Research Article

The CIMP Phenotype in BRAF Mutant Serrated Polyps from a Prospective Colonoscopy Patient Cohort

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Colorectal cancers arising via the serrated pathway are often associated with BRAF V600E mutation, CpG island methylator phenotype (CIMP), and microsatellite instability. Previous studies have shown a strong association between BRAF V600E mutation and serrated polyps. This study aims to evaluate CIMP status of all the serrated polyp subtypes and its association with functionally important genes such as MLH1, p16, and IGFBP7. CIMP status and methylation were evaluated using the real-time based MethyLight assay in 154 serrated polyps and 63 conventional adenomas. Results showed that CIMP-high serrated polyps were strongly associated with BRAF mutation and proximal colon. CIMP-high was uncommon in conventional adenomas (1.59%), occurred in 8.25% of hyperplastic polyps (HPs), and became common in sessile serrated adenomas (SSAs) (51.43%). MLH1 methylation was mainly observed in the proximal colon and was significantly associated with BRAF mutation and CIMP-high. The number of samples methylated for p16 and IGFBP7 was the highest in SSAs. The methylation panel we used to detect CIMP is highly specific for CIMP-high cancers. With this panel, we demonstrate that CIMP-high is much more common in SSAs than HPs. This suggests that CIMP-high correlates with increased risk of malignant transformation which was also observed in methylation of functionally important genes.

1. Introduction

Colorectal cancer (CRC) is a commonly occurring cancer with significant mortality in many countries. It is also a heterogeneous disease, which arises from two main groups of precursor lesions or polyps: conventional adenomas (CAs) and serrated polyps. Conventional adenomas are classified into tubular adenoma (TA), tubulovillous adenoma (TVA), and villous adenoma (VA) [1, 2]. Conventional adenomas give rise to CRC via the traditional pathway which is characterised by inactivation of the adenomatous polyposis coli (APC) gene, multiple allelic losses on chromosomes 5q, 17p, and 18q, inactivation of tumour suppressor genes such as p53 and epigenetic DNA hypomethylation [3, 4].

Serrated polyps were initially considered as lesions with no malignant potential; however, studies identified an alternate route to colorectal tumorigenesis arising from serrated polyps via the serrated neoplastic pathway [5–8]. Serrated polyps are classified into hyperplastic polyps (HPs) comprising microvesicular HP (MVHP), goblet cell HP (GCHP),
and mucin poor HP (MPHP), traditional serrated adenoma (TSA), and sessile serrated adenoma (SSA)—with cytological dysplasia and without cytological dysplasia [9, 10]. The colorectal tumours arising from serrated polyps have BRAF mutations, CpG island methylation, and often high levels of microsatellite instability (MSI-H) [11–13].

Hypermethylation is one of the key features of the serrated pathway and causes inactivation of multiple functionally important genes such as MLH1, p16, MGMT, TIMP3, and HLF [13, 14]. Toyota et al. classified methylation patterns in CRC into two categories-type A or age-specific methylation and type C or cancer-specific methylation. In this study, a group of colorectal tumours exhibited high type C methylation levels in 3 or more loci and this condition was described as CpG island methylator phenotype (CIMP). CIMP is an event in colorectal tumorigenesis and has the potential to silence key tumour suppressor genes [13]. CIMP was found to be strongly associated with BRAF mutation, MSI-H, and proximally located colorectal tumours [15, 16].

In 2006, Weisenberger et al. investigated CIMP in greater detail and developed a new panel of methylation markers to more specifically classify colorectal tumours based on their CIMP status. The MethyLight assay, a real-time based PCR technique, was employed to assess the methylation levels in the tumours. This study developed a panel of markers which more accurately defined tumours with high levels of CIMP (CIMP-H) and which were closely associated with BRAF mutation [17, 18]. In polyps, CIMP assessment using nonspecific markers and nonquantitative methylation-specific PCR (MSP) showed that CIMP was frequent in all serrated polyps, except for GCHPs [8]. It is likely that CIMP develops in serrated polyps and plays a role in their progression to malignancy. Although the Weisenberger et al. panel is more specific and quantitative, it has not been used previously to assess CIMP in all the serrated polyp types [8, 17, 19].

