Molecular biology of colorectal cancer

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Centenary Professor of Pathology
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Rapid pace of molecular change

Sequencing changes

2000

100,000 Genomes Project

“it is crucial that we continue to push the boundaries and this new plan will mean we are the first country in the world to use DNA codes in the mainstream of the health service”
The Rt Hon David Cameron MP
The Prime Minister
10 December 2012
Which technologies should we use?

- Genomics - DNA/RNA
- Proteomics
- Metabolome
- Pathways
- Phenotype

- Test
  - Fit for purpose
  - Cost
  - Changing
Molecular changes in bowel cancer

- DNA
  - Mutations – driver and passenger
  - Amplifications
  - Deletions
  - Chromosomal instability,
  - Microsatellite instability
  - Fusion genes
  - Methylation
Increasing complexity with time

Gradual increase in genomic changes
- Increasing mutations
- Increasing genomic instability
- Classical adenomas, Serrated pathway, mismatch repair
- Identification of subtypes

Early
- APC, Ras, PIK3CA, BRaf

Later
- p53, SMAD4 DNA repair, Copy number
Clinical uses

- Genetic syndromes
- Outcomes
  - DNA
    - Deficient mismatch repair
    - Kiras/Braf/PIK3CA
  - RNA –
    - oncotype Dx ?
    - RNA subtypes?

- Prediction of response
  - Anti-EGFr abs
    - Ras
    - Epiregulin/amphiregulin
    - Her3
  - Braf inhibitor
    - Braf
  - Her2
  - Aspirin PIK3CA
  - Immunotherapy
    - dMMR and anti PD1/PDL1
# Genetic syndromes

## Colorectal cancer syndromes

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Genes</th>
<th>Mode of inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lynch syndrome</td>
<td><em>MLH1, MSH2, MSH6, PMS2, or EpCAM</em></td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>(Attenuated) Familial adenomatous polyposis</td>
<td><em>APC</em></td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>MUTYH-associated polyposis</td>
<td><em>MUTYH (MYH)</em></td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>Peutz-Jeghers syndrome</td>
<td><em>LKB1 (STK11)</em></td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>Juvenile polyposis syndrome</td>
<td><em>SMAD4 (~30%) BMPR1A (~20%)</em></td>
<td>Autosomal dominant</td>
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<tr>
<td>Hereditary mixed polyposis syndrome</td>
<td><em>GREM1</em></td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>Serrated polyposis syndrome</td>
<td>unknown</td>
<td>unknown</td>
</tr>
</tbody>
</table>

Polymerase proof reading associated polyposis

| *POLE & POLD1* | Autosomal dominant |

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3% 1%
• HNPCC 3%
• Germ line mutations in DNA repair genes leads to hyper mutation
• Inactivates key genes leading to CRC and other cancers
• Truncated proteins appear on cell surface – immune response
• Screening via MSI or immunohistochemistry
  – dMMR proteins
    • hMSH2 (40%), hMLH1 (40%), hMSH6 (10%), PMS2 (6%) POLD (1%) and POLE (1%)
  – BRAF V600E wild type
• Germ line sequencing
The base case results in the economic evaluation suggest that screening for LS in CRC patients using IHC, BRAF V600E and MLH1 methylation testing would be cost-effective at a threshold of £20,000 per quality-adjusted life year (QALY). The incremental cost-effectiveness ratio for this strategy is £11,008 per QALY compared to no screening. Screening without tumour tests is not predicted to be cost-effective (more costly and less effective than another strategy).
Classification - DNA

- Prognosis
  - dMMR/hypermutated 12-15%
  - Genomic instability Rest

Comprehensive molecular characterization of human colon and rectal cancer

The Cancer Genome Atlas Network*

NATURE | VOL 487 | 19 JULY 2012

N= 276 with 97 undergoing WGS
Sporadic deficient Mismatch Repair (dMMR) Hyper mutated tumours

- Demographics
  - HNPCC dMMR/Braf wild type
  - Sporadic
  - Colon > rectum
  - Females
  - Stage II 12%  Stage III 7%, Stage 4 4%

- Prognosis
  - 50% lower recurrence risk in stage II dMMR tumours
  - Prognostic effect dMMR remains in treated group

- Prediction
  - US data suggests less responsive to chemotherapy
  - NO evidence of worse outcome on chemo in Quasar
Other mutations and prognosis

