Genetic associations between the miRNA polymorphisms miR-130b (rs373001), miR-200b (rs7549819), and miR-495 (rs2281611) and colorectal cancer susceptibility

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Abstract

Background: Recent studies have extensively investigated the role of miRNAs in colorectal cancer (CRC), and several associations have been reported. In addition, single nucleotide polymorphisms (SNPs) in promoter regions of miRNAs have been shown to affect miRNA expression. Therefore, we aimed to analyze the effect of miRNA polymorphisms on CRC susceptibility.

Methods: We conducted association studies on the relationships between the miRNA polymorphisms miR-130b T>C rs373001, miR-200b T>C rs7549819, and miR-495 A>C rs2281611 and CRC with 472 CRC patients and 399 control subjects in Korea.

Results: Multivariate logistic regressions of the CRC subgroups showed that the miR-495 CC genotype associated with rectal cancer (AA+AC vs. CC; adjusted odds ratio (AOR) for CC, 1.592; 95% confidence interval (CI), 1.071–2.368; \( P = 0.022 \)). The gene-environment combinatorial analysis showed that the combination of miR-495 A>C and low plasma folate contributed to an increased risk of rectal cancer (AA+AC vs. CC; AOR for CC, 3.829; 95% CI, 1.577–9.300; \( P = 0.003 \)). In the survival analysis, miR-200b T>C associated with CRC patient mortality (TT vs TC + CC; adjusted hazard ratio for TC + CC, 0.592; 95% CI, 0.373–0.940; \( P = 0.026 \)).

Conclusion: In this study, we found that miR-200b and miR-495 polymorphisms are involved in CRC susceptibility and prognosis.

Background

Colorectal cancer (CRC) is the third most prevalent cancer in the world with a high mortality rate [1], and eating habits and lifestyle patterns contribute to the high incidence in developed countries [2]. However, studies on dietary habits and lifestyle patterns have failed to sufficiently explain CRC disease outbreaks. Many groups have therefore focused on identifying the genetic causes of CRC, and molecular mechanisms such as microsatellite instability (MSI), CpG island methylator phenotype (CIMP), chromosomal instability (CIN), and KRAS or BRAF mutations have been described [3–7]. Recent studies indicate that microRNAs are potential prognostic biomarkers of CRC [8, 9].

MicroRNAs (miRNAs, miR) are small RNAs of ~22 bases, which bind to 3′-untranslated regions (UTRs) of target mRNAs to post-transcriptionally regulate the corresponding genes by silencing or degrading the mRNAs [10–12]. miRNAs are involved in many biochemical and metabolic pathways in many organisms, and most miRNAs exist in the noncoding regions of genes [13]. miRNA is firstly transcribed into primary miRNA (pri-miRNA) and then transformed into precursor miRNA (pre-miRNA) by the DGCR8-DROSHA complex. Pre-miRNA is transported to the cytoplasm by the

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RAN-GTP/exportin-5 complex, where it is processed into a mature miRNA by DICER. Mature miRNA functions in an RNA-induced silencing complex (RISC) complex that targets mRNA [14]. Previous studies have revealed associations between miRNA expression and various cancers, including leukemia [15], hepatocarcinoma [16], gastric cancer [17], bladder cancer [18], lung cancer [19], and breast cancer [20]. It has also been shown that polymorphisms in miRNA sequences regulate miRNA expression [21, 22]. Studies have confirmed associations between miRNA polymorphisms and cancer development, progression, and metastasis [23–25].

We previously demonstrated that miR-146a, miR-149, miR-196a2, and miR-499 single nucleotide polymorphisms (SNPs) associate with CRC [26]. However, because additional miRNA polymorphisms may associate with CRC, we asked whether miR-130b, miR-200b, and miR-495 SNPs also associate with CRC. MiR-130b has been shown to contribute to the occurrence of CRC and is involved in the PTEN/AKT signaling pathway [27, 28]. In addition, miR-200b has been shown to affect the breast cancer survival rate [29], to be involved in the regulation of c-Myc/PRDX2 in CRC [30], and to affect the migration, invasion, and epithelial mesenchymal transition (EMT) mechanisms of lung cancer [31]. miR-495 has been reported to reduce the proliferation of cancer cells in CRC and breast cancer [32, 33] and to affect cancer metastasis [34].

