Characterization of MLH1 and MSH2 DNA mismatch repair proteins in cell lines of the NCI anticancer drug screen

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Abstract Purpose and methods: The lack of a functional DNA mismatch repair (MMR) pathway has been recognized as a common characteristic of several different types of human cancers due to mutation affecting one of the MMR genes or due to promoter methylation gene silencing. These MMR-deficient cancers are frequently resistant to alkylating agent chemotherapy such as DNA-methylating or platinum-containing compounds. To correlate drug resistance with MMR status in a large panel of human tumor cell lines, we evaluated by Western blot the cellular levels of the two MMR proteins most commonly mutated in human cancers, MLH1 and MSH2, in the NCI human tumor cell line panel. This panel consists of 60 cell lines distributed among nine different neoplastic diseases. Results: We found that in most of these cell lines both MLH1 and MSH2 were expressed, although at variable levels. Five cell lines (leukemia CCRF-CEM, colon HCT 116 and KM12 and ovarian cancers SK-OV-3 and IGROV-1) showed complete deficiency in MLH1 protein. MSH2 protein was detected in all 57 cell lines studied. Absence of MLH1 protein was always linked to resistance to the methylating chemotherapeutic agent temozolomide. This resistance was independent of cellular levels of O6-alkylguanine DNA alkyltransferase. Based on data available for review in the NCI COMPARE database, cellular levels of MLH1 and MSH2 did not correlate significantly with sensitivity to any standard anticancer drug or with any characterized molecular target already tested against the same panel of cell lines. Conclusion: Based on evaluation of 60 tumor cell lines in the NCI anticancer drug screen, MLH1 deficiency was more common than MSH2 deficiency and was always associated with a high degree of temozolomide resistance. These data will enable correlations with other drug sensitivities and molecular targets in the COMPARE database to evaluate linked processes in tumor drug resistance.

Key words DNA mismatch repair · NCI anticancer drug screen · DNA repair · Drug resistance · Temozolomide

Introduction

DNA replication results in occasional base pair errors mainly due to the insertion of a mismatched base or the slippage of the replication complex in regions of simple nucleotide repeats. All organisms require a functional DNA mismatch repair (MMR) system able to correct the replicative mismatches. The central role played by this system in the maintenance of genomic stability is evident in the appearance of mutator phenotypes and microsatellite instability (MSI) in prokaryotic or eukaryotic cells carrying a defective MMR gene [1]. Interest in the role of MMR in the etiology and progression of cancer has increased because of the finding that defects in one of the five human MMR proteins are present in hereditary nonpolyposis colon cancer (HNPCC) [2–4], as well as many sporadic cancers [5–8].

Three polypeptides (MSH2, MSH3 and MSH6/GTBP) are homologues of the bacterial protein MutS and are all involved in the initial phase of recognizing and binding the targeted mismatch. MSH2 can heterodimerize either with MSH6 to give hMutSβ or with MSH3 to give hMutSβ, each of which is characterized by different substrate specificity [9, 10]. These heterodimers recruit to the repair reaction another heterodimer,
hMutLα, formed by two homologues of the bacterial protein MutL, MLH1 and PMS2. This heterodimer completes the assembly of the repair complex and proceeds with the excision and the resynthesis of the mismatched DNA region [11].

Beyond its role as a postreplicative repair system, MMR proteins appear to be able to recognize and bind a wide variety of DNA adducts including those induced by clinically used anticancer drugs such as simple methylating agents, platinum-containing compounds and several different alkylating agents. Cells lacking an efficient MMR pathway tolerate the presence in their DNA of adducts induced by cancer chemotherapy agents such as temozolomide (TMZ), cisplatin, 6-thioguanine, procarbazine, busulfan, doxorubicin and etoposide [12].

The correlation between MMR defects and drug resistance phenotypes has so far been investigated only in a limited number of human cancer cell lines or clinical specimens. Data have not been available concerning correlations between MMR defects and the outcome of the novel anticancer agent screen performed by NCI in its Anticancer Drug Screen Program. The Developmental Therapeutics Program (DTP) of the NCI has used 60 human cell lines derived from nine major histological types (bone marrow, lung, skin, ovary, colon, central nervous system, kidney, prostate and breast) to evaluate the eventual anticancer activity of new compounds from synthetic and natural origins [13]. To date, the approximate number of compounds tested by NCI is 60,000, and 10,000 new compounds are added to the program annually. For each compound tested in the assay, there is a pattern of anticancer activity represented by the GI50 values, which are the concentrations able to inhibit cell growth by 50% of the vehicle control sample after a 48-hour continuous exposure, obtained from different cell lines. The so-called mean graph represents the display technique used to characterize each compound's growth inhibitory potential [14]. The COM-PARE algorithm, developed by NCI, can be used to search the available database for compounds with similar activity pattern or compounds whose activity correlates to a certain extent with a molecular target measured in the same array of cell lines.

