Impact of microsatellite status on chemotherapy for colorectal cancer patients with *KRAS* or *BRAF* mutation

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Abstract. *KRAS* and *BRAF* mutations are frequently detected in cases of colorectal cancer (CRC). The microsatellite status of patients with CRC and mutated *KRAS*/*BRAF* is important when determining cancer therapy. In the present study, the microsatellite status and genetic polymorphisms of *KRAS* (codons 12 and 13) and *BRAF* (V600E) were characterized in CRC tissue. The mismatch repair activity and oncogenic potential of *KRAS* were assessed by immunoblots from two *KRAS*-mutated CRC cell lines, SW480 and HCT116, with different microsatellite statuses, following treatment with 5-fluorouracil (5-FU) and oxaliplatin. Of all the 205 patients with CRC enrolled in the present study, 31.2% (64 of 205) had a *KRAS* or *BRAF* mutation, and 79.7% (51 of 64) of these patients with a *KRAS*/*BRAF* mutation exhibited microsatellite stability (MSS), indicating that microsatellite status is correlated with *KRAS*/*BRAF* mutation (P=0.027). A higher proportion (39.0%, 41 of 105) of elderly patients (≥62.6 years) had mutated *KRAS* or *BRAF* than younger patients (<62.6 years; 23.0%, 23 of 100; P=0.013). In the subgroup of 154 patients with MSS, patients without the *KRAS* or *BRAF* mutation (n=110) had longer disease-specific survival rates (58.8±9.4%) than patients with *KRAS* or *BRAF* mutations (n=44; 50.6±11.0%; P=0.043). Cytoplasmic KRAS levels decreased whereas nuclear MutS protein homolog 2 (MSH2) levels increased slightly in CRC HCT116 cells that were microsatellite instable, following treatment with 76.9 μM 5-FU for 2 days. In microsatellite stable SW480 cells, MSH2 levels markedly increased in the nucleus following 150 μM oxaliplatin treatment for 3 days. However, no significant change was observed regarding KRAS distribution in these cells. The results of the present study suggest that it is important to identify patients with CRC who may benefit from adjuvant chemotherapy with 5-FU or oxaliplatin, particularly CRC patients with MSS and mutated *KRAS* or *BRAF,* who have poorer overall survival rates than patients with microsatellite instability. Knowledge of the microsatellite status of patients and whether they harbor *KRAS* or *BRAF* mutations may enable more effective therapeutic strategies to be developed. Further prospective studies are required to validate the findings of the current study.

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Abbreviations: CRC, colorectal cancer; MMR, mismatch repair; HNPCC, hereditary nonpolyposis colorectal cancer; MSI, microsatellite instability; MAPK pathway, mitogen-activated protein kinase pathway; MSS, microsatellite stability; DSS, disease-specific survival

Key words: colorectal cancer, microsatellite status, *KRAS*, *BRAF*, DNA mismatch repair, 5-fluorouracil, oxaliplatin

Introduction

Colorectal cancer (CRC) is a leading cause of cancer-associated mortality (1). CRC typically develops slowly by the progressive accumulation of genetic mutations, which cause the normal colonic epithelium to transform into adenocarcinoma (2,3).

Defects in the DNA mismatch repair (MMR) system may arise sporadically or in patients with hereditary nonpolyposis

colorectal cancer (HNPCC) syndrome, may be inherited in an autosomal-dominant manner (2-4). Tumors are characterized by the presence of microsatellite instability (MSI), caused either by genetic changes or by attenuating the expression of proteins in the DNA MMR pathway (5,6). Among MMR genes, abnormalities of MutL homolog 1 (*MLH1*) and MutS protein homolog 2 (*MSH2*) have been the subject of several studies investigating CRC (7,8), and it has been determined that the microsatellite status of CRC patients should be evaluated prior to chemotherapy (9). Furthermore, genetic studies have demonstrated that mutations of the GTPase oncogenes, *KRAS* and *BRAF* in the mitogen-activated protein kinase (MAPK) pathway, also known as the RAS-RAF-extracellular signal-regulated kinase (ERK)-MAPK/ERK kinase pathway, are detected in a high proportion of CRC patients, including those with defective MMR activity (10-12). Activation of the MAPK pathway is important in MSI CRC tumorigenesis (13). In addition, analysis of *KRAS* mutations has demonstrated an association with sporadic CRC (14,15). Elucidation of the microsatellite status of CRC patients may indicate what type of adjuvant chemotherapy is the most beneficial for a particular patient (9). Therefore, knowledge of MMR activity and *KRAS*/*BRAF* mutation status may provide further valuable guidance for planning therapeutic strategies (16).