As mentioned earlier, miR-130b, 200b, and 495 have been linked to CRC development and progression. We focused on three SNPs: miR-130b rs373001T > C, miR-200b rs7549819T > C, and miR-495 rs2281611A > C, all of which are regulatory regions of miRNA expression. We hypothesized that polymorphisms in these miRNAs would ultimately influence CRC susceptibility and mortality. There is no known genetic association of these SNPs with CRC. This study specifically examined whether miRNA polymorphisms are related to CRC susceptibility in Koreans.

Methods
Study population
For this case-control study, a total of 871 individuals were enrolled from June 2005 to January 2011, including 472 patients diagnosed with CRC at CHA Bundang Medical Center (Seongnam, South Korea) and 399 randomly selected non-CRC subjects who participated in a health-screening program. This case group included only CRC patients who had gone through surgery and who had confirmed to adenocarcinoma by histology. The case group included colon and rectal cancer patients (268 and 193 patients, respectively). Tumors were classified by their tumor, node, and metastasis classification (TNM) stage according to the 7th of the American joint committee on cancer (AJCC) staging manual as follows: stage I, n = 52 (11.02%); stage II, n = 191 (40.47%); stage III, n = 176 (37.29%); and stage IV, n = 47 (9.96%). Hypertension (HTN) and diabetes mellitus (DM) for overall participants were classified according to the criteria of the previous study [35]. We had provided written informed consent for all of the participants and the study protocol was approved by the Institutional Review Board of CHA Bundang Medical Center (IRB No. 2009–08-077) and followed the recommendations of the Declaration of Helsinki.

Genotyping
DNA was extracted from white blood cells using a “G-DEX”IIb For Blood kit” (iNtRON Biotechnology, South Korea). Genotyping of miR-130b rs373001T > C, miR-200b rs7549819T > C and miR-495 rs2281611A > C were performed by same protocol as in our previous study [36], and detailed PCR conditions were presented in Additional file 1: Table S1. We randomly repeated 10–15% of miR-130b rs373001T > C, miR-200b rs7549819T > C and miR-495 rs2281611A > C polymorphism genotyping results and confirmed the results with DNA sequencing [36]. The concordance between the experiment and randomly repeat was 100%.

Statistical analysis
To compare clinical characteristics between study groups, we used the χ² test and the two-tail t-test or Mann-Whitney test. The adjusted odds ratios (AORs) and 95% confidence intervals (CIs) for association with miRNAs polymorphisms in CRC risk were calculated by multivariate logistic regression adjusted for age, sex, HTN, and DM. The software program used for statistical analysis in this study were “GraphPad Prism 4.0” (GraphPad Software Inc., San Diego, CA, USA), “HAPSTAT 3.0” (University of North Carolina, Chapel Hill, NC, USA), and “Medcalc v.18.2.1” (Medcalc Software, Mariakerke, Belgium) and and the cut-off of statistically significant was considered was P values < 0.05. The false discovery rate (FDR) was calculated when performing multiple comparisons to estimate the overall experimental error rate resulting from false positives. Independent prognostic markers were investigated using the Cox proportional-hazards regression for mortality analysis, and the results were adjusted for age, sex, TNM stage, and chemotherapy. Hazard ratios (HRs) are shown with 95% CIs.

Results
Study subject characteristics
The 472 CRC cases included 212 males and 260 females with an overall mean age of 61.99 ± 12.32 years. There were no significant differences in the age and sex of the
CRC patients and the controls (P = 0.290 and 0.774, respectively). The baseline characteristics of patients with colon and rectal cancers, which are subgroups of CRC, showed no statistical differences when compared to the control group (Table 1).

