Serrated and non-serrated precursor lesions of colorectal cancer: implications for molecular pathology

7th International Course on Digestive Pathology
Bucharest Nov 7-8, 2014

Outline

- Pathogenesis of colorectal cancer
  - Classical adenomas (TA, TVA, VA)
  - Adenoma-carcinoma-sequence (sporadic and hereditary)
- Serrated polyps
  - Hyperplastic polyp (HP)
  - Sessile serrated adenoma / Polyp (SSA/P)
  - Traditional serrated adenoma (TSA)
- Molecular classification of colorectal cancer and the impact of BRAF (prognostic and predictive)
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Colorectal Adenoma

- Adenomas are defined by the presence of dysplastic epithelium. This is characterized (...) by enlarged, hyperchromatic nuclei, varying degrees of nuclear spindling and stratification.
Colorectal Adenoma

- Adenomas are defined by the presence of dysplastic epithelium. This is characterized (…) by enlarged, hyperchromatic nuclei, varying degrees of nuclear spindling and stratification.

- Most adenomas are <1cm in size and have tubular architecture.

- Villous architecture is defined as leaf- or finger-like projections of epithelium overlying a small amount of lamina propria.

- Tubulovillous adenomas are defined by a mixture of tubular and villous structures with arbitrary percentages in different studies, typically between 25% and 75% villous component.

  - <25% villous components = tubular adenoma
  - 25-75% villous components = tubulovillous adenoma
  - >75% villous components = villous adenoma

WHO Classification 2010
Post-polypectomy colonoscopy surveillance: European Society of Gastrointestinal Endoscopy (ESGE) Guideline

Advanced Adenoma („High Risk“) in Austria
Tubular Adenoma
Tubular Adenoma
Villous Adenoma

Villous Adenoma
Adenoma-Carcinoma-Sequence

APC/β-catenin → KRAS → TP53, PIK3CA

Normal mucosa → Aberrant crypt focus → Early adenoma → Late adenoma → Invasive Carcinoma

Increasing chromosomal instability

APC germline mutation = familial adenomatous polyposis (FAP)

Böcker, Denk, Heitz – Pathologie, 2004

Fearon und Vogelstein, Cell, 1990
Langner, Dig Dis, in press
Autosomal dominant cancer predisposition syndrome attributable to germline mutations in DNA mismatch repair (MMR) genes leading to non-corrected mutations in target genes, i.e. oncogenes, tumour suppressor genes (“mutator phenotype”)

Mutation carriers have a high risk of developing colorectal cancer from classical adenomas, but do not present with polyposis, a feature of other colorectal cancer syndromes such as familial adenomatous polyposis → HNPCC Syndrome

Approximately one in 500 members of the general population carries a pathogenetic mutation in an MMR gene

The MMR proteins function as heterodimers

The MSH2 (dominant) / MSH6 complex recognizes mispaired bases and insertion/deletion loops

The MSH2 / MSH6 complex recruits the MLH1 (dominant) / PMS2 complex which coordinates DNA repair, i.e. excision and re-synthesis

Microsatellites are simple repetitive DNA sequences composed of 1 to 6 base pair units – hypermutable due to their propensity for strand slippage during RNA replication: microsatellite instability (MSI) in MMR deficiency

Recognition of replication error

Excision and Resynthesis
Amsterdam Criteria

- **Clinical criteria for families with HNPCC (Lynch Syndrome): All criteria must be fulfilled**
  - ≥3 relatives with an histologically confirmed HNPCC-associated cancer (colorectal cancer or cancer of the endometrium, small bowel, ureter, or renal pelvis), one affected relative being a first-degree relative of the other two; FAP should be excluded
  - ≥2 successive generations affected
  - ≥1 relative diagnosed before the age of 50 years

Vasen et al. Gastroenterology 1999

Bethesda Guidelines

- **To guide clinical testing of colorectal tumours for MSI: One or more of the following criteria must be fulfilled**
  - Colorectal cancer before the age of 50 years
  - Synchronous or metachronous colorectal cancer or other HNPCC-related tumors, regardless of age
  - Colorectal cancer with MSI-high morphology before the age of 60 years
  - Colorectal cancer (regardless of age) and a first-degree relative with colorectal cancer or an HNPCC-related tumor before the age of 50 years
  - Colorectal cancer (regardless of age) and two or more first- or second-degree relatives diagnosed with colorectal cancer or an HNPCC-related tumor (regardless of age)

Umar et al. J Natl Cancer Inst 2004
**Lynch Syndrome: Tumour Spectrum and Lifetime Risks**