CRC patients with microsatellite stability (MSS) and *KRAS*/*BRAF* mutations usually have a poor prognosis (17). Therefore, personalizing treatment based on patient tumor characteristics is advantageous (18,19). However, only a few studies indicate that distinct chemotherapy is appropriate for CRC patients with different microsatellite status, MMR activity and *KRAS*/*BRAF* mutation (20). The present study aimed to assess the MSI status of CRC tumors and the presence of *KRAS*/*BRAF* mutation in patients with CRC, and to evaluate the outcome of treating cells from two CRC cell lines, HCT116 and SW480, with different microsatellite statuses, with the chemotherapeutic agents 5-fluorouracil (5-FU) and oxaliplatin (21-25).

Materials and methods

Patients and general data collection. A total of 205 patients with CRC (121 males and 84 females; mean age, 62.6 years; range, 25.4-90.1 years) from the Gastrointestinal Department of Cathay General Hospital (Taipei, Taiwan) were enrolled from January 2006 to December 2008 in the current study. Survival data were acquired from 176 patients and others were lost to follow-up due to referral. The mean follow-up time was 17.0±15.6 months (median, 10.5 months). Suspicious growths in patient colonic tissues were sampled with small biopsy forceps inserted through a colonoscope. The tissues were formalin-fixed, paraffin-embedded and cut into slices of 4-5-μm thickness for immunohistochemical staining, or immersed in RNAlater® solution (Thermo Fisher Scientific, Inc., Waltham, MA, USA) for genomic DNA preparation, according to the manufacturer's protocol. Presence of distant metastasis was routinely confirmed by abdominal computed tomography. In addition, blood samples were collected from each patient to serve as controls when determining the microsatellite status. The study protocol was approved by the Institutional Review Board of Cathay General Hospital, and informed consent was obtained from all patients prior to obtaining tissue samples.

Colonic cell lines, protein extraction, and western blotting. Cells from the human colorectal carcinoma HCT116 [American Type Culture Collection (ATCC) no. CCL-247; MSI) and SW480 (ATCC no. CCL-228; MSS) cell lines were purchased from the ATCC (Manassas, VA, USA) and maintained as recommended by their guidelines (www.atcc.org) (22,23). All cultured cells used in the current study were washed in ice-cold PBS (pH 7.4), scraped from culture dishes on ice using a plastic cell scraper and collected in 1.5-ml microcentrifuge tubes in 1 ml ice-cold PBS. Following a short centrifugation step (1,000 x *g* for 3 min at 4˚C) to pellet the cells, the supernatants were removed from each sample, and the cell pellets were resuspended in 900 μl ice-cold 0.1% NP-40 (Merck Millipore, Darmstadt, Germany) in PBS. Subsequently, a reagent-based protocol that enabled the stepwise lysis of cells, separation of cytoplasm from intact nuclei and extraction of nuclear proteins was performed, according to the protocol of the NE-PER™ Nuclear and Cytoplasmic Extraction kit (Thermo Fisher Scientific, Inc.). The cytoplasmic (10 μg) and nuclear (10 μg) fractions underwent 10% SDS-PAGE and were subsequently transferred to polyvinylidene difluoride membranes. Membranes were then blocked with 10% skimmed milk and 3% bovine serum albumin in TBS buffer containing Tween-20 [TBST; 20 mM Tris (pH 7.4), 150 mM NaCl and 0.05% Tween 20] for 1 h to prevent non-specific binding of antibodies. Mutated KRAS and MSH2 were immunoblotted with anti-KRAS antibody (1:2,000 in TBST; 05-516; Merck Millipore) and anti-MSH2 antibody (1:500 in TBST; ab52266; Abcam, Cambridge, MA, USA) for 1 h, respectively. In addition, membranes were incubated with an anti-α-tubulin antibody (1:500 in TBST; sc-5286; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) as a cytoplasmic marker, or with anti-lamin A/C antibody (1:500 in TBST; sc-7292; Santa Cruz Biotechnology, Inc.) as nuclear marker, for 1 h each. Protein signals were detected with Western Lightning Chemiluminescence Reagent Plus (PerkinElmer, Inc., Waltham, MA, USA) upon incubation with an appropriate secondary antibody conjugated with peroxidase (1:5,000 in TBST; Dako, Glostrup, Denmark) for 1 h. All incubations were performed at room temperature on a rocking platform and were followed by several washes with TBST buffer to remove the residual solution. Protein bands were quantified by densitometry using image processing AlphaView software version 3.2.2.0 for the FluorChem FC2 system (Cell Biosciences, Inc., Santa Clara, CA, USA). Relative protein levels were calculated and determined by normalizing their expression to that of a-tubulin or lamin A/C.

