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ORIGINAL ARTICLE

MSH3 expression does not influence the sensitivity of colon cancer HCT116 cell line to oxaliplatin and poly(ADP-ribose) polymerase (PARP) inhibitor as monotherapy or in combination

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Abstract

Purpose Defective expression of the mismatch repair protein MSH3 is frequently detected in colon cancer, and down-regulation of its expression was found to decrease sensitivity to platinum compounds or poly(ADP-ribose) polymerase inhibitors (PARPi) monotherapy. We have investigated whether MSH3 transfection in MSH3-deficient colon cancer cells confers resistance to oxaliplatin or PARPi and whether their combination restores chemosensitivity.

Methods MSH3-deficient/MLH1-proficient colon cancer HCT116^{MLH1} cells were transfected with the MSH3 cDNA cloned into the pcDNA3.1(−) vector. MSH3/MLH1-deficient HCT116, carrying MLH1 and MSH3 mutations on chromosome 3 and 5, respectively, and HCT116 in which wild-type MLH1 (HCT116+3), MSH3 (HCT116+5) or both genes (HCT116+3+5) were introduced by chromosome transfer were also tested. Sensitivity to oxaliplatin and to PARPi was evaluated by analysis of clonogenic survival, cell proliferation, apoptosis and cell cycle.

Results MSH3 transfection in HCT116 cells did not confer resistance to oxaliplatin or PARPi monotherapy. MSH3-proficient HCT116+5 or HCT116+3+5 cells, which were more resistant to oxaliplatin and PARPi in comparison with their MSH3-deficient counterparts, expressed higher levels of the nucleotide excision repair ERCC1 and XPF proteins, involved in the resistance to platinum compounds, and lower PARP-1 levels. In all cases, PARPi increased sensitivity to oxaliplatin.

Conclusions Restoring of MSH3 expression by cDNA transfection, rather than by chromosome transfer, did not affect colon cancer sensitivity to oxaliplatin or PARPi monotherapy; PARP-1 levels seemed to be more crucial for the outcome of PARPi monotherapy.

Keywords Colon cancer · Chemotherapy · Drug resistance · DNA repair · Poly(ADP-ribose) polymerase · Mismatch repair

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Introduction

Oxaliplatin is currently used in combination with 5-fluorouracil/leucovorin for the adjuvant treatment of stage III

colon cancer after resection of the primary tumour and for the treatment of the metastatic disease. Sensitivity to oxaliplatin is mostly limited by the expression of nucleotide excision repair components, whereas the functional status of the mismatch repair system (MMR), and in particular of MLH1, does not influence tumour response to this agent [1, 2]. On the other hand, MLH1 lack of function and the concomitant presence of microsatellite instability, frequently detected in colon cancer, have been associated with lower response to cisplatin, carboplatin or 5-FU [3–6].

MLH1 forms with PMS2 the MutLα heterodimer and interacts with the MutSα (MSH2/MSH6) or MutSβ (MSH2/MSH3) heterodimers to bind and repair mismatches during DNA replication. MLH1-deficient colon cancers often show a concomitant loss of MSH3 expression that favours tumour progression [7, 8]. MSH3 has been shown to indirectly interact with breast cancer susceptibility gene products (BRCA1/2) [8, 9], components of the homologous recombination (HR) that is involved in the repair of DNA double-strand breaks (DSB). BRCA1/2-mutated tumours are responsive to platinum agents, likely because DNA cross-links caused by platinum compounds eventually require HR to correct DNA damage [10, 11]. HR-defective tumours are also highly sensitive to poly (ADP-ribose) polymerase inhibitors (PARPi) monotherapy according to a synthetic lethality model [12–14]. The concept of synthetic lethality postulates that functional inhibition of two proteins leads to cell death, but blockade of either alone does not. PARPi mainly block the catalytic activity of PARP-1, a nuclear enzyme that synthesizes and transfers ADP-ribose polymers to acceptor proteins (including PARP-1 itself) using NAD^+ as a substrate. PARP-1 has a key role in the surveillance and maintenance of genome integrity, favouring the repair of DNA single-strand breaks (SSB). Cells with inhibited PARP activity may acquire more unrepaired SSB that can result in DSB formation when they encounter DNA replication forks. In normal cells that possess a functional HR, the DSB are repaired, whereas in HR-defective tumour cells, DSB persist and cause cell death. Thus, PARP-1 behaves as synthetic lethal partner of BRCA1/2 or of other HR components [14]. In regard to colon cancer, the PARPi olaparib is currently evaluated in monotherapy (www.clinicaltrials.gov) for the advanced disease with microsatellite instability.

Based on the interaction of MSH3 with BRCA1 and the observation that transient silencing of its expression in MSH3-proficient tumours increased sensitivity to oxaliplatin or PARPi, it has been hypothesized that MSH3 might play a role in tumour response to platinum compounds and might behave as synthetic lethal partner of PARP-1 [15].

Aim of the present study was to clarify whether MSH3 transfection in MSH3-deficient colon cancer cells confers resistance to oxaliplatin or to PARPi monotherapy and whether PARPi enhance susceptibility to the platinum compound depending on the expression levels of MSH3 or PARP-1.