The aim of our study was to evaluate CIMP status using the Weisenberger et al. panel and MethyLight assay in a well-characterised series of serrated polyps, which were used in an earlier study [20]. Our hypotheses were that (a) CIMP will be rare in HPs and CAs but will be more prevalent in SSAs which are the immediate precursors to CIMP-H and MSI-H CRC and (b) CIMP will be more prevalent in proximal lesions and (c) will be associated with the methylation of genes whose silencing is thought to play a functional role in the serrated neoplastic pathway (MLH1, p16, and IGFBP7) [21].

2. Materials and Methods

2.1. Patients and Colorectal Polyp Samples. A cohort of 154 serrated polyps and 63 conventional adenomas were used in this study. All of these polyps were obtained from 112 patients derived from a larger consecutive unselected series of 189 patients who underwent colonoscopy at the Royal Brisbane and Women’s Hospital (RBWH) in 2003 as previously described by Spring et al. [20]. All the patients gave written informed consent, and the Human Research Ethics Committee at the RBWH approved the study. Patients with familial adenomatous polyposis (FAP), hereditary nonpolyposis CRC (HNPPC) and hyperplastic polyposis (HPP) were excluded from this study. Polyp location, histology, gender, and size were recorded at the time of collection. The 217 polyps included in this study consisted of 35 sessile serrated adenomas (SSAs), 3 traditional serrated adenomas (TSAs), 7 mixed polyps (M Ps), 59 goblet cell (GC) hyperplastic polyps (H Ps), 50 microvesicular (MV) hyperplastic polyps (H Ps), 11 tubulovillous adenomas (TVAs), and 52 tubular adenomas (TAs). Mixed polyps consisted of 3 HPs with CA (2 GCHP and 1 MVHP), 3 HPs mixed with TSA (2 MVHP and 1 GCHP), and 1 TVA mixed with TSA. The 154 serrated polyps identified from the original series were from 75 patients, whilst the remaining patients (114) had conventional adenomas only. Of the 154 serrated polyps in this study, a further seven GCHPs, four MVH Ps, and one SSA were excluded due to failure of these samples to amplify in the MethyLight assay.

2.2. Methylation Analysis. DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tissues using the Chelex method as described in the Spring et al. study [20]. 500 ng of DNA was subjected to bisulfite modification using EpiTect Bisulfite kit (Qiagen, Limburg, The Netherlands), and the MethyLight assay was used to analyse the methylation levels in bisulfite modified samples [18]. CIMP status was assessed using the Weisenberger et al. panel of CIMP markers-IGF2, SOCS1, NEUROGI, RUNX3, and CACNAI G (Table 1) [17]. The methylation of MLH1, p16, and IGFBP7 was also analysed using the MethyLight assay using the following cycling conditions: ALU, IGFBP7, MLH1, NEUROGI, and CACNAIG: 15 sec at 95°C and 60 sec at 60°C; IGF2, SOCS1, and p16: 10 sec at 95°C and 30 sec at 60°C; and RUNX3: 15 sec at 95°C and 60 sec at 62°C. The level of methylation was calculated based on the percentage of methylated reference (PMR) of each sample with ALU as the standard, and samples with a PMR ≥ 7 were considered to be methylated. Polyps were designated as CIMP-H if three or more of the panel markers were methylated. The methylation and CIMP status of polyps were correlated with BRAF V600E and KRAS mutations, polyp location, size, histology, and patient gender.

2.3. Screening for BRAF V600E and KRAS Mutations. The polyps were screened for BRAF V600E and KRAS mutations (codons 12 and 13) using the allele-specific PCR and the MassARRAY system as previously described [20].