<table>
<thead>
<tr>
<th>Tumour</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorectal cancer</td>
<td>34-73%</td>
<td>32-59%</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td>-</td>
<td>39-50%</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>-</td>
<td>7-8%</td>
</tr>
<tr>
<td>Stomach cancer</td>
<td>1-6%</td>
<td></td>
</tr>
<tr>
<td>Cancer of renal pelvis / ureter</td>
<td>2-8%</td>
<td></td>
</tr>
<tr>
<td>Cancer of the bile ducts</td>
<td>1-4%</td>
<td></td>
</tr>
<tr>
<td>Cancer of the small bowel</td>
<td>1-4%</td>
<td></td>
</tr>
<tr>
<td>CNS tumours</td>
<td>approximately 2%</td>
<td></td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>approximately 4%</td>
<td></td>
</tr>
<tr>
<td>Tumours of the sebaceous glands (Muir-Torre)</td>
<td>depends on affected gene</td>
<td></td>
</tr>
</tbody>
</table>

Steinke Dtsch Arztebl Int. 2013

**Time-Related Differences in Cancer Development**

- **Sporadic colorectal carcinoma**
  - Normal → Adenoma → Carcinoma

- **Familial adenomatous polyposis**
  - Normal → Adenoma → Carcinoma

- **Lynch syndrome**
  - Normal → Adenoma → Carcinoma

Langner, Dig Dis, in press
Features of MSI-H CRC to Be Used in Pathology Routine Diagnosis

- **Clinical features**
  - Age < 50
  - Right-sided location (coecum to transverse colon)
  - Female sex (for sporadic MSI-H tumours)
  - Multiplicity (synchronous or metachronous)

- **Histological features**
  - **Histological subtype**: medullary carcinoma, mucinous adenocarcinoma ("any mucin"), signet-ring cell carcinoma ("any signet ring cell")
  - **Inflammation**: tumour infiltrating lymphocytes (TILs), peritumoral lymphocytes, Crohn-like reaction
  - **Growth features**: poor differentiation, expansive ("pushing") growth, lack of dirty necrosis, tumour heterogeneity

Jenkins et al. Gastroenterology 2007
CpG Island Methylator Phenotype (CIMP)

- There are CRC that lack both CIN and MSI
- MSI occurs more often in the sporadic setting than in Lynch Syndrome
- Some CRC are characterized by frequent epigenetic alterations, including aberrant methylation of 5’ promoter regions of genes with CpG islands (Cytosin → Methylcytosin)
- Methylation causes transcriptional silencing and can inactivate tumour suppressor genes (e.g. p16, MGMT, hMLH1 → MSI)
- CIMP CRC do not arise from classical adenomas

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  - Sessile serrated adenoma / Polyp (SSA/P)
  - Traditional serrated adenoma (TSA)

- Molecular classification of colorectal cancer and the impact of BRAF (prognostic and predictive)
Hyperplastic Polyp (HP)

- Incidence
  - >75% of all serrated polyps

- Morphology
  - Location: mainly distal colon
  - Microvesicular subtype (MHVP)
  - Goblet-cell rich subtype (GCHP)
  - Mucin-poor subtype (MPHP)

- Molecular Pathology
  - \textit{BRAF} mutation in up to 40% (MVHP)
  - \textit{KRAS} mutation in up to 40% (GCHP)
  - CIMP-phenotype (with methylation of MLH1 und MGMT) may be observed
**Morphogenesis of Hyperplastic Polyp (HP)**

- Upper third of the crypt
- Central portion of the crypt
- Lower third of the crypt


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**Incidence of Hyperplastic Polyp (HP)**

3060 HP in 226/263 (86%) Patients

<table>
<thead>
<tr>
<th>HP</th>
<th>N</th>
<th>Right Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 6 mm</td>
<td>3020</td>
<td>5%</td>
</tr>
<tr>
<td>6-10 mm</td>
<td>38 (8.7%)</td>
<td>42%</td>
</tr>
<tr>
<td>&gt;10 mm</td>
<td>2 (0.8%)</td>
<td>100%</td>
</tr>
<tr>
<td>Total</td>
<td>3060</td>
<td></td>
</tr>
</tbody>
</table>

Yano T et al. J Gastroenterol Hepatol 2005
Mixed hyperplastic adenomatous polyp - an underdiagnosed entity

Report of a case of adenocarcinoma arising within a mixed hyperplastic adenomatous polyp

- We report a case of colonic adenocarcinoma arising within a polyp with mixed morphology of a hyperplastic polyp and tubular adenoma. Despite the relatively small size of the polyp, two isolated foci of adenocarcinoma in situ were present and tumor islands invaded the submucosa. Isolated areas, morphologically resembling hyperplastic glands, and varying degrees of atypia. Though rare, some hyperplastic polyps may be precursors of adenomas.