Materials and methods

Cell lines and MSH3 transfection

The colon cancer HCT116 cell line has a hemizygous nonsense mutation in the MLH1 gene located on chromosome 3 and homozygous frameshift mutations of the [A]8 repeat in exon 7 of the MSH3 gene located on chromosome 5 [16, 17]. The MLH1-proficient HCT116/3–6 cell line (HCT116+3) was created by microcell chromosome transfer of a single normal human chromosome [18] and kindly provided by Dr. Giancarlo Marra (Institute of Molecular Cancer Research, University of Zürich, Switzerland). The HCT116 1–2 cells were generated by transfection with the full-length wild-type MLH1 cDNA cloned into the pcDNA3.1/Hygro vector (hereafter referred to as $HCT116^{MLH1}$), and the HCT116 0–1 cells transfected with the empty control vector (hereafter referred to as $HCT116^{hygro}$) [19]. The HCT116+5 and HCT116+3+5 cell lines, expressing MSH3, were generated by microcell chromosome 5 transfer [17] and kindly provided by Dr. Minoru Koi (Baylor University Medical Center, Dallas, TX, USA). Cell lines were cultured in DMEM, supplemented with 10 % foetal calf serum, 2 mM L-glutamine and antibiotics (Sigma-Aldrich, Milan, Italy). The HCT116+3 cells were cultured in the presence of 400 µg/ml geneticin, the $HCT116^{MLH1}$ and $HCT116^{hygro}$ cells in the presence of 100 µg/ml hygromycin, the HCT116+5 and HCT116+3+5 cells in the presence of 6 µg/ml blasticidin S.

The MSH3 cDNA was obtained from the pFastBac-MSH3 vector (kindly provided by Dr. Joseph Jiricny, Institute of Molecular Cancer Research, University of Zürich, Switzerland) and cloned into XhoI and HindIII cloning sites of the pcDNA3.1(−) vector. $HCT116^{MLH1}$ cells were transfected with the pcDNA3.1(−) or MSH3-pcDNA3.1(−) vectors, selected with 800 µg/ml geneticin and maintained in the presence of 400 µg/ml geneticin.

The human promyelocytic leukaemia cell line HL-60 and its methotrexate-resistant subline HL-60R, overexpressing MSH3, were a kind gift of Takashi Shimada (Nippon Medical School, Tokyo, Japan). The culture medium of HL-60R was supplemented with 1 µM methotrexate [20].

The pattern of expression of MSH3, MLH1 and PARP-1 in the transfected and chromosome reconstituted colon cancer cell lines, analysed in the present study, is summarized in Table 1.

Drugs

Oxaliplatin (Sanofi-Aventis, Milan, Italy) and cisplatin (Teva Pharma Italia, Milan, Italy) stock solutions (12.6 and 1.7 mM, respectively) were obtained by dissolving the drugs in saline. The PARP inhibitor GPI 15427 [10-(4-methyl-piperazin-1-ylmethyl)-2H-7-oxa-1,2-diaza-benzo-[de]anthracen-3-one, Eisai, Baltimore, MD, USA] stock solution (1 mM) was prepared by dissolving GPI 15427 in 70 mM PBS without potassium [21].

Cell growth assays and flow-cytometry analysis

Cell proliferation of colon cancer cell lines was evaluated by colony-formation assay. After 10 days of culture, colonies were fixed, stained with 2 % methylene blue in 95 % ethanol and counted. Only colonies comprising >50 cells were scored as survival colonies. Chemosensitivity was evaluated in terms of IC_{50}, that is, the concentration of the drug capable of inhibiting cell growth by 50 %.

Cell growth of HL-60 and HL-60R lines was evaluated in terms of number of viable cells, manually counted at 72 h after treatment. Viability was determined by trypan blue exclusion test. All determinations were made in triplicate.

Apoptosis and cell cycle perturbations induced by the drugs were evaluated by flow-cytometry analysis of the DNA content according to standard procedures [21]. The PI fluorescence was measured on a linear scale using a FACScan flow cytometer and the CellQuest software. Data collection was gated using forward light scatter and side scatter to exclude cell debris and aggregates. Apoptotic cells were represented by a broad hypodiploid peak easily distinguishable from the diploid DNA content in the red fluorescence channel. For cell cycle analysis, the Mod-Fit software version 3.0 was used (Becton and Dickinson, San Jose, CA, USA).

Table 1 Pattern of MSH3, MLH1 and PARP-1 expression in colon cancer cell lines

Cell line	MSH3	MLH1	PARP-1
HCT116	–	–	H[a]
HCT116+3	–	+	L[b]
HCT116+5	+	–	L
HCT116+3+5	+	+	L
HCT116^{hygro}	–	–	H
HCT116^{MLH1}	–	+	H
HCT116^{MLH1} pcDNA	–	+	H
HCT116^{MLH1} MSH3	+	+	H

[a] High expression

[b] Low expression

Western blot analysis

For immunoblot analysis, the following primary antibodies were used: rabbit polyclonal antihuman p53 and phosphorylated p53 (Ser15) (Cell Signaling Technology; Beverly, MA, USA; 1:1,000 dilution); rabbit polyclonal antihuman β-tubulin (clone H-235; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA; 1:400); rabbit polyclonal antihuman XPF (Abcam; Cambridge, UK; 1:2,000); monoclonal antihuman ERCC1 (clone 3H11, Novus Biologicals, Cambridge, UK, 1:1,000); monoclonal antihuman MLH1 (clone G168-15, BD Biosciences, San Jose, CA, USA; 1/500); monoclonal antihuman MSH3 (clone 52/MSH3, BD Biosciences; 1:500); monoclonal anti-calf PARP-1 (clone C2-10; Trevigen, Gaithersburg, MD, USA; 1:2,000 dilution). Goat anti-rabbit and anti-mouse IgG horseradish peroxidase (HRP)-conjugated secondary antibodies (Biorad, Milan, Italy) were used at the appropriate dilutions. Immunoreactive bands were detected by enhanced chemoluminescence (ECL) technique using the ECL Plus Western Blotting Substrate (Pierce, Rockford, IL USA). Signals were quantified using a Kodak densitometer (Rochester, NY, USA).

