Molecular markers in colorectal cancer

Wolfram Jochum

UniversitätsSpital Zürich

Departement Pathologie
Biomarkers in cancer

- Patient characteristics
  - Tumor tissue
  - Normal cells
  - Serum
  - Body fluids

- Diagnostic marker
  - Specific diagnosis
- Prognostic marker
  - Outcome
- Predictive marker
  - Therapy response

- Predisposition

- Early diagnosis

- Targeted therapy
  - Treatment modalities (surgery, radiotherapy, chemotherapy, immunotherapy)

- Individualized therapy
## Biomarkers in cancer

### Marker types

<table>
<thead>
<tr>
<th>Type of marker</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical</td>
<td>Score</td>
</tr>
<tr>
<td>Pathological</td>
<td>Stage, histological tumor type, grade, vascular invasion</td>
</tr>
<tr>
<td>Molecular</td>
<td>Proteins/peptides, DNA, mRNA, microRNA</td>
</tr>
</tbody>
</table>
Colorectal cancer

- Malignant epithelial tumor of the colon or rectum with invasion into submucosa
- Third most common cancer in industrialized nations
- Third most common cause of cancer-related mortality
- Sporadic (75%), hereditary (HNPCC, polyposis syndromes), chronic inflammatory bowel disease (ulcerative colitis)
- Localization: Sigmoid colon and rectum, less often in the proximal colon
Colorectal cancer

Histology

Adenocarcinoma

Tubular  Mucinous  Signet-ring
Biomarkers in cancer

Patient characteristics

Tumor tissue
Normal cells
Serum
Body fluids

Predisposition
Diagnostic marker
Specific diagnosis
Prognostic marker
Outcome
Predictive marker
Therapy response

Early diagnosis
Targeted therapy
Individualized therapy

Treatment modalities
(surgery, radiotherapy, chemotherapie, immunotherapie)
37-year-old patient, rectal polyp, transanal resection
Colorectal cancer
Pathogenesis

Adenoma with mild dysplasia

Adenoma with severe dysplasia

Normal

Adenoma-carcinoma sequence

Invasive carcinoma
Malignant polyp
(adenoma with carcinoma)

- Poor (G3)/signet cell differentiation of invasive carcinoma
- Vascular/lymphatic invasion
- Involvement of resection margin

Surgical resection

Polypectomy

High risk criteria for lymph node metastasis
# Colorectal cancer

## Prognostic marker after resection

<table>
<thead>
<tr>
<th>Pathologic adverse prognostic factors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pTNM stage</strong></td>
</tr>
<tr>
<td>- Tumor stage (pT4)</td>
</tr>
<tr>
<td>- Lymph node metastasis (pN1)</td>
</tr>
<tr>
<td>- Distant metastasis (pM1) (liver)</td>
</tr>
<tr>
<td><strong>Histological type</strong> (signet-ring/small cell)</td>
</tr>
<tr>
<td><strong>Tumor grade</strong></td>
</tr>
<tr>
<td><strong>Vascular invasion</strong> (venous, lymphatic)</td>
</tr>
<tr>
<td><strong>Tumor budding</strong></td>
</tr>
<tr>
<td><strong>Tumor border configuration</strong> (infiltrative)</td>
</tr>
</tbody>
</table>
Rectal carcinoma
Prognostic marker after TME

- Completeness of mesorectum (surgeon)
- Completeness of carcinoma resection: Involvement/distance from the circumferential resection margin (CRM)

<table>
<thead>
<tr>
<th>Distance from the CRM</th>
<th>Local recurrence</th>
<th>Distant metastasis</th>
<th>Survival (2 y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.1 cm</td>
<td>16.4 %</td>
<td>37.6 %</td>
<td>69.7 %</td>
</tr>
<tr>
<td>0.11-0.2 cm</td>
<td>14.9 %</td>
<td>21.0 %</td>
<td>84.8 %</td>
</tr>
<tr>
<td>0.21-0.5 cm</td>
<td>10.3 %</td>
<td>17.2 %</td>
<td>87.0 %</td>
</tr>
<tr>
<td>0.51-1.0 cm</td>
<td>6.0 %</td>
<td>8.2 %</td>
<td>91.2 %</td>
</tr>
<tr>
<td>&gt;1.0 cm</td>
<td>2.4 %</td>
<td>10.9 %</td>
<td>92.8 %</td>
</tr>
</tbody>
</table>

