Polymorphisms of cancer-related genes and risk of multiple primary malignancies involving colorectal cancer

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1. Introduction

Multiple primary cancers are defined as two or more independent primary malignancies occurring simultaneously or successively in the same or different organs or tissues of the same individual. Multiple primary malignancies involving colorectal cancer are multiple primary malignant tumors including primary colorectal cancer, and they can be divided into multiple primary colorectal cancers and multiple colorectal cancers associated with extracolonic primary cancer according to the second primary sites. In recent years, the incidence has shown a tendency to increase, with 17%–19% of cancer patients reported as having multiple primary cancers (1,2). Multiple primary malignancies involving colorectal cancers have received much attention because of the high incidence of these occurrences. According to reports from the Cancer Institute Hospital, Tokyo, Japan, a total of 24,498 cancer patients were recorded from 1986–1995, of whom 1281 (5.2%) had multiple primary cancers and 8.30% of whom were diagnosed with primary colorectal cancers (3). The causes of multiple primary malignancies involving colorectal cancers are poorly understood; however, they are thought to be complex, resulting from the interactions of many factors such as genetics, the environment, lifestyle, and benign colorectal lesions (4–6).

Single nucleotide polymorphisms (SNPs) are alterations in DNA sequences that are convenient genetic markers for association studies. Recent works have shown that certain SNPs are closely associated with malignant tumor development (7,8). We previously presented that the numbers of SNPs on some cancer-related genes are particularly associated with malignant tumors (9). Therefore, in the present study, we searched for SNPs in these same genes and explored their relationship with multiple primary malignancies involving colorectal cancers.
2. Materials and methods

2.1. Sample collection

A total of 1311 patients with colorectal cancers who had undergone surgery at the Third Xiangya Hospital of Central South University, Changsha, China, between August 2005 and August 2012 were enrolled in this study. According to the modification of the diagnostic criteria of Warren and Gates (10) and the diagnostic criteria of Lee et al. (11), in combination with clinical findings and pathologic and/or cytological examinations, 59 patients were confirmed to have multiple primary malignancies involving colorectal cancer; patients were excluded if the first primary cancer was not colorectal cancer and if no tissue sample was taken. Finally, 22 patients, 13 males and 9 females having a mean age of 63.83 ± 11.03 years (48–81 years), with multiple primary malignancies and first primary colorectal cancer, were evaluated. Seven patients were afflicted with multiple primary colorectal cancer, 15 with multiple colorectal cancer with extra colonic primary cancer, 6 with synchronous multiple primary cancer, and 16 with metachronous multiple primary cancer. Clinical characteristics of the patients are shown in Table 1.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All patients signed informed consent forms and the study received approval from the Xiangya Hospital Ethics Committee.

2.2. Single nucleotide polymorphism analysis

DNA was extracted from paraffin-embedded tissue samples using a commercial DNA extraction kit (QIAGEN, Hilden, Germany) in accordance with the manufacturer’s instructions. SNP detection was performed by using the Sequenom MassARRAY Analyzer 4 system (Sequenom, San Diego, CA, USA).

2.3. Data analysis

Genotype and allele frequencies at 116 genetic loci were calculated for the 22 samples of multiple primary malignancies involving colorectal cancer. The genotyping results for 116 loci of the control Chinese population were chosen from HapMap through the NCBI database. Differences in allele frequencies between the controls and the 22 patients with multiple primary malignancies involving colorectal cancer were compared using the chi-square test with P < 0.05 considered statistically significant. Statistical analysis was performed using SPSS 19.0 software.

3. Results

This analysis included 62 genes encoding proteins involved in DNA repair mechanisms and in other cellular signaling pathways (Table 2). Eighty-one of the 116 loci presented no significant difference in the allele frequencies between patients and the control Chinese population (data not shown); significant differences were identified at the remaining 35 (P < 0.05), including TNFRSF1B (rs1061624, rs1061622, rs3397), TP53 (rs1625895), EDN1 (rs1800541, rs2071942), p73 (rs1801173), ERCC6 (rs2228526), ERCC1 (rs3212986), CASP8 (rs3834129, rs3769818), XRCC5 (rs3835), CANX (rs7566), WNT2 (rs887574), CCND1 (rs9344), APEX1 (rs1130409), REL (rs13031237), RECQL (rs13035), XRCC4 (rs1805377), LIN28B (rs314276), TRAF6 (rs4755453, rs5030437), XRCC3 (rs861539), CCL2 (rs1024611), VCP (rs2074549), NFKB1 (rs28362491), CTNNB1 (rs4135385), SOD2 (rs4880), EDNRA (rs5333, rs5343), P21 (rs1059234), ATM (rs1799757), XRCC1 (rs3213245), CASP3 (rs4647693), and CAT (rs7943316).

