Molecular pathological epidemiology of colorectal cancer in Chinese patients with KRAS and BRAF mutations

Wenbin Li¹, Tian Qiu¹, Yun Ling¹, Lei Guo¹, Lin Li¹, Jianming Ying¹

¹Department of Pathology, Cancer Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China

Correspondence to: Jianming Ying, e-mail: jmying@hotmail.com

Keywords: colorectal cancer, KRAS, BRAF, mutation, molecular pathological epidemiology

Received: July 27, 2015  Accepted: October 09, 2015  Published: October 22, 2015

ABSTRACT

An investigation of interactive effects of exogenous and endogenous factors and tumor molecular changes can lead to a better understanding of tumor molecular signatures in colorectal cancer. We here report a molecular pathological epidemiology study in a large cohort of 945 colorectal cancer patients. Mutations of KRAS (36.6%) and BRAF (3.46%) were nearly mutually exclusive. KRAS-mutated tumors were more common in female patients (odds ratio [OR] = 1.68; P = 0.0001) and never smokers (OR = 1.60; P = 0.001). Whereas BRAF-mutated tumors demonstrated no discrepancy in aspects of gender and smoking status compared with wild-type tumors. In addition, tumors with BRAF or KRAS mutations were in correlation with elevated serum level of carbohydrate antigen (CA19-9) and carcinoma embryonic antigen (CEA) and the combination of serum biomarkers and molecular mutation status may enhance the more precise risk stratification of CRC patients. Further studies are needed to define the mechanism brought about by the aforementioned epidemiologic and clinicopathologic characteristics that may help optimize cancer prevention and precision therapy.

INTRODUCTION

Colorectal cancer (CRC) is a heterogenous disease evolving from the accumulation of genetic and epigenetic modifications [1–3]. Mutations within the KRAS and BRAF oncogenes lead to constitutive activation of epidermal growth factor receptor (EGFR) signaling pathway. KRAS is mutated in 35%–40% CRC and with more than 95% mutations in codons 12 and 13 [4]. Mutation of KRAS oncogene is an early event in development of these cancers, exerting a strong influence on the growth of colonic polyps and early cancers [5]. Robust evidence suggests the predictive value of KRAS mutation in metastatic CRC treated with anti-EGFR targeted therapy [6, 7]. However, the biological and functional consequences of KRAS mutations at codon 12 may be different from those at codon 13 [8, 9]. It has been suggested that patients whose tumors harbor a KRAS Gly13Asp mutation may benefit from anti-EGFR mAb therapy [10–12]. Our previous reports have also demonstrated that KRAS codon 12 mutation, but not codon 13 mutation, is associated with more positive lymph nodes and higher pTNM stages in colorectal cancer [13]. On the other hand, BRAF c.1799T > A (p.V600E) mutation occurs in less than 10% of patients and are a strong negative prognostic marker [14, 15].

To date, few studies have evaluated the associations of epidemiologic factors and tumor molecular features. Consequently, using collected patient questionnaire data from the database of the Department of Pathology, Cancer Hospital, along with the corresponding KRAS and BRAF mutational status, we evaluated the associations between tumor molecular and epidemiological features.

RESULTS

Epidemiologic characteristics

A total of the 945 cases were analyzed for KRAS, BRAF gene mutations, MMR status and completed patient questionnaire registration. Of these, 945 (100%) and 924 (97.8%) yielded KRAS and BRAF mutation
status, respectively. There were 346 (36.6%) tumors that had KRAS mutations, whereas 32 (3.46%) tumors had a BRAF mutation. The distribution and frequencies of the epidemiological characteristics are summarized in Table 1.

