Overexpression of MutL homolog 1 and MutS homolog 2 proteins have reversed prognostic implications for stage I–II colon cancer patients

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Background: The outcome of colon cancer patients without lymph node metastasis is heterogeneous. Searching for new prognostic markers is warranted.

Methods: One hundred twenty stage I–II colon cancer patients who received complete surgical excision during 1995–2004 were selected for this biomarker study. Immunohistochemical method was used to assess p53, epidermal growth factor receptor, MLH1, and MSH2 status. KRAS mutation was examined by direct sequencing.

Results: Thirty three patients (27.5%) developed metachronous metastasis during follow up. By multivariate analysis, only female gender (p = 0.03), high serum carcinoembryonic antigen (CEA) level (≥5 ng/ml) (p = 0.04), and MLH1 overexpression (p = 0.003) were associated with the metastasis group. The 5-year-survival rate were also significantly lower for female gender (71.7% versus 88.9%, p = 0.025), high CEA level (64.9% versus 92.4%, p < 0.001), and MLH1 overexpression (77.5% versus 94.4%, p = 0.039). In contrast, MSH2 overexpression was associated with better survival, 95.1% versus 75.5% (p = 0.024).

Conclusions: The reversed prognostic implications in the overexpression of MLH1 and MSH2 for stage I–II colon cancer patients is a novel finding and worthy of further confirmation.
Colorectal cancer (CRC) remains the third place in cancer incidence around the world and affects more than 1 million individuals annually with nearly 33% disease-related mortality rate in developed countries [1,2]. The therapeutic strategies for patients with CRC are mainly guided by adequate tumor staging. Localized tumor diseases, i.e., tumors of AJCC/UICC (American Joint Committee on Cancer/Union for International Cancer Control) stage I and II (T1-4N0M0), are considered to be associated with shorter survival and overexpression of MSH2 associated with longer survival. The serum CEA level combined with MLH1 and MSH2 expression status could help in selecting stage I/II colon cancer patients with higher risk for tumor recurrence.

Aside from the clinical parameters, much effort have been applied in searching for molecular biomarkers of CRC, which could help for selecting the high-risk patients who might be benefit from receiving postoperative chemotherapy [7,8]. Among the various biomarkers, p53 gene status, epidermal growth factor receptor (EGFR) expression, V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) mutation, and microsatellite instability (MSI) genotype have been studied most extensively. However, the significance of these biomarkers is still controversial. A meta-analysis for p53 protein expression and mutation reported that both were only associated with borderline increased risk of death for CRC patients [9]. EGFR expression determined by immunohistochemistry (IHC) also have no correlation with the therapeutic response to EGFR antibody therapy nor prognosis in some reports [10–13]. As for KRAS mutation, the results from the two clinical trials: Cancer and Leukemia Group B (CALGB) 89803 (for stage III CRC patients) and Pan European Trial Adjuvant Colon Cancer (PETACC)-3 (for stage II and III CRC patients) all have demonstrated that KRAS mutation itself was not a major prognostic factor for patients treated with adjuvant 5-fluorouracil-based chemotherapy [14,15].

In contrast, the association of MSI phenotype and survival in CRC patients has been more consistent. A meta-analysis of 32 studies including 7642 CRC patients demonstrated that patients with MSI-high tumors treated by adjuvant fluorouracil had prognostic advantage (HR = 0.65) [16]. This survival benefit was maintained when restricted to patients with stage II or III disease (HR = 0.75). The subsequent large clinical trial, PETACC-3, also confirmed that MSI phenotype is a strong prognostic factors for relapse-free and overall survival in stage II and III CRC [17].

About 85% of sporadic CRC have chromosomal instability with complex chromosomal alterations and the remaining 15% have MSI with frequent mutations in the short tandemly repeated nucleotide sequences (microsatellites) [2]. CRC with MSI is due to dysfunction of DNA mismatch repair (MMR) system, which is caused by mutations or epigenetic methylations of the MMR genes. Lynch syndrome, also named hereditary nonpolyposis CRC, is the inherited prototype for MSI and most were resulted from MLH1 (MutL homolog 1), and MSH2 (MutS protein homolog 2) gene abnormality [18]. The standard method of MSI detection is genetic analysis by a panel of microsatellite markers. The Bethesda consensus defined five microsatellite loci (BAT25, BAT26, DSS346, D2S123, and D17S250), and instability at two or more loci (or >30% of loci) is considered to be MSI-high phenotype [19]. On the other hand, loss of MMR proteins, which include MLH1, PMS1 (Postmeiotic segregation increased 1), PMS2, MSH2, MSH3 and MSH6, determined by IHC stain also can have comparable sensitivity for detection of MSI and can provide additional functional status of individual proteins [20].

