COLON CANCER: “PAST, PRESENT, AND FUTURE” AND FAMILIAL SYNDROMES

Eun Lee, MD, Professor & Director of Anatomic Pathology
MCC Affiliate Network Meeting

- Etiology for colon cancer
- “Past, Present, and Future” for colon cancer
- Familial syndromes
- New universal IHC screening protocol for Lynch syndrome
Cancer Incidence & Death Rate in USA

A. 2008 ESTIMATED CANCER INCIDENCE BY SITE AND SEX*

- Melanoma of skin 5%
- Oropharynx 3%
- Lung 15%
- Pancreas 3%
- Kidney 4%
- Colon and rectum 10%
- Urinary bladder 7%
- Prostate 25%
- Leukemia 3%
- Non-Hodgkin lymphoma 5%
- All others 20%

B. 2008 ESTIMATED CANCER DEATHS BY SITE AND SEX*

- Lung 31%
- Esophagus 4%
- Liver 4%
- Pancreas 6%
- Kidney 3%
- Colon and rectum 8%
- Urinary bladder 3%
- Prostate 10%
- Leukemia 4%
- Non-Hodgkin lymphoma 3%
- All others 24%

3rd for Incidence & Death in both Male & Female
Colon cancer in Kentucky

- Colon cancer incidence
  - #1 in the nation in 2009
  - 9% higher than the national rate

- Increased statewide colorectal cancer screening (colonoscopy)
  - 34.7% in 2001
  - 63.7% in 2010
  - led by Dr. Thomas Tucker
  - statewide joint effort
Dietary ("Western diet")

- Low fiber diet
- High fat diet
- Decreased intake of vitamins A, C, E
Colon Cancer in Korea

Uncommon in 70’s

2nd M/C for male & 4th M/C for female
Immigrants from Japan to the United States

![Graph showing death rates from cancers](https://example.com/graph.png)

© Elsevier 2005
Japan in 2012

- Colon cancer: #2
- Gastric cancer: #3
Etiology

- Hereditary
  - 1st degree relative w/ cancer: 2-4 fold ↑ risk
  - Lynch syndrome
  - Familial adenomatous polyposis (FAP)

- Ulcerative colitis

- Colorectal adenomas
Colonic polyps

A. Non-neoplastic polyps (NO malignant potential)
   1. Hyperplastic polyp
   2. Juvenile polyp
   3. Hamartomatous polyp
   4. Inflammatory polyp (pseudopolyp)
   5. Lymphoid polyps

B. Neoplastic polyps (malignant potential)
   1. Tubular adenoma
   2. Villous adenoma
   3. Tubulovillous adenoma
   4. Traditional serrated adenoma
   5. Sessile serrated adenoma
Adenomatous change
Adenomatous change
Tubular adenoma
Tubular adenoma
Villous adenoma
Other etiologies

- Heavy metals (e.g., Arsenic) in Appalachia
  (Drs. M. Dignan, T. Tucker, X. Shi, G. M. Li)
 Colon Cancer in 80’s

- Hyperplastic polyps: M/C (not true anymore)
- Adenomas
  - tubular, tubulovillous, villous
- Colon cancer
  - diagnosis, histologic type, grading
  - staging (Dukes classification)
- High grade dysplasia (instead of carcinoma-in-situ)
Invasive CA in adenoma ("malignant polyp"): up to 5%
Dr. Vogelstein
“Multi-step carcinogenesis”
Adenomatous Polyposis Coli (APC) Gene

- Promote cell adhesion and regulate cell proliferation
- Increased & unregulated proliferation
APC/β-catenin pathway

Classical pathway
Chromosomal instability pathway
Lynch Syndrome (HNPCC)

- 3-5% (M/C hereditary) of all colon cancers
- Younger age of onset, right colon, synchronous cancer, strong family history
- Germline (inherited) mutations of DNA mismatch repair genes leading to microsatellite instability -“automatic spelling checker”
- Small increases or decreases in the size of microsatellite throughout the genome ("MSI-H tumor")
Lynch Syndrome

- **Lifetime cancer risks:**
  - Colorectal: 80%
  - Endometrial: 20-60%
  - Gastric: 13-19%
  - Ovarian: 9-12%
  - Biliary tract: 2%
  - Urinary tract: 4%
  - Small bowel: 1-4%
  - Brain/CNS: 1-3%
American Founder Mutation
(76 families; 12 families in Kentucky)

MSI-H Colon Cancers

- Also in 10% of sporadic colon cancers
Microsatellite Instability & Prognosis

A. All Patients with Colorectal Cancer

Survival (%)

Years after Diagnosis

- MSI
- MSS

Overall survival

- MSI
- No MSI

n=656
p=0.043

NEJM 342:71, 2000

Lancet 355:1748, 2000
Adjuvant Therapy in MSI-H CRC (stage II & III)