2-year follow-up

Genetic mechanisms of tumorigenesis

Onkogene

Growth-promoting signals

Gain of function

Cell transformation

Proliferation, apoptosis, invasive growth, angiogenesis

Tumor suppressor gene

Growth-inhibitory signals

Loss of function

Chemicals

Chronic infection (HPV, HBV, HCV, EBV)

Radiation (UV, ionizing)

Genetic disposition
# Colorectal carcinoma

**Putative tissue biomarker for prognosis**

<table>
<thead>
<tr>
<th>Type of alteration</th>
<th>DNA</th>
<th>mRNA</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss of heterozygosity, amplification, mutation, DNA content</td>
<td>Loss of expression, overexpression, expression profile</td>
<td>Loss of expression, overexpression</td>
<td></td>
</tr>
<tr>
<td>FISH, PCR, DNA cytometry</td>
<td>RT-PCR, ISH, cDNA microarray</td>
<td>Immuno-histochemistry</td>
<td></td>
</tr>
<tr>
<td>TP53, DCC, MYC, K-RAS, etc.</td>
<td>LISCH7, MATS1, etc.</td>
<td>p21, p27, Cyclin D1, Bcl-2, Bax, etc.</td>
<td></td>
</tr>
</tbody>
</table>

**Under evaluation:** microRNA expression
Colorectal carcinoma
Putative tissue biomarker for prognosis

Clinical usefulness limited by:

• Controversial results
• Retrospective study design
• Not validated for patient care
Biomarkers in cancer

**Predisposition**

**Diagnostic marker**
- Specific diagnosis

**Prognostic marker**
- Outcome

**Predictive marker**
- Therapy response

**Patient characteristics**
- Tumor tissue
- Normal cells
- Serum
- Body fluids

**Clinical settings:**
- Adjuvant
- Neoadjuvant
- Systemic disease

**Early diagnosis**

**Targeted therapy**
- Treatment modalities (surgery, radiotherapy, chemotherapy, immunotherapy)

**Individualized therapy**
## Systemic therapy for colorectal cancer

<table>
<thead>
<tr>
<th>Drug</th>
<th>Target structure</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cytotoxic agents</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-FU/Folinic acid</td>
<td>Thymidylate synthase</td>
<td>Inhibition of DNA replication</td>
</tr>
<tr>
<td>Oxaliplatin (Eloxatin®)</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Irinotecan (Campto®)</td>
<td>DNA topoisomerase I</td>
<td>Induction of apoptosis</td>
</tr>
</tbody>
</table>
Microsatellite instability
Clinical significance

15-20% of colorectal carcinomas

Mechanism:
- Loss of MLH1 expression due to promoter hypermethylation
- Mutation of DNA mismatch repair genes (hMLH1, hMSH2, hMSH6, hPMS2)

Resistance against adjuvant chemotherapy
5-Fluorouracil (5-FU)
Better prognosis
HNPCC screening
Colorectal cancer with microsatellite instability (HNPCC)

<table>
<thead>
<tr>
<th>Feature</th>
<th>High level of microsatellite instability</th>
<th>No microsatellite instability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Localization</td>
<td>proximal</td>
<td></td>
</tr>
<tr>
<td>Mucinous component</td>
<td>22 %</td>
<td>5-7 %</td>
</tr>
<tr>
<td>Poor differentiation (G3)</td>
<td>34-57 %</td>
<td>13 %</td>
</tr>
<tr>
<td>Numerous tumor-infiltrating lymphocytes (TIL)</td>
<td>60-70%</td>
<td>3 %</td>
</tr>
</tbody>
</table>
Microsatellite instability

Microsatellite: Repeating units of 1-5 base pairs in length in the genome, 10-100 times

MLH1
MSH2
MSH6
PSM2
DNA mismatch repair proteins

Defects of DNA mismatch repair → microsatellite instability

DNA replication

CACACA
GTGTGTGTGTGT
6 repeats
Template strand

Loop of mismatched DNA from slippage

CACACACACACA
GTGTGTGTGTGT

DNA mismatch repair intact (MSS)