Compared with patients with wild-type KRAS tumors, those with mutant KRAS tumors were more likely to be female (49.4% vs 36.7%; OR = 1.68; 95% CI = 1.29 to 2.20; P = 0.0001), to be never smoker (73.4% vs 63.2%; OR = 1.60; 95% CI = 1.19 to 2.15; P = 0.001), to have elevated CA19-9 serum concentrations (29.8% vs 13.5%; OR = 2.71; 95% CI = 1.95 to 3.76; P = 0.0001) and to have elevated CEA serum concentrations (51.7% vs 31.9%; OR = 2.29; 95% CI = 1.74 to 3.01; P = 0.0001). However, there were no significant differences in aspects of age, overweight, alcohol intake, diabetes mellitus, hypertension and chronic GI conditions between mutant KRAS and wild-type KRAS groups. Moreover, compared with wild-type KRAS patients, HDL-cholesterol, LDL-cholesterol and triglycerides were not risk factors for mutant KRAS patients.

When compared with those without BRAF mutated tumors, patients with BRAF mutated tumors were more likely to have elevated CA19-9 serum concentrations (37.5% vs 17.5%; OR = 2.83; 95% CI = 1.35 to 5.91; P = 0.004) and to have elevated CEA serum concentrations (59.4% vs 38.5%; OR = 2.34; 95% CI = 1.14 to 4.79; P = 0.02). Patients with BRAF mutations were more likely to be less overweight compared with wild-type BRAF cases (81.3% vs 63.6%; OR = 0.40; 95% CI = 0.16 to 0.99; P = 0.04). Moreover, compared with wild-type BRAF patients, smoking status, alcohol intake, diabetes mellitus, hypertension and chronic GI conditions were not risk factors for mutant BRAF patients.

**Associations between factors and mutation status**

Univariate logistic regression models identified the following factors as statically significantly associated with having mutant KRAS status: female, never smokers as well as elevated CA19–9 and CEA serum concentrations (Figure 1A). BRAF mutated tumors were statistically significantly associated with less overweight and elevated CA19–9 and CEA serum concentrations (Figure 1B).

In the analysis using multivariable logistic regression models, we reviewed epidemiological characteristics in Table 2. As shown multivariably, tumors with KRAS mutation were statistically associated with female patients, never smokers and elevated serum level of CA19-9 and CEA. In addition, patients with BRAF-mutated tumors were statistically significantly to have elevated serum level of CA19-9.

**DISCUSSION**

Molecular pathological epidemiology, which was first consolidated by Shuji Ogino and his colleagues, is a relatively new field of epidemiology based on molecular classification of cancer [16]. In recent years, there has been a new direction of this field where we examine an interactive effect of tumor molecular features and lifestyle or other exposure factor on tumor behavior [16–19]. Furthermore, molecular pathological epidemiology has specific strengths on optimizing colorectal cancer prevention and precision therapy. To the best of our knowledge, this is the first study to summarize epidemiologic (i.e., cigarette smoking and alcohol drinking) features associated with the KRAS and BRAF mutations status of tumors in a large cohort of Chinese patients.

Cigarette smoking history is a known risk factor for developing colon cancer [20]. Studies have shown that carcinogens found in tobacco smoke can induce cancer-related base substitutions, such as G:C → A:T transitions in RAS oncogenes [21]. However, several large studies have indicated that cigarette smoking was more closely associated with incident CRCs characterized by KRAS mutation-negative rather than KRAS mutation-positive status [22–24]. This is in line with our observations that colorectal cancers from patients with a history of current or former smoking were less likely to harbor a KRAS mutation. Although smoking is not associated with the risk for colorectal cancer with KRAS oncogene mutations, it may be an early event in the development of colorectal cancers that arise through other underlying genetic pathways, such as mutations in the adenomatous polyposis coli (APC) tumor suppressor gene, P53 over-expression or absence of MLH1 protein expression [25, 26]. Recently studies from the large case-control study suggested that smoking is related to CIMP (CpG Island Methylator Phenotype) and BRAF mutations in colon cancer, rather than with microsatellite-unstable cancer [18, 27, 28]. Our study reveals an association between current or former smoking history with the presence of BRAF mutations in tumors (43.7% vs 31.9%), although this did not reach a significant difference due to small sample size. Tobacco exposure has been shown to stimulate DNA methyltransferase activity that is associated with CIMP and BRAF mutations [29, 30]. Previous data indicate that the CIMP-high subgroup, which exhibits a very high frequency of cancer-specific DNA hypermethylation, is strongly associated with epigenetic inactivation of MLH1 and BRAF mutation. [31] Therefore, the BRAF mutation can serve as a surrogate marker for the CIMP-high group showing sporadic dMMR status.