**Genotype frequencies**
The distributions of genotypes for the miRNA polymorphisms miR-130b T>C, miR-200b T>C, and miR-495 A>C in CRC patients and control subjects are shown in Table 2. The genotype frequencies of CRC and control groups were in Hardy-Weinberg equilibrium (HWE). There was no statistically significant difference in the distribution of miR-130b T>C, miR-200b T>C, and miR-495 A>C SNPs between the CRC and control groups. In a subgroup analysis, we observed that the miR-495CC genotype was more frequent in rectal cancer patients than in the control group (AA+AC vs. CC; AOR for CC, 1.592; 95% CI, 1.071–2.368; Table 3). However, this statistical significance was lost after correcting for multiple comparisons using the FDR method (P = 0.065). There were no statistically significant differences in the distributions of the other miRNA SNPs between the CRC subgroups and the control group. We also confirmed that these SNPs are not associated to the MSI status (Additional file 1: Table S2).

**Combinatorial effects of miRNA polymorphisms and environmental factors**
Because CRC has been shown to be influenced by various environmental factors, we performed a stratified analysis of age, sex, HTN, DM, and test levels of peripheral blood factors (homocysteine, folate, TG, HDL) to determine whether there was an association between miRNA polymorphisms and CRC risk (Additional file 1: Table S3). We did not find any associations between miRNA polymorphisms and CRC risk in the high-risk groups for each variable.

We then conducted a gene-environment analysis to assess the combined effects of miR-130b T>C, miR-200b T>C, or miR-495 A>C polymorphisms and clinical factors on CRC risk.

### Table 1 Baseline characteristics between controls and CRC patients

| Characteristic          | Controls (n = 399) | CRC Patients (n = 472) | P     | Colon cancer (n = 268) | P     | Rectal cancer (n = 193) | P     |
|-------------------------|-------------------|-----------------------|-------|------------------------|-------|-------------------------|-------|
| Age (years, mean ± SD)  | 61.15 ± 10.93     | 61.99 ± 12.32         | 0.129 | 61.44 ± 12.88          | 0.464 | 62.28 ± 11.54           | 0.153 |
| Male (%)                | 173 (43.4)        | 212 (44.9)            | 0.645 | 118 (44.0)             | 0.915 | 88 (45.6)               | 0.750 |
| Hypertension (%)        | 155 (38.8)        | 281 (59.5)            | <0.0001 | 157 (58.6)            | 0.003 | 117 (60.6)             | 0.003 |
| HDL-C (mg/dL, mean ± SD)| 45.91 ± 13.48     | 42.18 ± 13.05         | 0.001 | 42.82 ± 13.00          | 0.013 | 41.27 ± 13.07           | 0.001 |
| LDL-C (mg/dL, mean ± SD)| 115.87 ± 40.28    | 101.31 ± 28.62        | 0.003 | 98.55 ± 28.01          | 0.002 | 104.32 ± 29.54          | 0.142 |
| Diabetes mellitus (%)   | 52 (13.0)         | 156 (33.1)            | <0.0001 | 92 (34.3)            | <0.0001 | 64 (33.2)             | <0.0001 |
| Smoking (%)             | 138 (34.6)        | 92 (19.5)             | <0.0001 | 55 (20.5)            | 0.003 | 35 (18.1)              | 0.001 |
| Folate (nmol/L, mean ± SD)| 8.64 ± 6.13     | 7.94 ± 7.13           | <0.0001 | 8.12 ± 7.36          | 0.001 | 7.70 ± 6.86             | 0.000 |
| Triglyceride (mg/dL, mean ± SD)| 146.79 ± 89.33 | 129.00 ± 86.30 | 0.0003 | 126.93 ± 84.48 | 0.001 | 132.48 ± 90.86 | 0.015 |
| Homocysteine (μmol/L, mean ± SD)| 9.96 ± 4.27 | 10.68 ± 7.83 | 0.671 | 10.47 ± 8.21 | 0.572 | 10.88 ± 7.32 | 0.215 |
| Total cholesterol (mg/dL, mean ± SD)| 192.00 ± 37.32 | 178.76 ± 40.56 | 0.0001 | 178.73 ± 38.88 | 0.001 | 176.69 ± 42.89 | 0.002 |

| Tumor size (%)         |       |                      |       |                       |       |                       |       |
|-------------------------|-------|----------------------|-------|-----------------------|-------|-----------------------|-------|
| < 5 cm                  | 208 (44.1) | 106 (39.6)          | 93 (48.2) |
| ≥ 5 cm                  | 264 (55.9) | 162 (60.4)          | 100 (51.8) |