DNA mismatch repair deficient (MSI)

CACACACACACA
GTGTGTGTGTGT
6 repeats

7 repeats

Defects of DNA mismatch repair → microsatellite instability
Immunohistochemical staining for DNA mismatch repair proteins

**Colorectal carcinoma**
Microsatellite instability
Analysis

Microdissection of carcinoma cells

Tumor DNA

Normal DNA

PCR
Gel electrophoresis

Microsatellites
(BAT25, BAT26, D5S346, D2S123, D17S250)

Instable

<table>
<thead>
<tr>
<th>Instability</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>No marker</td>
<td>No MSI</td>
</tr>
<tr>
<td>1 marker</td>
<td>Low level of MSI (MSI-L)</td>
</tr>
<tr>
<td>( \geq 2 ) marker</td>
<td>High level of MSI (MSI-H)</td>
</tr>
</tbody>
</table>
# Systemic therapy for colorectal cancer

<table>
<thead>
<tr>
<th>Drug</th>
<th>Target structure</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cytotoxic agents</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-FU/Folinic acid</td>
<td>Thymidylate synthase</td>
<td>Inhibition of DNA replication</td>
</tr>
<tr>
<td>Oxaliplatin (Eloxatin®)</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Irinotecan (Campto®)</td>
<td>DNA topoisomerase I</td>
<td>Induction of apoptosis</td>
</tr>
<tr>
<td><strong>Targeted therapy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bevacizumab (Avastin®)</td>
<td>VEGF</td>
<td>Inhibition of angiogenesis</td>
</tr>
</tbody>
</table>

No predictive marker for Bevacizumab available
# Systemic therapy for colorectal cancer

<table>
<thead>
<tr>
<th>Drug</th>
<th>Target structure</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cytotoxic agents</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-FU/Folinic acid</td>
<td>Thymidylate synthase</td>
<td>Inhibition of DNA replication</td>
</tr>
<tr>
<td>Oxaliplatin (Eloxatin®)</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Irinotecan (Campto®)</td>
<td>DNA topoisomerase I</td>
<td>Induction of apoptosis</td>
</tr>
<tr>
<td><strong>Targeted therapy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bevacizumab (Avastin®)</td>
<td>VEGF</td>
<td>Inhibition of angiogenesis</td>
</tr>
<tr>
<td>Cetuximab (Erbitux®)</td>
<td>EGFR</td>
<td>Inhibition of EGFR signal transduction</td>
</tr>
<tr>
<td>Panitumumab (Vectibix®)</td>
<td>EGFR</td>
<td>Inhibition of EGFR signal transduction</td>
</tr>
</tbody>
</table>

**Under evaluation**: EGFR kinase inhibitors (gefitinib, erlotinib, etc.)
Epidermal growth factor receptor (EGFR)
Anti-EGFR antibody

Mechanism of action

Binding to EGFR leads to:

- Inhibition of proliferation
- Induction of apoptosis
- EGFR internalization and degradation
- Inhibition of angiogenesis
- Antibody-dependent, cell mediated cytotoxicity
Panitumumab in metastatic CRC

- Open-label phase III trial: panitumumab plus best supportive care (BSC) vs. BSC alone
- 463 Patients with metastatic CRC with progression after standard chemotherapy
- **Endpoints**: Progression-free survival (PFS), objective response, overall survival
- **Results**:

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Pan + BSC</th>
<th>BSC alone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median PFS time</td>
<td>8 weeks</td>
<td>7.3 weeks</td>
</tr>
<tr>
<td>Mean PFS time</td>
<td>13.8 weeks</td>
<td>8.5 weeks</td>
</tr>
<tr>
<td>Objective response</td>
<td>10%</td>
<td>0%</td>
</tr>
<tr>
<td>Overall survival</td>
<td>No difference</td>
<td></td>
</tr>
</tbody>
</table>