To our knowledge, this is the first study to suggest that patients with BRAF or KRAS mutated tumors were more likely to have an elevated preoperative serum level of CA19–9 and CEA. CA19–9 and CEA are widely accepted tumor serum biomarkers for CRC and elevated preoperative CA19–9 and CEA level have been considered as an independent prognostic factor for DFS (Disease Free Survival) in CRC patients [32]. Recent studies have been suggested that a high preoperative serum CA19-9 level was
| Characteristics               | Mutant KRAS (n = 346) | Wild-type KRAS (n = 599) | P-value | Mutant BRAF (n = 32) | Wild-type BRAF (n = 892) | P-value |
|-------------------------------|-----------------------|--------------------------|---------|----------------------|--------------------------|---------|
| Sex                           |                       |                          |         |                      |                          |         |
| Male                          | 175 (50.6%)           | 379 (63.3%)              | 0.0001  | 15 (46.9%)           | 524 (58.7%)              | 0.18    |
| Female                        | 171 (49.4%)           | 220 (36.7%)              |         | 17 (53.1%)           | 368 (41.3%)              |         |
| BMI (kg/m²)                   |                       |                          | 0.13    |                      |                          | 0.04    |
| <25                           | 232 (67.1%)           | 372 (62.1%)              |         | 26 (81.3%)           | 567 (63.6%)              |         |
| ≥25                           | 114 (32.9%)           | 227 (37.9%)              |         | 6 (18.7%)            | 325 (36.4%)              |         |
| Smoking status                |                       |                          | 0.001   |                      |                          | 0.16    |
| Never                         | 251 (73.4%)           | 373 (63.2%)              |         | 18 (56.3%)           | 602 (68.1%)              |         |
| Former/current                | 91 (26.6%)            | 217 (36.8%)              |         | 14 (43.7%)           | 282 (31.9%)              |         |
| Missing                       | 4                     | 9                        |         | 0                    | 8                        |         |
| Alcohol intake                |                       |                          | 0.09    |                      |                          | 0.53    |
| Never                         | 249 (73.2%)           | 405 (68.0%)              |         | 24 (75.0%)           | 617 (69.9%)              |         |
| Former/current                | 91 (26.8%)            | 191 (32.0%)              |         | 8 (25.0%)            | 266 (30.1%)              |         |
| Missing                       | 6                     | 3                        |         | 0                    | 9                        |         |
| Diabetes mellitus             |                       |                          | 0.84    |                      |                          | 0.86    |
| Yes                           | 47 (13.7%)            | 79 (13.2%)               |         | 4 (12.5%)            | 119 (13.4%)              |         |
| No                            | 297 (86.3%)           | 520 (86.8%)              |         | 28 (87.5%)           | 770 (86.6%)              |         |
| Missing                       | 2                     | 0                        |         | 0                    | 3                        |         |
| Hypertension                  |                       |                          | 0.93    |                      |                          | 0.38    |
| Yes                           | 88 (26.0%)            | 152 (25.7%)              |         | 6 (18.8%)            | 227 (25.6%)              |         |
| No                            | 251 (74.0%)           | 439 (74.3%)              |         | 26 (81.3%)           | 659 (74.4%)              |         |
| Missing                       | 7                     | 8                        |         | 0                    | 6                        |         |
| Chronic GI conditions         |                       |                          | 0.74    |                      |                          | 0.65    |
| Yes                           | 43 (12.4%)            | 79 (13.2%)               |         | 5 (15.6%)            | 115 (12.9%)              |         |
| No                            | 303 (87.6%)           | 520 (86.8%)              |         | 27 (84.4%)           | 777 (87.1%)              |         |
| CA19-9 (U/ml)                 |                       |                          | 0.0001  |                      |                          | 0.004   |
| <37                           | 243 (70.2%)           | 518 (86.5%)              |         | 20 (62.5%)           | 736 (82.5%)              |         |
| ≥37                           | 103 (29.8%)           | 81 (13.5%)               |         | 12 (37.5%)           | 156 (17.5%)              |         |
| CEA (ng/ml)                   |                       |                          | 0.0001  |                      |                          | 0.02    |
| <5                            | 167 (48.3%)           | 408 (68.1%)              |         | 13 (40.6%)           | 549 (61.5%)              |         |
| ≥5                            | 179 (51.7%)           | 191 (31.9%)              |         | 19 (59.4%)           | 343 (38.5%)              |         |
| Total cholesterol (mmol/L)    | 5.46 ± 0.94           | 4.53 ± 0.97              | 0.18†   | 4.29 ± 0.90          | 4.89 ± 0.97              | 0.21†   |
| HDL-cholesterol (mmol/L)      | 1.21 ± 0.41           | 1.16 ± 0.40              | 0.06†   | 1.08 ± 0.31          | 1.39 ± 0.41              | 0.17†   |