Mixed hyperplastic adenomatous polyps/serrated adenomas. A distinct form of colorectal neoplasia.

- 110 colorectal mixed hyperplastic adenomatous polyps (MHAP) that exhibited the architectural but not the cytologic features of a hyperplastic polyp.
- MHAP measured 0.2-7.5 cm in diameter. They were distributed throughout the colorectum, but a slight preponderance of large lesions (more than 1.0 cm) occurred in the coecum and appendix.
- All MHAP were characterized by a serrated glandular pattern simulating that seen in hyperplasia (27% of MHAP were initially diagnosed as hyperplastic polyps).
- 37% of MHAP contained foci of significant dysplasia, and 11% contained areas of intramucosal carcinoma.

Serrated Polyps with "Abnormal Proliferation" Part III

- Hyperplastic (WHO 2010: serrated) polyposis
  - At least 5 serrated polyps proximal to the sigmoid colon with two or more of these being > 10 mm
  - Any number of serrated polyps proximal to the sigmoid colon in an individual who has a first-degree relative with hyperplastic (serrated) polyposis
  - More than 20 serrated polyps of any size, but distributed throughout the colon

- Two variants
  - Type 1: Multiple lesions (larger and often more proximal) → substantial cancer risk
  - Type 2: Numerous small (< 5 mm) lesions distributed throughout the colon → modest cancer risk

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Hyperplastic Polyp?

Torlakovic und Snover. Gastroenterology 1996
Snover et al. WHO Classification 2010
Hyperplastic Polyp?

SSP: Sessile serrated polyp
SSA: Sessile serrated adenoma
SSL: Sessile serrated lesion
WHO 2010: SSA/P

Incidence
- 15-25% of all serrated polyps

Morphology
- Location: right > left (coecum to transverse colon)
- Macroscopy / Endoscopy: sessile (non-polypoid) lesion (> 5 mm)
- Histology:
  - (Hyper)Serration in the lower third of the crypts (with and without branching)
  - T- and L-shaped crypts above the muscularis mucosae
  - Inverted crypts (pseudoinvasion) below the muscularis mucosae
  - Columnar dilatation in the lower third of the crypts (with or without mucus → "mucus cap")
  - Bland cytology (no classical dysplasia)
Sessile Serrated Adenoma / Polyp (SSA/P)

- Cytological dysplasia is not present in uncomplicated SSA/P but develops with progression towards carcinoma (often in conjunction with methylation of MLH1 and MSI)
  - SSA/P with dysplasia (formerly “mixed polyp”: SSA with TA, TVA, VA, TSA)
  - SSA/P as indicator of “advanced neoplasia” and/or cancer in follow-up endoscopies

SSA/P Molecular Pathology

- Normal mucosa
- Microvesicular hyperplastic polyp
  - BRAF mutation
  - Promoter hypermethylation
  - hMLH1 methylation
  - Increased rate of mutations in tumour suppressor genes and oncogenes

- Sessile serrated adenoma/polyp
  - Microsatellite instability
  - Variable rate of progression

- Sessile serrated adenoma/polyp with cytological dysplasia
  - Rapid rate of progression similar to Lynch syndrome polyps

Schreiner et al. Gastroenterology 2010
Lu et al. Am J Surg Pathol 2010
Rex et al. Am J Gastroenterol 2012

Langner, Dig Dis, in press
**SSA/P Molecular Pathology**

Clinical Subtypes and Molecular Characteristics of Serrated Polyposis Syndrome

**BACKGROUND & AIMS:** We investigated clinical and molecular differences between the different phenotypes of serrated polyposis syndrome (SPS) and the frequency of mutations in BRAF or KRAS in polyposis from patients with SPS.

**METHODS:** We collected data on clinical and demographic characteristics of 38 patients who fulfilled the criteria for SPS. Polyposis chain reaction and sequencing analysis were used to identify BRAF and KRAS mutations in 472 polyposis collected from 38 patients; we analyzed Cpg island methylator phenotypes in 272 of these polyposis.

**RESULTS:** Fifteen patients (39%) had type 1 SPS and 23 had type 2 SPS. There were no significant differences in age at diagnosis, sex, smoking frequency, body mass index, or colorectal cancer predisposition between groups of patients, or in the pathologic or molecular characteristics of their polyposis. A familial history of colorectal cancer or colorectal polyposis was more common in patients with type 2 SPS. BRAF mutations were found in 68% of polyposis and in 52% of polyposis from patients with type 2 SPS. KRAS mutations were found in 4.4% of polyposis and in 4.4% of polyposis from patients with type 2 SPS. In addition, we found a significant correlation between the presence of BRAF and KRAS mutations in polyposis from patients with type 2 SPS.

