Methylation Patterns In Dysplasia
In Inflammatory Bowel Disease Patients

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Methylation Patterns
In Dysplasia
In Inflammatory Bowel Disease Patients

Overview

1. Introduction
2. Aim
3. Methods
4. Results
5. Discussion/Conclusion
6. Questions
Inflammatory bowel disease (IBD) with colonic involvement increases colorectal cancer (CRC) risk.

Colitis-related dysplasia differs from conventional dysplasia, and both may occur in IBD patients.
## Methylation Patterns in Dysplasia in Inflammatory Bowel Disease Patients

### 1. Introduction

<table>
<thead>
<tr>
<th>Colitis-related dysplasia</th>
<th>Conventional dysplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammation &gt; Dysplasia &gt; Carcinoma</td>
<td>Adenoma &gt; Carcinoma</td>
</tr>
<tr>
<td>Multifocal</td>
<td>Focal</td>
</tr>
<tr>
<td>Non adenoma-like</td>
<td>Adenoma-like</td>
</tr>
<tr>
<td>TP53 mutation frequent / initiating</td>
<td>TP53 mutation frequent / late stage</td>
</tr>
<tr>
<td>Low rate of KRAS and APC mutations</td>
<td>High rate of APC (initiating) and KRAS mutations</td>
</tr>
</tbody>
</table>
Methylation Patterns In Dysplasia In Inflammatory Bowel Disease Patients

1. Introduction

Dysplasia in IBD patients

Colitis-related dysplasia

- Endoscopic resection: Polypoid +/- Flat lesions
- Colectomy: Flat lesions

Conventional dysplasia

- Managed as general population
Good marker specific for the type of dysplasia (Colitis-related / Conventional)
Some data favors the importance of abnormal DNA methylation in colitis-related carcinogenesis.

Hypothesis: Lesions that evolved through different pathways would show different methylation patterns.

Aim: Identify methylation markers that could later be prospectively evaluated.
Patients with colonic IBD

Samples of colonic mucosa

Paraffin-embedded biopsies and surgical specimens

3. Methods

Methylation Patterns In Dysplasia In Inflammatory Bowel Disease Patients

A: With dysplasia/CRC
B: <10cm from dysplasia/CRC, without IBD-colitis
C: >10cm from dysplasia/CRC, with inactive IBD-colitis
D: >10cm from dysplasia/CRC, with active IBD-colitis
E: >10cm from dysplasia/CRC, without IBD-colitis

Inclusion criteria:
A + (B and/or C and/or D and/or E)
3. Methods

Methylation Patterns In Dysplasia In Inflammatory Bowel Disease Patients

Patients with colonic IBD

Samples of colonic mucosa
Paraffin-embedded biopsies and surgical specimens

Without dysplasia/CRC (B, C, D, E)

With dysplasia/CRC (A)

Colitis-related (Cases)
Random biopsies of flat mucosa
Non adenoma-like lesion

Conventional (Controls)
Dysplasia in non-IBD affected area
Adenoma-like lesion
3. Methods

Methylation Patterns In Dysplasia In Inflammatory Bowel Disease Patients

Patients with colonic IBD

Samples of colonic mucosa

With dysplasia/CRC (A)
  - Conventional (Controls)
  - Colitis-related (Cases)

Without dysplasia/CRC (B, C, D, E)

Methylation patterns of CpG islands in the promoter regions of 67 genes implicated in CRC carcinogenesis

RUNX3, CDH1, SOCS1, ESR, CDKN2A, APC, SFRP1, MLH1, TGFβ, BMP, WNT, IGF2, RARB, ESR1, CHFR, CDH13, WT1, GATA5, WIF1, TIMP3, MSH6, MSH3, CRABP1, TP73, RARB, CDH13, PAX5, WT1, THBS1, TP53, SFRP1, WIF1, APAF1, BCL2, ...
Methylation Patterns In Dysplasia In Inflammatory Bowel Disease Patients

4. Results

Patients with colonic IBD

N = 35
29 UC, 6 CD

Samples

N = 91
2-6 samples per patient

No dysplasia/CRC (B, C, D, E)

N = 56

Dysplasia/CRC (A)

N = 35

Colitis-related (Cases)

N = 9

Conventional (Controls)

