DNA Repair 35 (2015) 126–136

Contents lists available at ScienceDirect

DNA Repair

journal homepage: www.elsevier.com/locate/dnarepair

Role of mismatch repair proteins in the processing of cisplatin interstrand cross-links

Akshada Sawant[a], Anbarasi Kothandapani[a], Anatoly Zhitkovich[b], Robert W. Sobol[c,d,e,f], Steve M. Patrick[g,*]

[a] Department of Biochemistry and Cancer Biology, University of Toledo—Health Science Campus, Toledo, OH 43614, USA
[b] Department of Pathology and Laboratory Medicine, Alpert Medical School, Brown University, Providence, RI 02912, USA
[c] Department of Pharmacology & Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213, USA
[d] University of Pittsburgh Cancer Institute, Hillman Cancer Center, Pittsburgh, PA 15213, USA
[e] Department of Human Genetics, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA 15213, USA
[f] Department of Oncologic Sciences, Molecular & Metabolic Oncology Program, University of South Alabama Mitchell Cancer Institute, 1660 Springhill Avenue, Mobile, AL 36604, USA
[g] Department of Oncology, Karmanos Cancer Institute, Wayne State University, Detroit, MI 48201, USA

ARTICLE INFO

Article history:
Received 12 February 2015
Received in revised form 9 October 2015
Accepted 10 October 2015

Keywords:
BER
Cisplatin
MMR
Resistance
ATPase

ABSTRACT

Mismatch repair (MMR) deficiency gives rise to cisplatin resistance and can lead to poor prognosis in cancers. Various models have been proposed to explain this low level of resistance caused due to loss of MMR proteins. We have shown that MMR proteins are required to maintain cisplatin interstrand cross-links (ICLs) on the DNA leading to increased cellular sensitivity. In our previous studies, we have shown that BER processing of the cisplatin ICLs is mutagenic. Polymerase β (Polβ) can generate mismatches which leads to the activation and the recruitment of mismatch repair proteins. In this paper, we distinguished between the requirement of different downstream MMR proteins for maintaining cisplatin sensitivity. We show that the MutSα (MSH2–MSH6) heterocomplex is required to maintain cisplatin sensitivity, whereas the Mutsβ complex has no effect. These results can be correlated with the increased repair of cisplatin ICLs and ICL induced DNA double strand breaks (DSBs) in the resistant cells. Moreover, we show that MLH1 proficient cells displayed a cisplatin sensitive phenotype when compared with the MLH1 deficient cells and the ATPase activity of MLH1 is essential to mediate this effect. Based on these results, we propose that MutSα as well as the downstream MMR pathway proteins are essential to maintain a cisplatin sensitive phenotype as a consequence of processing Polβ induced mismatches at sites flanking cisplatin ICLs.

1. Introduction

The DNA mismatch repair (MMR) system which is involved in the post replicative repair of mismatches plays a crucial role in the maintenance of genomic stability [1]. In addition to the recognition of mismatches, MMR proteins have also been involved in the recognition and processing of DNA damage inflicted by a number of chemotherapeutic agents like cisplatin, carboplatin, alkylating agents and 5-fluorouracil [2–6]. The MMR pathway is composed of recognition proteins with MSH2 as a common partner in two heterocomplexes namely MutSα (MSH2–MSH6) and MutSβ (MSH2–MSH3) [7]. MutSα is required for the repair of base–base mismatches and one base pair insertion deletion loops (IDLs). On the other hand, MutSβ carries out the repair of IDLs with single or multiple base pairs. The mismatch recognition step is followed by the recruitment of downstream MMR proteins including MutLα (MLH1–PMS2), exonuclease I, DNA polymerase δ and DNA ligase.

MMR has also been shown to participate in the DNA damage response after treatment with certain chemotherapeutic agents. Loss of MMR proteins has been associated with resistance to a number of anti-cancer agents (e.g., alkylating agents and cisplatin) [5,6]. Various models have been proposed for the possible role of MMR in maintaining drug sensitivity. Adducts formed by alkylating agents can result in the generation of mismatched bases. It has been suggested that MMR proteins take part in futile cycles of repair of these mismatches in the daughter strand. The resulting strand breaks signal apoptosis and loss of this function can

* Corresponding author at: 4100 John R, Detroit, MI 48201, USA. Fax: +1 313 576 8928.
E-mail address: patricks@karmanos.org (S.M. Patrick).

http://dx.doi.org/10.1016/j.dnarep.2015.10.003
1568-7864/

give rise to drug resistance [8–10]. In addition, MMR proteins have been shown to directly signal the DNA damage, eventually resulting in cell death [3,11]. These studies, however, did not differentiate between the different types of DNA adducts that are formed by cisplatin, namely intrastrand adducts which are formed within the same DNA strand versus interstrand crosslinks (ICLs) which are formed between adjoining strands of DNA. MutSα proteins have been shown to recognize cisplatin intrastrand adducts [10]. In addition, MutSβ was found to be one of the proteins that interact with cisplatin ICLs [12]. However, the exact role of MSH3 in the processing of cisplatin adducts has not been clearly evaluated.

Recent studies have shown that MSH3 is required for the repair of DNA double strand breaks (DSBs) induced during cisplatin and oxaliplatin treatment [13,14]. Thus, the MMR pathway has been shown to be required for the sensitization of colorectal cancer cells to cisplatin and oxaliplatin, and this effect is believed to be independent of the canonical MMR processing. However, other studies have shown that MSH3 proficient cells, which were more resistant to chemotherapy, expressed higher levels of NER proteins which could explain the reason for increased resistance [15]. Thus, the exact role of MSH3 in modulating platinum cytotoxicity remains to be determined.