2.4. Statistical Analysis. GraphPad Prism 6 V 6.02 software was used to perform the statistical analysis. Fisher’s exact test and Chi-square test were used to calculate P values (two-tailed) depending on the data analyzed.

3. Results

3.1. CIMP and Polyp Type. Serrated polyps were found to be significantly associated with CIMP-H status (Figure I and
Table 1: Primers and probes for methylation, BRAF, and KRAS mutation analysis.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers</th>
<th>Probe</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ALU</strong></td>
<td>FP: GGTTAGGTATAGGTGTATTATTTTGTAATTT</td>
<td>6FAM-CCITACCTTAACCTCGCC-MGBNFQ</td>
</tr>
<tr>
<td><strong>IGF2</strong></td>
<td>FP: GAGCGGTTCGTTGATTT</td>
<td>6FAM-CCITACCTTAACCTCGCC-MGBNFQ</td>
</tr>
<tr>
<td><strong>SOCS1</strong></td>
<td>FP: GGCTCGATTCGTTGATTT</td>
<td>6FAM-CCITACCTTAACCTCGCC-MGBNFQ</td>
</tr>
<tr>
<td><strong>NEUROGI</strong></td>
<td>FP: CGTGTCGGTTGATTT</td>
<td>6FAM-CCITACCTTAACCTCGCC-MGBNFQ</td>
</tr>
<tr>
<td><strong>RUNX3</strong></td>
<td>FP: TTTTTTCTGGTGTTGATTT</td>
<td>6FAM-CCITACCTTAACCTCGCC-MGBNFQ</td>
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<tr>
<td><strong>CACNA1G</strong></td>
<td>FP: AGGAAGGCGGATACGTTT</td>
<td>6FAM-CCITACCTTAACCTCGCC-MGBNFQ</td>
</tr>
<tr>
<td><strong>MLH1</strong></td>
<td>FP: TAGCCTCAATTCTTACCATCCAC</td>
<td>6FAM-CCITACCTTAACCTCGCC-MGBNFQ</td>
</tr>
<tr>
<td><strong>p16</strong></td>
<td>FP: TGGAGTTTTCGGTTGATTGT</td>
<td>6FAM-CCITACCTTAACCTCGCC-MGBNFQ</td>
</tr>
<tr>
<td><strong>IGFBP7</strong></td>
<td>FP: GTGACTTCTGAACCTCAATTCTT</td>
<td>6FAM-CCITACCTTAACCTCGCC-MGBNFQ</td>
</tr>
<tr>
<td><strong>BRAF</strong></td>
<td>V: GTGATTTCCTATTACCTCAGGCTG</td>
<td>6FAM-CCITACCTTAACCTCGCC-MGBNFQ</td>
</tr>
<tr>
<td><strong>KRAS 189-bp</strong></td>
<td>FP: TCATTATTATTATTAAAGGCTGTT</td>
<td>6FAM-CCITACCTTAACCTCGCC-MGBNFQ</td>
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<tr>
<td><strong>KRAS 92-bp</strong></td>
<td>FP: ttataaGCGTGTAAAATGTAGCTGA</td>
<td>6FAM-CCITACCTTAACCTCGCC-MGBNFQ</td>
</tr>
</tbody>
</table>

Table 2). The SSAs showed the highest percentage of CIMP-H samples (51.43%), while only nine out of 109 HPs (8.26%) were CIMP-H. The number of CIMP-H HPs was similar in GCHPs and MVHPs with 6.78% and 10%, respectively. In TVAs, CIMP-H samples were absent and they accounted for only a minor portion in TAs, one out of 52 TAs (1.92%).