N = 26

Methylation patterns of promotors of 67 genes involved in CRC carcinogenesis

N = 56/91

No adenoma-like and non-adenoma-like lesions in the same patient.
4. Results

Methylation Patterns In Dysplasia In Inflammatory Bowel Disease Patients

<table>
<thead>
<tr>
<th>Patient’s characteristics</th>
<th>Colitis-related dysplasia/CRC (index lesion)</th>
<th>Significance (X²/Exact/T tests)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>9</td>
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<tr>
<td>Diagnosis</td>
<td>Crohn’s Disease</td>
<td>4</td>
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<tr>
<td></td>
<td>Ulcerative Colitis</td>
<td>22</td>
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<tr>
<td>Age at IBD diagnosis</td>
<td>46.73 +/- 13.81 (22-65)</td>
<td>29.22 +/- 16.08 (14-64)</td>
</tr>
<tr>
<td>Age at dysplasia/cancer diagnosis</td>
<td>58.50 +/- 12.92 (31-84)</td>
<td>47.67 +/- 13.30 (30-71)</td>
</tr>
<tr>
<td>Active inflammation in the dysplasia/CRC area</td>
<td>No</td>
<td>22</td>
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<tr>
<td></td>
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</tr>
</tbody>
</table>

Patients with colitis-related dysplasia/CRC were **younger** at IBD diagnosis and dysplasia/CRC diagnosis.

Colitis-related dysplasia/CRC were more common in areas with **active inflammation**.
4. Results

Methylation Patterns In Dysplasia
In Inflammatory Bowel Disease Patients

67 genes involved in CRC carcinogenesis

23 without promoters’ hypermethylation

44 with promoters’ hypermethylation
4. Results

44 genes with promoters’ hypermethylation

7 More common in dysplastic/CRC (A) vs not-dysplastic samples (B.C.D.E) (p< 0.001 – p= 0.043)
IGF2. RARB. ESR1. CHFR. WT1. GATA5. WIF1

15 More common in high-grade dysplasia/CRC (subset of A) vs LG-dysplastic/not-dysplastic samples (B.C.D.E) (p= 0.001 – p= 0.038)
MSH6. MSH3. MGMT. RUNX3. CDKN2A. IGF2. RARB. ESR1. CADM1. TIMP3. PAX5. PAX6. WT1. THBS1. SFRP1

15 More common in active-IBD (D) vs inactive or non-affected IBD (B.C.E) (p= 0.001 – p= 0.038)
MSH6. MSH3. RUNX3. CRABP1. TP73. RARB. CDH13. PAX5. WT1. THBS1. TP53. SFRP1. WIF1. APAF1. BCL2
4. Results

**Methylation Patterns In Dysplasia In Inflammatory Bowel Disease Patients**

**44 genes with** promoters’ hypermethylation

Aim: Identify methylation markers (for dysplasia pathways in IBD patients) that could later be prospectively evaluated.

(MSH6, TIMP3)

More common in colitis-related dysplastic/CRC (subset of A) than conventional dysplastic/CRC samples (subset of A) (p= 0.002 and p= 0.012)

**MSH6**

Multivariate analysis (p= 0.029)
<table>
<thead>
<tr>
<th>Gene</th>
<th>Methylation</th>
<th>Inflammation</th>
<th>Significance (X²/Exact)</th>
<th>Dysplasia</th>
<th>Significance (X²/Exact)</th>
<th>HGD/cancer</th>
<th>Significance level (X²/Exact)</th>
<th>IBD-related dysplasia/cancer</th>
<th>Significance (X²/Exact)</th>
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<td></td>
<td></td>
<td>No</td>
<td>Yes</td>
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<td>MSH6</td>
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<td>64</td>
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<td>38</td>
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<td>MGMT</td>
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<td>67</td>
<td>21</td>
<td>p=0.569</td>
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<td>12</td>
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<td>CDKN2A</td>
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<td>CRABP1</td>
<td>No</td>
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<td>CACNA1G</td>
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</tr>
</tbody>
</table>
DNA methylation occurs in genes implicated in CRC carcinogenesis

Inflammation alters DNA methylation in IBD mucosa

Methylation of \textit{MSH6} promoter region is significantly associated to colitis-related dysplasia/CRC, irrespective of the grade (multivariate analysis).

15\% of colitis-related CRC carcinogenesis is MSI, some cases due to MSH6\textsuperscript{1}

\textsuperscript{1}Harpaz and Polydorides (2010).
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5. Discussion/Conclusion

Methylation of MSH6 promoter region

may contribute to the classification of dysplastic lesions in IBD patients and eventually identify patients at risk for multifocal dysplastic/cancer lesions.
THANK YOU!

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