For patients with CRC, even in AJCC stage I or II (excluding pT1 tumors), a small fraction of patients remained suffering from local recurrence or distant metastatic disease after radical resection. Various studies have tried to identify the potential risk factors by analyzing important clinical, pathological, and molecular factors, but the results remained inconclusive [3,4,21–23]. In this study, we intend to examine all of the above mentioned biomarkers of colon cancers in a homogeneous cohort of stage I–II colon cancer patients to search for prognostic markers significantly associated with tumor recurrence and survival. These colon cancer patients all received complete radical resection by the same surgeon and none received adjuvant therapy.

Materials and methods

Patients

In this study, we have reviewed the surgical pathology reports of the colon cancer (adenocarcinoma) patients (rectal cancers were not included), who were all operated by one single surgeon (JSC) during 1995–2004 at Chang Gung Memorial Hospital, Taoyuan, Taiwan. After patients with pT1 tumors were excluded, there were 549 colon cancer patients who had received a complete tumor resection (resection margins were all free). Among them, there were 296 patients with no lymph node metastasis.
node or distant metastasis at the time of resection (stage I or II). During follow up, 38 of the 296 patients developed tumor recurrence or distant metastasis. Among the 296 patients, there were 112 patients with sigmoid colon cancers. Ninety one of these 112 patients had no tumor recurrence or distant metastasis during the follow up period. These 91 patients were belonged to “non-metastasis group”, and the remaining 21 patients were “metastasis group”. To increase the patient number of the metastasis group, all of the 38 colon cancer patients who developed tumor recurrence or distant metastasis were included in the “metastasis group” (21 were sigmoid colon cancer patients, 8 were ascending colon, 3 were transverse colon, and 6 were descending colon). Unstained formalin-fixed paraffin-embedded (FFPE) tumor tissue sections of these 2 groups of patients were prepared for genetic and IHC studies. The clinical and pathologic features, including age, gender, serum carcinoembryonic antigen (CEA) level, tumor size, main histopathological pattern (tubular versus mucinous), tumor grade [24], tumor stage (AJCC, 6th ed.) [25], numbers of dissected regional lymph nodes, and outcome were obtained from the medical records. There was no patient with family history of colon cancers in this study cohort. The study protocol was approved by the Institutional Reviewing Board of Chang Gung Memorial Hospital prior to the study (96-1459B).

**Immunohistochemistry (IHC) study**

Unstained FFPE tumor tissue sections of 3–4 μm in thickness were used for IHC study. The source, clone, and dilution of antibodies were shown as below: p53 (DakoCytomation Denmark A/S, Glostrup, Denmark, clone DO-7, 1:50, antigen retrieval by heat denature), EGFR (DakoCytomation Denmark A/S, Glostrup, Denmark, clone H11, 1: 50, antigen retrieval by proteinase K for 8 min), MLH1 (BD Biosciences, San Jose, CA, clone G168-15, 1:1, antigen retrieval by heat denature), and MSH2 (Calbiochem Inc., Darmstadt, Germany, clone DO-7, 1:50, antigen retrieval by heat denature). The detection was performed as instructed. The IHC stains were read by two experience pathologists according to the manufacturer. Only coding sequences of exon 2 and K-ras were amplified and subjected to direct sequencing. The PCR primers used were: E2F: 5’-GCT ACT GGT GGA GTA TTT GAT AG-3’; E2R: 5’-CAA AGA ATG GTC CTG CAC CAG-3’; E3F: 5’-GGA GCA GGA ACA ATG TCT TTT C-3’; E3R: 5’-GCA TGG CAT TAG CAA AGA CTC-3’. The PCR was performed according to the protocol published previously [29]. Forward and reverse sequencing reactions were performed using the same primers for PCR on an ABI3730 genetic analyzer (Applied Biosystems, CA, USA). The sequences were determined by the Seqscape software (Applied Biosystems). The KRAS reference sequence is based on NM_004985 from the NCBI database. All mutations were verified on a second independent PCR product. In order to increase the sensitivity, HybProbe assay for analyzing KRAS codon 12 and 13 mutations was also performed. The LightMix® Kit k-ras Mutation Codon 12/13 (TIB MOLBIOL Syntheselabor GmbH, Berlin, Germany) were used and performed according to the manufacturer’s protocol.