| TNM stage (%)           |       |                      |       |                       |       |                       |       |
|-------------------------|-------|----------------------|-------|-----------------------|-------|-----------------------|-------|
| I                       | 52 (11.2) | 26 (9.7)           | 26 (13.5) |
| II                      | 191 (41.0) | 118 (44.2)      | 70 (36.3) |
| III                     | 176 (37.8) | 94 (35.2)         | 81 (42.0) |
| IV                      | 47 (10.1) | 29 (10.9)         | 16 (8.3) |
| N.A.                    | 6     | 1                    | 0     |

| MSI (%)                 |       |                      |       |                       |       |                       |       |
|-------------------------|-------|----------------------|-------|-----------------------|-------|-----------------------|-------|
| MSI-high (%)            | 61 (15.6) | 49 (22.0)         | 12 (7.3) |
| MSI-low (%)             | 15 (3.8) | 11 (4.9)          | 4 (2.4) |
| N.A.                    | 82    | 45                  | 29    |

*P*-values were calculated by Man whithney U test for continuous variables and chi-square test for categorical variables. TNM stage, TNM classification of malignant tumours; MSI, microsatellite instability; N.A. row, missing data.
Table 2 Genotype frequencies of microRNA polymorphisms in CRC patients and control subjects

| Genotypes               | Controls (n = 399) | Patients (n = 472) | AOR (95% CI)     | P     | FDR-P |
|-------------------------|-------------------|-------------------|------------------|-------|-------|
| miR-130b rs373001T > C  |                   |                   |                  |       |       |
| TT                      | 216 (54.2)        | 269 (57.0)        | 1.000 (reference) |       |       |
| TC                      | 157 (39.3)        | 168 (35.6)        | 0.825 (0.610–1.115) | 0.210 | 0.416 |
| CC                      | 26 (6.5)          | 35 (7.4)          | 0.943 (0.532–1.670) | 0.840 | 0.840 |
| Dominant (TT vs TC + CC)|                   |                   | 0.846 (0.635–1.127) | 0.254 | 0.398 |
| Recessive (TT + TC vs CC)|                 |                   | 1.028 (0.590–1.792) | 0.923 | 0.923 |
| HWE P                   | 0.723             | 0.222             |                  |       |       |
| miR-200b rs7549819T > C |                   |                   |                  |       |       |
| TT                      | 171 (42.9)        | 216 (45.7)        | 1.000 (reference) |       |       |
| TC                      | 176 (44.1)        | 200 (42.4)        | 0.882 (0.652–1.194) | 0.416 | 0.416 |
| CC                      | 52 (13.0)         | 56 (11.9)         | 0.758 (0.481–1.194) | 0.232 | 0.696 |
| Dominant (TT vs TC + CC)|                   |                   | 0.850 (0.638–1.132) | 0.266 | 0.398 |
| Recessive (TT + TC vs CC)|                 |                   | 0.789 (0.512–1.215) | 0.281 | 0.422 |
| HWE P                   | 0.527             | 0.356             |                  |       |       |
| miR-495 rs2281611A > C  |                   |                   |                  |       |       |
| AA                      | 103 (25.8)        | 125 (26.5)        | 1.000 (reference) |       |       |
| AC                      | 194 (48.6)        | 222 (47.0)        | 0.829 (0.584–1.176) | 0.292 | 0.416 |
| CC                      | 102 (25.6)        | 125 (26.5)        | 1.080 (0.734–1.590) | 0.696 | 0.840 |
| Dominant (AA vs AC + CC)|                   |                   | 0.919 (0.666–1.268) | 0.660 | 0.660 |
| Recessive (AA+AC vs CC) |                   |                   | 1.208 (0.897–1.626) | 0.214 | 0.422 |
| HWE P                   | 0.582             | 0.197             |                  |       |       |

AOR, adjusted odds ratio (adjusted for age, gender, hypertension, diabetes mellitus); CI, confidence interval; FDR, false discovery ratio; HWE, Hardy-Weinberg equilibrium