Results

Influence of MSH3 expression on sensitivity to oxaliplatin as single agent or in combination with PARPi

A recent study indicated that transient down-regulation of the MSH3 protein increased sensitivity to oxaliplatin in HCT116+3+5 cells, expressing both MLH1 and MSH3 after chromosome 3 and 5 transfer [15]. Here, we have investigated whether reconstitution of MSH3 expression by transfection of the corresponding cDNA, rather than by chromosome transfer, into MSH3-deficient HCT116^{MLH1} cells, might confer resistance to oxaliplatin. Chemosensitivity to oxaliplatin of control (HCT116^{MLH1} pcDNA) or MSH3 transfected (HCT116^{MLH1} MSH3) clones was analysed and compared to that of chromosome reconstituted HCT116+3+5 cells and in HCT116+5 cells, expressing only MSH3. The results of immunoblot analysis showed that the MSH3 protein was expressed in HCT116^{MLH1} MSH3 cells at a higher level than in HCT116+3+5 and HCT116+5 cells (Fig. 1a). The methotrexate-resistant

HL-60R cell line, in which the amplification of the dihydrofolate reductase gene is associated with that of the MSH3 gene, leading to overexpression of MSH3 protein and its sensitive counterpart HL-60, expressing very low MSH3 levels, were also assayed (Fig. 1a). The data of clonogenic assay indicated that MSH3 transfection did not confer resistance to oxaliplatin or cisplatin, whereas lack of MLH1 resulted in reduced HCT116 sensitivity to cisplatin (Fig. 1b). On the other hand, HCT116+5 and HCT116+3+5 cells were more resistant to oxaliplatin than their MSH3-deficient counterparts or MSH3 transfected cells (Fig. 1c). Anyway, it is important to remark that the addition of PARPi increased the sensitivity to oxaliplatin of about 2–3-fold in all cell lines. Moreover, overexpression of MSH3 in HL-60R cells did not confer resistance to oxaliplatin since the drug IC_{50} value was similar to that of control HL-60 cells (data not shown).

Treatment with oxaliplatin caused similar p53 activation in control or MSH3 transfected cells, whereas the same drug concentrations did not induce significant p53 activation in chromosome 5 reconstituted HCT116+3+5 and HCT116+5 cell lines (Fig. 2a). Flow-cytometry analysis indicated that oxaliplatin-induced apoptosis in MLH1-deficient HCT116 cells (41 ± 1 % and 51 ± 1.9 % at 1.2 and 2.5 μM, respectively), whereas the drug provoked G2/M arrest in MLH1-proficient cells (Figure S1).

In order to investigate whether the lower sensitivity of HCT116+5 and HCT116+3+5 cell lines to oxaliplatin could be attributed to differential expression of DNA repair proteins involved in the resistance to platinum compounds, we analysed the expression pattern of excision repair cross-complementation group 1 (ERCC1) and xeroderma pigmentosum complementation group F (XPF). The ERCC1 and XPF proteins are both components of the nucleotide excision repair; they form a heterodimer that behaves as an endonuclease and plays an important role in the repair of interstrand cross-links generated by platinum compounds [3]. The results of immunoblot analysis revealed that HCT116+3+5 and HCT116+5 cell lines were characterized by constitutive higher levels of ERCC1 and XPF in comparison with HCT116 or HCT116+3 cells and with $HCT116^{MLH1}$ pcDNA or $HCT116^{MLH1}$ MSH3 cells that were all equally sensitive to oxaliplatin (Fig. 2b).

Influence of MSH3 and PARP-1 expression on sensitivity to PARPi as monotherapy

In order to investigate whether MSH3 might behave as synthetic lethal partner of PARP-1 and whether the level of PARP-1 protein might influence the susceptibility to PARPi monotherapy, the colon cancer cell lines with differential MSH3 and/or MLH1 expression were subjected to immunoblot analysis of PARP-1 and to clonogenic assay after treatment with the PARPi GPI 15427 as single agent. The results indicated that MLH1/MSH3-deficient HCT116 or $HCT116^{hygro}$, MLH1-proficient/MSH3-deficient $HCT116^{MLH1}$ or $HCT116^{MLH1}$ pcDNA and MLH1-proficient/MSH3-proficient $HCT116^{MLH1}$ MSH3, which possessed comparable high levels of PARP-1 expression (Fig. 3a), showed similar GPI 15427 IC_{50} values (Fig. 3b), independently on MSH3 or MLH1 expression. Nevertheless, in MLH1-deficient $HCT116^{hygro}$ cells, the PARPi was found to cause G1 arrest, whereas in MLH1-proficient $HCT116^{MLH1}$ cells the inhibitor-induced G2/M arrest (Fig. 4). Interestingly, also MSH3 overexpressing HL-60R cells showed PARPi sensitivity similar to that of control HL-60 cells; both cell lines were characterized by high PARP-1 levels (data not shown). On the other hand, the chromosome reconstituted HCT116+3, HCT116+3+5 and HCT116+5 lines expressing low PARP-1 levels were more resistant to the PARPi than HCT116 cells or transfected clones, characterized by high PARP-1 expression (Fig. 3a, b). FACS analysis revealed that treatment with PARPi induced an accumulation in G1 phase in sensitive HCT116 cells that was not observed in HCT116+5 (Fig. 4), HCT116+3 and HCT116+3+5 cells (Figure S2).