Recurrence Quasar1 – by KRas 12,13,61

N=789 MRC Focus stage IV

<table>
<thead>
<tr>
<th>Marker</th>
<th>Endpoint</th>
<th>Group</th>
<th>n</th>
<th>Hazard Ratio* (95% CI)</th>
<th>p-value</th>
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<tbody>
<tr>
<td>KRAS</td>
<td>PFS</td>
<td>W/T KRAS</td>
<td>322</td>
<td>1.0</td>
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<tr>
<td></td>
<td></td>
<td>Any KRAS mutation</td>
<td>311</td>
<td>1.17 (1.00, 1.36)</td>
<td>0.06</td>
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<td></td>
<td>OS</td>
<td>W/T KRAS</td>
<td>324</td>
<td>1.0</td>
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<tr>
<td></td>
<td></td>
<td>Any KRAS mutation</td>
<td>314</td>
<td>1.23 (1.04, 1.44)</td>
<td>0.02</td>
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</table>

<table>
<thead>
<tr>
<th>Marker</th>
<th>Endpoint</th>
<th>Group</th>
<th>n</th>
<th>Hazard Ratio* (95% CI)</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>BRAF</td>
<td>PFS</td>
<td>W/T BRAF</td>
<td>679</td>
<td>1.0</td>
<td>0.80</td>
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<td></td>
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<td>BRAF mutation</td>
<td>54</td>
<td>1.04 (0.78, 1.38)</td>
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<tr>
<td></td>
<td>OS</td>
<td>W/T BRAF</td>
<td>684</td>
<td>1.0</td>
<td>&lt;0.0001</td>
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<tr>
<td></td>
<td></td>
<td>BRAF mutation</td>
<td>54</td>
<td>1.69 (1.26, 2.27)</td>
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</tr>
</tbody>
</table>

BRAF/dMMR
3063 cases
Braf poor prognosis

Braf V600E not prognostic
PIK3CA mutations not prognostic
Multi-Gene RT-PCR Colon Cancer Assay – Oncotype Dx colon

Colon Cancer Technical Feasibility

Development Studies
- Surgery Alone
  - NSABP C-01/C-02 (n=270)
  - Cleveland Clinic (n = 765)
- Development Studies
  - Surgery + 5FU/LV
  - NSABP C-04 (n=308)
  - NSABP C-06 (n=508)

Selection of Final Gene List & Algorithm

Standardization and Validation of Analytical Methods

Clinical Validation Study – Stage II Colon Cancer
- QUASAR (n=1,436)
- Test Prognosis and Treatment Benefit

Fig 3. Kaplan-Meier estimates of 3-year recurrence in surgery-alone patients by risk group. Obs. observed; Exp. experienced.

HR = 1.47 (p=0.046)
New RNA array patterns

The consensus molecular subtypes of colorectal cancer

A pathology atlas of the human cancer transcriptome

Mathias Uhlen1,3,5 Cheng Zhang1 Sunjae Lee1, Evelina Sjostedt1,4 Linn Fagerberg3, Gholamreza Bidkhori1 Rui Benfeitas3, Muhammad Arif1, Zhengtao Liu1, Friedrik Edfors1, Kemal Sanli1, Kalle von Feilitzen1, Per Oksvold1, Emma Lundberg1, Sophia Hofer3, Peter Nilsson1, Johanna Mattsson, Jochen M. Schwens1, Hans Brunström5, Bengt Glimelius, Tobias Sjöblom, Per-Henrik Edqvist4, Dijana Djureinovic, Patrick Miche4, Cecilia Lindskog4, Adi Mardinoglu1,3,5 Fredrik Ponten4

Cancer is one of the leading causes of death, and there is great interest in understanding the underlying molecular mechanisms involved in the pathogenesis and progression of individual tumors. We used systems-level approaches to analyze the genome-wide transcriptome of the protein-coding genes of 17 major cancer types with respect to clinical outcome. A general pattern emerged: Shorter patient survival was associated with up-regulation of genes involved in cell growth and down-regulation of genes involved in cellular differentiation. Using genome-scale metabolic models, we show that cancer patients have widespread metabolic heterogeneity, highlighting the need for precise and personalized medicine for cancer treatment. All data are presented in an interactive open-access database (www.proteinatlas.org/pathology) to allow genome-wide exploration of the impact of individual proteins on clinical outcomes.

Fig. 1. Analysis of the global expression patterns of protein-coding genes in human cancers. (A) Schematic drawing of the Human Pathology Atlas effort described herein. (B) Principal components analysis (PCA) showing the similarities in expression of 13,557 protein-coding genes among 17 cancer types. See fig. 5.4 for additional PCA analysis with more stratified patient cohorts. (C) PCA plot showing the individual differences in the genome-wide global expression profiles among the 17 cancer types in 9,665 individual patients.