**CONCLUSIONS:** For a greater likelihood of familial history of colorectal cancer or polyposis in patients with type 2 SPS, we found no significant difference in age, sex, smoking frequency, or body mass index between patients with type 2 SPS and those with type 1 SPS. However, patients with type 2 SPS were more likely to have a familial history of colorectal cancer or polyposis.


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**SSA/P with Dysplasia**
About 1 in 27 (95% CI = 20 – 36) CRCs are interval cancers, developing within 6 – 36 months of a colonoscopy, and are 2.4 times more likely to arise in the proximal colon.

Patient (older age, diverticulosis, presence of comorbidities), endoscopy (low polypectomy rate, low procedure completion rate, procedural performance by a non-gastroenterologist), and tumor biology-related factors (microsatellite instability and CpG island methylator phenotype positive) are associated with interval CRC.
Sessile Serrated Adenomas: prevalence of dysplasia and carcinoma in 2139 patients

<table>
<thead>
<tr>
<th>Location</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right</td>
<td>81%</td>
</tr>
<tr>
<td>Left</td>
<td>11%</td>
</tr>
<tr>
<td>Right and left</td>
<td>3%</td>
</tr>
<tr>
<td>Unknown</td>
<td>4%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSA without Dysplasia</td>
<td>85%</td>
</tr>
<tr>
<td>SSA with LG Dysplasia</td>
<td>12%</td>
</tr>
<tr>
<td>SSA with HG Dysplasia</td>
<td>2%</td>
</tr>
<tr>
<td>SSA with Carcinoma</td>
<td>1%</td>
</tr>
</tbody>
</table>
Sessile Serrated Adenoma / Polyp (SSA/P)

- Cytological dysplasia is not present in uncomplicated SSA/P but develops with progression towards carcinoma (often in conjunction with methylation of MLH1 and MSI)
  - SSA/P with dysplasia (formerly "mixed polyp": SSA with TA, TVA, VA, TSA)
  - SSA/P as indicator of "advanced neoplasia" and/or cancer in follow-up endoscopies

- **Minimum Morphological Criteria (DD HP)**
  - If more than two or three contiguous crypts show features of SSA/P the lesion should be classified as SSA/P (WHO 2010)
  - "We recommend that the presence of at least one unequivocal architecturally distorted, dilated, and/or horizontally branched crypt, particularly if it is associated with inverted maturation, is sufficient for a diagnosis of SSA/P" (Rex et al.)

Schreiner et al. Gastroenterology 2010
Lu et al. Am J Surg Pathol 2010
Rex et al. Am J Gastroenterol 2012

**Box 1 Questions asked in the survey and responses from the 43 participants**

Question 4: Do you diagnose SSA/P in only the right or left colon, or both?
- I make the diagnosis only in the right colon: 8
- I make the diagnosis only in the left colon: 0
- I make the diagnosis in the right and left colon: 35

Question 6: What are the minimum histological criteria that you accept for a diagnosis of SSA/P?
- A single crypt showing one of the characteristic features is sufficient: 5
- A single crypt is sufficient if it shows >1 of the characteristic features: 8
- Several crypts showing at least one of the characteristic features are required: 30
Critical Appraisal of the Diagnosis of the Sessile Serrated Adenoma

Mark Bettinton, FRCPA.* † † Neal Walker, FRCPA, MD.† †
Christophe Rosy, FRCPA, PhD.‡ ‡ Ian Brown, FRCPA.‡ ‡
Andrew Clouston, FRCPA, PhD.‡ ‡ Leesa Wockner, PhD.‡
Vicki Whitehall, PhD.‡ ‡ and Barbara Leggett, FRACP, MD.‡ ‡

Cross-sectional study of 6340 colorectal polyps received at a high-volume community-based pathology practice over a 3-month period

Table 1. Diagnostic Subcategories for MVHPs and SSAs

<table>
<thead>
<tr>
<th>Subcategory</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVHP</td>
<td>No SSA-type crypts</td>
</tr>
<tr>
<td>pSSA (type 1)</td>
<td>One SSA-type crypt</td>
</tr>
<tr>
<td>pSSA (type 2)</td>
<td>Two nonadjacent SSA-type crypts</td>
</tr>
<tr>
<td>pSSA (type 3)</td>
<td>Multiple crypts with poorly developed SSA-type features</td>
</tr>
<tr>
<td>SSA (type 1)</td>
<td>Minimal WHO criteria to 4 SSA-type crypts</td>
</tr>
<tr>
<td>SSA (type 2)</td>
<td>5 to 9 SSA-type crypts</td>
</tr>
<tr>
<td>SSA (type 3)</td>
<td>10 or more SSA-type crypts</td>
</tr>
</tbody>
</table>