**Statistical and survival analysis**

Statistical analysis was performed using SPSS software (version 20; IBM, New York, NY, United States). The associations between metastasis, clinicopathological features, immunohistochemical reactivity, and KRAS gene mutation were evaluated by Pearson’s χ² test or Fisher exact tests. Variables with p value less than 0.10 in univariate analysis were re-assessed in a multivariate logistic model. Kaplan–Meier estimates and log-rank analyses were done for comparison of overall survival in different subgroups. The Cox proportional hazard regression model was undertaken to determine the consistency of prognostic effect. Two-sided p values were calculated and p < 0.05 was considered to be significant for all statistical analyses.

**Results**

**Patient characteristics**

Among the 129 colon cancer patients, IHC stains were not successful in 9 patients’ tumor tissue due to lack of residual
tumor tissue in the sections or staining failure. Thus, only 120 colon cancer patients were included for this study. The follow up period ranged from 1.0 to 135.9 months (mean: 73.3 months). Except some patients who died of other diseases, all of the patients alive in the “non-meta” group were disease free for >5 years, and 16 patients have been disease free for >10 years. There were 33 patients (33/120, 27.5%) developed distant metastasis during the follow up period. The time to metastasis ranged from 4 to 83.9 months (median 15.9 months). Except for the 6 patients with no available data, the most common metastatic site was liver (6/27, 22.2%), intraabdominal distant lymph nodes (6/27, 22.2%), lung (4/27, 14.8%), and ovary (1/27, 3.7%). Five patients had distant metastases in two or more organs at the same time. This study included 14 (11.7%) pT2 tumors, 50 (41.7%) pT3 tumors, and 56 (46.7%) pT4 tumors. When comparing the clinicopathological features between the two subgroups, only gender and serum CEA level had significant difference by univariate analysis [Table 1]. For the metastasis and non-metastatic groups, the female patients were 55.5% (18/33) versus 31.0% (27/87), respectively (p = 0.02). For patients with high serum CEA level (≥5 ng/ml), it was 51.5% (17/32) versus 32.2% (28/86), respectively (p = 0.04). The age distribution, tumor size, T stage, main histology patterns, tumor grades, and total harvested lymph node numbers all showed no significant differences. By multivariate analyses, the gender (p = 0.03) and high serum CEA level (p = 0.04) remained significant.

Expression of MLH1, MSH2, p53 and EGFR

For MLH1 protein, none of the tumor had negative expression in the metastasis group, while 6 patients were negative in the non-metastatic group. In addition, up to 30 patients (30/33, Table 1 The clinicopathological characteristics of the 120 colon cancer patients.