Uhlen et al., Science 357, eaan2507 (2017) 18 August 2017
Predictors

- Targeted therapies CRC
  - Anti EGFr ab’s
  - Braf inhibitors
  - Anti PD1 and dMMR
  - Others
    - Herceptin – anti Her2
    - Aspirin – PIK3CA mutations

Table 1. Targeted Therapies in Cancer.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genetic Alteration</th>
<th>Tumor Type</th>
<th>Therapeutic Agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR</td>
<td>Mutation, amplification</td>
<td>Lung cancer, glioblastoma</td>
<td>Gefitinib, erlotinib</td>
</tr>
<tr>
<td>ERBB2</td>
<td>Amplification</td>
<td>Breast cancer</td>
<td>Lapatinib</td>
</tr>
<tr>
<td>FGFR1</td>
<td>Translocation</td>
<td>Chronic myeloid leukemia</td>
<td>PKC412, BIBF-1220</td>
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<tr>
<td>FGFR2</td>
<td>Amplification, mutation</td>
<td>Gastric, breast, endometrial cancer</td>
<td>PKC412, BIBF-1220</td>
</tr>
<tr>
<td>FGFR3</td>
<td>Translocation, mutation</td>
<td>Multiple myeloma</td>
<td>PKC412, BIBF-1220</td>
</tr>
<tr>
<td>FGFR4</td>
<td>Mutation</td>
<td>Glioblastoma, gastrointestinal stromal tumor</td>
<td>Sunitinib, sorafenib, imatinib</td>
</tr>
<tr>
<td>FGFR8</td>
<td>Translocation</td>
<td>Chronic myelomonocytic leukemia</td>
<td>Sunitinib, sorafenib, imatinib</td>
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<tr>
<td>ALK</td>
<td>Mutation or amplification</td>
<td>Lung cancer, neuroblastoma, anaplastic large-cell lymphoma</td>
<td>Crizotinib</td>
</tr>
<tr>
<td>c-MET</td>
<td>Amplification</td>
<td>Gefitinib-resistant non-small-cell lung cancer, gastric cancer</td>
<td>Crizotinib, XL184, SU1274</td>
</tr>
<tr>
<td>JGFR1</td>
<td>Activation by insulin-like growth factor II ligand</td>
<td>Colorectal, pancreatic cancer</td>
<td>CP-751,871, AMG479</td>
</tr>
<tr>
<td>CDK4/6</td>
<td>Mutation</td>
<td>Gastrointestinal stromal tumor</td>
<td>Sunitinib, imatinib</td>
</tr>
<tr>
<td>FLT3</td>
<td>Internal tandem duplication</td>
<td>Acute myeloid leukemia</td>
<td>Lestaurtinib, XL599</td>
</tr>
<tr>
<td>RET</td>
<td>Mutation, translocation</td>
<td>Thyroid medullary carcinoma</td>
<td>XL184</td>
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<tr>
<td>Non-receptor tyrosine kinase</td>
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<td></td>
<td></td>
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<tr>
<td>ABL</td>
<td>Translocation (BCR-ABL)</td>
<td>Chronic myeloid leukemia</td>
<td>Imatinib</td>
</tr>
<tr>
<td>JAK2</td>
<td>Mutation (V617F), translocation</td>
<td>Chronic myeloid leukemia, myeloproliferative disorders</td>
<td>Lestaurtinib, INC001842</td>
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<tr>
<td>SRC</td>
<td>Overexpression</td>
<td>Non-small-cell lung cancer; ovarian, breast cancer; sarcoma</td>
<td>KI201992, dasatinib, AZD0530</td>
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<tr>
<td>Serine-threonine lipid kinase</td>
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<tr>
<td>B-Raf</td>
<td>Mutation (V600E)</td>
<td>Melanoma; colon, thyroid cancer</td>
<td>SB-590885, PLX-4032, RAF265, XL1281</td>
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<tr>
<td>Aurora A and B kinases</td>
<td>Overexpression</td>
<td>Breast, colon cancer, leukemia</td>
<td>MK-5108 (VX-809)</td>
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<tr>
<td>Polo-like kinases</td>
<td>Overexpression</td>
<td>Breast, lung, colon cancer, lymphoma</td>
<td>BZ536, GSK46164</td>
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<tr>
<td>mTOR</td>
<td>Increased activation</td>
<td>Renal cell carcinoma</td>
<td>Temsirolimus (CCI-779), BEZ235</td>
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<td>PI3K</td>
<td>PIK3CA mutations</td>
<td>Colorectal, breast, gastric cancer; glioblastoma</td>
<td>BEZ235</td>
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<tr>
<td>DNA damage or repair</td>
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<tr>
<td>BRCA1 and BRCA2</td>
<td>Mutation (synthetic lethal-effect)</td>
<td>Breast, ovarian cancer</td>
<td>Olaparib, MK-4827 (PARP inhibitors)</td>
</tr>
</tbody>
</table>

* PARP denotes poly(adenosine diphosphate-ribose) polymerase.
KRAS and the EGFr signalling pathway in KRAS-wild type and KRAS-mutant patients

**KRAS-WT**

1. EGFR monoclonal antibody blocks EGFR dimerization
2. Gene transcription
3. Cell-cycle progression
4. Angiogenesis
5. Survival
6. Proliferation
7. Metastasis