Table 2. Number, Location, and Average Size of the Polyps by Type Using WHO Diagnostic Criteria*

<table>
<thead>
<tr>
<th>Polyp Type (n = 6340)</th>
<th>Subtype</th>
<th>Total Number</th>
<th>Proximal</th>
<th>Distal</th>
<th>Rectum</th>
<th>Mean Size (SD) (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All adenomatous polyps</td>
<td>MVHP</td>
<td>825 (13)</td>
<td>129 (16)</td>
<td>418 (51)</td>
<td>266 (12)</td>
<td>4.5 (2.4)</td>
</tr>
<tr>
<td>Thyroid (LDG)</td>
<td>MVHP</td>
<td>1343 (21.2)</td>
<td>202 (15)</td>
<td>593 (44)</td>
<td>533 (40)</td>
<td>4.6 (2.4)</td>
</tr>
<tr>
<td>SSA</td>
<td></td>
<td>741 (11.7)</td>
<td>394 (60)</td>
<td>128 (17)</td>
<td>11 (1)</td>
<td>3.5 (1.4)</td>
</tr>
<tr>
<td>SSAAD</td>
<td></td>
<td>27 (0.4)</td>
<td>21 (78)</td>
<td>3 (11)</td>
<td>0 (0)</td>
<td>7.8 (3.6)</td>
</tr>
<tr>
<td>Transitional serrated adenoma</td>
<td></td>
<td>37 (0.6)</td>
<td>18 (52)</td>
<td>22 (68)</td>
<td>17 (30)</td>
<td>10.6 (6.8)</td>
</tr>
<tr>
<td>Serrated polyp undiscernible</td>
<td></td>
<td>26 (0.3)</td>
<td>14 (70)</td>
<td>6 (30)</td>
<td>0 (0)</td>
<td>4.7 (1.5)</td>
</tr>
<tr>
<td>Malignant polyp</td>
<td></td>
<td>23 (0.4)</td>
<td>8 (55)</td>
<td>12 (52)</td>
<td>3 (13)</td>
<td>20 (12.7)</td>
</tr>
</tbody>
</table>

*Some percentages do not add to 100 as site data were not supplied in all cases.
HGD indicates high-grade dysplasia; LGD, low-grade dysplasia.
We found that serrated polyps (MVHPs or SSAs) with any SSA-like crypts had clinical features more in common with the SSA than the MVHP and that this diagnostic cutoff showed good reproducibility between pathologists. This supports the position of a recent consensus publication proposing that polyps with as few as 1 SSA-type crypt should be diagnosed as an SSA.
Traditional Serrated Adenoma (TSA)

- **Incidence**
  - Rare: <1% of all colorectal polyps

- **Morphology**
  - Location: left > right (distal colon and rectum)
  - Macroscopy / Endoscopy: polypoid > flat lesion

- **Histology:**
  - Complex villiform growth pattern with prominent serration
  - Ectopic crypt foci (ECF)
  - Cytological dysplasia (intraepithelial neoplasia) – „serrated dysplasia“
  - Diffuse eosinophilic cytoplasm
The study cohort comprised 60 traditional serrated adenomas with cytologic dysplasia and/or invasive carcinoma.

On the basis of morphological features, 16 cases (27%) were categorized as traditional serrated adenoma with serrated dysplasia and 25 cases (42%) as traditional serrated adenoma with conventional adenomatous dysplasia.
Of the polyps, 71% were distal. Advanced histology (overt dysplasia or carcinoma) was present in 19% of cases. BRAF mutation was present in 67% and KRAS mutation in 22%.
Table 2: Clinicopathological features by mutation status

<table>
<thead>
<tr>
<th>Feature</th>
<th>BRAF mutation (n = 134)</th>
<th>KRAS mutation (n = 43)</th>
<th>BRAF/KRAS wild type (n = 23)</th>
<th>P-value (BRAF versus KRAS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>64 (27–49)</td>
<td>65 (36–60)</td>
<td>62 (36–67)</td>
<td>0.8611</td>
</tr>
<tr>
<td>Female</td>
<td>49%</td>
<td>49%</td>
<td>57%</td>
<td>1.000</td>
</tr>
<tr>
<td>Mean size (mm)</td>
<td>14 (3–70) (median 33)</td>
<td>18 (7–60) (median 33)</td>
<td>20 (4–63) (median 33)</td>
<td>0.050</td>
</tr>
<tr>
<td>Distal location</td>
<td>61%</td>
<td>38%</td>
<td>74%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Precursor polyp</td>
<td>57%</td>
<td>0%</td>
<td>0%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sessile serrated adenoma</td>
<td>46%</td>
<td>0%</td>
<td>0%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Microvascular hyperplastic polyp</td>
<td>10%</td>
<td>0%</td>
<td>0%</td>
<td>0.0233</td>
</tr>
</tbody>
</table>

P-values < 0.05 are indicated in bold.