Immunohistochemical staining for CRC tissues. Sections were dewaxed in xylene and rehydrated through a graded series of ethanol concentrations (100, 95 and 70%) to water. Antigen retrieval was performed by immersing the slides in BD Retrievagen A (pH 6.5 for MLH1 and pH 6.0 for MSH2; BD Biosciences, San Jose, CA, USA) and heating the slides in a microwave oven for 30 min at 95˚C. Sections were treated with 3% hydrogen peroxide for 5 min to block endogenous peroxidase activity. Sections were washed in PBS and subsequently placed in 20% normal goat serum (G9023; Sigma-Aldrich; Merck Millipore)

in PBS for 20 min to reduce non-specific staining. Sections were then incubated with monoclonal antibodies raised against human (h) MLH1 (1:10; clone G168-15; BD Biosciences) for 1 h 32 min at 42˚C or against MSH2 (1:25; clone 25D12; Thermo Fisher Scientific, Inc.) for 1 h at room temperature. Subsequently, visualization was performed using the EnVision+Dual Link system-HRP (Dako) according to the manufacturer's protocol, using 3,3'-diaminobenzidine as a chromogen. Finally, slides were counterstained with hematoxylin solution. Both MLH1 and MSH2 were scored as either negative or positive staining. Tissue specimens were analyzed by two independent pathologists blinded to the conditions.

Determining microsatellite status and detecting mutations of KRAS and BRAF. Colonic tissues pathologically diagnosed as CRC were subjected to the assessment of microsatellite status and identification of hotspot mutations of *KRAS* and *BRAF*. Peripheral blood samples from patients diagnosed with CRC were collected in anticoagulant tubes containing EDTA. Genomic DNA was extracted from blood and tissue samples according to a standard protocol (26). To determine the microsatellite status of the enrolled CRC patients, a reference panel of five fluorescent dye-labeled microsatellite primers (BAT25, BAT26, D2S123, D5S346 and D17S250) purchased from Thermo Fisher Scientific, Inc., was used (27). Primer sets for the BAT25, BAT26 and D5S346 loci, and for the D2S123 and D17S250 loci, were respectively combined for the multiplex polymerase chain reaction (PCR) amplifications in a volume of 5.5 μl containing 20.0 ng genomic DNA. Denatured PCR products were analyzed by capillary electrophoresis in an ABI Prism® 3100 Genetic Analyzer (Applied Biosciences; Thermo Fisher Scientific, Inc.). Raw data were collected using Data Collection Software version 1.1 (Applied Biosciences), and marker performance was evaluated using GeneScan® Analysis Software version 3.7 (Applied Biosciences) electropherograms in full-view display for each dye color. For the purpose of prognostic evaluation, microsatellite status was categorized as MSS or MSI according to whether instability was evident for no markers or for ≥1 marker, respectively (28). To detect *KRAS* mutations at codons 12 and 13, a restriction enzyme-based analysis (*Bst*NI for codon 12 and *Xcm*I for codon 13) following appropriate PCR amplifications was employed, as previously described (29,30). Briefly, the cycling conditions for codon 12 were 35 cycles of amplification (93˚C for 35 sec, 56˚C for 50 sec and 72˚C for 50 sec), and for codon 13 were 40 cycles of amplification (93˚C for 60 sec, 52˚C for 60 sec and 72˚C for 60 sec). The sequences of the PCR primers used were: *Bst*NI forward, 5'-ACTGAATATAAACTTGTGGTA GTTGGACCT-3' and reverse, 5'-TCATGAAAATGGTCAGAG AAACC-3'; and *Xcm*I, forward, 5'-ACTGAATATAAACTT GTGGTCCATGGA-3' and reverse, 5'-TATCTGTATCAAAGA ATGGTCCTGCACCAG-3' (30,31). The presence of the *BRAF* V600E mutation was determined using an allele-specific PCR assay (32). Depending on the genotypes, either allele-specific reaction could amplify the target sequence. The cycling conditions used for PCR were 10 min at 95˚C for initial activation and 40 cycles of amplification (92˚C for 15 sec and 60˚C for 60 sec).