Table 3 Genotype frequencies of microRNA polymorphisms in CRC subgroups and control subjects

| Genotypes               | Controls (n = 399) | Colon (n = 268) | AOR (95% CI)     | P     | FDR-P |
|-------------------------|-------------------|----------------|------------------|-------|-------|
| miR-130b rs373001T > C  |                   |                |                  |       |       |
| TT                      | 216 (54.2)        | 156 (58.2)     | 1.000 (reference) |       |       |
| TC                      | 157 (39.3)        | 109 (43.0)     | 0.825 (0.580–1.177) | 0.295 | 0.443 |
| CC                      | 26 (6.5)          | 15 (6.3)       | 0.671 (0.327–1.377) | 0.717 | 1.166 |
| Dominant (TT vs TC + CC)|                   |                | 0.805 (0.575–1.126) | 0.205 | 0.371 |
| Recessive (TT + TC vs CC)|                 |                | 0.740 (0.369–1.486) | 0.398 | 0.909 |
| HWE P                   | 0.527             | 0.356          |                  |       |       |
| miR-200b rs7549819T > C |                   |                |                  |       |       |
| TT                      | 171 (42.9)        | 126 (47.0)     | 1.000 (reference) |       |       |
| TC                      | 176 (44.1)        | 109 (40.7)     | 0.826 (0.580–1.177) | 0.290 | 0.443 |
| CC                      | 52 (13.0)         | 33 (12.3)      | 0.835 (0.493–1.412) | 0.500 | 0.717 |
| Dominant (TT vs TC + CC)|                   |                | 0.822 (0.589–1.146) | 0.248 | 0.371 |
| Recessive (TT + TC vs CC)|                 |                | 0.876 (0.531–1.447) | 0.606 | 0.909 |
| HWE P                   | 0.582             | 0.197          |                  |       |       |
| miR-495 rs2281611A > C  |                   |                |                  |       |       |
| AA                      | 103 (25.8)        | 72 (26.9)      | 1.000 (reference) |       |       |
| AC                      | 194 (48.6)        | 135 (50.4)     | 0.881 (0.537–1.321) | 0.540 | 0.717 |
| CC                      | 102 (25.6)        | 61 (22.8)      | 0.919 (0.583–1.450) | 0.717 | 1.319 |
| Dominant (AA vs AC + CC)|                   |                | 0.900 (0.618–1.310) | 0.581 | 0.940 |
| Recessive (AA+AC vs CC) |                   |                | 0.991 (0.675–1.543) | 0.961 | 1.592 |

CRC, colorectal cancer; AOR, adjusted odds ratio (adjusted for age, gender, hypertension, diabetes mellitus); CI, confidence interval; FDR, false discovery ratio; HWE, Hardy-Weinberg equilibrium
and CRC subgroup susceptibility. The combination of \( miR-495A > C \) and low plasma folate level contributed to an increased risk for CRC (AA+AC vs. CC; AOR, 3.119; 95% CI, 1.432–6.791; Additional file 1: Table S4). In addition, the \( miR-495CC \) genotype exhibited an increased risk in rectal cancer patients with HTN (AOR, 3.404; 95% CI, 1.902–6.092, \( P < 0.001 \)), DM (AOR, 3.758; 95% CI, 1.685–8.383; \( P = 0.001 \)), and in rectal cancer patients with low plasma folate levels (AOR, 3.829; 95% CI, 1.577–9.300; \( P = 0.003 \) Table 4 and Fig. 1).

**Associations of miRNA SNPs with CRC survival**

Associations between miRNA polymorphisms and CRC survival are shown in Table 5. Multivariate Cox proportional analysis showed that the \( miR-200bTC \) and TC + CC genotypes associated with survival in CRC patients (adjusted HR = 0.522; 95% CI, 0.307–0.888; \( P = 0.017 \) and adjusted HR = 0.522; 95% CI, 0.307–0.888; \( P = 0.017 \), respectively; Fig. 2).