Table 4: Flat morphology relative to location and mutation status

<table>
<thead>
<tr>
<th>Flat morphology</th>
<th>All cases</th>
<th>Proximal location</th>
<th>Distal location</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cases</td>
<td>76/200 (38%)</td>
<td>37/59 (63%)</td>
<td>39/141 (28%)</td>
</tr>
<tr>
<td>BRAF mutant</td>
<td>57/134 (43%)</td>
<td>33/52 (64%)</td>
<td>24/82 (29%)</td>
</tr>
<tr>
<td>KRAS mutant*</td>
<td>19/41 (48%)</td>
<td>0/3 (0%)</td>
<td>10/40 (25%)</td>
</tr>
<tr>
<td>Wild type</td>
<td>9/23 (39%)</td>
<td>4/6 (67%)</td>
<td>5/17 (28%)</td>
</tr>
<tr>
<td>P-value (BRAF versus KRAS mutant)</td>
<td>0.0030</td>
<td>0.3774</td>
<td>0.6726</td>
</tr>
</tbody>
</table>

P-values < 0.05 are indicated in bold.

*Location was not available for two of the KRAS mutant TISAs.

Figure 4: Proposed molecular pathways of malignant progression in BRAF and KRAS mutant traditional serrated adenoma.
Outline

- Pathogenesis of colorectal cancer
  - Classical adenomas (TA, TVA, VA)
  - Adenoma-carcinoma-sequence (sporadic and hereditary)
- Serrated polyps
  - Hyperplastic polyp (HP)
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  - Traditional serrated adenoma (TSA)
- Molecular classification of colorectal cancer and the impact of BRAF (prognostic and predictive)

Case Report
80-year-old male

UICC2009: pT3b(m); G3; L1; N1a; (1/32); R0
Tumour C

Molecular Analysis: MSI

- Normal Tissue
- Tumour A: MSI-H
- Tumour B: MSI-H
- Tumour C: MSI-H
- Lymph Node: MSI-H
Molecular Analysis: BRAF

Normal Tissue

Tumour A  
BRAF wt

Tumour B  
BRAF V600E mut

Tumour C  
BRAF V600E mut

Lymph Node  
BRAF V600E mut

What is the most probable diagnosis?

- **Mucinous adenocarcinoma** in the transverse colon. Loss of MSH2 (and MSH6). MSI-H and BRAF wild type.
  → **Most probably Lynch Syndrome**

- **Two mucinous adenocarcinomas** in the transverse and ascending colon. Loss of MLH1 (and PMS2). MSI-H and BRAF mutation.
  → **Sporadic MSH-H CRC** (serrated pathway)
## Immunohistochemical Analysis of MSI Colorectal Cancer

- **Markers**
  - MLH1, MSH2, MSH6, PMS2
  - MSH6 and PMS2 proteins are instable in the absence of their respective dominant partner
  - Combined staining for MLH1 and MSH2 achieve 92% sensitivity and 100% specificity for the identification of MSI-H tumours

- **Possible staining results**
  - Loss of MSH2 and/or MSH6 (and also isolated loss of PMS2) is highly suggestive for hereditary tumours (Lynch Syndrome)
  - Loss of MLH1 occurs more often in sporadic tumours than in hereditary, i.e. Lynch Syndrome-related tumours (serrated route: transcriptional silencing, i.e. promoter methylation of hMLH1 in BRAF mutated tumours, but MLH1 silencing may also occur also in sporadic BRAF wild type tumours)

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## MSI Testing in Colorectal Cancer

- Molecular analysis using the 1997 NCI MS reference panel (Roche): two mononucleotide repeats (BAT25, BAT26) and three dinucleotide repeats (D5S346, D2S123, D17S250)
  - **MSI High (MSI-H):** instability at ≥ 2 loci
  - **MSI Low (MSI-L):** instability at 1 locus

- Molecular analysis using only mono-nucleotide repeats (e.g. BAT 25, BAT26, NR-21, NR-24, NR-27; "Pentaplex PCR")
  - **MSI High (MSI-H):** instability at ≥ 2 loci