In our previous studies, we have shown that loss of base excision repair (BER) and MMR proteins gives rise to resistance to cisplatin and these two pathways take part in the same mechanistic pathway to mediate cisplatin sensitivity [16,17]. In the absence of these proteins, increased repair of cisplatin ICLs was observed which leads to decreased cellular cytotoxicity. We also showed that this mechanism is dependent upon the low fidelity of DNA polymerase β (Polβ), which leads to mis-incorporation of bases and generation of mismatches at sites flanking a cisplatin ICL. This mismatch in turn activates the MMR pathway. In this report, we distinguish between the requirement of different downstream MMR proteins to mediate this effect, and we show that in contrast to previous studies, there is a clear distinction between the initial MMR recognition heterocomplexes. MutSα is required to maintain cisplatin sensitivity while MutSβ plays no role at least in breast cancer cell lines and mouse embryonic fibroblasts in mediating cisplatin cytotoxicity. Moreover, we show that the ATPase activity of MLH1 is required for maintaining a cisplatin sensitive phenotype highlighting the importance of the MMR pathway in the non-productive processing of cisplatin ICLs and not just shielding of the DNA damage by MutSα.

2. Materials and methods

2.1. Chemicals and antibodies

Cisplatin, oxaliplatin and myricetin were purchased from Sigma–Aldrich. All other chemicals and reagents were from standard suppliers. Antibodies directed against MSH3, MSH6 and MLH1 were from BD Pharmigen and α-tubulin was from Sigma–Aldrich. For the stock preparation, cisplatin and oxaliplatin were diluted in 1× PBS and vortexed vigorously until the drug dissolved completely. The stock concentration was 1 mM. Cisplatin was prepared fresh before each experiment. Stock solutions for oxaliplatin were stored at −80 °C for up to 6 months and thawed at room temperature (RT) when needed.

2.2. Cell lines

The human breast adenocarcinoma MDA-MB-231 cells were grown in RPMI 1640 containing 10% FBS and geneticin (700 μg/ml). MDA-MB-231 Polβ knockdown cells (Polβ lentiviral shRNA) were grown in the presence of 0.5 μg/ml puromycin. The development and characterization of the MDAMB-231/Polβ-KD cells were described previously [18]. MLH1-null HCT116 cells were used for complementation with wt-MLH1 and its S44L and S44P ATPase mutants. A site-specific mutagenesis was performed using the QuikChange multi site-directed mutagenesis kit from Stratagene. MLH1 constructs were inserted into the pQCXIN retroviral vector. Infected cells with stable expression of the vector sequences were selected in the presence of geneticin [19]. The HCT116 cells were grown in DMEM F-12 media with 10% FBS, antibiotics and 600 μg/ml of geneticin. The DLD-1 and DLD-1 +chr 2 cells were kindly provided by Dr. Thomas Kunkel (NIH) and were maintained in DMEM F-12 media with 10% FBS, antibiotics. The chromosome complemented DLD-1 +chr 2 cells were maintained in 400 μg/ml of geneticin.

2.3. shRNA transfection

Mission shRNA plasmid bacterial stocks directed against human MSH6 and MSH3 were obtained from Sigma–Aldrich. The plasmid DNA was purified using a plasmid purification maxi prep kit from Qiagen. Lentiviral particles were packaged using 293FT cells with the help of 3rd generation packaging plasmids PMD2G, PMDLG/RRE and PRSV/RRE. Lipofectamine 2000 reagent (Invitrogen) was used for the transfection of the plasmid DNA. The media was changed after 24 h of transfection. The viral particles were harvested 48 h and 72 h after the transfection by centrifugation followed by filtration through 0.2 micron filters. The viral stocks were stored as aliquots at −80 °C for future use. At the time of the experiment, the viral stocks were used along with polybrene (Sigma–Aldrich) for the knock down of proteins of interest. Cells were harvested at the 72 h timepoint post transduction to check for protein and transcript expression.

2.4. siRNA transfection

ON-TARGET plus SMART pool siRNAs specific for human MSH3 and MLH1 were purchased from Dharmacon RNAi technologies, Thermo Scientific. The non-targeting control siRNA was used as a control for non-specific effects. siRNA transfection was carried out as per the manufacturer's protocol. Briefly, the cells were plated in 6 well plates in the antibiotic free media. At the time of transfection, the cell density was maintained at 60–70% and two transfections were done with an interval of 24 h. Dharmafect transfection reagent 1 and 4 were used for MEFs and MDA-MB-231 cells, respectively. The cells were harvested at 48 and 72 h timepoints after transfection for the detection of protein and transcript expression.

2.5. Western blot analysis

Cells were harvested at 96, 120 and 144 h after the infection, washed with PBS and lysed in lysis buffer (10 mM Tris pH 8.0, 120 mM NaCl, 0.5% NP-40, 1 mM EDTA) containing protease inhibitors (0.5 M phenyl methyl sulphonyl fluoride PMSF, 1 mg/ml, Leupeptin and 1 mg/ml pepstatin A). The proteins were separated on 8% SDS-polyacrylamide gels and transferred onto Immobilon-P transfer membranes (Millipore). After blocking (2% non-fat dry milk), the membranes were probed with primary antibodies recognizing human MLH1, with α-tubulin as a loading control. The membranes were incubated with appropriate secondary antibodies and the signal was detected by using Enhanced chemiluminescence detection system.

2.6. Real time PCR for the measurement of transcript levels

At indicated post-transfection time points, cells were harvested and pelleted. RNA was isolated using TRIzol reagent (Invitrogen) by