Discussion

In the present study, we demonstrated, for the first time, that reconstitution of MSH3 expression in colon cancer cells by transfection of the corresponding cDNA did not reduce sensitivity to oxaliplatin or to PARPi monotherapy. Cells with low levels of PARP-1 protein were less susceptible to the anti-proliferative effects of PARPi monotherapy; nevertheless, they could be still sensitized by PARPi to oxaliplatin.

The MSH3-containing MutSβ complex appeared to be dispensable for oxaliplatin toxicity [2]. However, a recent study demonstrated that MSH3 expression might be involved in the resistance of colon cancer to platinum agents, since transient MSH3 silencing increased sensitivity to oxaliplatin in HCT116+3+5 cells, in which MSH3 expression derived from chromosome 5 transfer [15]. This effect was attributed by the authors to the down-regulation of DSB repair by HR. In contrast, we found that transfection of the wild-type MSH3 cDNA in $HCT116^{MLH1}$ cells did not affect their susceptibility to oxaliplatin. The lower sensitivity to oxaliplatin of MSH3-proficient HCT116+5 and HCT116+3+5 cells might be attributed to the higher levels of nucleotide excision repair ERCC1 and XPF proteins present in these cells, in comparison with HCT116 cells or MLH1/MSH3 transfected clones. In fact,

