Hypermethylation of Hedgehog signalling regulatory protein \textit{TUBB6} is a highly sensitive and specific biomarker for ulcerative colitis associated neoplasia

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Declarations & conflicts of interest

• I declare I have no conflicts of interest

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  • Cancer Research UK
  • Academy of Medical Sciences
  • Illumina UK
The clinical problem

- UC is associated with a significantly higher than background risk of colorectal cancer
  - Recent meta-analysis by Jess et al demonstrated 2.4x increased risk of colorectal cancer
  - Increases with severity of colitis
The clinical problem

Normal epithelium → Hyperproliferative epithelium → Adenoma → Invasive Carcinoma

- APC
- k-RAS, SMAD2/4, BRAF
- p53

Promoter methylation
Microsatellite instability
Current screening regimes for UC associated cancer

- British Society of Gastroenterology guidelines suggest:
  - Serial colonoscopies at defined time points
  - Using dye spray if at all possible
  - Serial biopsies every 5-10cm
  - Goal is to identify areas of dysplasia
Previous biomarker attempts

- Multiple different methods attempted:
  - FISH for chromosomal number
  - TP53 mutation screening
  - γ-H2AX immunohistochemistry
  - Telomere length
  - HPLC for 8-hydroxydeoxyguanosine
Aims of study

1. To carry out a genome-wide methylation study of UC associated dysplasia and neoplasia

2. To develop a potential methylation biomarker based on these results

3. To validate this biomarker on a separate sample set
Methodology – discovery cohort

- Sample set chosen consisting of:
  1. Control normal mucosa from patients without UC
  2. Normal mucosa from patients with UC who developed cancer
  3. UC associated cancer from patients in (2)

- Analysed on Illumina HumanMethylation450 array platform

- Bayesian statistical methodology
Results – discovery cohort

- Five top differentially methylated biomarkers identified

<table>
<thead>
<tr>
<th>Gene</th>
<th>Log (Fold Change)</th>
<th>Bonferroni adjusted P-value</th>
<th>Bayes factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZNF583</td>
<td>5.82</td>
<td>0.001</td>
<td>8.11</td>
</tr>
<tr>
<td>KIF5C</td>
<td>3.99</td>
<td>0.001</td>
<td>7.69</td>
</tr>
<tr>
<td>PIK3R5</td>
<td>4.15</td>
<td>0.001</td>
<td>7.47</td>
</tr>
<tr>
<td>RNF150</td>
<td>2.67</td>
<td>0.031</td>
<td>6.12</td>
</tr>
<tr>
<td>TUBB6</td>
<td>2.51</td>
<td>0.031</td>
<td>5.67</td>
</tr>
</tbody>
</table>
Methodology – validation cohort

- Validation cohort consisted of multiple tissue types:
  1. Control normal mucosa, no hx UC (n=6)
  2. Acute colitis (n=17)
  3. Chronic > 3yrs colitis (n=23)
  4. “Normal” mucosa from colons of patients with dysplasia or neoplasia (n=19)
  5. UC associated dysplasia (n=10)
  6. UC associated cancers (n=16)
### Results – validation cohort

<table>
<thead>
<tr>
<th>Condition</th>
<th>ZNF583</th>
<th>KIF5C</th>
<th>PIK3R5</th>
<th>RNF1</th>
<th>TUBB6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normal tissue</strong></td>
<td>2%</td>
<td>2%</td>
<td>3%</td>
<td>2%</td>
<td>8%</td>
</tr>
<tr>
<td><strong>Acute colitis</strong></td>
<td>2%</td>
<td>2%</td>
<td>3%</td>
<td>4%</td>
<td>8%</td>
</tr>
<tr>
<td><strong>Chronic colitis</strong></td>
<td>2%</td>
<td>7%</td>
<td>10%</td>
<td>2%</td>
<td>11%</td>
</tr>
<tr>
<td><strong>Field mucosa</strong></td>
<td>5%</td>
<td>12%</td>
<td>5%</td>
<td>1%</td>
<td>20%</td>
</tr>
<tr>
<td><strong>Dysplasia</strong></td>
<td>5%</td>
<td>12%</td>
<td>1%</td>
<td>3%</td>
<td>21%</td>
</tr>
<tr>
<td><strong>Cancer</strong></td>
<td>12%</td>
<td>14%</td>
<td>8%</td>
<td>5%</td>
<td>26%</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>0.23</td>
<td>0.11</td>
<td>0.76</td>
<td>0.98</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Results – validation cohort

Plot showing methylation at TUBB6 by tissue type

- Normal colon
- Acute colitis
- Chronic colitis
- Field mucosa
- Dysplasia
- Cancer

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University of Birmingham
Results – TUBB6

- Tubulin, beta 6 class V
- Located on 8p11.21
- Multiple studies have implicated decreased expression of TUBB6 as a marker of malignancy:
  - Chao et al, Cytoskeleton 2012
  - Mariani et al, Clin Can Res 2012
  - Leandro-Garcia et al, Cytoskeleton 2012
- Negative regulator of Shh (sonic hedgehog) pathway
Results – model performance

• ROC 0.86

• Sensitivity 70.1%, Specificity 98.7%

• PPV 85.4%, NPV 96.7%

• Post test odds of positivity 5.84

Area under ROC curve = 0.8638
Results – model performance

• Repeated on new set of patients to assess if model valid
  • Dysplasia (n=15)
  • Cancer (n=2)
  • Field mucosa (n=32)
  • Normal control mucosa (n=38)

• Similar model performance in predicting dys/neoplasia
  • Sensitivity 75%, Specificity 97.6%
  • PPV 78%, NPV 97%
  • ROC 0.86 (95% CI 0.80-0.93)
Conclusions

• Whole genome methylation analysis to identify biomarkers has been shown to be robust.

• Hypermethylation of *TUBB6* seems to be a highly accurate biomarker for the detection of UC associated dysplasia/neoplasia.

• It can detect that a patient has dysplasia/neoplasia somewhere in their colon based on a biopsy of “normal” mucosa.
Next steps

• Clinical trial:
  • Prospective blinded study of the accuracy of methylated biomarkers in predicting dysplasia
  • “ENDCAP-C” currently underway

• Other possibilities:
  • Detection of methylated biomarker in stool/plasma
  • We have validated a methodology in lab and have a working assay
Acknowledgements

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Department of Surgery

• Prof Dion Morton

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