Ribic et al, NEJM 2003;349:247-257
MSI-high Tumors

- Peritumoral lymphoid reaction ("Crohn’s-like")
- ↑ intratumoral lymphocytes
- Medullary histology
- Prominent mucinous differentiation
- NO "dirty necrosis"
Evolution

- Changes for AJCC (TMN) staging
  - New AJCC, new WHO Classification next year
  - Molecular information in certain tumors

- Evolution of reporting
  - CAP (College of American Pathologists) cancer protocol/checklist for synoptic reporting
  - Mandatory documentation of all pertinent pathologic findings in pathology reports
New adenomas (5-6 years ago)

- Tubular adenoma
- Tubulovillous adenoma
- Villous adenoma
- Sessile serrated adenoma (SSA)
- Traditional serrated adenoma (TSA)
HP vs Sessile serrated adenoma
Traditional serrated adenomas (TSA): mixed adenomatous/hyperplastic polyp or serrated adenoma
Extensive DNA methylation

- About 50% of genes have clusters of dinucleotide cytosine guanine (CpG) within their promoter regions: CpG islands
- Normally unmethylated
- Transcriptional inactivation of genes
- Suppressor genes: BRAF, TGFβRII, BAX, IGF2R
- Epigenetic changes: changes in gene activity
- CpG island methylator phenotype (CIMP or CIMP-high)
Mutually Exclusive Relationships

Chromosomal instability pathway

Microsatellite instability pathway

Epigenetic instability pathway

Partially Overlapping Two Pathways
Major Pathways for Colorectal Cancer

**Classic APC Pathway**
- MSS: 60%

**CIMP Pathway**
- MSS: 25%
- MSI: 10%

**Classic Lynch Pathway**
- MSI: 5%

Four Molecular Subtypes of Colorectal Cancers according to CIMP and MSI status

1. CIMP-/MSI+, 8%
2. CIMP+/MSI+, 5%
3. CIMP+/MSI-, 8%
4. CIMP-/MSI-, 79%

MSI+ CRC, 1+3, 13%
CIMP+ CRC, 2+3, 13%

$P=0.0006$
# Molecular Classification for Colorectal Cancer

<table>
<thead>
<tr>
<th>Group 1</th>
<th>CIMP-high/MSI-H/BRAF mutation</th>
<th>Serrated polyps</th>
<th>12%</th>
<th>Good</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 2</td>
<td>CIMP-high/MSI-L or MSS/BRAF mutation</td>
<td>Serrated polyps</td>
<td>8%</td>
<td>Bad</td>
</tr>
<tr>
<td>Group 3</td>
<td>CIMP-low/MSS or MSI-L/KRAS mutation</td>
<td>Adenomas or serrated</td>
<td>20%</td>
<td>?</td>
</tr>
<tr>
<td>Group 4</td>
<td>CIMP-neg/MSS</td>
<td>Adenomas</td>
<td>57%</td>
<td>Average</td>
</tr>
<tr>
<td>Group 5 (Lynch)</td>
<td>CIMP-neg/MSI-H</td>
<td>Adenomas</td>
<td>3%</td>
<td>Good</td>
</tr>
</tbody>
</table>

J R Jass, Histopathology 2007
### Table. Estimated Drug Costs for Eight Weeks of Treatment for Metastatic Colorectal Cancer.

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Drugs and Schedule of Administration</th>
<th>Drug Costs*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Regimens containing fluorouracil</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mayo Clinic</td>
<td>Monthly bolus of fluorouracil plus leucovorin</td>
<td>63</td>
</tr>
<tr>
<td>Roswell Park</td>
<td>Weekly bolus of fluorouracil plus leucovorin</td>
<td>304</td>
</tr>
<tr>
<td>LV5FU2</td>
<td>Biweekly fluorouracil plus leucovorin in a 48-hr infusion</td>
<td>263</td>
</tr>
<tr>
<td><strong>Regimens containing irinotecan or oxaliplatin</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Irinotecan alone</td>
<td>Weekly bolus</td>
<td>9,497</td>
</tr>
<tr>
<td>IFL</td>
<td>Weekly bolus of fluorouracil plus irinotecan</td>
<td>9,539</td>
</tr>
<tr>
<td>FOLFIRI</td>
<td>LV5FU2 with biweekly irinotecan</td>
<td>9,381</td>
</tr>
<tr>
<td>FOLFOX</td>
<td>LV5FU2 with biweekly oxaliplatin</td>
<td>11,889</td>
</tr>
<tr>
<td><strong>Regimens containing bevacizumab or cetuximab</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FOLFIRI with bevacizumab</td>
<td>FOLFIRI with fortnightly bevacizumab</td>
<td>21,399</td>
</tr>
<tr>
<td>FOLFOX with bevacizumab</td>
<td>FOLFOX with biweekly bevacizumab</td>
<td>21,033</td>
</tr>
<tr>
<td>Irinotecan with cetuximab</td>
<td>Weekly irinotecan plus cetuximab</td>
<td>30,790</td>
</tr>
<tr>
<td>FOLFIRI with cetuximab</td>
<td>FOLFIRI and weekly cetuximab</td>
<td>30,675</td>
</tr>
</tbody>
</table>

* Costs represent 95 percent of the average wholesale price in May 2004.

