cisplatin and oxaliplatin treatment. In addition, instability at one locus was observed for the cisplatin-selected HCT15-CP200 cells. In contrast, no MIN was detected in the resistant subpopulations obtained from the four other parental cell lines (HT29, Caco2, SW620, SW480).

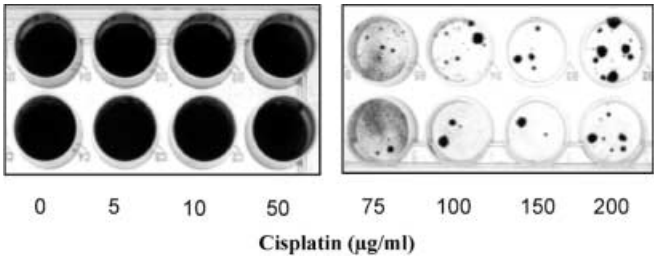

Fig. 1. Isolation of resistant colonies. Cultures were maintained for 3 to 4 weeks after a 3-h exposure of HT29 subconfluent cells to various concentrations of cisplatin (0–200 μg/ml). Surviving colonies were fixed and stained with crystal violet

To study cells from independent colonies, five resistant clones were isolated after two successive cisplatin treatments of the HT29 cell line at 200 μg/ml for 3 h at an interval of 1 month. These independent clones were designed HT29-CP^2 A, B, C, D and E. No MIN was found in the five different HT29-CP^2 resistant clones (A to E) isolated from parental HT29 cells after two exposures to high-dose cisplatin (Table 3).

Expression of MMR proteins

We compared the expression of five MMR proteins in parental and cisplatin- or oxaliplatin-selected cells from the six colon cancer cell lines. Western blot analysis did not reveal any significant differences either in the amounts of protein or in the size of any of the tested MMR proteins (hMLH1, hMSH2, hMSH6, hPMS1 and hPMS2) between parental and drug-surviving cells (Fig. 4a). As reported previously [13, 21], the hMLH1 protein was present in the HCT116 line as a truncated and probably inactive form, attributed to a mutation in the *hMLH1* gene. The parental HCT15 cell line also showed a mutation for hMSH6, as reported previously

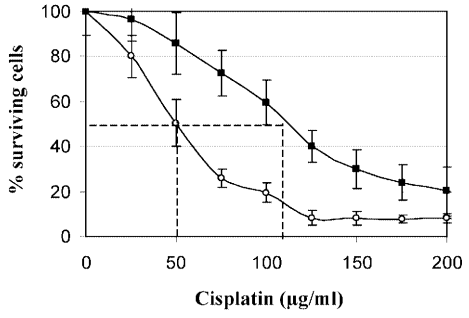

Fig. 2. Drug sensitivity assays. Cisplatin cytotoxicity was evaluated in HT29 (*open squares*) and HT29-CP200 (*solid squares*) cell lines. Surviving cells were fixed and stained with crystal violet. Optical density was measured at 540 nm to determine the percentage of surviving cells. The *dotted lines* indicate the IC_{50} values (IC_{50} 50 and 110 μg/ml, respectively). The values shown are the means ± SD of triplicate assays

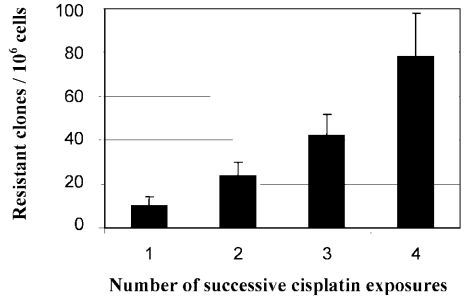

Fig. 3. Clonogenic assays. The clonogenicity of HT29-CP200 cells was evaluated after two, three or four successive exposures of the cells to cisplatin. Surviving colonies were fixed and stained with crystal violet before counting. The *bars* indicate the mean ± SD of three wells

Table 2. Microsatellite mutation analysis of nine loci in the six parental and resistant subpopulations (*cisplatin resistant subpopulations* Caco2-CP50, and HT29-, HCT15-, HCT116-, SW480- and SW620-CP200; *oxaliplatin resistant subpopulations* -OX200). MIN was scored when novel alleles were observed (+ MIN, – no MIN)

