The silence of the genes: clinical applications of (colorectal) cancer epigenetics

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Colorectal cancer

• Major health problem in the western world
  • Second leading cause of cancer death

• Most studied and best characterised cancer type

• Hereditary forms of colorectal cancer

• Well described model systems (in vitro and in vivo)
Colorectal cancer is a multistep process

Adapted from: Fearon & Vogelstein, Cell 1990
Posttranslational histone modifications
Spatial chromatin organisation at GATA4 locus

Tiwari et al., PLOS Biology 2008
Nucleosomal occupancy *MLH1* locus

Lin et al., Cancer Cell 2007
Initial consequence: transcriptional silencing

Normal tissue

Methylated promoter

Unmethylated promoter

Cancer tissue

Methylated promoter

Unmethylated promoter

Jones and Baylin, Nat Rev Genet, 2002
DNA METHYLATION

NORMAL CELL

Correct Organization of Chromatin in Active and Inactive States
X-Chromosome Inactivation
Silencing of Parasitic Sequences

CANCER CELL

Hypermethylation of CpG Islands of Tumour Suppressor Genes
Disruption of the p16INK4a/Rb, p53/p14ARF and APC/β-catenin Pathways
Defects in Mutation Repair Networks (hMLH1, BRCA1, MGMT) and Production of Mutations Loss of Apoptosis and Adherence Mechanisms

Global Genomic Hypomethylation
Chromosomal Instability Aneuploidy
Activation of Transposons Gene Up-Regulation

Persistence of m5C Residues
Generation of Spontaneous m5C to T Mutations

Tissue Specific-Methylation
Genetic Imprinting
CpG island methylator phenotype (CIMP)

- Subgroup of CRCs with significantly more methylation
  - Older age
  - Female
  - Proximal location
  - Mucinous differentiation
  - Specific precursor lesions (serrated adenomas)
  - Smoking
  - Bad prognosis
Why study DNA methylation?

• Methylation as a process (causes)
• Methylation to define pathways involved in tumorigenesis (consequences)
• Methylation as a marker for the presence of cancer cells (applications)
• Methylation as a target for clinical intervention (interference)
5-methylcytosine: the fifth base

- Molecular weight
- Sodium bisulfite mediated conversion to uracil
- Restriction enzymes
- Gene expression
Expression based detection of methylated tumor suppressor genes

5-methylcytosine → methylated CRC cell line

Demethylation by inhibition of DNMTs:
- chemically (5-azacytidine)
- genetically (KO of DNMT1/3b)
Rhee et al., Nature 2002

cytosine

demethylated CRC cell line

Suzuki et al., Nature Genetics 2002
Genome-wide screen for hypermethylated genes in CRC

Schuebel et al., PLOS Genetics, 2007
The Consensus Coding Sequences of Human Breast and Colorectal Cancers


The elucidation of the human genome sequence has made it possible to identify genetic alterations in cancers in unprecedented detail. To begin a systematic analysis of such alterations, we determined the sequence of well-annotated human protein-coding genes in two common tumor types. Analysis of 13,023 genes in 11 breast and 11 colorectal cancers revealed that individual tumors accumulate an average of ~90 mutant genes but that only a subset of these contribute to the neoplastic process. Using stringent criteria to delineate this subset, we identified 189 genes (average of 11 per tumor) that were mutated at significant frequency. The vast majority of these genes were not known to be genetically altered in tumors and are predicted to affect a wide range of cellular functions, including transcription, adhesion, and invasion. These data define the genetic landscape of two human cancer types, provide new targets for diagnostic and therapeutic intervention, and open fertile avenues for basic research in tumor biology.
Schuebel et al., PLOS Genetics, 2007
N-Myc Downstream-Regulated Gene 4 (NDRG4): A Candidate Tumor Suppressor Gene and Potential Biomarker for Colorectal Cancer

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Hospital-based series†</th>
<th>Population-based series‡</th>
</tr>
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<tbody>
<tr>
<td><strong>TNM stage§</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>11/12 (92)</td>
<td>30/42 (71)</td>
</tr>
<tr>
<td>II</td>
<td>23/28 (82)</td>
<td>42/57 (74)</td>
</tr>
<tr>
<td>III</td>
<td>29/32 (91)</td>
<td>39/56 (70)</td>
</tr>
<tr>
<td>IV</td>
<td>8/11 (72)</td>
<td>17/21 (81)</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>.431</td>
<td>.790</td>
</tr>
<tr>
<td><strong>Tumor location</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal</td>
<td>34/39 (87)</td>
<td>47/58 (81)</td>
</tr>
<tr>
<td>Distal</td>
<td>37/42 (89)</td>
<td>81/118 (69)</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>1.00</td>
<td>.141</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>34/41 (83)</td>
<td>71/95 (75)</td>
</tr>
<tr>
<td>Female</td>
<td>37/42 (88)</td>
<td>57/81 (70)</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>.548</td>
<td>.611</td>
</tr>
<tr>
<td><strong>Age at diagnosis, y</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤70</td>
<td>30/32 (94)</td>
<td>83/117 (71)</td>
</tr>
<tr>
<td>&gt;70</td>
<td>41/51 (80)</td>
<td>45/59 (76)</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>.117</td>
<td>.453</td>
</tr>
</tbody>
</table>
Melotte et al., JNCI 2009
Clinical applications
Colorectal cancer (CRC)