**Table 4** Combinatorial effects of miRNA polymorphisms and environmental factors on rectal cancer risk

| Characteristics | \( miR-130bTT \) | \( miR-130bTC + CC \) | \( miR-200bTT \) | \( miR-200bTC + CC \) | \( miR-495AA + AC \) | \( miR-495CC \) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Age             |                 |                 |                 |                 |                 |                 |
| < 63 years      | 1.000 (reference) | 1.222 (0.706–2.115) | 1.000 (reference) | 1.032 (0.599–1.780) | 1.000 (reference) | 1.563 (0.874–2.793) |
| \( \geq 63 \) years | 1.097 (0.672–1.790) | 0.696 (0.412–1.176) | 0.826 (0.475–1.436) | 0.854 (0.515–1.415) | 1.107 (0.706–1.736) | 1.784 (1.038–3.064) |
| Gender          |                 |                 |                 |                 |                 |                 |
| Male            | 1.000 (reference) | 0.799 (0.469–1.362) | 1.000 (reference) | 1.214 (0.707–2.085) | 1.000 (reference) | 1.772 (0.975–3.221) |
| Female          | 1.087 (0.664–1.782) | 0.966 (0.570–1.636) | 1.454 (0.835–2.531) | 1.303 (0.753–2.255) | 0.885 (0.565–1.387) | 1.273 (0.730–2.222) |
| Hypertension    |                 |                 |                 |                 |                 |                 |
| No              | 1.000 (reference) | 0.924 (0.531–1.610) | 1.000 (reference) | 0.799 (0.457–1.399) | 1.000 (reference) | 1.906 (1.050–3.461) |
| Yes             | 2.539 (1.535–4.200) | 1.854 (1.076–3.196) | 1.921 (1.101–3.350) | 2.171 (1.279–3.683) | 2.362 (1.496–3.727) | 3.404 (1.902–6.092) |
| Diabetes mellitus |               |                 |                 |                 |                 |                 |
| No              | 1.000 (reference) | 0.832 (0.544–1.274) | 1.000 (reference) | 0.872 (0.571–1.332) | 1.000 (reference) | 1.686 (1.077–2.642) |
| Yes             | 2.535 (1.382–4.651) | 2.545 (1.385–4.676) | 1.946 (0.998–3.793) | 3.261 (1.798–5.913) | 3.088 (1.851–5.152) | 3.758 (1.685–8.383) |
| Homocysteine (\( \mu \)mol/L) |                 |                 |                 |                 |                 |                 |
| < 13.3          | 1.000 (reference) | 0.795 (0.531–1.191) | 1.000 (reference) | 1.191 (0.794–1.787) | 1.000 (reference) | 1.641 (1.069–2.518) |
| \( \geq 13.3 \) | 0.936 (0.451–1.943) | 1.199 (0.579–2.484) | 1.938 (0.904–4.152) | 0.820 (0.394–1.708) | 1.211 (0.653–2.248) | 1.619 (0.612–4.282) |
| Folate (nmol/L) |                 |                 |                 |                 |                 |                 |
| > 3.7           | 1.000 (reference) | 0.853 (0.571–1.272) | 1.000 (reference) | 0.977 (0.654–1.458) | 1.000 (reference) | 1.478 (0.956–2.286) |
| \( \leq 3.7 \)  | 2.427 (1.152–5.114) | 2.193 (0.948–5.076) | 1.645 (0.685–3.953) | 2.512 (1.228–5.138) | 2.069 (1.016–4.216) | 3.829 (1.577–9.300) |
| Triglyceride (mg/dL) |                 |                 |                 |                 |                 |                 |
| < 150           | 1.000 (reference) | 0.843 (0.545–1.303) | 1.000 (reference) | 0.902 (0.584–1.394) | 1.000 (reference) | 0.934 (0.567–1.538) |
| \( \geq 150 \)  | 0.524 (0.300–0.914) | 0.426 (0.227–0.799) | 0.373 (0.187–0.745) | 0.609 (0.352–1.055) | 0.457 (0.196–1.066) | 0.359 (0.179–0.718) |
| HDL-C (mg/dL)   |                 |                 |                 |                 |                 |                 |
| \( \geq 40 \)   | 1.000 (reference) | 0.839 (0.542–1.299) | 1.000 (reference) | 0.965 (0.624–1.492) | 1.000 (reference) | 1.119 (0.683–1.836) |
| < 40            | 2.706 (1.500–4.882) | 2.259 (1.162–4.394) | 2.575 (1.312–5.053) | 2.624 (1.442–4.775) | 4.639 (1.799–11.961) | 2.137 (1.141–4.001) |

Upper and lower 15% cut-off values of homocysteine and folate were 13.3 \( \mu \)mol/L and 3.7 ng/mL, respectively.