Cell line	Locus								
	BAT26	D8S256	D10S197	D11S2179	THO1	AR	D17S855	D3S1514	TP53
HT29	–	–	–	–	–	–	–	–	–
HT29-CP200	–	–	–	–	–	–	–	–	–
HT29-OX200	–	–	–	–	–	–	–	–	–
HCT15	–	–	–	–	–	–	–	–	–
HCT15-CP200	–	–	–	+	–	–	–	–	–
HCT15-OX200	–	–	–	–	–	–	–	–	–
HCT116	–	–	–	+	–	+	–	–	–
HCT116-CP200	–	+	+	+	–	+	+	–	+
HCT116-OX200	–	+	+	+	–	+	+	–	+
Caco2	–	–	–	–	–	–	–	–	–
Caco2-CP50	–	–	–	–	–	–	–	–	–
Caco2-OX200	–	–	–	–	–	–	–	–	–
SW480	–	–	–	–	–	–	–	–	–
SW480-CP200	–	–	–	–	–	–	–	–	–
SW480-OX200	–	–	–	–	–	–	–	–	–
SW620	–	–	–	–	–	–	–	–	–
SW620-CP200	–	–	–	–	–	–	–	–	–
SW620-OX200	–	–	–	–	–	–	–	–	–

[23]. In the same way, no alteration in MMR proteins were found in the five independent HT29-CP^2 resistant clones (A to E) (Fig. 4b).

Discussion

The clinical response to cisplatin or oxaliplatin monotherapy in CRC is low, suggesting the presence of innately resistant cell populations in patients with these cancers. Furthermore, the initial response is often followed by relapse due to further selection of these resistant cells. We showed that a spontaneous resistance to platinum derivatives is indeed frequent in most human colon cancer cell lines. Moreover an increase in the number of resistant colonies was found after each new treatment and the global level of resistance increased in the selected subpopulations. Drug concentrations (ranging from 50 to 200 μg/ml) used in this study for 3 h were far above those reached in plasma of patients receiving cisplatin or oxaliplatin. For example, the peak serum concentration of free cisplatin ranges from 1 to 3 μg/ml after a bolus administration of 50 to 80 mg/m^2 cisplatin, with a short initial half-life of 0.3 h [24]. In the same way, the peak serum concentration of oxaliplatin is 1.6 μg/ml at the end of a 4-h infusion of the maximal tolerated dose of 130 mg/m^2 oxaliplatin, with a rapid decrease and an initial half-life of 0.09 h [25]. The fact that some unselected cells can survive cisplatin and oxaliplatin at concentrations about 100-fold higher than peak serum concentration reached in patients fully explains why human CRC are so resistant to platinum derivatives.

Putative mechanisms of the high intrinsic resistance of human colon cancer cells to platinum derivatives are numerous. We did not observe any difference in the cellular accumulation of cisplatin, in the expression of the antiapoptotic proteins Bcl2, Mcl1 and BclxL, or the proapoptotic proteins Bax, Bak and Bad, between the HT29 parental cells or the subpopulation derived from cisplatin-surviving colonies (unpublished results). Cisplatin resistance has been hypothesized to develop as a result of the absence of a p53-mediated apoptotic signal [26]. However, we obtained as many surviving colonies in the HCT116 cells, in which the p53 pathway is intact [27], as in the HT29, Caco2, SW480 and SW620 cell lines in which the p53 protein is mutated or truncated [28, 29, 30].