| Variables                        | Patient no. (%) | Distant metastasis | Univariate analysis | Multivariate analysis |
|----------------------------------|-----------------|--------------------|---------------------|----------------------|
|                                  |                 | Positive no. (%)   | Negative no. (%)    | p value              | Odds ratio (95% CI) |
| Age (yr)                         |                 |                    |                     |                      |                     |
| >60                              | 84 (70.0)       | 23 (69.7)          | 61 (70.1)           | 0.964                |                     |
| ≤60                              | 36 (30.0)       | 10 (30.3)          | 26 (29.9)           |                      |                     |
| Gender                           |                 |                    |                     |                      |                     |
| Male                             | 75 (62.5)       | 15 (45.5)          | 60 (69.0)           | 0.018                | 0.035               |
| Female                           | 45 (37.5)       | 18 (54.5)          | 27 (31.0)           |                      |                     |
| CEA (ng/ml)                      |                 |                    |                     |                      |                     |
| <5                               | 73 (61.9)       | 15 (45.5)          | 58 (66.7)           | 0.041                | 0.041               |
| ≥5                               | 45 (38.1)       | 17 (51.5)          | 28 (32.2)           |                      |                     |
| Tumor size (cm)                  |                 |                    |                     |                      |                     |
| ≤5                               | 84 (70.0)       | 23 (69.7)          | 61 (70.1)           | 0.964                |                     |
| >5                               | 36 (30.0)       | 10 (30.3)          | 26 (29.9)           |                      |                     |
| Tumor stage                      |                 |                    |                     |                      |                     |
| pT2-3                            | 64 (53.4)       | 18 (54.6)          | 46 (52.9)           | 0.870                |                     |
| pT4                              | 56 (46.6)       | 15 (45.4)          | 41 (47.1)           |                      |                     |
| Main histology pattern           |                 |                    |                     |                      |                     |
| Tubular                          | 113 (94.2)      | 31 (93.9)          | 82 (94.3)           | 1.000                |                     |
| Mucinous                         | 7 (5.8)         | 2 (6.7)            | 5 (5.7)             | 0.355                |                     |
| Tumor grade                      |                 |                    |                     |                      |                     |
| I/II                             | 110 (91.7)      | 29 (87.9)          | 81 (93.1)           | 0.420                |                     |
| III                              | 10 (8.3)        | 4 (12.1)           | 6 (6.9)             |                      |                     |
| No. of dissected LN              |                 |                    |                     |                      |                     |
| <12                              | 47 (39.2)       | 11 (33.3)          | 36 (41.4)           | 0.402                |                     |
| ≥12                              | 73 (60.8)       | 22 (66.7)          | 51 (58.6)           |                      |                     |
| MLH1 over-expression             |                 |                    |                     |                      |                     |
| Negative                         | 36 (30.0)       | 3 (9.1)            | 33 (37.9)           | 0.002                | 0.003               |
| Positive                         | 84 (70.0)       | 30 (90.9)          | 54 (62.1)           | 10.459 (2.266–48.264) |                     |
| MSH2 overexpression              |                 |                    |                     |                      |                     |
| Negative                         | 79 (65.8)       | 24 (72.7)          | 55 (63.2)           | 0.327                |                     |
| Positive                         | 41 (34.2)       | 9 (27.3)           | 32 (36.8)           |                      |                     |
| p53 status                       |                 |                    |                     |                      |                     |
| Wild type                        | 28 (23.3)       | 5 (15.1)           | 23 (26.4)           | 0.233                |                     |
| Mutated                          | 92 (76.7)       | 28 (84.9)          | 64 (73.6)           |                      |                     |
| EGFR overexpression              |                 |                    |                     |                      |                     |
| Negative                         | 104 (86.7)      | 28 (84.9)          | 76 (87.4)           | 0.766                |                     |
| Positive                         | 16 (13.3)       | 5 (15.1)           | 11 (12.6)           |                      |                     |
| KRAS mutation                    |                 |                    |                     |                      |                     |
| Absent                           | 104 (86.7)      | 26 (78.8)          | 78 (89.7)           | 0.118                |                     |
| Present                          | 16 (13.3)       | 7 (21.2)           | 9 (10.3)            |                      |                     |

Abbreviations: CEA: carcinoembryonic antigen; EGFR: epidermal growth factor receptor; LN: regional lymph node.

a Serum CEA data was unavailable in one patient for each subgroup, respectively.
90.9%) in the metastatic group had overexpression of MLH1, but only 54 patients (54/87, 62.1%) had overexpression in the non-metastatic group. The difference was statistically significant by both univariate and multivariate analyses [Table 1]. For MSH2 protein, the metastasis group also had no tumor with negative expression, while 3 patients were negative in the non-metastatic group. MSH2 overexpression was found in 9 (27.3%) and 32 (36.8%) patients in the metastatic and the non-metastatic group, respectively. The differences were non-significant. The expression patterns of representative cases for MLH1 and MSH2 are shown in Fig. 1. p53 overexpression was found in 19 (57.6%) and 64 (73.5%) patients in the metastatic and the non-metastatic group, respectively. EGFR overexpression was recognized in 5 (15.1%) and 11 (12.6%) patients in the metastatic and the non-metastatic group, respectively. Alteration of the above two proteins all had no significant differences between the two study groups.

**KRAS gene mutation analysis**

KRAS mutations of codon 12 and 13 were identified in 16 patients (13.3%), including 13 patients with mutations in codon 12 and 3 patients in codon 13. Seven patients were in the metastasis group and 9 in the non-metastasis group. The difference was non-significant (p = 0.12).