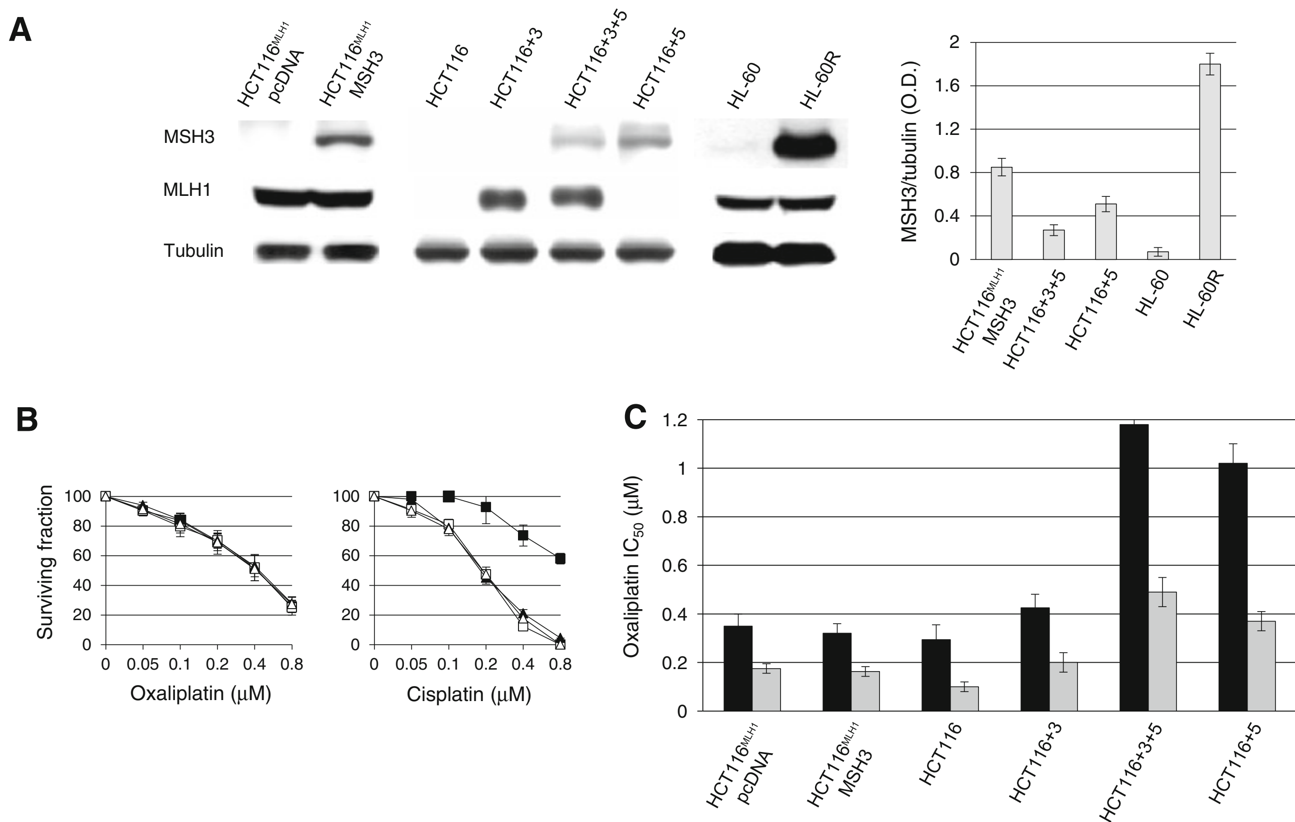

Fig. 1 Sensitivity to oxaliplatin in cells expressing MSH3 after cDNA transfection or chromosome transfer. **a** Immunoblot analysis of MSH3 and MLH1. Cellular extracts from HCT116^{MLH1} cells transfected with control pcDNA3.1(−) vector (HCT116^{MLH1} pcDNA) or with MSH3-pcDNA3.1(−) vector (HCT116^{MLH1} MSH3), HCT116, HCT116+3, HCT116+3+5, HCT116+5, HL-60 and HL-60R cells were tested for MSH3, MLH1 and tubulin expression. *Bar graphs* represent the mean ratios between the optical densities (O.D.) of MSH3 and those of tubulin in MSH3-proficient cells from three independent experiments. **b** In vitro sensitivity to oxaliplatin or cisplatin of HCT116 cells transfected with MLH1 or with MLH1 and MSH3. HCT116^{hygro}, HCT116^{MLH1}, HCT116^{MLH1} pcDNA and HCT116^{MLH1} MSH3 cells were treated with graded concentrations of the platinating agents and analysed by colony-formation assay. The results are expressed as clonogenic survival fraction and are the mean (±SD) of three independent experiments. Cisplatin IC$_{50}$s are as follows: HCT116^{hygro}, 0.95 μM ± 0.17; HCT116^{MLH1}, 0.21 ± 0.04; HCT116^{MLH1} pcDNA, 0.18 ± 0.01; HCT116^{MLH1} MSH3, 0.18 ± 0.03; oxaliplatin IC$_{50}$s are as follows: HCT116^{hygro}, 0.32 μM ± 0.06; HCT116^{MLH1}, 0.35 ± 0.06; HCT116^{MLH1} pcDNA, 0.35 ± 0.05; HCT116^{MLH1} MSH3, 0.32 ± 0.04. Statistical analysis indicated that the differences between cisplatin IC$_{50}$s of HCT116^{hygro} and those of the other cell lines are statistically significant [$P < 0.0001$ according to ANOVA ($\alpha = 0.05$) and post-hoc Bonferroni test; $P < 0.01$ according to the non-parametric Kruskal–Wallis analysis and post-hoc Dunn's test]. **c** Sensitivity to oxaliplatin in combination with PARPi. Cells were treated with oxaliplatin in the absence (*black columns*) or in the presence of a fixed concentration (0.3 μM) of the PARPi GPI 15427 (*grey columns*) and analysed by colony-formation assay. The results are expressed as IC$_{50}$ and are the mean (±SD) from five independent experiments. Statistical analysis using the ANOVA and post-test Bonferroni method for multiple comparison indicated that the differences between oxaliplatin IC$_{50}$s of HCT116+3+5 or HCT116+5 cells and all other cell lines are statistically significant ($P < 0.0001$), whereas differences between HCT116^{MLH1} pcDNA, HCT116^{MLH1} MSH3, HCT116 and HCT116+3 are not significant. The non-parametric Kruskal–Wallis analysis followed by post-hoc Dunn's test indicated that the differences between the oxaliplatin IC$_{50}$s of HCT116+3+5 or HCT116+5 and those of HCT116 are statistically significant ($P < 0.0001$ and $P < 0.01$, respectively). The results of statistical analysis by Student's *t* test of the differences in sensitivity between cells treated with oxaliplatin and cells treated with oxaliplatin + PARPi are as follows: HCT116^{MLH1} pcDNA, oxaliplatin versus oxaliplatin + PARPi, $P = 0.004$; HCT116^{MLH1} MSH3, oxaliplatin versus oxaliplatin + PARPi, $P = 0.002$; HCT116, oxaliplatin versus oxaliplatin + PARPi, $P = 0.001$; HCT116+3, oxaliplatin versus oxaliplatin + PARPi, $P = 0.0004$; HCT116+3+5, oxaliplatin versus oxaliplatin + PARPi, $P < 0.0001$; HCT116+5, oxaliplatin versus oxaliplatin + PARPi, $P < 0.0001$

these proteins have been found to contribute to low responsiveness to platinum compounds, including oxaliplatin [3]. It is likely that when MSH3 is replaced by chromosome transfer, the expression of other proteins may change and influence tumour response to drug treatment.

MLH1-deficient HCT116 cells showed susceptibility to oxaliplatin similar to that of their MLH1-proficient drug-sensitive counterparts. On the other hand, MLH1-deficient HCT116 cells were more resistant to cisplatin. This is consistent with the knowledge that the MLH-1 containing