AOR, adjusted odds ratio (adjusted for age, gender, hypertension, diabetes mellitus); CI, confidence interval

**Discussion**

In this study, we investigated whether the miRNA polymorphisms \( miR-130bT > C \) rs377001, \( miR-200bT > C \) rs7549819, and \( miR-495A > C \) rs2281611 associate with susceptibility for CRC or a CRC subgroup in Korean subjects. These three SNPs are regulatory SNPs located in the promoter regions of the miRNA genes. SNPs in the promoter regions of miRNAs have been shown to affect the expression of mature miRNAs that regulate target genes [24, 25].

\( miR-495 \) has been shown to play a tumor suppressor role in many cancers, including gastric cancer [37], non-small cell lung cancer [38], glioma [39], and CRC [40]. In particular, \( miR-495 \) has been shown to regulate expression of genes involved in cellular processes, including mTOR, Akt, and PRL-3 [37, 41, 42]. Our data suggest that the \( miR-495CC \) genotype associates with an increased risk for rectal cancer when compared with the other genotypes. Therefore, we assume that substitution of the C allele with the rs2281611 A allele in the
The promoter region of the miR-495 gene leads to a reduction in miRNA expression, which then affects CRC susceptibility. In the combinatorial gene-environment analysis, the miR-495CC genotype combined with folate exhibited a significantly increased risk of CRC. Folic acid is an essential factor involved in one-carbon metabolism, including DNA synthesis, repair, and methylation [43–45]. When the folate level is insufficient, DNA is abnormally replicated during cell division [46], DNA is degraded, and mutagenesis increases [43]. In addition,

**Table 5** Multivariate survival analysis of polymorphisms in CRC patients

| Genotype          | CRC(n = 472) | Death(n = 85) | Adjusted HR*(95% CI) | P     |
|-------------------|--------------|--------------|----------------------|-------|
| miR-130b rs373001T > C |
| TT                | 269 (57.0)   | 47 (55.3)    | 1.000 (reference)    | 1.000 |
| TC                | 168 (35.6)   | 29 (34.1)    | 0.810 (0.491–1.338)  | 0.411 |
| CC                | 35 (7.4)     | 9 (10.6)     | 1.345 (0.632–2.864)  | 0.442 |
| Dominant (TT vs TC + CC) | 0.910 (0.575–1.438) | 0.685 |
| Recessive (TT + TC vs CC) | 1.435 (0.688–2.990) | 0.336 |
| miR-200b rs7549819T > C |
| TT                | 216 (45.7)   | 48 (56.5)    | 1.000 (reference)    | 1.000 |
| TC                | 200 (42.4)   | 26 (30.6)    | 0.522 (0.307–0.888)  | 0.017 |
| CC                | 56 (11.9)    | 11 (12.9)    | 0.781 (0.393–1.555)  | 0.482 |
| Dominant (TT vs TC + CC) | 0.592 (0.373–0.940) | 0.026 |
| Recessive (TT + TC vs CC) | 0.994 (0.509–1.944) | 0.987 |
| miR-495 rs2281611A > C |
| AA                | 125 (26.5)   | 23 (27.1)    | 1.000 (reference)    | 1.000 |
| AC                | 222 (47.0)   | 37 (43.5)    | 1.077 (0.618–1.879)  | 0.794 |
| CC                | 125 (26.5)   | 25 (29.4)    | 1.167 (0.628–2.170)  | 0.625 |
| Dominant (AA vs AC + CC) | 1.126 (0.672–1.886) | 0.652 |
| Recessive (AA+AC vs CC) | 1.147 (0.691–1.903) | 0.595 |

*HR estimates with 95% CI and P-values from the Cox-proportional hazard model on overall survival. HR, hazard ratio (adjusted for age, gender, chemotherapy, TNM stage); CI, confidence interval
uracil misincorporation and double-strand breaks have been observed in tumor cells cultured in low folate conditions [43, 47]. Low folate levels have also been associated with breast cancer [48], CRC [49], and gastric cancer [50]. Thus, the effects of the miR-495CC genotype and low folate concentration appear to be synergistic.