**Risk assessment of metachronous distant metastasis**

Univariate analysis of the clinicopathological features revealed that only female gender, high serum CEA level (≥5 ng/ml), and MLH1 overexpression were significantly associated with the metastasis group. The above three features remained significant by multivariate analysis. For female patients, the OR (odds ratio) was 2.653 and 95% CI (confidence interval) was 1.070–6.579 (p = 0.03). For high CEA level, the OR was 2.582 and 95% CI was 1.040–6.410 (p = 0.04). For MLH1 overexpression, the OR was 10.459 and 95% CI was 2.266–48.264 (p = 0.003) [Table 1].

**Survival analysis**

The overall survival (OS) was analyzed, which was defined as the interval from the operation date till the date of death or last follow up. In the metastasis group, the mean following duration was 45.7 months (5.3–109 months). The median survival was 47.9 months. In the non-metastasis group, the mean follow-up time was 83.8 months (1.0–135.9 months). The median survival was not reached yet. As expected, the OS and 5-year survival rate were both significantly different between the metastasis and non-metastasis group (p < 0.001). The variables associated with the OS and 5-year survival rate were analyzed and shown in Table 2. The MLH1 and MSH2 expression were categorized as overexpression and non-overexpression. The latter was for tumors with negative or intermediate expression levels. Only gender, serum CEA level, and MLH1 and MSH2 overexpression were significantly associated with the 5 year survival and OS, respectively. The Cox proportional hazard regression model confirmed that female gender was also a poor prognostic factor for OS. The HR was 2.383 (95% CI 1.009–5.629) with a borderline p value (p = 0.048) [Fig. 2A]. High CEA level [p < 0.001, HR: 4.525, 95% CI 1.923–11.765] was an independent poor prognostic factor for OS [Fig. 2B]. MLH1 and MSH2 overexpression all showed significant association with OS, respectively. MLH1 overexpression was an indicator for shorter survival, when compared with patients with non-overexpression (p = 0.01, HR 6.173, 95% CI 1.425–26.316) [Fig. 2C]. In contrast, MSH2 overexpression was associated

![Fig. 1 Immunohistochemical satin for MLH1 protein expression in the colon adenocarcinoma: (A) Intermediate expression (200×), (B) Overexpression (400×); for MSH2 protein expression: (D) Intermediate expression (200×), (E) Overexpression (400×), (F) Negative expression (400×).](image-url)
with longer survival ($p = 0.02$, HR $0.253$, 95% CI $0.083–0.7$) [Fig. 2D]. If the protein expression was divided in 3 levels (negative, intermediate and overexpression), and log-rank test was applied, MSH2 protein retained its statistic significance ($p = 0.04$) but MLH1 protein became non-significant ($p = 0.11$) [Fig. 3]. Other clinicopathological factors including p53 status, EGFR overexpression and KRAS mutation all showed no significant influence on survival.

### Discussion

In the present study, we have narrowed down the study cohort to only radically resected pN0 colon adenocarcinoma without chemotherapy. Detailed clinical, pathological, and various biomarkers were studied simultaneously. The tumor differentiation, pT4 stage, and inadequate lymph node dissections all showed no predictive or prognostic significance in this cohort, suggesting staging and histopathology are not useful prognostic factors for this group of early stage colon cancers. Only female gender, high CEA level ($>5$ ng/ml), and MLH1 overexpression were significantly associated with metachronous distant metastasis. The above three factors were also significantly associated with shorter survival. In contrast, MSH2 overexpression emerged as an indicator of better survival with statistical significance. EGFR overexpression, p53 status, and KRAS mutation all were not significantly associated with outcome, which are similar to previous reports [7,8].

It is uncertain why female gender had higher metastasis rate and shorter survival in this cohort. We do find some
reports which described shorter survival in female patients with metastatic CRC. For example, in a recent trial, panitumumab added to FOLFOX significantly prolonged progression-free survival in males but not in females with metastatic CRC [30]. In a meta-analysis, which included 345 females and 497 males CRC patients, they also found gender was a robust determinant of the chemotherapy delivery schedule and resulted in survival differences [31].