The components of the reaction for the different genotypes were identical except for the respective allele-specific probes, as described below. The primer sequences were: Forward, 5'-CATGAAGACCTC ACAGTAAAAATAGGTGAT-3' and reverse, 5'-TGGGACCCA CTCCATCGA-3'. Allele-specific TaqMan® probes (VIC®-labeled reporter T allele, 5'-CTAGCTACAG [T] GAAATC-3' and 6-carboxyfluorescein-labeled reporter A allele, 5'-TAGCTACAG [A] GAAATC-3') (Applied Biosciences) and TaqMan Genotyping Master Mix (Applied Biosciences) were used in a 7300 Real-Time PCR system (Applied Biosciences; Thermo Fisher Scientific, Inc.) (32). All identified *KRAS*/*BRAF* mutations were then validated using a BigDye® Terminator v3.1 Cycle Sequencing kit (Applied Biosciences; Thermo Fisher Scientific, Inc.).

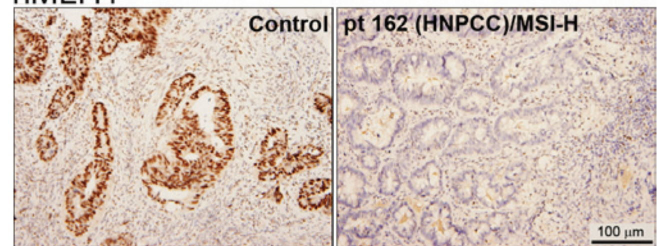

Figure 1. Immunohistochemical stain of CRC tissue for hMLH1 expression. The left panel presents a positive control for hMLH1 expression. The right panel presents the immunohistochemical staining results of a paraffin-embedded CRC tissue section acquired from a patient with HNPCC (pt 162). Both slides were counterstained with hematoxylin. Bar, 100 μm. HNPCC, hereditary nonpolyposis colorectal cancer; CRC, colorectal cancer; MSI-H, high microsatellite instability; pt, patient; hMLH1, human MutL homolog 1.

Statistical analysis. The association between tumor microsatellite status and patient clinicopathological features was analyzed using a χ^2 test. Patient survival time was calculated from the date of complete resection of CRC tumors to the date of last follow-up, with the only patient who succumbed to CRC being excluded from counting towards disease-specific survival (DSS). DSS distributions were estimated using a Kaplan-Meier method and compared using a log-rank test. P<0.05 was considered to indicate a statistically significant difference. All calculations were performed using IBM SPSS Statistics for Windows version 22.0 (IBM SPSS, Armonk, NY, USA).

Results

Microsatellite status and mutation rates of KRAS and BRAF. One patient (age, 58.4 years; female) out of the 205 enrolled patients met the clinical diagnostic criteria for HNPCC (33,34). Immunohistochemical results indicated that staining for hMLH1 was negative for this patient (Fig. 1). As presented in Table I, 12.7% of the tumors (26 of 205) exhibited MSI, including the tumor in the patient with HNPCC. The mean age of the 26 patients with MSI (58.2 years; range, 29.0-81.6 years) was lower than that of the other 179 patients with MSS (63.3 years; range: 25.4-90.1 years). Furthermore, a *KRAS* or *BRAF* mutation (5 for *BRAF* V600E, 24 for *KRAS* codon 12 and 4 for *KRAS* codon 13) was detected in 31.2% of patients (64 of 205). A total of 79.7% (51 of 64) of patients with *KRAS*/*BRAF* mutations had MSS (P=0.027), demonstrating that a correlation exists between MSS and *KRAS*/*BRAF* mutations (Table II). When comprehensively considering the microsatellite status of patients and the site of the tumors, tumors at distal/rectal

Table I. Clinicopathological characteristics of patients.

Feature	Number of patients[a]
Age, years	
≤62.6	100
>62.6	105
Gender	
Male	121
Female	84
AJCC staging	
I+II	73
III+IV	88
Microsatellite status	
MSS	179
MSI	26
KRAS/*BRAF* mutation	
Wild type	141
Mutant	64
Differentiation	
Well/moderate	157
Poor	15
Tumor location	
Right	48
Left	91
Rectum	66
Tumor size, cm	
≤4.3	89
>4.3	82

[a]The number of cases that were assessed in each category was dependent on the number of available cases. All information on female patients includes clinical information regarding the aforementioned patient with hereditary nonpolyposis colorectal cancer. AJCC, American joint committee on cancer; MSS, microsatellite stability; MSI, microsatellite instability.

Table II. High percentage of patients with MSS in different CRC groups.

Feature	Percentage, % (n)	P-value
Microsatellite status vs. *KRAS*/*BRAF* mutation		
MSS	79.7 (51/64)	0.027
MSI	20.3 (13/64)	
Microsatellite status vs. distal/rectal sites		
MSS	90.4 (142/157)	0.015
MSI	9.6 (15/157)	

MSS, microsatellite stability; MSI, microsatellite instability.

areas were significantly diagnosed in patients with MSS (90.4%, 142 of 157; P=0.015).