3.2. CIMP and Anatomic Location, Polyp Size, and Gender. CIMP-H was significantly associated with proximal colonic location - 30 out of 112 (26.79%) polyps in the proximal colon were CIMP-H. A majority of the SSAs were proximally located - 27 out of 35 (77.14%); 17 of 27 proximal SSAs were CIMP-H (62.96%). The remaining eight SSAs were located in the distal colon and only one was CIMP-H (12.5%). In the HPs, a similar pattern was observed - all the CIMP-H samples (four GCHPs and five MVHPs) were located in the proximal colon.

Overall, the size of the polyp also had an effect on the CIMP status. CIMP-H increased significantly \((P = 0.0035)\) with an increase in polyp size as shown in Figure 2(b). However, this was not the case for SSAs, which were just as likely to be CIMP-H even if they were small. In the size range of 1–5 mm, six of 12 SSAs (50%) were CIMP-H. In SSAs > 5 mm, 12 of 23 (52.17%) were CIMP-H.

There was no significant association between CIMP-H polyps and gender. Females showed a slightly higher incidence of CIMP-H polyps as seen in Figure 2(c) with 19 CIMP-H samples out of 112 (16.52%), while males had 12 CIMP-H polyps out of a total of 102 (11.76%).

3.3. BRAF Mutant/CIMP-H Status and Polyp Type with respect to Location. As reported in Spring et al., BRAF mutation was strongly associated with MVHPs and SSAs and KRAS mutation was associated with GCHPs and TVAs [20]. The
<table>
<thead>
<tr>
<th>Clinical, pathological, and molecular features</th>
<th>All cases</th>
<th>BRAF mutant</th>
<th>KRAS mutant</th>
<th>CIMP-H</th>
<th>MLH1 methylation</th>
<th>p16 methylation</th>
<th>IGFBP7 methylation</th>
<th>IGFBP7 and p16 methylation</th>
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<tr>
<td>All colorectal polyps</td>
<td>217</td>
<td>83 (38.25%)</td>
<td>51 (23.5%)</td>
<td>31 (14.29%)</td>
<td>8 (3.69%)</td>
<td>45 (20.74%)</td>
<td>74 (34.1%)</td>
<td>20 (9.22%)</td>
</tr>
<tr>
<td>Patient age (years, SD)</td>
<td>62.36 ± 13.54</td>
<td>58.8 ± 13.48</td>
<td>61.67 ± 13.87</td>
<td>64 ± 13.51</td>
<td>71 ± 6.05</td>
<td>63.4 ± 14.17</td>
<td>58.45 ± 13.3</td>
<td>58.8 ± 14.98</td>
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<tr>
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</tr>
<tr>
<td>Female</td>
<td>115</td>
<td>50 (43.48%)</td>
<td>26 (22.61%)</td>
<td>19 (16.52%)</td>
<td>4 (3.48%)</td>
<td>28 (24.35%)</td>
<td>49 (42.61%)</td>
<td>16 (13.91%)</td>
</tr>
<tr>
<td>Male</td>
<td>102</td>
<td>33 (32.35%)</td>
<td>25 (24.51%)</td>
<td>12 (11.76%)</td>
<td>4 (3.92%)</td>
<td>17 (16.67%)</td>
<td>25 (24.51%)</td>
<td>4 (3.92%)</td>
</tr>
<tr>
<td>Location</td>
<td></td>
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<tr>
<td>Proximal</td>
<td>112</td>
<td>44 (39.29%)</td>
<td>18 (16.07%)</td>
<td>30 (26.79%)</td>
<td>7 (6.25%)</td>
<td>38 (33.93%)</td>
<td>43 (38.39%)</td>
<td>18 (16.07%)</td>
</tr>
<tr>
<td>Distal</td>
<td>105</td>
<td>39 (37.14%)</td>
<td>33 (31.43%)</td>
<td>1 (0.95%)</td>
<td>1 (0.95%)</td>
<td>7 (6.67%)</td>
<td>31 (29.52%)</td>
<td>2 (1.9%)</td>
</tr>
<tr>
<td>Polyp type</td>
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<tr>
<td>Serrated polyps</td>
<td>154</td>
<td>82 (53.25%)</td>
<td>40 (25.97%)</td>