(Continued)
a significant marker of poor prognosis in patients with all stages of CRC [33]. Strong prognostic effect of KRAS and BRAF mutations were previously reported by Maughan et al. in COIN trial and the OS (Overall Survival) was shorter for patients with any mutation of the two oncogenes compared with all wild-type, irrespective of treatment received [34]. Therefore, tumors with BRAF or KRAS mutations were in correlation with elevated serum level of tumor biomarkers of CRC and the association of tumor biomarkers and molecular status may indicate the poor prognosis of these patients. Further work needs to estimate the more precise risk stratification of CRC patients based on the combination of serum biomarkers and molecular mutation status.

Our study had several limitations associated with its retrospective nature and single center design. Patients recollected the answers to several questions from memory when filling out the form, hence possibly introducing reporting errors while classifying several patient risk characteristics. Moreover, we did not examine rare

| Characteristics                          | Mutant KRAS (n = 346) | Wild-type KRAS (n = 599) | P-value | Mutant BRAF (n = 32) | Wild-type BRAF (n = 892) | P-value |
|------------------------------------------|-----------------------|--------------------------|---------|----------------------|--------------------------|---------|
| LDL-cholesterol (mmol/L)                 | 2.73 ± 0.85           | 2.81 ± 0.75              | 0.08†   | 2.71 ± 0.73          | 2.78 ± 0.86              | 0.65†   |
| Triglycerides (mmol/L)                   | 1.27 ± 0.69           | 1.36 ± 0.47              | 0.17†   | 1.34 ± 0.71          | 1.32 ± 0.74              | 0.92†   |

Abbreviations: MMR = mismatch repair; SD = standard deviation.
†Two-sided Kruskal Wallis test
‡Two-sided χ² test with continuity correction
§Fischer’s exact test
Others are two-sided χ² test

Figure 1: Forest plots of univariate logistic model associations with KRAS A. and BRAFV600E B. mutation status features. P values are for two-sided Pearson χ² test. CI = confidence interval; dMMR = deficient mismatch repair; LCL = lower confidence limit; UCL = upper confidence limit; OR = odds ratio.
common mutations in \textit{KRAS} codons 61, 146 and \textit{NRAS} mutations, which seemed to be negative predictive factors to anti-EGFR therapies.

In conclusion, our study suggests that specific epidemiologic characteristics are associated with \textit{KRAS} and \textit{BRAF} mutations in a large cohort of Chinese CRC patients. \textit{KRAS}-mutated tumors are more common in female patients and never smokers. Tumors with \textit{BRAF} or \textit{KRAS} mutations were in correlation with elevated serum level of tumor biomarkers of CRC and the combination of serum biomarkers and molecular mutation status may enhance the more precise risk stratification of CRC patients. Further studies are needed to define the mechanism brought about by the aforementioned epidemiologic and clinicopathologic characteristics that may help optimize cancer prevention and precision therapy.