Significantly different mutation rates of *KRAS* or *BRAF* were observed in patients of different ages (P=0.013; Table III). A higher proportion of elderly patients (≥62.6 years) had mutated *KRAS* or *BRAF* (39.0%, 41 of 105), whereas only 23.0% (23 of 100) of younger patients (<62.6 years) harbored these mutations. The odds ratio for *KRAS* or *BRAF* mutations in the elderly patients was 2.15 (95% confidence interval, 1.17-3.94). In addition, of the 172 patients with available data for tumor differentiation, 15 were diagnosed with poorly differentiated tumors, and 53.3% (n=8) of these patients harbored a *KRAS* or *BRAF* mutation (P=0.036).

Different survival rates according to KRAS or BRAF mutations in patients with MSS. In the present study, survival data were available for 176 patients with CRC (154 patients with MSS and 22 patients with MSI). Patients with MSS tended to have poorer DSS (57.4±7.0%) compared with patients with MSI, although this difference was not significant (P=0.065; Fig. 2). In addition, it has been demonstrated that *BRAF* and *KRAS* mutations are frequently associated with CRC and influence prognosis (35). Therefore, the current study assessed the survival rates of patients with MSS according to their different KRAS/RAS mutations. In the subgroup of 154 patients with MSS, patients without *KRAS* or *BRAF* mutations (n=110) had better DSS (58.8±9.4%) than those with *KRAS* or *BRAF* mutations (n=44; 50.6±11.0%; P=0.043; Fig. 3).

Different responses to 5-FU- or oxaliplatin-based adjuvant treatment in CRC cell lines. Due to the clinical significance of microsatellite status and *KRAS*/*BRAF* mutations in CRC, MSS (SW480 cells; Fig. 4) and MSI (HCT116 cells; Fig. 5) cell lines were employed in the present study (22,23). Both SW480 and HCT116 cells had mutated *KRAS* but wild-type *BRAF* (36). In SW480 cells treated with 17.5 μM 5-FU for 2 days or 150 mM oxaliplatin for 3 days, the levels of KRAS detected did not significantly differ between the nucleus and the cytoplasm (Fig. 4A and B). However, under the same treatment conditions, the expression of the DNA MMR protein, MSH2, varied in SW480 cells (Fig. 4C). MSH2 expression increased in the cytoplasm and decreased in the nucleus of SW480 cells following treatment with 5-FU (both P<0.001). By contrast, MSH2 expression decreased in the cytoplasm and increased in the nucleus of SW480 cells treated with oxaliplatin (both P<0.001; Fig. 4B and D).

In addition, distinct expression patterns for KRAS and MSH2 in HCT116 cells treated with 76.9 μM 5-FU for 2 days or 1.4 μM oxaliplatin for 3 days were observed (Fig. 5). The levels of cytoplasmic and nuclear KRAS significantly decreased in HCT116 cells following 5-FU treatment (P=0.050 for cytoplasm and P=0.030 for nucleus; Fig. 5A). However, in cells with treated with oxaliplatin, the levels of nuclear KRAS decreased significantly (P<0.001), while the levels of cytoplasmic KRAS increased but not significantly (P=0.216; Fig. 5B). MSH2 expression decreased in the cytoplasm (P<0.001) and increased in the nucleus (P=0.027) of 5-FU-treated HCT116 cells compared with control cells (Fig. 5C). However, oxaliplatin treatment did not increase the expression of MSH2 in the nucleus of HCT116 cells (Fig. 5D).

Table III. Mutation rate of *KRAS*/*BRAF* according to patient's age and tumor differentiation.

Feature	*KRAS*/*BRAF* mutant, % (n)	P-value	Odds ratio	95% CI
Age, years[a]				
≤62.6	23.0 (23/100)	0.013	2.15	1.17-3.94
>62.6	39.0 (41/105)			
Differentiation[b]				
Well/moderate	27.4 (43/157)	0.036	3.03	1.04-8.86
Poor	53.3 (8/15)			

[a]Mean age, 62.6 years. [b]The number of assessed cases was dependent on the number of available cases. CI, confidence interval.