Table 3. Microsatellite mutation analysis of nine loci in the five independent resistant HT29 clones -CP^2A, B, C, D and E (isolated after two successive exposures to cisplatin). MIN was scored when novel alleles were observed (– no MIN)

HT29-CP^2 clone	Locus								
	BAT26	D8S256	D10S197	D11S2179	THO1	AR	D17S855	D3S1514	TP53
A	–	–	–	–	–	–	–	–	–
B	–	–	–	–	–	–	–	–	–
C	–	–	–	–	–	–	–	–	–
D	–	–	–	–	–	–	–	–	–
E	–	–	–	–	–	–	–	–	–

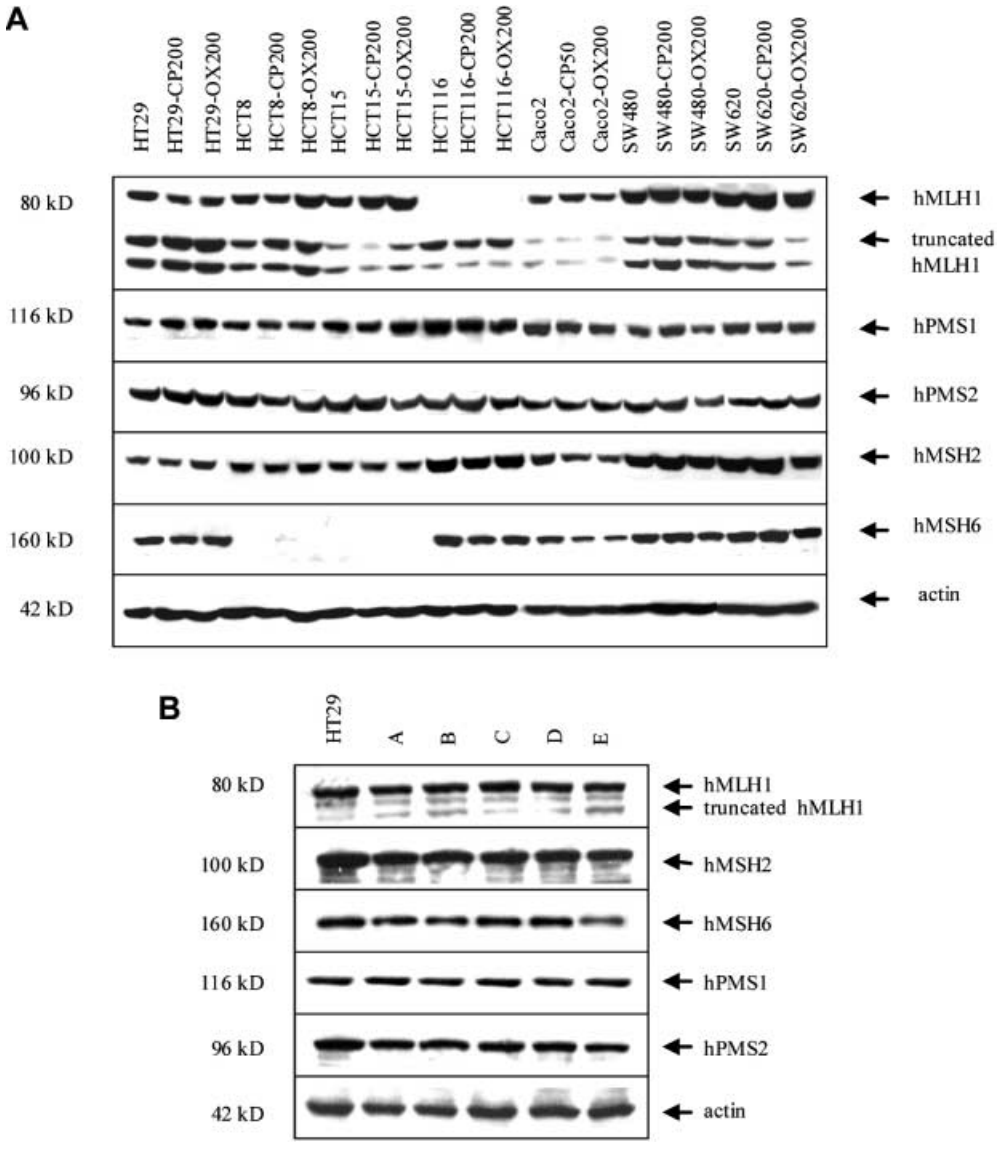

Fig. 4a, b Western blot analysis of MMR proteins. **a** hMLH1, hMSH2, hMSH6, hPMS1 and hPMS2 expression in six parental human colon cancer cell lines and the resistant subpopulations (*CP50* and *CP200* populations surviving 50 and 200 μg/ml cisplatin for 3 h; *OX200* populations surviving 200 μg/ml oxaliplatin for 3 h). **b** Western blot analysis of hMLH1, hMSH2, hMSH6, hPMS1 and hPMS2 expressions in the HT29 parental line and the five independent resistant clones HT29-CP^2 A, B, C, D and E (isolated after two successive exposures to cisplatin)