\textbf{MATERIALS AND METHODS}

\textbf{Study population}

The tumor molecular and epidemiological records of 945 patients with corresponding paraffin-embedded material available for molecular analysis were retrospectively collected from the Department of Pathology, Cancer Hospital, Chinese Academy of Medical Sciences, Beijing, China from December 2011 to December 2013. Patients who had a history of preoperative radiochemotherapy or gastrointestinal surgical resection were excluded. The results of pathological characteristics between \textit{KRAS} and \textit{BRAF} mutations of these patients were published in the previous study [13]. The study was approved by the Institute Review Board of the Cancer Hospital, Chinese Academy of Medical Sciences. The methods were carried out in accordance with the approved guidelines. Each participant signed an Institutional Review Board approved informed consent in accordance with current guidelines.

\textbf{Risk factor assessment}

The following data fields were recorded and included in the analysis: smoking history (never smoker; former smoker: used to smoking but has quit; current: still smoking), alcohol intake (never drink; former: used to drink but has quit; current: still drink), body mass index (BMI, obese: $\geq 30$ kg/m$^2$; overweight: $25$–$29.9$ kg/m$^2$; normal: $18$–$24.9$ kg/m$^2$; underweight: $< 18$ kg/m$^2$) and history of chronic gastrointestinal diseases (Crohn’s disease, ulcerative colitis or microscopic colitis). All patients had determined serum concentrations of preoperative CEA (Carcinoma embryonic antigen) and CA19–9 (Carbohydrate antigen), which were performed on a Cobas e601 Immunology Analyzer (Roche, Basel, Switzerland). Serum CEA concentrations $\geq 5.0$ ng/ml and CA19–9 concentrations $\geq 37$ U/ml were regarded as elevated. Serum total cholesterol and triglycerides were quantitatively determined by a colorimetric method and high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol were determined in a homogenous assay with a colorimetric end point. All measurements were performed on a P8000 Chemistry Analyzer (Roche, Basel, Switzerland).

\textbf{KRAS and BRAF mutation analysis}

Assessment of \textit{KRAS} and \textit{BRAF} c.1799T $>$ A (p.V600E) mutational status was performed in the Molecular Pathology Laboratory of Department of Pathology, CICAMS as previously reported [13].

\textbf{Statistical analysis}

The primary objective of this study was to identify distinct epidemiological features associated with specific \textit{KRAS} and \textit{BRAF}\textsuperscript{V600E} mutation status. Differences of patient characteristics and epidemiological factors in the two-dimensional cross-comparison were evaluated statistically by Pearson’s $\chi^2$-test or Fischer’s exact test. Statistical tests were two-sided, and $P < 0.05$ were considered significant. Logistic regression models were used to detect associations of these characteristics with each of the specific \textit{KRAS} mutations and provided estimates of odds ratio (ORs) and confidence intervals (CIs). Statistics were carried out using SPSS software (version 16.0 of SPSS, Chicago, IL, USA).

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|}
\hline
\textbf{Characteristics} & \textbf{Mutant KRAS} & \textbf{Mutant BRAF} & \textbf{V600E} \\
& \textbf{OR (95\% CI)} & \textbf{P} & \textbf{OR (95\% CI)} & \textbf{P} \\
\hline
Female (referent: male) & 2.14 (1.07 to 3.86) & 0.001 & — & — \\
BMI $<$ 25 (referent: $\geq 25$) & — & — & 2.35 (0.92 to 3.58) & 0.11 \\
Never smoker (referent: Former/current) & 1.92 (1.36 to 3.90) & 0.004 & — & — \\
CA19-9 $\geq 37$ (referent: $<$ 37 U/ml) & 3.35 (2.46 to 5.17) & 0.001 & 2.03 (1.04 to 3.98) & 0.006 \\
CEA $\geq 5$ (referent: $<$ 5 ng/ml) & 2.46 (1.55 to 4.60) & 0.001 & 1.52 (0.63 to 2.82) & 0.17 \\
\hline
\end{tabular}
\caption{Multivariate logistic regression model associations between patient, tumor and \textit{KRAS} or \textit{BRAF}\textsuperscript{V600E} mutation status}
\end{table}

CI = confidence interval; pMMR = proficient mismatch repair; OR = odds ratio.
ACKNOWLEDGMENTS AND GRANT SUPPORT

We thank all study participants of the Department of Pathology for their contributions to this project.