The present study is the first characterization of two components of the MMR pathway most frequently defective in human malignancies, MLH1 and MSH2, in the NCI anticancer drug screen cell lines. Not only did we want to correlate MMR deficiency with drug resistance to methylation agents, as we have previously reported with two MMR-deficient cell lines [15], but we also wanted to determine whether resistance or sensitivity to other drugs would be correlated with deficiency or relative expression of MLH1 and MSH2. In addition, since this panel has been characterized for several other molecular pathways important in drug resistance [16–21], our study was also designed to correlate MLH1 and MSH2 levels with other molecular targets.

Materials and methods

Cell lines and cell survival assay

Cell pellets (1 × 10^7 cells each) from the 58 cell lines included in the study were obtained from Dr. D. Scudiero and Mr. R. Camalier of the NCI DTP. For cytotoxicity experiments, cells were grown in RPMI-1640 supplemented with 5% fetal bovine serum and 5 mM l-glutamine. TMZ cytotoxicity was evaluated by plating, according to the inoculation density suggested by NCI-DTP, the different cell lines in six-well clusters 16 h before exposure to the drug. Cells were exposed to TMZ for 2 h, and incubated for 5 days at 37 °C in drug-free medium. Some samples were pretreated for 2 h with O6-benzylguanine (BG) (kindly provided by Dr. R. Moschel, FCRDC-NCI) and further incubated for 5 days in the presence of BG after the removal of the drug. Cells were trypsinized and counted with a hemocytometer.

Western blotting

Cell pellets were resuspended in ice-cold lysis buffer containing 0.5% sodium deoxycholate, 0.1% SDS, 1% Triton X-100, 1% NP40 and 1 mM EDTA, 1 mM PMSF, 0.01 mg/ml aprotinin and 0.01 mg/ml leupeptin. Samples were sonicated twice, 10 s each time, and the total protein concentration evaluated by the Bradford assay (Bio-Rad, Hercules, Calif.). Total protein (20 μg for each cell line) was separated on an 8% SDS-polyacrylamide gel and transferred to a PVDF Immobilon membrane (Millipore, Bedford, MA). Detection of MLH1 and MSH2 proteins was performed by using specific monoclonal antibodies recognizing the two human mismatch proteins: anti-MLH1 no. 13271A (Pharmingen, San Diego, Calif.) and anti-MSH2 no. 26-100U/G (Oncogene Research/Calbiochem, Cambridge, Mass). Equal loading of the different samples was monitored by probing the filters with monoclonal anti-actin antibody no. N350 (Amersham Piscataway, NJ). Relevant bands were visualized by hybridization of the membranes to an anti-mouse IgG antibody (Amersham) which was peroxidase-linked and detected with the ECL system (Amersham). Comparison between individual blots was accomplished including in each gel 20 μg of total cell protein extracted from the MMR wild-type colon cancer cell line, SW480. MLH1 and MSH2 levels in SW480 cell extracts were found to vary by no more than 10% between the different blots analyzed (Fig. 1). MLH1 and MSH2 levels are expressed throughout as SW480-relative densitometry units. Similar standard errors were found when the MLH1 and MSH2 ratios between individual cell line extracts and SW480 were determined in at least two independent experiments.

COMPARE methodology

The available data concerning the chemosensitivity profiles of the cell lines from the NCI anticancer drug screen can be accessed through the world-wide web at http://dtp.nci.nih.gov//. The NCI screening procedures have been already described [22]. Briefly, each compound included in the screening was evaluated for its cell growth inhibition activity using a 48-h assay with sulforhabdamine B [23]. The growth-inhibitory power of each agent is expressed as the GI50 value. All the GI50 values obtained for a given compound can be graphically represented through the mean graph created by plotting positive and negative values from a set of GI50 values along a vertical line representing the mean response of all the cell lines in the panel to the test agent. Positive values represent cellular sensitivities to the test agent that exceed the mean, whereas negative values represent sensitivities to the test agent that are less than the mean.

The expression of molecular targets in the cell lines can be correlated with the effects of the test compounds through the aid of the COMPARE algorithm [14], which analyzes the different databases (standard anticancer agents, synthetic compounds, natural compounds and molecular targets) to search for the highest ranked