We sought to determine the implications of faulty MMR in our cisplatin-selected populations, based on the facts that MMR defects occur in approximately 20% of patients with CRC [31, 32, 33] and that recent studies have indicated that a defective DNA MMR system is a dominant mechanism of cisplatin resistance in several cancer cell lines [4, 8, 9, 10, 21]. In contrast with the previously reported studies, we did not observe any significant alteration in the expression of MMR proteins, or any difference in the MIN between the HT29, HCT15, Caco2, SW480 and SW620 parental human colon cancer cells or the platinum-derivative-selected sublines. The HCT116 drug-selected sublines displayed instabilities at five loci, including the two unstable loci known in the parental line HCT116. MIN of HCT116 parental cells was attributed to a mutation in the *hMLH1* gene, as described previously [21, 34].

Despite MIN and mutated hMLH1 protein, there was no difference in the number of clonogenic cells emerging after cisplatin exposure of the HCT116 parental cells, compared to the five other human colon cancer cell lines studied. The HCT15 cell line, which is deficient in hMSH6 and also mutated in DNA polymerase delta, is considered to be highly mutative and potentially resistant to drug treatment [23, 35]. Besides *hMSH6* mutations often occur in "atypical" HNPCC, although they generally do not lead to any MIN [16, 35]. This is in agreement with the fact that no MIN was detected here for the HCT15 cell line and its drug-selected counterpart. Clonogenicity of the HCT15 cells was of the same degree as other cell lines with intact hMSH6 proteins. Taken together, these data suggest that clonogenicity of colon cancer cells was not related to hMSH6 or hMLH1 mutation.

In order to mimic the clinical situation, we briefly exposed unselected colon cancer cells to a very high dose of cisplatin (200 μg/ml) and expanded the rare surviving colonies. Our method only selected inherently resistant

cells in a parental population that needs to exceed 10^6 cells to obtain surviving clones. The precise mechanisms of the intrinsic resistance in these cells remains unknown, but does not appear to be related to defects in DNA MMR proteins. There could be several explanations for the discrepancy between our results and those of other studies that have demonstrated alterations in MMR proteins and MIN in cisplatin-resistant cells. The method of drug selection and the cell line used could have an influence on the biochemical pathways of resistance. These authors used ovarian cancer, Hela, or embryonic stem cells, progressively selected with low cisplatin concentrations. As MMR alterations play a role in DNA instability and genetic mutations, a progressive selection could favour MMR-defective cells, that adapt better to the drug.

The high intrinsic resistance to oxaliplatin of the six human colon cancer cell lines in the absence of defective MMR is another indication of the lack of involvement of defective MMR in this phenomenon. Oxaliplatin produces types of DNA adducts different from those produced by cisplatin which are not recognized by the MMR system [4]. This lack of recognition could involve steric hindrance by the DACH carrier ligand or a distortion in DNA that is distinct from that produced by cisplatin. Loss of DNA MMR due to a lack of either hMSH2 or hMLH1 activity results in low-level resistance to cisplatin but not to oxaliplatin. $hMSH2^{-/-}$ tumours are significantly less responsive to cisplatin than $hMSH2^{+/+}$ tumours in transgenic mice, whereas no difference has been recorded in sensitivity to oxaliplatin. However, we observed that intrinsic resistance to oxaliplatin in human colon cancer cells was as high as that for cisplatin. Moreover, no MIN or defect in MMR proteins was detected in oxaliplatin-resistant populations.

In conclusion, high intrinsic resistance to cisplatin and oxaliplatin of clonogenic cells from unselected colon cancer cell lines was not related to defects in the DNA MMR proteins. In order to investigate other mechanisms of resistance, a non-gene-oriented approach, using microarrays between parental and platinum-surviving cells, is presently being carried out.

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