<td>30 (19.48%)</td>
<td>8 (5.19%)</td>
<td>35 (22.73%)</td>
<td>62 (40.26%)</td>
<td>19 (12.34%)</td>
</tr>
<tr>
<td>HP</td>
<td>109</td>
<td>48 (44.04%)</td>
<td>35 (32.11%)</td>
<td>9 (8.26%)</td>
<td>1 (0.92%)</td>
<td>16 (14.68%)</td>
<td>35 (32.11%)</td>
<td>4 (3.67%)</td>
</tr>
<tr>
<td>GCHP</td>
<td>59</td>
<td>12 (20.34%)</td>
<td>30 (50.85%)</td>
<td>4 (6.78%)</td>
<td>0</td>
<td>7 (11.86%)</td>
<td>19 (32.2%)</td>
<td>2 (3.39%)</td>
</tr>
<tr>
<td>MVHP</td>
<td>50</td>
<td>36 (72%)</td>
<td>5 (10%)</td>
<td>5 (10%)</td>
<td>1 (2%)</td>
<td>9 (18%)</td>
<td>16 (32%)</td>
<td>2 (4%)</td>
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<tr>
<td>TSA</td>
<td>3</td>
<td>2 (66.67%)</td>
<td>0</td>
<td>1 (33.33%)</td>
<td>0</td>
<td>0</td>
<td>2 (66.67%)</td>
<td>0</td>
</tr>
<tr>
<td>MP</td>
<td>7</td>
<td>4 (57.14%)</td>
<td>2 (28.57%)</td>
<td>2 (28.57%)</td>
<td>0</td>
<td>2 (28.57%)</td>
<td>3 (42.86%)</td>
<td>1 (14.29%)</td>
</tr>
<tr>
<td>SSA</td>
<td>35</td>
<td>28 (80%)</td>
<td>3 (8.57%)</td>
<td>18 (51.43%)</td>
<td>7 (20%)</td>
<td>17 (48.57%)</td>
<td>22 (62.86%)</td>
<td>14 (40%)</td>
</tr>
<tr>
<td>Conventional adenomas</td>
<td>63</td>
<td>1 (1.59%)</td>
<td>11 (17.46%)</td>
<td>1 (1.59%)</td>
<td>0</td>
<td>10 (15.87%)</td>
<td>12 (19.05%)</td>
<td>1 (1.59%)</td>
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<tr>
<td>TA</td>
<td>52</td>
<td>1 (1.92%)</td>
<td>4 (7.69%)</td>
<td>1 (1.92%)</td>
<td>0</td>
<td>6 (11.54%)</td>
<td>10 (19.23%)</td>
<td>1 (1.92%)</td>
</tr>
<tr>
<td>TVA</td>
<td>0</td>
<td>7 (63.64%)</td>
<td>0</td>
<td>0</td>
<td>4 (36.36%)</td>
<td>2 (18.18%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Size</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1–5 mm</td>
<td>144</td>
<td>50 (34.72%)</td>
<td>37 (25.69%)</td>
<td>13 (9.03%)</td>
<td>3 (2.08%)</td>
<td>22 (15.28%)</td>
<td>44 (30.56%)</td>
<td>8 (5.56%)</td>
</tr>
<tr>
<td>&gt;5 mm</td>
<td>73</td>
<td>33 (45.21%)</td>
<td>14 (19.18%)</td>
<td>18 (24.66%)</td>
<td>5 (6.85%)</td>
<td>23 (31.51%)</td>
<td>30 (41.1%)</td>
<td>12 (16.44%)</td>
</tr>
<tr>
<td>BRAF status</td>
<td></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>BRAF mutant</td>
<td>83</td>
<td>0</td>
<td>28 (33.73%)</td>
<td>7 (8.43%)</td>
<td>27 (32.53%)</td>
<td>44 (53.01%)</td>
<td>17 (20.48%)</td>
<td></td>
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<tr>
<td>BRAF wild</td>
<td>134</td>
<td>51 (38.06%)</td>
<td>3 (2.24%)</td>
<td>1 (0.75%)</td>
<td>18 (13.43%)</td>
<td>30 (22.39%)</td>
<td>3 (2.24%)</td>
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<tr>
<td>KRAS status</td>
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<tr>
<td>KRAS mutant</td>
<td>51</td>
<td>0</td>
<td>0</td>
<td>7 (13.7%)</td>
<td>8 (15.69%)</td>
<td>1 (1.96%)</td>
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<tr>
<td>KRAS wild</td>
<td>166</td>
<td>83 (50%)</td>
<td>31 (18.67%)</td>
<td>8 (4.82%)</td>
<td>38 (22.89%)</td>
<td>66 (39.76%)</td>
<td>19 (11.45%)</td>
<td></td>
</tr>
</tbody>
</table>
only TA with a BRAF mutation was a <5 mm polyp occurring in the setting of multiple SSAs and was the only TA to show CIMP-H. There were no TVAs with BRAF mutation or CIMP-H status (Table 2).