In the survival analysis, the miR-200bTC and TC + CC genotypes associated with the survival rate of patients who had undergone CRC resection. The miR-200 family has been shown to inhibit EMT, which shares many similarities with cancer progression [51], and to associate with poor prognoses, including metastasis, invasion, and chemoresistance in gastric cancer [52], bladder cancer [53], and CRC [54]. The miR-200 family has also been implicated in CRC survival [55]. Abnormal miR-200b expression moderates the poor prognosis and progression of CRC, and these factors may affect patient survival rate.

There are several limitations to our study. The first is that expression differences in mature miRNAs due to SNPs in the regulatory regions of miRNA genes have not been confirmed at the molecular and functional levels. Therefore, we are inferring that expression of the altered miR-495 relates directly to CRC risk by targeting the tumor suppressor gene. The second limitation is that the sample size may be insufficient to draw any conclusions from the stratified analysis. Future studies should include more than 1000 ethnically homogeneous people. Lastly, this study only included Koreans who visited CHA Bundang Medical Center. Although our findings provide the first evidence that miRNA polymorphisms could be potential biomarkers of CRC prevention and prognosis, significant results should be identified in independent populations to confirm the validity of these results.

**Conclusion**

In conclusion, we investigated the relationship between CRC susceptibility and the miRNA polymorphisms miR-130b rs373001, miR-200b rs7549819, and miR-495 rs2281611. We found that miR-200b and miR-495 associated with CRC susceptibility and survival of CRC patients, respectively. Although there have been many studies that have described the relationships between miR-200b and miR-495 and CRC susceptibility, no associations between the miR-200b and miR-495 polymorphisms and CRC have been reported. Thus, our results provide evidence that miR-200b and miR-495 polymorphisms may be potential biomarkers for CRC diagnosis and prevention.

**Additional file**

Additional file 1: Table S1. Information of miR-200 and 495 polymorphisms for PCR-RFLP. Table S2. Comparison of genotype frequencies of microRNA polymorphisms between colorectal cancer subtype and control. Table S3. Stratified effects of miR-130b T>C, miR-200b T>C, and miR-495 C>A polymorphisms on CRC susceptibility. Table S4. Combinatorial effects of miRNA polymorphisms and environmental factors on CRC risk. (DOCX 26 kb)

**Abbreviations**

AJCC: American joint committee on cancer; AOR: Adjusted odds ratio; CI: Confidence interval; CIMP: CpG island methylator phenotype; CIN: Chromosomal instability; CRC: Colorectal cancer; DM: Diabetes mellitus; EMT: Epithelial-mesenchymal transition; FDR: False discovery rate; HR: Hazard ratio; HTN: Hypertension; HWE: Hardy-weinberg equilibrium; miRNA: microRNA; MSI: Microsatellite instability; pre-miRNA: precursor miRNA; pri-miRNA: primary miRNA; RISC: RNA-induced silencing complex; SNP: Single nucleotide polymorphism; TNM: Tumor, node and metastasis classification; UTR: Untranslated region

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Availability of data and materials
The data supporting the conclusions of this article are available from the authors on request.

Authors’ contributions
Conceived and designed the experiments: JWK and NKK. Performed the experiments: EGK, JOK, HSP, CSR, JYJ, and HHJ. Analyzed the data and statistical analyses: EGK, JOK, HSP, CSR. Contributed reagents/material/analysis tools: JOK, HHJ, JWK, and NKK. Wrote the main manuscript text: EGK and NKK. All authors reviewed the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
All of the study subjects were ethnic Koreans and provided written informed consent. Ethics approval and consent to participate were obtained from the Institutional Review Board of CHA Bundang Medical Center (IRB No. 2009–08-077) and followed the recommendations of the Declaration of Helsinki.

Consent for publication
Not applicable.

Competing interests
The authors have no conflicts of interest to declare.

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