- **MSI-high tumours are more often sporadic than hereditary**
  - Additional testing for BRAF mutation (most common in V600E)
  - Additional immunohistochemical staining
In 181 of 232 patients MMR germline gene mutations and somatic MLH1 promoter methylation were identified as underlying cause. In the remaining 51 patients (22%) the situation was unclear. In 25 cases of these deep sequencing was performed. In 17 of 18 MLH1-deficient (94%) and 6 of 7 MSH2-deficient tumors (86%), somatic events were detected: In 8 MLH1- and 2 MSH2-deficient tumors, loss of heterozygosity (LOH) was detected. In 13 (52%) cases (8 MLH1- and 5 MSH2-deficient tumors), 2 somatic were identified, which were considered to explain the MMR-deficient phenotype.
Accepted Manuscript

Colon and Endometrial Cancers with Mismatch Repair Deficiency can Arise from Somatic, Rather Than Germline, Mutations

Sigurdur Haraldsdottir, MD MS- Heather Hampai, MS Jemima Tomacz, PhD Wendy L. Frankel, MD Rachel Pearlman, MS Albert de la Chapelle, MD PhD Colin C. Pritchard, MD PhD

Pit: 55019-55865(14)/01086-4
DOI: 10.1053/gastro.2014.09.041
Reference: YGAST 59324

To appear in: Gastroenterology
Accepted Date: 27 August 2014

Results: Twenty-two of 32 patients (69%) were found to have two somatic (tumor) mutations in MMR genes encoding proteins that were lost from tumor samples, based on immunohistochemistry. Of the 10 tumors without somatic mutations in MMR genes, 3 had somatic mutations with possible loss of heterozygosity that could lead to MMR deficiency, 6 were found to be false-positive results (19%), and 1 had no mutations known to be associated with MMR deficiency. All of the tumors found to have somatic MMR mutations were of the hypermutated phenotype (>12 mutations/Mb); 6 had mutation frequencies >200 per Mb, and 5 of these had somatic mutations in POLE, which encodes a DNA polymerase.

BRIEF COMMUNICATION

Microsatellite Instability and BRAF Mutation Testing in Colorectal Cancer Prognostication

Paul Lochhead, Aya Kuchiba, Yu Imamura, Xiaoyun Liao, Mai Yamauchi, Reiko Nishihara, Zhi Rong Qian, Teppi Monikawa, Jeanne Shen, Jeffrey A. Meyerhardt, Charles S. Fuchs, Shuji Ogino

Lochhead et al. J Natl Cancer Inst 2013
BRAFV600E immunohistochemistry in conjunction with mismatch repair status predicts survival in patients with colorectal cancer

Christopher W Toon1,2,3, Angela Chou4, Keshani DeSilva5, Joseph Chan5,6, Jillian Patterson7, Adele Clarkson1,3, Loretta Sioson1,3, Lucy Jankova1,6 and Anthony J Gill1,3,5

![Graphs and tables]