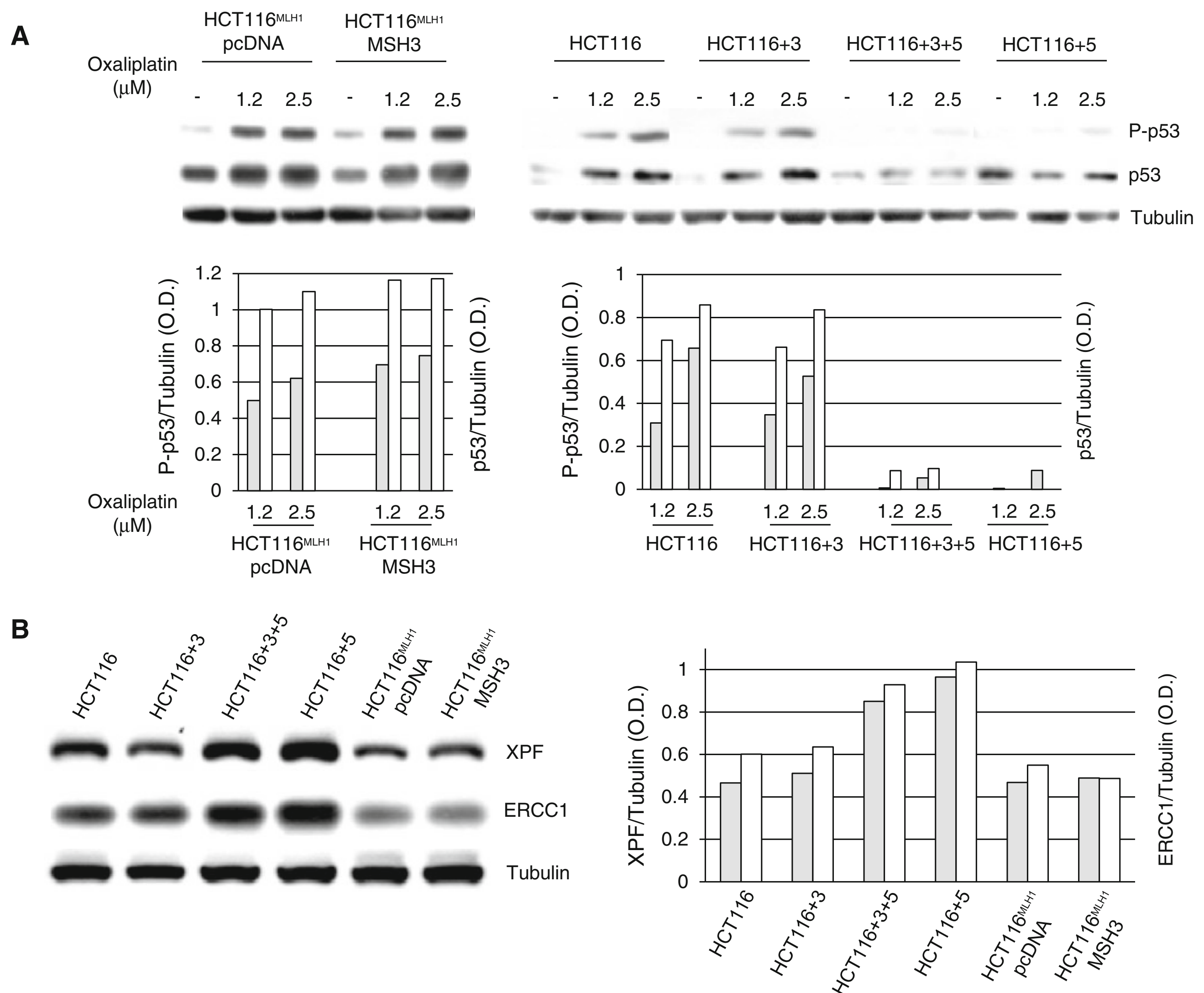

Fig. 2 Analysis of p53 activation and of DNA repair proteins involved in resistance to oxaliplatin in HCT116 cells expressing MSH3 after cDNA transfection or chromosome transfer. **a** Analysis of phosphorylated and total p53 in HCT116^{MLH1} pcDNA and HCT116^{MLH1} MSH3 cells or in HCT116, HCT116+3, HCT116+3+5 and HCT116+5 cells treated with oxaliplatin (1.2 and 2.5 μM) for 6 h. *Histograms* represent the ratios between the O.D. of phosphorylated p53 (P-p53) (*grey columns*) or total p53 (*white columns*) and tubulin in oxaliplatin treated groups (after subtraction of untreated control O.D.). The results are representative of one out of two experiments with similar results. **b** Immunoblot analysis of XPF and ERCC1. *Bars* represent the ratios between the O.D. of the indicated proteins and tubulin. The results are representative of one out of two experiments with similar results

heterodimer MutLα does not recognize adducts formed by oxaliplatin, whereas it is required for the detection of damaged DNA generated by cisplatin and carboplatin [1]. Our data also indicated that the expression of MLH1 favoured the induction of G2/M arrest over apoptosis. In fact, MLH1 is known to be involved in DNA damage-induced checkpoint, favouring G2/M arrest through Chk1 phosphorylation [22].

Inhibition of PARP activity counteracted resistance to the platinum agent, restoring sensitivity of HCT116+5 and HCT116+3+5 to oxaliplatin at levels similar to those of HCT116 or HCT116+3, but it also increased the sensitivity of the other cell lines with higher intrinsic susceptibility to oxaliplatin monotherapy. Even though the mechanism underlying the chemosensitizing effect of PARPi in combination with platinum compounds has not been clarified yet, it likely involves a reduced repair of cross-links by nucleotide excision repair [23]. Moreover, PARP-1 has been shown to participate in platinum–DNA damage response, being capable of binding to platinum-derived cross-links [24].