For CRC, measurement of serum CEA level is a widely accepted tumor marker for monitoring tumor response and recurrence. Our results is quite consistent with previous reports, which also found CEA to be a significant factor for predicting distant metastasis or survival in pathologically T1 or T2 CRC [21,32], and stage II or III CRC, respectively [33]. Currently the American Society of Clinical Oncology did not recommend the use of preoperative CEA levels to determine whether patients with CRC were candidates for adjuvant therapy [5]. But the European Society for Medical Oncology has added high CEA level as a risk factor for a subgroup of stage II colorectal cancer [6].

Fig. 2 Kaplan–Meier overall survival curve of the 120 colon adenocarcinoma patients according to different variables: (A) gender, (B) carcinoembryonic antigen (CEA) level, (C) MLH1 expression, and (D) MSH2 expression.
For MSI phenotype, the current study demonstrated MLH1 overexpression was not only a predictor of metachronous distant metastasis, but also a poor prognostic factor. MSH2 overexpression was only significantly associated with better OS, but not predictive for distant metastasis. To our best knowledge, MMR protein overexpressions have never been reported to be significantly associated with the prognosis of CRC patients before. Currently, only deficiency of MMR protein in the colon cancers is considered to be clinically important. The pathologists do not pay attention to high or low expression of MMR protein in the tumors, since any unequivocally positivity is interpreted as positive of the MMR proteins. Actually, the IHC stain for those tumor with no MMR proteins deficiency does not always have (+) stain in near 100% of the tumor cells. So different intensities of the IHC stains for MMR proteins in different colon cancer tumor cells do exist [34].

In this study, either MLH1-negative or MSH2-negative patients all had longest OS [Fig. 3]. Since MLH1-negative or MSH2-negative tumors would result in MSI, our result is consistent with the reports that CRC with MSI-high tumors would have better survival [16,17].

The overexpression of MMR protein logistically represents enhanced DNA repair capability and, therefore, should also confer a good prognosis, which could be the reason why patients with MSH2 overexpression had longer survival than those with non-overexpression in the current study. On the other hand, previous studies have disclosed that MLH1, PMS1, or PMS2 overexpression could increase spontaneous MLH1 gene mutation rate and inactivation of DNA mismatch repair [35–37]. This mechanism of inactivation of DNA mismatch repair genes is probably different from MSI caused by MMR protein deficiency, so it cannot confer the same favorable outcome. This could be the reason why MLH1 overexpression was a poor prognostic factor for distant metastasis and overall survival in the current report. Scherbakova et al. have demonstrated that MLH1 protein overexpression in yeast could lead to formation of nonfunctional MMR complexes containing MLH1 homodimers [36]. Similar phenomenon also have been found in other MMR proteins. For examples, overexpression of MSH3 protein could reduced the MMR efficacy by increased formation of MSH2/MSH3 heterodimer at the expense of MSH2/MSH6 heterodimer [38]. Norris and his co-workers also have discovered prostatic cancer with high PMS2 protein levels had short disease-free period after radical prostatectomy [39], which is similar to our result for MLH1 overexpression.

The limitation of this study is its small patient population. Since this study series only focused on the stage I–II patients and had a very long follow up time, the study result should be still quite valuable. Although patients of the metastasis group were not limited to sigmoid colon cancer as in the non-metastasis group. It should have no significant impact. The AJCC staging for colon cancers does not need to include the location in colon, either.

In summary, we have demonstrated that high CEA level and overexpression of MLH1 were associated with shorter survival and overexpression of MSH2 were associated with longer survival in this study cohort. The reversed prognostic implications in the overexpression of MLH1 and MSH2 for stage I–II colon cancer patients has never been reported, which is worthy of further confirmation with larger patient numbers.

**Conflicts of interest**

All authors declared no conflicts of interest.
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REFERENCES