# EGFR protein/gene alterations in colorectal cancer

<table>
<thead>
<tr>
<th>Type of alteration</th>
<th>Method</th>
<th>Frequency</th>
<th>Predictive value #</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR protein expression</td>
<td>IHC</td>
<td>-80%</td>
<td></td>
</tr>
<tr>
<td>EGFR gene amplification</td>
<td>FISH, PCR</td>
<td>25-40%</td>
<td></td>
</tr>
<tr>
<td>Activating EGFR gene mutation</td>
<td>PCR, sequence analysis</td>
<td>Rare (0.3-12%)</td>
<td></td>
</tr>
</tbody>
</table>

# response to treatment with anti-EGFR drugs
**EGFR** protein expression in colorectal cancer

Problems:
- EGFR antibody (clone)
- Intratumoral heterogeneity
- Scoring system
  (intensity, number of tumor cells)
- Cut-off
Her2/neu in breast carcinoma

Problems:
- Intratumoral heterogeneity
- Scoring system (intensity, number of tumor cells)
- Cut-off
EGFR copy number and response to Panitumumab in CRC

EGFR mutations in lung cancer

EGFR protein/gene alterations in colorectal cancer

<table>
<thead>
<tr>
<th>Type of alteration</th>
<th>Method</th>
<th>Frequency</th>
<th>Predictive value #</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR protein expression</td>
<td>IHC</td>
<td>-80%</td>
<td>No 1</td>
</tr>
<tr>
<td>EGFR gene amplification</td>
<td>FISH, PCR</td>
<td>25-40%</td>
<td>Yes 2</td>
</tr>
<tr>
<td>Activating EGFR gene mutation (Exon 18-21)</td>
<td>PCR, sequence analysis</td>
<td>Rare (0.3-12%)</td>
<td>?</td>
</tr>
</tbody>
</table>

# response to treatment with anti-EGFR drugs

1 Cunningham D et al. 2004, Chung KY et al. 2005; 2 Sartore-Bianchi A et al., 2007
K-RAS

- GDP/GTP-binding protein
- Intracellular signal transducer of the EGFR pathway (among others)
- One of the most frequently mutated oncogenes
- Constitutive activation
- Mutations cluster in codons 12 and 13 (exon 2)
- 30-50% of primary colorectal adenocarcinomas
Sporadic colorectal carcinoma
Pathogenesis

Adenoma-carcinoma sequence

Chromosomal instability (CIN) pathway

Normal mucosa

Early adenoma

Late adenoma

Colorectal carcinoma

Metastasis

APC loss-of-function

K-RAS activation

DCC loss-of-function

TP53 loss-of-function

Additional genetic alterations

75-85% CRC

Chromosomal instability (CIN) pathway
Intrinsic resistance to EGFR targeted treatment (Panitumumab) due to K-RAS mutations

K-RAS mutation analysis

Work flow

1. Paraffin block
2. H&E stained section
3. DNA extraction + PCR
4. DNA sequence analysis
K-RAS mutations in colorectal carcinoma

**Tu 1**
Wild type
Codon 12 and 13

**Tu 2**
GGT -> TGT
Gly -> Cys
p.G13C

**Tu 3**
GGT -> GCT
Gly -> Ala
p.G13A
Molecular markers in colorectal carcinoma

Summary

• Conventional pathological parameters remain to provide most reliable prognostic information in CRC
• High level microsatellite instability predicts resistance to 5-FU based chemotherapy
• EGFR gene copy number (FISH) and K-RAS mutations are predictive markers for anti-EGFR antibody treatment (cetuximab, panitumumab)
• All markers can be retrospectively analysed on FFPE-tumor tissues
Mechanisms of oncogene activation

**Mutation**
- Growth factor receptor
  - Ligand binding
  - Tyrosine kinase domain
  - Plasma membrane
  - Ligand-dependent firing
  - Normal receptor
  - Mutations
  - Ligand-independent firing

**Translocation**
- Transcription factor
  - Normal chromosomes
  - Burkitt's lymphoma t(8;14)

**Gene amplification**
- Deregulated expression

Weinberg RA, 2007
Microsatellite analysis of a HNPCC-associated CRC

BAT-25

Normal

Tumor

D2S123

Normal

Tumor

D17S250

Normal

Tumor

BAT-26

Normal

Tumor

D5S346

Normal

Tumor

M06.197