This work was supported by a grant from Youth Backbone Program (to Jianming Ying) of Cancer Hospital, CAMS, Beijing, China, the Natural Basic Research Program of China (973 program 2014CB542002) and the National Natural Science Foundation of China (81401984).

CONFLICTS OF INTEREST

The authors declare no competing financial interests.

Abbreviations

CRC, Colorectal cancer; KRAS, kirsten rat sarcoma viral oncogene homolog; IHC, Immunohistochemistry; OR, Odds ratio; CEA, carcinoembryonic antigen; CA 19-9, carbohydrate antigen; CI, confidence interval; EGFR, epidermal growth factor receptor; MMR, mismatch repair.

Authors’ contributions

Conceived and designed the experiments: Jianming Ying. Performed the experiments: Wenbin Li, Tian Qiu, Yun Ling and Lin Li. Analyzed the data: Wenbin Li. Performed the experiments: Wenbin Li, Tian Qiu, Zhi W, Shi S, Zou S, Ling Y, Shan L, Ying J, Lu N. Contributed reagents/materials/analysis tools: Lei Guo. Wrote the paper: Wenbin Li and Jianming Ying.

REFERENCES

1. Ogino S, Goel A. Molecular classification and correlates in colorectal cancer. J Mol Diagn. 2008; 10:13–27.
2. Fearon ER. Molecular genetics of colorectal cancer. Annu Rev Pathol. 2011; 6:479–507.
3. Comprehensive molecular characterization of human colon and rectal cancer . Nature. 2012; 487:330–337.
4. Zlobec I, Kovac M, Erzberger P, Molinari F, Bihl MP, Rufle A, Foerster A, Frattini M, Terracciano L, Heinimann K, Lugli A. Combined analysis of specific KRAS mutation, BRAF and microsatellite instability identifies prognostic subgroups of sporadic and hereditary colorectal cancer. Int J Cancer. 2010; 127:2569–2575.
5. Ward RL, Todd AV, Santiago F, O’Connor T, Hawkins NJ. Activation of the K-ras oncogene in colorectal neoplasms is associated with decreased apoptosis. Cancer. 1997; 79:1106–1113.
6. Jonker DJ, O’Callaghan CJ, Karapetis CS, Zalcberg JR, Tu D, Au HJ, Berry SR, Krahm M, Price T, Simes RJ, Tebbutt NC, van Hazel G, Wierzbicki R, Langer C, Moore MJ. Cetuximab for the treatment of colorectal cancer. N Engl J Med. 2007; 357:2040–2048.
7. Karapetis CS, Khambata-Ford S, Jonker DJ, O’Callaghan CJ, Tu D, Tebbutt NC, Simes RJ, Chalchal H, Shapiro JD, Robitaille S, Price TJ, Shepherd L, Au HJ, Langer C, Moore MJ, Zalcberg JR. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. N Engl J Med. 2008; 359:1757–1765.
8. Bazan V, Migliavacca M, Zanna I, Tubiolo C, Grassi N, Latteri MA, La Farina M, Albanese I, Dardanoni G, Salerno S, Tomasino RM, Labianca R, Gebbia N, Russo A. Specific codon 13 K-ras mutations are predictive of clinical outcome in colorectal cancer patients, whereas codon 12 K-ras mutations are associated with mucinous histotype. Ann Oncol. 2002; 13:1438–1446.
9. Chen CC, Er TK, Liu YY, Kwang JK, Barrio MJ, Rodrigo M, Garcia-Toro E, Herreros-Villanueva M. Computational analysis of KRAS mutations: implications for different effects on the KRAS p.G12D and p.G13D mutations. PLoS One. 2013; 8:e55793.