BRAF mutations were common in MVHPs-36 BRAF mutant polyps out of 50 and three were BRAF mutant/CIMP-H. The other two CIMP-H MVHPs were wild-type for both BRAF and Kras. Although Kras mutation was predominant in GCHPs and only 20.34% had BRAF mutations, three out of the four CIMP-H samples were BRAF mutant and the remaining CIMP-H sample was wild-type for both BRAF and Kras. TSAs and MPs had the same proportion of BRAF mutant/CIMP-H polyps (50%). SSAs had a higher percentage of BRAF mutant polyps (80%) and had the highest number of BRAF mutant/CIMP-H polyps - 18 out of a total of 28 BRAF mutant samples (64.29%) (see Table S1 in the Supplementary Material available online at http://dx.doi.org/10.1155/2014/374926). Overall, there was a very significant association between BRAF mutation and CIMP-H ($P < 0.0001$) and a negative association between Kras mutation and CIMP-H (Supplementary Table S2).

The distribution of BRAF mutant/CIMP-H in serrated polyps was assessed with respect to anatomic location (Figure 3). In the proximal region, SSAs had a significant ($P = 0.0335$) number of BRAF mutated polyps with 24 out of 28 samples (85.71%), out of which 17 SSAs were CIMP-H (70.83%). The SSAs had the least number of distally located BRAF mutant samples with only four out of 28 polyps (14.29%) and only one BRAF mutant/CIMP-H SSA in the distal colon. In MPs and TSAs, all the BRAF mutant and BRAF mutant/CIMP-H polyps were proximal, except for one BRAF mutant distal MP. Although BRAF mutant HPs were more common distally, only proximal HPs showed CIMP-H (Figure 3).

3.4. MLH1 Methylation. MLH1 methylation was not observed in conventional adenomas and was present in only one MVHP out of 109 HPs (2%). The SSAs had the highest number of MLH1 methylated samples with seven out of 35 (20%) and it was absent in MPs and TSAs. A significant association between methylation of MLH1 and CIMP-H status was observed ($P = 0.0001$). BRAF V600E mutation was also significantly associated with MLH1 methylation ($P = 0.0056$) and was present in seven out of eight MLH1 methylated samples. All the polyps with MLH1 methylation were located in the proximal region except for the one MVHP.