The potential role of MSH3 in the repair of DSB was further explored analysing the susceptibility of MSH3-

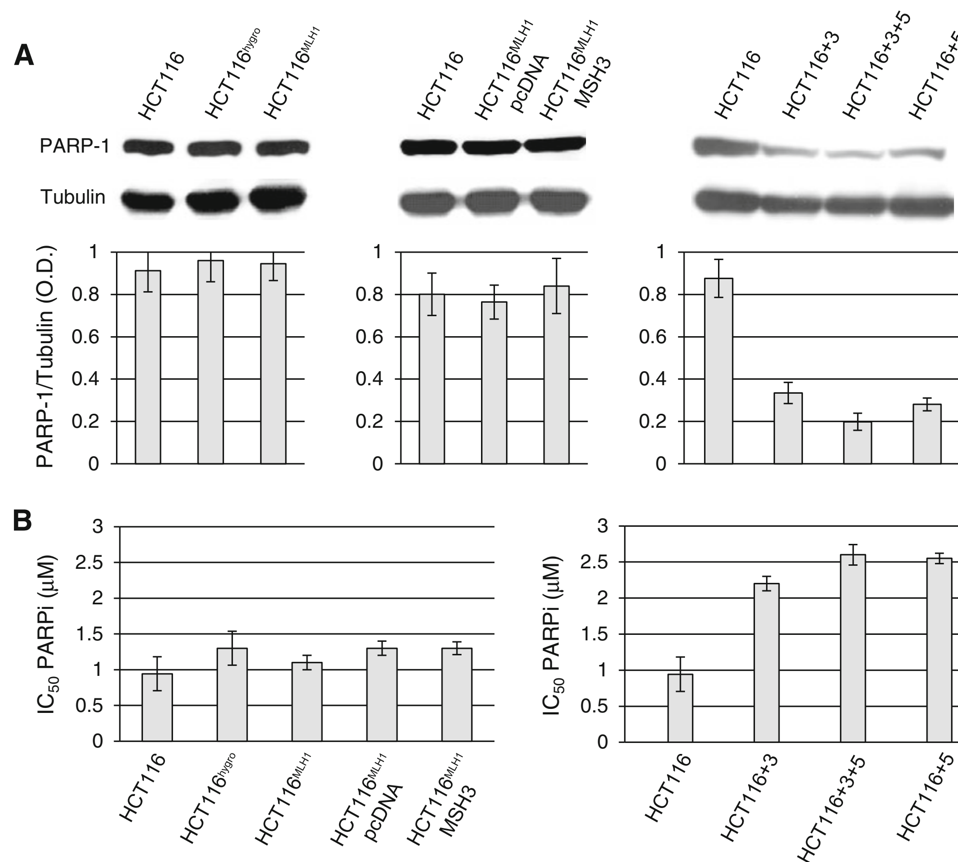

Fig. 3 Influence of MLH1, MSH3 and PARP-1 expression on sensitivity to PARPi as monotherapy. **a** Analysis of PARP-1 expression. Cell extracts from HCT116, HCT116^{hygro}, HCT116^{MLH1}, HCT116^{MLH1} pcDNA, HCT116^{MLH1} MSH3, HCT116+3, HCT116+3+5 and HCT116+5 cells were tested for immunoblot analysis of PARP-1 and tubulin expression. *Bars* represent the mean ratios between the O.D. of PARP-1 and those of tubulin from three independent experiments. **b** In vitro sensitivity to the PARPi GPI 15427. Tumour cells were treated with graded concentrations of GPI 15427 (0.01–6 μM) and analysed by colony-formation assay. The results are the mean (±SD) from three independent experiments. The results of statistical analysis by Student's *t* test of the differences in sensitivity to GPI 15427 are as follows: HCT116 versus HCT116^{hygro}, HCT 116^{MLH1}, HCT116^{MLH1} pcDNA or HCT116^{MLH1} MSH3, not significant; HCT116 versus HCT116+3, HCT116+3+5 or HCT116+5, $P < 0.0001$

proficient and MSH3-deficient cells to PARPi. The MSH3-proficient HCT116+5 and HCT116+3+5 cells were more resistant to PARPi monotherapy as compared to HCT116 cells. The absence of an efficient MRE11–RAD50–NBS1 (MRN) protein complex, which is regarded as the primary sensor of DBS, seems to contribute to PARPi sensitivity of HCT116 cells [25, 26]. However, HCT116+3 cells, which also showed a very low expression of RAD50 (data not shown) and lack of MSH3 expression, were more resistant to PARPi than HCT116 cells. In regard to whether PARP-1 expression might influence the response to PARPi, it has been reported that PARP-1 is hyperactivated in BRCA1/2-mutated tumour cells, suggesting the occurrence in HR-defective cancers of a compensatory mechanism involving PARP-1. Thus, high PARP-1 levels might predict sensitivity to PARPi monotherapy and, accordingly, down-regulation of PARP-1 expression might contribute to resistance [27]. In line with this hypothesis, HCT116+3, HCT116+3+5 and HCT116+5 cells expressed PARP-1 levels lower than PARPi sensitive HCT116 cells. Indeed, HCT116, HCT116^{hygro} and HCT116^{MLH1} cells, devoid of MSH3 protein but with the same pattern of PARP-1 expression, were equally sensitive to GPI 15427. Transfection of HCT116^{MLH1} cells with MSH3 did not substantially change the sensitivity to GPI 15427, suggesting that PARP-1 levels rather than MSH3 expression influenced the response to PARPi. The expression of MLH1 in HCT116^{MLH1} cells favoured cell cycle arrest at the G2/M phase, whereas MLH1-deficient HCT116^{hygro} or HCT116 cells underwent G1 arrest upon treatment with the PARPi.