10. Mao C, Huang YF, Yang ZY, Zheng DY, Chen JZ, Tang JLP. KRAS p.G13D mutation and codon 12 mutations are not created equal in predicting clinical outcomes of cetuximab in metastatic colorectal cancer: a systematic review and meta-analysis. Cancer. 2013; 119:714–721.
11. Yoon HH, Tougeron D, Shi Q, Alberts SR, Mahoney MR, Nelson GD, Nair SG, Thibodeau SN, Goldberg RM, Sargent DJ, Sinicrope FA. KRAS codon 12 and 13 mutations in relation to disease-free survival in BRAF-wild-type stage III colon cancers from an adjuvant chemotherapy trial (N0147 alliance). Clin Cancer Res. 2014; 20:3033–3043.
12. Imamura Y, Morikawa T, Liao X, Lochhead P, Kuchiba A, Yamauchi M, Qian ZR, Nishihara R, Meyerhardt JA, Haigis KM, Fuchs CS, Ogino S. Specific mutations in KRAS codons 12 and 13, and patient prognosis in 1075 BRAF wild-type colorectal cancers. Clin Cancer Res. 2012; 18:4753–4763.
13. Li W, Qiu T, Zhi W, Shi S, Zou S, Ling Y, Shan L, Ying J, Lu N. Colorectal carcinomas with KRAS codon 12 mutations are associated with more advanced tumor stages. BMC Cancer. 2015; 15:340.
14. Tol J, Nagtegaal ID, Punt CJ. BRAF mutation in metastatic colorectal cancer. N Engl J Med. 2009; 361:98–99.
15. Yaeger R, Cercek A, Chou JF, Sylvester BE, Kemeny NE, Hechtman JF, Ladanyi M, Rosen N, Weiser MR, Capanu M, Solit DB, D’Angelica MI, Vakiani E, Saltz LB. BRAF mutation predicts for poor outcomes after metastasectomy in patients with metastatic colorectal cancer. Cancer. 2014; 120:2316–2324.
16. Ogino S, Stampfer M. Lifestyle factors and microsatellite instability in colorectal cancer: the evolving field of molecular pathological epidemiology. J Natl Cancer Inst. 2010; 102:365–367.
17. Ogino S, Chan AT, Fuchs CS, Giovannucci E. Molecular pathological epidemiology of colorectal neoplasia: an emerging transdisciplinary and interdisciplinary field. Gut. 2011; 60:397–411.
18. Ogino S, Lochhead P, Chan AT, Nishihara R, Cho E, Wolpin BM, Meyerhardt JA, Meissner A, Schernhammer ES, Fuchs CS, Giovannucci E. Molecular pathological epidemiology of epigenetics: emerging integrative science to analyze environment, host, and disease. Mod Pathol. 2013; 26:465–484.

19. Bishehsari F, Mahdavinia M, Vacca M, Malekzadeh R, Mariani-Costantini R. Epidemiological transition of colorectal cancer in developing countries: environmental factors, molecular pathways, and opportunities for prevention. World J Gastroenterol. 2014; 20:6055–6072.

20. Liang PS, Chen TY, Giovannucci E. Cigarette smoking and colorectal cancer incidence and mortality: systematic review and meta-analysis. Int J Cancer. 2009; 124:2406–2415.

21. Porta M, Crous-Bou M, Wark PA, Vincis P, Real FX, Malats N, Kampman E. Cigarette smoking and K-ras mutations in pancreas, lung and colorectal adenocarcinomas: etiopathogenic similarities, differences and paradoxes. Mutat Res. 2009; 682:83–93.