Table 1 Clinical and pathological characteristics of 1469 consecutive colorectal cancer patients (2004–2009)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Count (%) unless otherwise stated</th>
<th>Single variable P-value</th>
<th>Univariate analysis HR (95% CI) P-value</th>
<th>Multivariate analysis HR (95% CI) P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Female</td>
<td>740 (52.1)</td>
<td>0.13</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Male</td>
<td>729 (47.9)</td>
<td></td>
<td>0.62 (0.67–1.01)</td>
<td>0.07</td>
</tr>
<tr>
<td>Age at diagnosis, median (range)</td>
<td>37 (17–100)</td>
<td>&lt;0.01</td>
<td>1.03 (1.00–1.06), &lt;0.01</td>
<td>1.04 (1.00–1.06), &lt;0.01</td>
</tr>
<tr>
<td>Histologic grade</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Low</td>
<td>626 (57.9)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>High</td>
<td>214 (15.0)</td>
<td>1.87 (1.42–2.47)</td>
<td>&lt;0.01</td>
<td>1.27 (0.67–2.44)</td>
</tr>
<tr>
<td>Lymphovascular space invasion</td>
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<tr>
<td>Absent</td>
<td>830 (57.7)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
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<tr>
<td>Present</td>
<td>59 (32.3)</td>
<td>&lt;0.01</td>
<td>2.26 (1.72–2.95), &lt;0.01</td>
<td>1.57 (1.13–2.19), &lt;0.01</td>
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<tr>
<td>Overall stage AJCC/TNM 7th edition</td>
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<tr>
<td>I</td>
<td>355 (16.5)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
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<tr>
<td>II</td>
<td>415 (29.1)</td>
<td></td>
<td>2.00 (1.30–3.17), &lt;0.01</td>
<td>2.21 (1.20–4.15), 0.01</td>
</tr>
<tr>
<td>III</td>
<td>85 (6.0)</td>
<td>2.44 (1.34–4.42), &lt;0.01</td>
<td>&lt;0.01</td>
<td>2.60 (1.20–5.69), 0.02</td>
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<tr>
<td>IV</td>
<td>15 (1.1)</td>
<td>0.60 (0.17–2.24)</td>
<td>&lt;0.01</td>
<td>1.00</td>
</tr>
<tr>
<td>IIIA</td>
<td>91 (6.5)</td>
<td>1.10 (0.90–2.43), 0.82</td>
<td>&lt;0.01</td>
<td>0.94 (0.57–1.53), 0.89</td>
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<td>IIIB</td>
<td>376 (26.0)</td>
<td>3.05 (1.97–4.72), &lt;0.01</td>
<td>&lt;0.01</td>
<td>2.75 (1.49–5.10), &lt;0.01</td>
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<tr>
<td>IIIC</td>
<td>176 (11.2)</td>
<td>6.68 (4.12–11.21), &lt;0.01</td>
<td>&lt;0.01</td>
<td>5.85 (3.51–10.12), &lt;0.01</td>
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<td>IVB</td>
<td>32 (2.1)</td>
<td>8.00 (4.24–15.31), &lt;0.01</td>
<td>&lt;0.01</td>
<td>11.76 (4.98–29.32), &lt;0.01</td>
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<tr>
<td>IIIF</td>
<td>90 (6.3)</td>
<td>14.39 (7.47–28.94), &lt;0.01</td>
<td>&lt;0.01</td>
<td>15.86 (6.73–37.48), &lt;0.01</td>
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<tr>
<td>Mismatch repair HCC status</td>
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<tr>
<td>Proficient</td>
<td>1148 (90.3)</td>
<td>&lt;0.01</td>
<td>1.00</td>
<td>1.00</td>
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<tr>
<td>Deficient</td>
<td>271 (19.7)</td>
<td></td>
<td>0.74 (0.36–1.56), &lt;0.01</td>
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<tr>
<td>BRAFV600E mutation immunohistochemistry status</td>
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<tr>
<td>Wild type</td>
<td>1131 (80.7)</td>
<td>&lt;0.01</td>
<td>1.00</td>
<td>1.00</td>
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<tr>
<td>Mutant</td>
<td>277 (19.3)</td>
<td>1.14 (0.60–2.09), 0.32</td>
<td>&lt;0.01</td>
<td>1.10 (0.60–1.76), 0.66</td>
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<tr>
<td>Immunohistochemistry</td>
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</tr>
<tr>
<td>types</td>
<td></td>
<td></td>
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<tr>
<td>BRAF wild type</td>
<td>1057 (74.1)</td>
<td>&lt;0.01</td>
<td>1.00</td>
<td>1.00</td>
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<tr>
<td>Mismatch repair-proficient/</td>
<td></td>
<td></td>
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<tr>
<td>BRAF wild type</td>
<td>1014 (12.9)</td>
<td>0.04 (0.00–1.39), 0.32</td>
<td>0.57 (0.35–0.94), 0.03</td>
<td></td>
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<tr>
<td>Mismatch repair-deficient/</td>
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</tr>
<tr>
<td>BRAF wild type</td>
<td>46 (6.6)</td>
<td>0.60 (0.40–1.00), 0.10</td>
<td>0.65 (0.34–1.30), 0.23</td>
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</tr>
<tr>
<td>Mismatch repair-proficient/</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRAF wild type</td>
<td>91 (6.4)</td>
<td>1.79 (1.24–2.60), &lt;0.01</td>
<td>&lt;0.01</td>
<td>1.18 (0.69–1.96), 0.66</td>
</tr>
</tbody>
</table>
Take Home Message

- Non-serrated adenomatous lesions (TA TVA, VA) provide the basis for the classical adenoma-carcinoma-sequence
- The diagnosis of an advanced adenoma has deep impact on follow-up strategies
- CRC in Lynch Syndrome (hereditary MSI-H CRC) develop from classical adenomas, preferably in the right colon
- Serrated lesions are important new players in the group of colorectal polyps
- The serrated pathway involves a sequence of genetic and epigenetic alterations that lead to sporadic MSI-H CRC (CIMP phenotype of CRC)
- Molecular CRC subtyping in the routine setting follows a two-step approach: immunohistochemistry and molecular analysis (MSI, BRAF)

Thank you very much for your kind attention!

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