3.5. Methylation of p16 and IGFBP7. The number of p16 methylated samples was the highest in SSAs with 17 of 35 (48.57%) [Figure 4(a)]. In the conventional adenomas only ten samples were methylated for p16 (15.87%) with the majority being TAs. The levels of IGFBP7 methylation were similar to the p16 methylation analysis with the highest number of IGFBP7 methylated samples in the SSAs with 22 out of 35 samples (62.86%) followed by HPs-35 IGFBP7 methylated samples out of 109 (32.11%). In conventional adenomas, only 10 out of 63 samples (19.01%) were methylated for IGFBP7 [Figure 4(b)].

3.6. p16 and IGFBP7 Methylation in BRAF Mutant and CIMP-H Polyps. BRAF mutant SSAs had the highest number of p16 methylated (53.57%), IGFBP7 methylated (71.43%), and p16/IGFBP7 methylated (46.43%) samples [Figure 5(a)]. In the BRAF mutant HPs, 20 out of 48 samples (41.67%) were IGFBP7 methylated, but only nine out of 48 samples were methylated for p16. The combined p16/IGFBP7 methylation percentage in the HPs was quite low with only two methylated samples compared to the SSAs with combined p16/IGFBP7 methylation in 13 out of 28 BRAF mutant polyps. The analysis of p16 and IGFBP7 methylation in CIMP-H polyps was shown in Figure 5(b). In CIMP-H SSAs, 77.78% were methylated for p16 and IGFBP7 and combined p16/IGFBP7 methylation was 66.67%. In the CIMP-H HPs, out of nine samples, six and four samples were methylated for p16 and IGFBP7, respectively (Supplementary Table S1). IGFBP7 methylation was also common in CIMP-negative HPs.

4. Discussion

Toyota et al. identified the existence of CIMP in colorectal tumours and preneoplastic adenomatous polyps [13, 22]. There is also a significant association between CIMP-H, MSI, and BRAF mutations in colorectal cancers [23]. In colorectal polyps, CIMP was significantly associated with serrated polyps. CIMP-H conventional adenomas constituted a minor proportion and were dependent on the polyp size (>2 cm) and percentage of villous component (>80%) [24, 25]. A variety of methylation markers have been employed to determine CIMP status in polyps including the Toyota et al. and Weisenberger et al. panels, HIC1, MGMT, and RASSF2.
Figure 2: Correlation between CIMP and location, polyp size, and gender. CIMP-H polyps displayed a significant correlation with proximal location (a) and large polyp size (>5 mm) (b). However, there was no significant correlation between CIMP-H and gender, although females had a higher number of CIMP-H polyps than males (c).

Figure 3: BRAF mutation and CIMP in serrated polyps. A comparative analysis of the distribution of BRAF mutant/CIMP-H samples in serrated polyps with respect to the anatomical location in the human colon.
and increase in polyp size. This study shows a progressive increase in CIMP as the malignant potential of serrated polyps increases (HP versus SSA). CIMP seems more likely to develop when there is a BRAF mutation and the polyp is in the proximal colonic environment. SSAs had the highest number of CIMP-H polyps (51.43%), while the HPs and CAs showed a very low frequency of CIMP-H with 8.25% and 1.59%, respectively. A majority of other studies showed that SSAs were predominantly CIMP-H, followed by HPs with a higher frequency compared to our study. This could be due to the classification of serrated polyps based on CIMP-H status using MSP and the Toyota et al. methylation markers [8, 19, 32].

In our study, 62.96% of proximal SSAs were CIMP-H and only one CIMP-H SSA was located in the distal colon, while all the CIMP-H HPs were proximal. This was similar to other reports where a majority of CIMP-H SSAs and HPs were proximally located [19, 27, 33]. We found that CIMP-H was significantly associated with BRAF mutant serrated polyps and a negative association existed between KRAS mutation and CIMP-H which was also observed in studies by O’Brien et al. and Yang et al. [19, 29]. Based on our results and similar
studies, BRAF mutant, CIMP-H SSAs which are proximally located provide further support for SSAs as potential precursors of BRAF mutant, CIMP-H CRCs. A recent retrospective CIMP study by Burnett-Hartman et al. was carried out on 359 serrated polyps using the Weisenberger et al. methylation panel and MethylLight assay. Results showed that CIMP-H was more prevalent in SSAs, proximal colon, and BRAF mutant serrated lesions, which confirms our findings [31].