- Global economic burden: $14-22 billion yearly
  - (hospitalization, chemo- and radiation therapy and related side-effects, supportive care,…)

- Early stage CRCs: well treatable and often cured by surgical resection
  - Cure rates (5-years post-diagnosis):
    - >90% for early stage disease
    - < 5% for advanced disease

- CRC screening tests will reduce:
  - > 50% of CRC-related morbidity and mortality
  - a significant part of the health care costs related to treatment of terminal patients
Current screening tools (people > 50 years of age/high risk individuals)

- Colonoscopy/sigmoidoscopy
  - High sensitivity and specificity
  - Small risks of complications: bleeding and bowel perforation
  - Burdening diagnostic procedure (bowel preparation) → low participation level
  - Capacity problems

  → preselection for colonoscopy is necessary

- Fecal Occult blood test (FOBT)
  - Lack of sensitivity and specificity

- Non-invasive (blood/stool) molecular marker based screening test?
Power and promise of methylation markers

- DNA methylation is a stable source of molecular diagnostic information
- Single (multiplex) assay using sensitive Q-MSP (tumor derived free DNA (serum, stool etc.)
- High throughput analysis
Promoter CpG island methylation markers in stool

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Sensitivity* (stool)</th>
<th>Specificity (stool)</th>
</tr>
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<tbody>
<tr>
<td>GATA4: training set¹</td>
<td>20/28 (71%)</td>
<td>38/45 (84%)</td>
</tr>
<tr>
<td>GATA4: test set¹</td>
<td>24/47 (51%)</td>
<td>28/30 (93%)</td>
</tr>
<tr>
<td>NDRG4: training set²</td>
<td>17/28 (61%)</td>
<td>42/45 (97%)</td>
</tr>
<tr>
<td>NDRG4: test set²</td>
<td>25/47 (53%)</td>
<td>30/30 (100%)</td>
</tr>
<tr>
<td>TFPI2: training set³</td>
<td>23/26 (89%)</td>
<td>35/45 (78%)</td>
</tr>
<tr>
<td>TFPI2: test set³</td>
<td>35/46 (76%)</td>
<td>28/30 (93%)</td>
</tr>
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Hellebrekers et al., Clinical Cancer Research 2009¹; Melotte et al., JNCI 2009²; Glockner et al., Cancer Research 2009³
Methylation markers for detection of CRC in blood

<table>
<thead>
<tr>
<th>Sample groups (plasma training set 2)</th>
<th>Plasma gene panel: OSMR, GATA5, NDRG4 and ADAM23</th>
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<tbody>
<tr>
<td></td>
<td>Optimized for sensitivity</td>
</tr>
<tr>
<td></td>
<td>Sensitivity % (# detected / # total) [95% CI]</td>
</tr>
<tr>
<td>Early stages CRC: 0, I, and II</td>
<td>70% (23/33) [54-86]</td>
</tr>
<tr>
<td>All stages CRC</td>
<td>74% (54/73) [64-84]</td>
</tr>
<tr>
<td>Adenomas</td>
<td>10% (4/39)</td>
</tr>
<tr>
<td>Controls</td>
<td>92% (6/77) [80-98]</td>
</tr>
</tbody>
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Conclusions

• Epigenetic screens have identified novel CRC candidate tumor suppressor genes

• Methylated tumor suppressor genes are promising biomarkers for non-invasive detection of CRC
  • Multi-marker assay
  • Sensitive assay (nano-technology)

• Cost-effectiveness analysis

• Causes of altered methylation need to be unraveled in order to understand CRC biology and to interfere with this process
Causes of altered DNA methylation

- Genetic alterations
  - Oncogenic signaling (RAS, MYC)
  - Double strand DNA breaks (SIRT1)
  - SNPs/mutations in genes encoding epigenetic ($DNMT$, $SMYD3$) and folate metabolizing ($MTHFR$, $MTR$) enzymes

- Inflammation

- Lifestyle
  - Carcinogens (smoking etc.)
  - Diet (methyl donor intake)
  - Energy balance