**KRAS-MUTANT**

1. EGFR monoclonal antibody blocks EGFR dimerization
2. Gene transcription
3. Cell-cycle progression
4. Angiogenesis
5. Survival
6. Proliferation
7. Metastasis

Amphiregulin
Epiregulin

**Anti-EGFr monoclonal Abs block signalling**
BRAF mutants

- BRAFi inhibitors negative
- Block BRAF and others?
- Early reports
  - No
  - BRAFi and MEK
  - Yes 26-35% RR
    - BRAFi, anti EGFr and anti PIK3CA
    - BRAFi, anti EGFr and Mek inhibitor
    - BRAFi, anti EGFr and Irinotecan

- Looks likely that dual inhibition BRAFi and antiEGFr works
**PD-1 Blockade in Tumors with Mismatch-Repair Deficiency**


<table>
<thead>
<tr>
<th>Progression-free Survival in Cohorts with Colorectal Cancer</th>
<th>Overall Survival in Cohorts with Colorectal Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td><strong>B</strong></td>
</tr>
<tr>
<td><img src="image1" alt="Graph" /></td>
<td><img src="image2" alt="Graph" /></td>
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</table>

**Biochemical Response**

- Mismatch repair–proficient colorectal cancer
- Mismatch repair–deficient colorectal cancer
- Mismatch repair–deficient noncolorectal cancer

**Change in Tumor Marker Level (%)**

<table>
<thead>
<tr>
<th>Days</th>
<th>0% (no change)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
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<tr>
<td>100</td>
<td>50</td>
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<tr>
<td>200</td>
<td>25</td>
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<tr>
<td>300</td>
<td>10</td>
</tr>
<tr>
<td>400</td>
<td>0</td>
</tr>
</tbody>
</table>

**Radiographic Response**

- Mismatch repair–proficient colorectal cancer
- Mismatch repair–deficient colorectal cancer
- Mismatch repair–deficient noncolorectal cancer

**Change from Baseline in the Sum of Longest Diameters (%)**

- 20% increase (progressive disease)
- 30% decrease (partial response)
Reapplying drugs from other sites - small populations

- **Challenge**
  - Agents positive in other cancers
    - **Her-2**
      - Stage II Quasar 25/1767 (1.4%) all pMMR
      - Stage IV CRC 29/1340 (2.2%)
        - Ki ras/Braf wild type 24/461 (5.2%)
        - Ki ras/Braf mutant 5/527 (0.95%)
    - 96.4% amplified
  - ? Others –fusion genes

Susan Richman
Katie Southward
Gemma Hemmings
PIK3CA mutations

- **Quasar1**
  - 874 cases PIK3CA mutations 13.9 %. 83 exon 9 (9.5%) exon 20 (4.4%).
  - No prognostic effect in stage II

- **Aspirin**
  - PIK3CA mutations reduced recurrence
    - Victor HR 0.11 J Clin Oncol. 2013 Dec 1;31(34):4297-305.

Add Aspirin trials
EME/CRUK Focus 4  
\( n = 3,500 \)

Testing NGS  
Kras \(12,13, 59, 61, 146\)  
Nras \(12,13, 61\)  
PIK3CA exon 9 & 20  
Braf V600E  
p53

PTEN immuno  
dMMR immuno

Labs  
Leeds and Cardiff  
Susan Richman  
Gemma Hemmings

Changing clinical trials – Focus 4

-aspirin  
oral Her 1,2,3 inhibitor (AZD8931)  
WEE-1 inhibitor
Following disease
Resistance develops on therapy

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Emerging mutational Resistance Ras in CRC with anti-EGF-R Rx

Excite phase 2 Rectal cancer 19% gained mutations 35% lost mutations after treatment
Screening - DNA

- Screening/diagnosis
- Cologuard
- DNA mutations in stool


Kate Sutton
Summary

- Diagnosis of hereditary disease
- Routine screening for HNPCC
- DNA subtypes
  - Hypermutators/dMMR are important 12%
  - Worse prognosis RAS mutants and BRAF in stage 4
  - RNA profiles not yet clinically usable
- Predictors
  - K and NRas mutations present insensitive to any
    - Anti PD1/PDL1 and dMMR story developing
    - Braf - anti EGFr and Braf
    - Her-2 and anti-Her2 therapy
    - PIK3CA,
- New trial methods
Thanks to:

- Yorkshire Cancer Research
- Quasar1 Richard Gray, Kelly Handley, Gordon Hutchins
- Focus 1-4, Piccolo, Susan Richman, Matt Seymour, Jenny Barrett, Gemma Hemmings
- Tim Palmer, Susan Richman, Morag Taylor
- Trialists, collaborators and colleagues
- MRC, CRUK and Yorkshire Trials Units