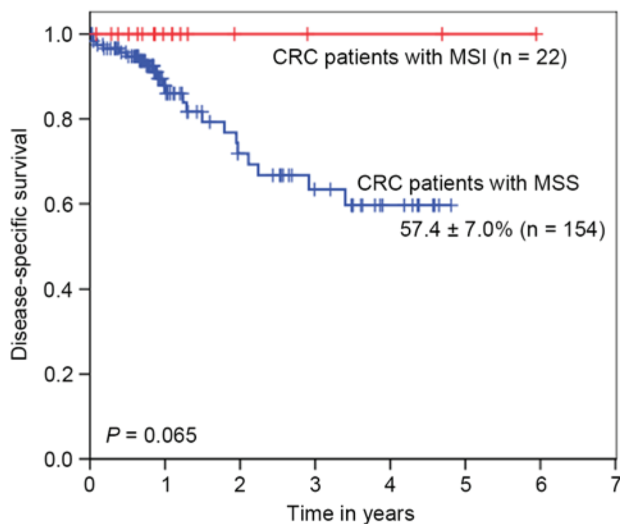

Figure 2. Disease-specific survival of non-HNPCC patients according to MSS. Disease-specific survival was defined as the time from surgery to either CRC-associated mortality or end of follow-up. Survival probabilities were estimated by the Kaplan-Meier method and compared using the log-rank test according to MSS in non-HNPCC patients. Patients with available survival data were stratified into two groups: MSI (n=22) and MSS (n=154) (P=0.065). CRC, colorectal cancer; MSI, microsatellite instability; MSS, microsatellite stability; HNPCC, hereditary nonpolyposis colorectal cancer.

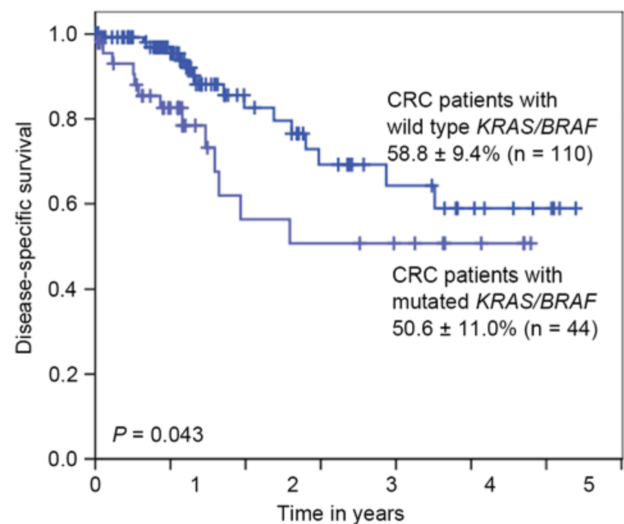

Figure 3. Disease-specific survival of 154 MSS patients according to *KRAS*/*BRAF* mutation status. Disease-specific survival was defined as the time from surgery to either CRC-associated mortality or end of follow-up. Survival probabilities were estimated by the Kaplan-Meier method and compared using the log-rank test according to those patients with or without *KRAS* or *BRAF* mutation (P=0.043). MSS, microsatellite stability; CRC, colorectal cancer.

Discussion

It has previously been demonstrated that the microsatellite status of CRC patients responds to specific chemotherapy (37). Furthermore, in combination with other genes, assessing the mutation status of *KRAS* or *BRAF* may provide guidance during the planning of therapeutic strategies (16,25). In the present study, the majority of enrolled patients were MSS, and their colorectal tumors frequently arose in the distal/rectal colon, similarly to the study by Boland and Goel (24). Another previous study indicated that the highest survival rate was detected in patients with MSI CRC, followed by those with MSS CRC, whereas the lowest survival rate was detected in the subgroup of MSS patients with *KRAS* or *BRAF* mutations (38). In the present study, *KRAS* or *BRAF* mutations were frequently detected in the tumors of elderly patients, as has previously been demonstrated in the feces of CRC patients (39). Thus, it is important to improve the efficacy of CRC treatment if patients have MSS and *KRAS*/*BRAF* mutations (35).

It has been demonstrated that patients with different microsatellite statuses respond differently to chemotherapeutics (24). The results of the current study strongly suggest that more complex tumorigenic pathways exist in MSS CRC than in MSI CRC (40). This may explain why MSS CRC patients have a poorer prognosis than MSI patients, even though they do not have unstable microsatellites (35,41). CRC patients with MSS may slowly and progressively accumulate genetic mutations, including *BRAF* and *KRAS* mutations, in the MAPK pathway (3,13). Although the current study demonstrated that the mutation rate of *KRAS*/*BRAF* was lower in MSS CRC than in MSI CRC (data not shown), such mutations coupled with the MSS phenotype correlate with metastatic CRC and with high mortality rate (42,43). CRC patients with MSS have aberrant activation of the MAPK pathway (12,13,17). MAPK activation, due to mutational *KRAS* or *BRAF* hotspots, has been identified in CRC tumorigenesis (17,44). Mutually exclusive *KRAS* and *BRAF* mutations provide useful additional risk stratification of CRC to guide the use of chemotherapy (13,45,46). Therefore, in order to perform appropriate chemotherapy, it is important