In conclusion, these data indicated that MSH3 expression itself does not affect sensitivity to oxaliplatin, for which is instead more critical the expression of ERCC1-XPF. Inhibition of PARP-1 function increases the antitumour activity of oxaliplatin even in the presence of low

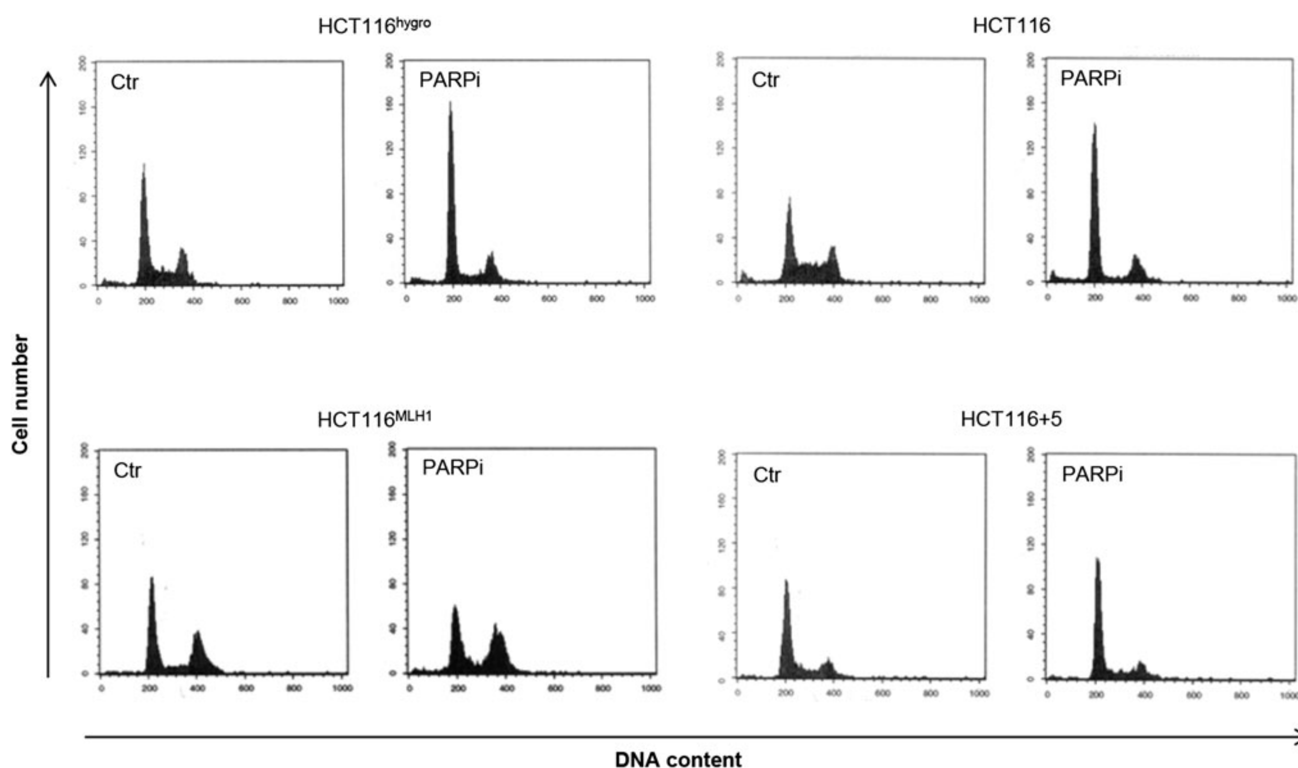

Fig. 4 Analysis of cell cycle of $HCT116^{hygro}$, HCT 116^{MLH1}, HCT116 and HC116+5 cells treated with PARPi. Cells were exposed to GPI 15427 (5 μM) and analysed by flow cytometry at 72 h after treatment. The results of the mean percentages (±SD; $n = 3$) of cells in the different phases of cell cycle are as follows: $HCT116^{hygro}$, untreated control (Ctr), G1 40 ± 8, S 39 ± 9, G2/M 21 ± 5; $HCT116^{hygro}$, PARPi, G1 68 ± 2, S 10 ± 2, G2/M 22 ± 7; $HCT116^{MLH1}$, Ctr, G1 45 ± 6, S 26 ± 5, G2/M 29 ± 4; $HCT116^{MLH1}$, PARPi, G1 37 ± 6, S 13 ± 5, G2/M 50 ± 5; HCT116, Ctr, G1 42 ± 8, S 37 ± 10, G2/M 21 ± 5; HCT116, PARPi, G1 74 ± 4, S 8 ± 4, G2/M 18 ± 5; HCT116+5, Ctr, G1 50 ± 4, S 27 ± 3, G2/M 23 ± 6; HCT116+5, PARPi, G1 57 ± 7, S 24 ± 10, G2/M 19 ± 5

PARP-1 expression. On the other hand, PARP-1 levels, rather than MSH3 function, seem to be more crucial for the outcome of a treatment based on PARPi monotherapy.

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Conflict of interest The authors declare that they have no competing interests.

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