MLH1 and p16 along with MINT1, MINT2, and MINT31 constitute the Toyota et al. panel [13]. p16 and MLH1 methylation have been evaluated in numerous studies in colorectal tumours and polyps. In tumours, MLH1 methylation showed a significant association with CIMP, MSI-H and was mainly localized to the proximal colon [13, 34]. In HPs, 3–21% of the samples were methylated for MLH1, while the frequency varied from 16 to 23.2% in SSAs [27, 32, 35, 36]. Only 7% of the TAs were methylated for MLH1, while this was completely absent in the TVAs [25, 35]. Out of 217 polyps in our study, only 3.69% of the samples were methylated for MLH1 and all the samples were proximally located except for one MVHP. MLH1 methylation was detected in 4% of HPs and 17% of SSAs and was completely absent in conventional adenomas and these results were found to be similar to reports discussed above [27, 31].

p16 methylation is also closely linked to CIMP-H and MSI-H CRCs [13, 37, 38]. The results from our study indicated that p16 methylation was correlated with serrated polyp type, proximal location, BRAF V600E mutations, and CIMP-H status. In a study by Dhir et al., p16 along with MLH1, CDX2, and TLR2 showed the highest frequency of methylation in SSAs based on an unsupervised clustering analysis [39]. A similar trend was observed in other studies with the frequency of p16 methylation being the highest in SSAs with 76.8%, while it was 48% in TSAs and 29% in TAs [24, 32]. Our results showed a comparatively lower percentage of p16 methylated samples in SSAs (48.57%) and TAs (11.53%). There was no increase in p16 methylation from TAs to TVAs as was observed by Psofaki et al. [38].

IGFBP7 has been implicated as an early event in CRC progression via the serrated pathway. Methylation of IGFBP7, a potential tumour suppressor gene, was also evaluated in different polyp types. Similar to p16 methylation, IGFBP7 methylated polyps were mainly serrated, closely linked to BRAF V600E mutations and CIMP-H status. A study by Suzuki et al. also observed similar correlations between IGFBP7 methylation and BRAF mutation and p16 methylation and CIMP and an inverse correlation with KRAS mutation in colorectal tumours. IGFBP7 methylation was also detected in 18% of conventional adenomas, which was similar to our results (19%) [40]. In a recent study by Kaji et al., it was proposed that IGFBP7 methylation did not affect HPs due to lower PMRs when compared to TSAs or SSAs [41]. This was also observed in our study with only 32.11% of HPs methylated for IGFBP7 which was lower than TSAs (66.67%) and SSAs (62.86%) [40, 41].

5. Conclusion

To date, the serrated pathway has not yet been fully characterised. Our study showed that CIMP-H was significantly associated with serrated polyps, mainly the SSA subgroup. Only a minority of HPs and CAs were CIMP-H. All the CIMP-H HPs and a significant proportion of CIMP-H SSAs were located in the proximal colon. There was a strong association between BRAF V600E mutation and CIMP-H polyps. Methylation of the functionally important genes MLH1, p16, and IGFBP7 was the highest in SSAs and was associated with BRAF V600E mutation, CIMP-H, and proximal location. Our study using a stringent definition of CIMP has highlighted the association of increasing levels of methylation in serrated polyps as their malignant potential increases. The application of advanced methodology such as methylation and expression arrays will help to evaluate more genes/markers and hopefully contribute towards a more defined understanding of the various changes causing the transformation of a serrated precursor lesion into CRC.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References


H. Suzuki, S. Igarashi, M. Nojima et al., “IGFBP7 is a p53-responsive gene specifically silenced in colorectal cancer with...