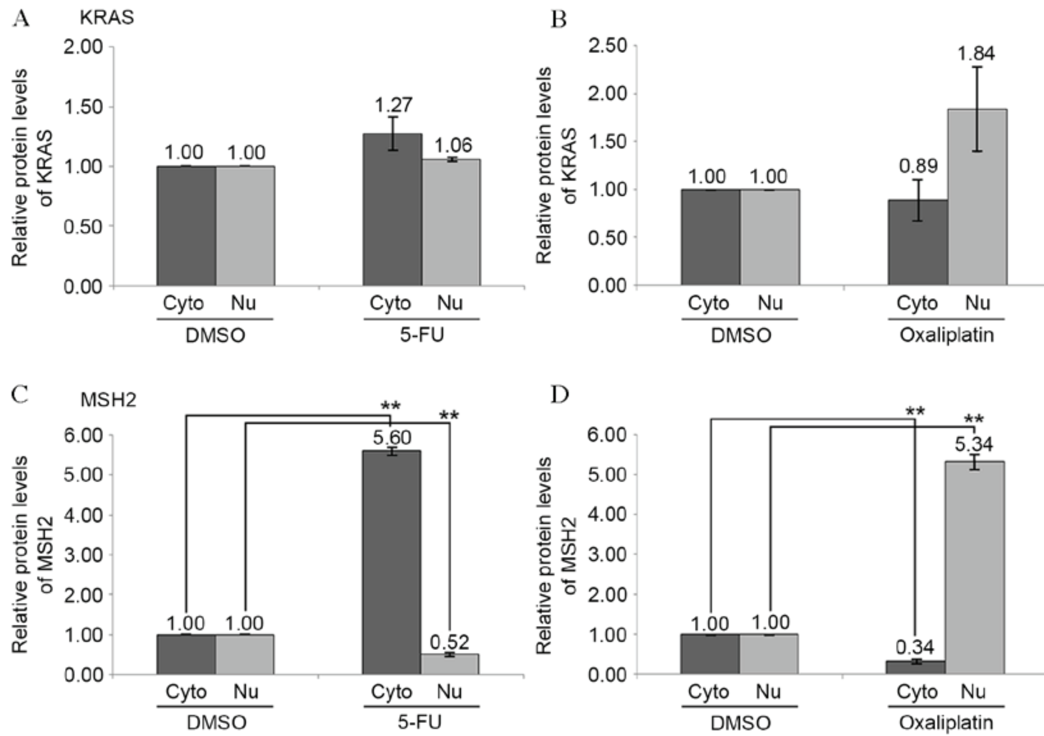

Figure 4. Relative protein quantitation in different cellular compartments of SW480 cells following treatment with chemotherapy agents. Determination of KRAS expression under (A) 5-FU and (B) oxaliplatin treatment. Determination of MSH2 expression under (C) 5-FU and (D) oxaliplatin treatment. SW480 cells were treated with 17.5 μM 5-FU or 150 μM oxaliplatin. The different cellular fractions (cytoplasmic and nuclear) were separately harvested. A 10-μg sample of each fraction was electrophoresed, and each protein band was quantified by densitometry using image processing FluorChem FC2 software. Relative protein levels were determined by normalizing their expression to that of α-tubulin (for the cytoplasmic fraction) or lamin A/C (for the nuclear fraction) (**P<0.001). Cyto, cytoplasm; Nu, nucleus; DMSO, dimethyl sulfoxide; 5-FU, 5-fluorouracil; MSH2, MutS protein homolog 2.