Table 1. Clinical characteristics of 22 patients with multiple primary malignancies involving colorectal cancer.

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>&lt;65 years</td>
<td>11 (50.00%)</td>
</tr>
<tr>
<td>&gt;65 years</td>
<td>11 (50.00%)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>13 (59.09%)</td>
</tr>
<tr>
<td>Female</td>
<td>9 (40.91%)</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
</tr>
<tr>
<td>Synchronous multiple primary cancers</td>
<td>6 (27.27%)</td>
</tr>
<tr>
<td>Metachronous multiple primary cancer</td>
<td>16 (72.73%)</td>
</tr>
</tbody>
</table>
Table 2. Information of the 62 genes analyzed.

<table>
<thead>
<tr>
<th>Signal pathway</th>
<th>Gene name</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA repair genes</td>
<td>ERCC1, ERCC2, ERCC4, ERCC5, ERCC6, XRCC1, XRCC2, XRCC3, XRCC4, XRCC5, XRCC6, XPA, XPC, RECOQL, ATM, ATR, APE1, OGG1, NBN</td>
</tr>
<tr>
<td>Apoptosis-related genes</td>
<td>TP53, P73, TNFRSF1B</td>
</tr>
<tr>
<td>Cell cycle-related genes</td>
<td>CCND1, CDKN2A, CDKN1A, CDKN1B, CDKN2B, P21</td>
</tr>
<tr>
<td>Antioxidant-related genes</td>
<td>CAT, SOD2, NQO1</td>
</tr>
<tr>
<td>Detoxification enzyme-related genes</td>
<td>GSTP1</td>
</tr>
<tr>
<td>Fibrosis and inflammatory cytokines</td>
<td>TGF-β1, LIN28B</td>
</tr>
<tr>
<td>Angiogenesis-related genes</td>
<td>VEGF, EDN1, EDNRA</td>
</tr>
<tr>
<td>NF-κB signaling pathway</td>
<td>NFKB1, REL, NFKB1I, IKBK3, TRAF6, TNFAIP3, TNIP1</td>
</tr>
<tr>
<td>ER stress signaling pathways-related genes</td>
<td>VCP, XBP1, HSP90B1, CALCR, CANX, HSPA2</td>
</tr>
<tr>
<td>Wnt signaling pathways-related genes</td>
<td>WNT2, GSK-3b, APC, CTNNB1</td>
</tr>
<tr>
<td>Caspase apoptotic pathway-related genes</td>
<td>CASP3, CASP8</td>
</tr>
<tr>
<td>Chemokine genes</td>
<td>CCL2, CCL8, CXCL12</td>
</tr>
<tr>
<td>Cell transcription and translation genes</td>
<td>EIF3A</td>
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<tr>
<td>mTOR signaling pathways</td>
<td>RICTOR</td>
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</table>

(Table 3). Of these, five loci were previously reported to be associated with the pathogenesis of colorectal cancer, including TNFRSF1B (rs1061624) (12), P21 (rs1059234) (13), CCND1 (rs9344) (14), NFKB1 (rs28362491) (15), and ERCC1 (rs3212986) (16) (Table 4).

4. Discussion

Incidences of multiple primary cancer have increased in recent years. The colon and rectum are the most commonly reported organs for multiple primary cancer development, and multiple primary malignancy involving colorectal cancer is the most frequently seen form of multiple primary cancer (17). Patients with multiple primary colorectal cancers have been shown to have a significantly worse prognosis than those with single primary colorectal cancers (18). However, few studies have investigated the cause of multiple primary malignancies involving colorectal cancer.