[1] Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74–108.
[2] Cunningham D, Atkin W, Lenz HJ, Lynch HT, Minsky B, Nordlinger B, et al. Colorectal cancer. Lancet 2010;375:1030–47.
[3] Gertler R, Rosenberg R, Schuster T, Friess H. Defining a high-risk subgroup with colon cancer stages I and II for possible adjuvant therapy. Eur J Cancer 2009;45:2992–9.
[4] Mroczykowski P, Schmidt U, Sahm M, Gastinger I, Lippert H, Kube R. Prognostic factors assessed for 15,096 patients with colon cancer in stages I and II. World J Surg 2012;36:1693–8.
[5] Benson 3rd AB, Schrag D, Somerfield MR, Cohen AM, Figueroed AT, Flynn PJ, et al. American Society of Clinical Oncology recommendations on adjuvant chemotherapy for stage II colon cancer. J Clin Oncol 2004;22:3408–19.
[6] Van Cutsem E, Oliveira J. Primary colon cancer: ESMO clinical recommendations for diagnosis, adjuvant treatment and follow-up. Ann Oncol 2009;20(Suppl. 4):49–50.
[7] Tejpar S, Bertagnolli M, Bosman F, Lenz HJ, Garraway L, Waldman F, et al. Prognostic and predictive biomarkers in resected colon cancer: current status and future perspectives for integrating genomics into biomarker discovery. Oncologist 2010;15:390–404.
[8] Walther A, Johnstone E, Swanton C, Midgley R, Tomlinson I, Kerr D. Genetic prognostic and predictive markers in colorectal cancer. Nat Rev Cancer 2009;9:489–99.
[9] Munro AJ, Lain S, Lane DP. PS3 abnormalities and outcomes in colorectal cancer: a systematic review. Br J Cancer 2005;92:434–44.
[10] Heinemann V, Stintzing S, Kirchner T, Boeck S, Jung A. Clinical relevance of EGFR- and KRAS-status in colorectal cancer patients treated with monoclonal antibodies directed against the EGFR. Cancer Treat Rev 2009;35:262–71.
[11] Nicholson RI, Gee JM, Harper ME. EGFR and cancer prognosis. Eur J Cancer 2001;37(Suppl. 4):59–15.
[12] Huang CW, Tsai HL, Chen YT, Huang CM, Ma CJ, Lu CY, et al. The prognostic values of EGFR expression and KRAS mutation in patients with synchronous or metachronous metastatic colorectal cancer. BMC Cancer 2013;13:595.
[13] Ljuslinder I, Melin B, Henriksson ML, Oberg A, Palmqvist R. Increased epidermal growth factor receptor expression at the invasive margin is a negative prognostic factor in colorectal cancer. Int J Cancer 2011;128:2031–7.
[14] Roth AD, Tejpar S, Delorenzi M, Yan P, Fiocca R, Klingbiel D, et al. Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: results of the translational study on the PETACC-3, EORTC 40993, SAKK 60-00 trial. J Clin Oncol 2010;28:466–74.
[15] Ogino S, Meyerhardt JA, Barbeito D, Niedzwiecki D, Hollis D, Saltz LB, et al. KRAS mutation in stage III colon cancer and clinical outcome following intergroup trial CALGB 89803. Clin Cancer Res 2009;15:7322–9.
[16] Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. J Clin Oncol 2005;23:609–18.
[17] Roth AD, Delorenzi M, Tejpar S, Yan P, Klingbiel D, Fiocca R, et al. Integrated analysis of molecular and clinical prognostic factors in stage II/III colon cancer. J Natl Cancer Inst 2012;104:1635–46.
[18] Boland CR, Goel A. Microsatellite instability in colorectal cancer. Gastroenterology 2010;138:2073–87. e3.
[19] Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, et al. A National Cancer Institute Workshop on microsatellite instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 1998;58:5248–57.
[20] Shia J. Immunohistochemistry versus microsatellite instability testing for screening colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome. Part I. The utility of immunohistochemistry. J Mol Diagn 2008;10:293–300.
[21] Lou Z, Meng RG, Zhang W, Yu ED, Fu CG. Preoperative carcinoembryonic antibody is predictive of distant metastasis in pathologically T1 colorectal cancer after radical surgery. World J Gastroenterol 2013;19:389–93.
[22] Nitsche U, Rosenberg R, Balment A, Schuster T, Siotta-Huspenina J, Herrmann P, et al. Integrative marker analysis allows risk assessment for metastasis in stage II colon cancer. Ann Surg 2012;256:763–71. discussion 71.
[23] Biffi R, Botteri E, Bertani E, Zampino MG, Cenciarelli S, Luca F, et al. Factors predicting worse prognosis in patients affected by pT3 N0 colon cancer: long-term results of a monocentric series of 137 radically resected patients in a 5-year period. Int J Colorectal Dis 2013;28:207–15.
[24] Chang HC, Huang SC, Chen JS, Tang R, Changchien CR, Chang JM, et al. Risk factors for lymph node metastasis in pT1 and pT2 rectal cancer: a single-institute experience in 943 patients and literature review. Ann Surg Oncol 2012;19:2477–84.
[25] Greene FL, Page DL, Fleming ID, Fritz AG, Balch CM, Haller DG, et al., editors. AJCC cancer staging manual. 6th ed. New York: Springer; 2002.
[26] McCluggage WG, Soslow RA, Gilks CB. Patterns of p53 immunoreactivity in endometrial carcinomas: ‘all or nothing’ staining is of importance. Histopathology 2011;59:786–8.
[27] Abd El All HS, Mishrikiy AM, Mohamed FA. Epidermal growth factor receptor in colorectal carcinoma: correlation with clinico-pathological prognostic factors. Colorectal Dis 2008;10:170–8.
[28] Allred DC, Harvey JM, Berardo M, Clark GM. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol 1998;11:155–68.
[29] Wu CC, Hsu HY, Liu HP, Chang JW, Chen YT, Hsieh WY, et al. Reversed mutation rates of KRAS and EGFR genes in adenocarcinoma of the lung in Taiwan and their implications. Cancer 2008;113:3199–208.
[30] Doollard JY, Siena S, Cassidy J, Tabernero J, Burkes R, Barigel M, et al. Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. J Clin Oncol 2010;28:4697–705.
[31] Giacchetti S, Dugue PA, Innominato PF, Bjarnason GA, Elia GR, Allegri E, et al., editors. AJCC cancer staging manual. 7th ed. New York: Springer; 2017.
[32] Abad AI, El AAL HS, Mishrikiy AM, Mohamed FA. Epidermal growth factor receptor in colorectal carcinoma: correlation with clinico-pathological prognostic factors. Colorectal Dis 2012;14:1151–8.
[33] Vasiliev BL, Ljuslinder I, Melin B, Henriksson ML, Oberg A, Palmqvist R. Increased epidermal growth factor receptor expression at the invasive margin is a negative prognostic factor in colorectal cancer. Int J Cancer 2011;128:2031–7.
[34] Detterbeck FC, Schiller JH, Winkler B, Fossella FV, Cagle PT, Henschke CI, et al. Randomized, phase III study of paclitaxel versus fluorouracil, leucovorin, and cisplatin in resected non-small cell lung cancer: results of CALGB 30610. J Clin Oncol 2003;21:2974–83.
curative resection of stage II colon cancer. J Surg Oncol 2009;99:65–70.