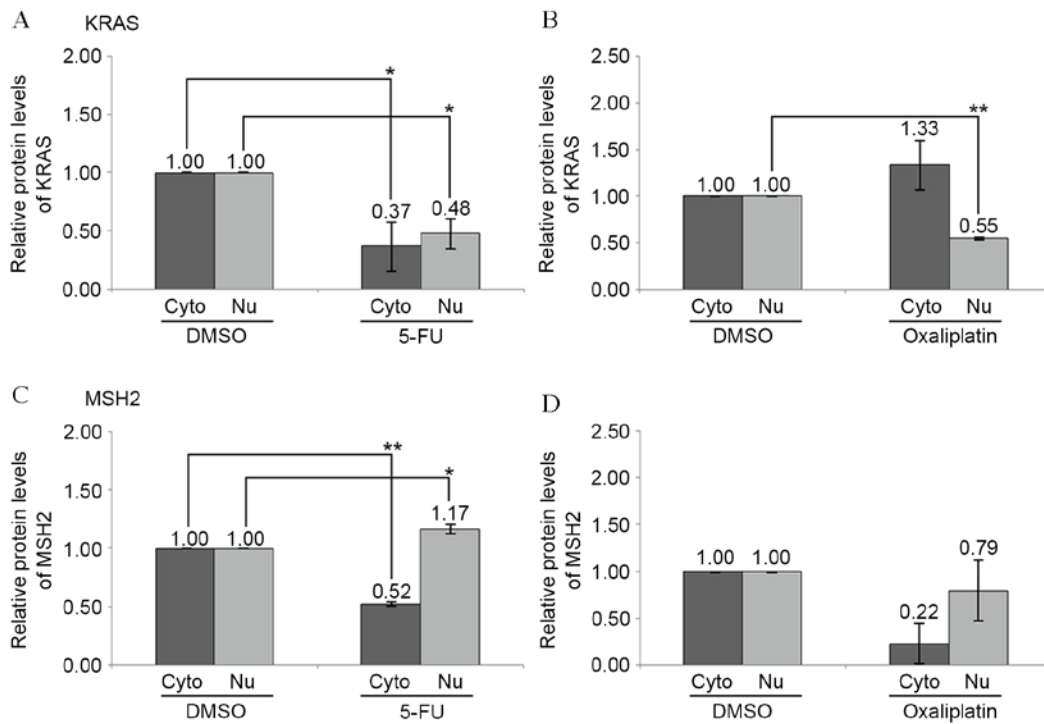

Figure 5. Relative protein quantitation in different cellular compartments of HCT116 cells under treatment with chemotherapeutic agents. (A) Determination of KRAS expression under 5-FU treatment. (B) Determination of KRAS expression under oxaliplatin treatment. (C) Determination of MSH2 expression under 5-FU treatment. (D) Determination of MSH2 expression under oxaliplatin treatment. HCT116 cells were treated with 76.9 μM 5-FU or 1.4 μM oxaliplatin. Different cellular fractions (cytoplasmic and nuclear) were separately harvested. A 10-μg sample of each fraction was electrophoresed, and each protein band was quantified by densitometry using image processing FluorChem FC2 software. Relative protein levels were determined by normalizing their expression to that of α-tubulin (for the cytoplasmic fraction) or lamin A/C (for the nuclear fraction) (*P<0.05, **P<0.001). Cyto, cytoplasm; Nu, nucleus; DMSO, dimethyl sulfoxide; 5-FU, 5-fluorouracil; MSH2, MutS protein homolog 2.

to identify *KRAS* or *BRAF* mutations to treat CRC patients with different microsatellite statuses (18,43,44,47,48).

Two first-line chemotherapeutic agents, 5-FU and oxaliplatin, induce a cytotoxic response, and may be used to treat CRC cells through the stable correction of MMR activity (21,24,25). Combined microsatellite and *KRAS*/*BRAF* mutation status provides significant prognostic stratification (16). In the current study, oxaliplatin slightly decreased the level of oncogenic KRAS in the cytoplasm while significantly increased the level of MSH2 in the nucleus of SW480 cells. Similarly, 5-FU was able to induce the same changes in HCT116 cells. Previous studies have demonstrated that decreasing the level of KRAS expression in the cytoplasm would reduce its oncogenic potential (49), and that the translocation of MMR proteins into the nucleus may be induced by increased MMR activity (50,51). Furthermore, Ooki *et al* (16) have determined that 5-FU is an insufficient treatment for CRC patients with MSS and mutated *KRAS* or *BRAF*. Together with the results of the current study, this indicates that oxaliplatin may be an efficient chemotherapeutic agent for certain CRC patients with specific microsatellite statuses. Therefore, identifying CRC patients who would benefit from adjuvant chemotherapy with 5-FU or oxaliplatin by determining their microsatellite and *KRAS*/*BRAF* mutation statuses is necessary (52,53). This may enable practitioners to employ more intensive chemotherapy or molecular targeting drugs (52,54).

In conclusion, the microsatellite status and the mutation of *KRAS* or *BRAF* should be determined prior to therapeutic decision making. For CRC patients with MSI and *KRAS*/*BRAF* mutations, 5-FU treatment is recommended. Otherwise, it is better to perform treatment with oxaliplatin for patients with MSS and *KRAS*/*BRAF* mutations. The molecular diagnosis for these CRC patients should be individualized following evaluation of the relevant genetic conditions.

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