22. Samadder NJ, Vierkant RA, Tillmans LS, Wang AH, Lynch CF, Anderson KE, French AJ, Haile RW, Harnack LJ, Potter JD, Slager SL, Smyrk TC, Thibodeau SN, Cerhan JR, Limburg PJ. Cigarette smoking and colorectal cancer risk by KRAS mutation status among older women. Am J Gastroenterol. 2012; 107:782–789.

23. Wark PA, Van der Kuil W, Ploemacher J, Van Muijen GN, Mulder CJ, Weijenberg MP, Kok FJ, Kampman E. Diet, lifestyle and risk of K-ras mutation-positive and -negative colorectal adenomas. Int J Cancer. 2006; 119:398–405.

24. Weijenberg MP, Aardenning PW, de Kok TM, de Goeij AF, van den Brandt PA. Cigarette smoking and KRAS oncogene mutations in sporadic colorectal cancer: results from the Netherlands Cohort Study. Mutat Res. 2008; 652:54–64.

25. Curtin K, Samowitz WS, Wolff RK, Herrick J, Caan BJ, Slattery ML. Somatic alterations, metabolizing genes and smoking in rectal cancer. Int J Cancer. 2009; 125:158–164.

26. Martinez ME, Maltzman T, Marshall JR, Einspahr J, Reid ME, Sampliner R, Ahnen DJ, Hamilton SR, Alberts DS. Risk factors for Ki-ras protooncogene mutation in sporadic colorectal adenomas. Cancer Res. 1999; 59:5181–5185.

27. Samowitz WS, Albertsen H, Sweeney C, Herrick J, Caan BJ, Anderson KE, Wolff RK, Slattery ML. Association of smoking, CpG island methylator phenotype, and V600E BRAF mutations in colon cancer. J Natl Cancer Inst. 2006; 98:1731–1738.

28. Limsiu D, Vierkant RA, Tillmans LS, Wang AH, Weisenberger DJ, Laird PW, Lynch CF, Anderson KE, French AJ, Haile RW, Harnack LJ, Potter JD, Slager SL, Smyrk TC, Thibodeau SN, Cerhan JR, et al. Cigarette smoking and colorectal cancer risk by molecularly defined subtypes. J Natl Cancer Inst. 2010; 102:1012–1022.

29. Wan ES, Qiu W, Bacciarelli A, Carey VJ, Bachereman H, Rennard SI, Agusti A, Anderson W, Lomas DA, Demeo DL. Cigarette smoking behaviors and time since quitting are associated with differential DNA methylation across the human genome. Hum Mol Genet. 2012; 21:3073–3082.

30. Lee KW, Pausova Z. Cigarette smoking and DNA methylation. Front Genet. 2013; 4:132.

31. Hinoue T, Weisenberger DJ, Lange CP, Shen H, Byun HM, Van Den Berg D, Malik S, Fan P, Noushmehr H, van Dijk CM, Tollenaar RA, Laird PW. Genome-scale analysis of aberrant DNA methylation in colorectal cancer. Genome Res. 2012; 22:271–282.

32. Vukobrat-Bijedic Z, Husic-Selimovic A, Sofic A, Bijedic N, Bjelogrlic I, Gogov B, Mehmedovic A. Cancer Antigens (CEA and CA 19-9) as Markers of Advanced Stage of Colorectal Carcinoma. Med Arch. 2013; 67:397–401.

33. Takakura Y, Ikeda S, Imaoka Y, Urushihara T, Itamoto T. An elevated preoperative serum carbohydrate antigen 19–9 level is a significant predictor for peritoneal dissemination and poor survival in colorectal cancer. Colorectal Dis. 2015; 17:417–425.

34. Smith CG, Fisher D, Claes B, Maughan TS, Iduiaszczysk S, Peuteman G, Harris R, James MD, Meade A, Jasani B, Adams RA, Kenny S, Kaplan R, Lambrechts D, Cheadle JP. Somatic profiling of the epidermal growth factor receptor pathway in tumors from patients with advanced colorectal cancer treated with chemotherapy +/- cetuximab. Clin Cancer Res. 2013; 19:4104–4113.