[33] Kim CH, Huh JW, Kim HJ, Lim SW, Song SY, Kim HR, et al. Factors influencing oncological outcomes in patients who develop pulmonary metastases after curative resection of colorectal cancer. Dis Colon Rectum 2012;55:459–64.

[34] Shia J, Stadler Z, Weiser MR, Rentz M, Gonen M, Tang LH, et al. Immunohistochemical staining for DNA mismatch repair proteins in intestinal tract carcinoma: how reliable are biopsy samples? Am J Surg Pathol 2011;35:447–54.

[35] Shcherbakova PV, Kunkel TA. Mutator phenotypes conferred by MLH1 overexpression and by heterozygosity for MLH1 mutations. Mol Cell Biol 1999;19:3177–83.

[36] Shcherbakova PV, Hall MC, Lewis MS, Bennett SE, Martin KJ, Bushel PR, et al. Inactivation of DNA mismatch repair by increased expression of yeast MLH1. Mol Cell Biol 2001;21:940–51.

[37] Gibson SL, Narayanan L, Hegan DC, Buermeyer AB, Liskay RM, Glazer PM. Overexpression of the DNA mismatch repair factor, PMS2, confers hypermutability and DNA damage tolerance. Cancer Lett 2006;244:195–202.

[38] Marra G, Iaccarino I, Lettieri T, Roscilli G, Delmastro P, Jiricny J. Mismatch repair deficiency associated with overexpression of the MSH3 gene. Proc Natl Acad Sci U S A 1998;95:8568–73.

[39] Norris AM, Gentry M, Peehl DM, D’Agostino Jr R, Scarpinato KD. The elevated expression of a mismatch repair protein is a predictor for biochemical recurrence after radical prostatectomy. Cancer Epidemiol Biomark Prev 2009;18:57–64.