In this study, we performed SNP genotyping of 116 SNPs from 62 tumor-associated genes in 22 patients with multiple primary malignancies involving primary colorectal cancer. We identified significant differences in the allele frequency distribution at 35 loci of 31 genes between patients and the control Chinese population. This indicated that alterations at these loci may be associated with the pathogenesis of multiple primary malignancies involving colorectal cancer. Five of these loci were previously reported to have variable association with the risk of single primary colorectal cancer; rs1061624 of TNFRSF1B, rs1059234 of P21, and rs9344 of CCND1 were found to reduce the risk of colorectal cancer and exert protective effects, while rs28362491 of NFKB1 and rs3212986 of ERCC1 increased the risk of colorectal cancer.

Among the other 30 loci, five (rs7566, rs887574, rs13035, rs1799757, and rs3534) are reported for the first time in this study, while 25 were associated with malignant tumor development and the response to chemoradiotherapy (19–33), inflammatory bowel disease (34), autoimmune diseases (35,36), vitiligo (37), insulin resistance (38), coronary heart disease, stroke, and other types of cardiovascular and cerebrovascular disease (39,40).

From this study, we found that alterations at 35 gene loci (TNFRSF1B (rs1061624, rs1061622, rs3397), TP53 (rs16255895), EDN1 (rs1800541, rs2071942), etc.) may be associated with the risk of multiple primary malignancies involving colorectal cancer by performing SNP genotyping assay and comparing with the SNP data of a control Chinese population from HapMap. However, the relationship between differences in allele distributions of these alleles and the development of multiple primary colorectal cancer is still unclear because the current sample sizes are insufficient, and studies also lack comparisons between patients with a single cancer and the normal healthy population, and between phenotypic

Table 3. Description of the 62 genes.

<table>
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<tbody>
<tr>
<td>DNA repair genes</td>
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<tr>
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<td>Chemokine genes</td>
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<td>Cell transcription and translation genes</td>
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<td>mTOR signaling pathways</td>
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<tr>
<td>Gene</td>
<td>Genotyped SNP ID</td>
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<td>TNFRSF1B</td>
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</table>

*P < 0.05 is considered statistically significant.
differences within patients with different SNPs of the same gene. Large-scale scale genetic screening and in vitro and in vivo experiments are therefore required to further explore the molecular mechanisms of mutations at the 35 gene loci involved in the development of multiple primary cancer. Such analyses, which may be expected to identify novel pathogenic genes and the molecular genetic basis of multiple primary malignancies involving colorectal cancer, could provide theoretical guidance for the diagnosis and treatment of multiple primary colorectal cancer.

Acknowledgments
This work was supported by the National Natural Science Foundation of China (Grant Nos. 81301688, 81272192, 81572965); the PhD Programs Foundation of the Ministry of Education of China (Nos. 20130162110050 and 20130162120093); the Natural Science Foundation of Hunan Province (Grant No. 2015J4053); the Post-Doctoral Foundation of Central South University (No.131425); the HuXiang Youth Talent Project (Grant No.2016RS3022); and the 125 Talent Project/New Xiangya Project of the Third Xiangya Hospital of Central South University.

Table 4. Five loci associated with colorectal cancer.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Gene locus</th>
<th>Major allele</th>
<th>Altered allele</th>
<th>The relationship between genes and colorectal cancer</th>
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<tbody>
<tr>
<td>TNFRSF1B</td>
<td>rs1061624</td>
<td>AA</td>
<td>AG/GG</td>
<td>G allele is a protective factor for colorectal cancer</td>
</tr>
<tr>
<td>P21</td>
<td>rs1059234</td>
<td>CC</td>
<td>CT/TT</td>
<td>T allele is a protective factor for colorectal cancer</td>
</tr>
<tr>
<td>CCND1</td>
<td>rs9344</td>
<td>AA</td>
<td>AG/GG</td>
<td>G allele is a protective factor for colorectal cancer</td>
</tr>
<tr>
<td>NFKB1</td>
<td>rs28362491</td>
<td>Ins/Ins</td>
<td>Ins/Del Del/Del</td>
<td>Del allele has an increased risk of colorectal cancer in Danish population</td>
</tr>
<tr>
<td>ERCCI</td>
<td>rs3212986</td>
<td>GG</td>
<td>GT/TT</td>
<td>T allele has an increased risk of colorectal cancer</td>
</tr>
</tbody>
</table>

References