For 8 weeks course; $161,000 for 1 year treatment
KRAS testing before Anti-EGFR therapy

- Second & third line therapy for metastatic CRC
- Only effective if KRAS is wild type
- ASCO guideline in 2009
- KRAS mutation: 30-40%
- Substantial saving for health care system
  - KRAS testing: <$500/test
  - Erbitux: $2,491 x 24 infusions = $60,000
- Other mutations in EGFR pathway:
  - BRAF, PIK3CA
Aspirin use, tumor \textit{PIK3CA} mutation, and colorectal cancer survival

Figure 1. Mortality among Patients with Colorectal Cancer, According to Regular Use or Nonuse of Aspirin after Diagnosis and \textit{PIK3CA} Mutation Status.

Panels A and B show colorectal cancer–specific mortality among patients with mutant-\textit{PIK3CA} tumors and those with wild-type \textit{PIK3CA} tumors, respectively, and Panels C and D show overall mortality in the respective subgroups of patients.

Next Generation Sequencing (NGS) for CRC

MI-ONCOSEQ:
The Michigan Oncology Sequencing Center

Tumor Biopsy → Informed Consent → Buccal swab or Blood → Sequencing → Analysis

Sequencing Tumor Board

1) Actionable Results?
2) Incidental Results?

Disclosure of Results

Genetic Counselor
Cost for NGS/whole genomic sequencing

- 1990: 8 years, 3 billions
- 2010: 3-4 months, $100,000
- Near future: several days, >$1,000
- Future: <24 hours, <$1,000
Promise of Genomic Medicine

- Personalized medicine
- Improves patient outcome
  - more effective treatment
  - less toxic drugs
- Reduces cost
Personalized Medicine

- Molecular characteristics of DISEASE
  - molecular classification of tumor
  - characterization of tumor heterogeneity
  - characterization of therapeutic targets

- Molecular characteristics of HOST
  - disease susceptibility
  - treatment efficacy (e.g., pharmacogenomics)
Other -omics Techniques

- Transcripomics (mRNA)
- Interferomics (iRNA)
- Epigenomics
- Tumor cells (oncomics) vs stroma (stromics)
- Proteins vs nucleic acids (e.g., proteomics and metabolomics)
“Specialty Pharmacy” services

- “Accredited Specialty Pharmacy” at UK
- FDA approval for 900 new drugs by 2016
- 40%: specialty medications
  - requires associated genetic lab tests
  - majority are cancer drugs
- **Pre-authorization** for specialty medications and associated genetic lab tests by **insurances**
What will the pathology report in the future look like?

- Site of biopsy
- Morphology
- Point mutations
- Germline alterations
- Copy number aberrations
- Gene rearrangements
- Gene expression (pathway activation)
- Drugs matched to mutation
"Information Overload"

"I think we over-ordered"
New prognostic DNA tests for colon cancers

- On their way to market
- Oncotype DX Colon Cancer Assay, ColoPrint
- Similar to Oncotype DX & Mammaprint for breast cancer
- NONE have shown any proven utility
Familial syndromes

- Familial adenomatous polyposis (FAP)
  a) Classic FAP
  b) Attenuated FAP
  c) Gardner syndrome
  d) Turcot syndrome
- Lynch syndrome (HNPCC)
- Others (e.g.: Serrated adenomatous polyposis, Juvenile polyposis syndrome, etc.)
Familial adenomatous polyposis (FAP)
Classic FAP syndrome

- Usually 1000, at least 100 adenomas
- Colon, small intestine (ampulla), stomach
- Relatively uncommon
- Germ-line (inherited) APC gene mutation ("first hit")
- Adenomas in 2nd and 3rd decades
Classic FAP syndrome

- Colon cancer in 100% of untreated patients
- 10% after 10 years, 100% after 40 years
- Often before age of 30
- Family screening and early detection
- DNA marker (APC gene mutation)
- Flexible sigmoidoscopy at age of 12
- Prophylactic proctocolectomy
Diagnosis of Lynch Syndrome

- For other family members
  - Early screening program for CRC
  - Endometrial cancer
- Second tumor in colon (50% within 15 years)
- Differences in prognosis and therapy
Tools to detect MSI (and exclude sporadic tumors)

- Immunohistochemistry
  - mismatch repair proteins: MLH1, MSH2, MSH6, PMS2

- PCR
  - to detect MSI (MSI testing)

- Sequencing of suspected gene (Genetic testing)
  - to confirm the diagnosis of Lynch syndrome

- BRAF mutational analysis (or IHC for BRAF)
  - BRAF mutation = sporadic CRC

- MLH1 methylation assay
  - MLH1 promoter hypermethylation = sporadic CRC
Two Molecular Pathways of MSI

HNPCC
- MSH2, MLH1 mutation
  - CIMP-/MSI+

Sporadic
- MLH1 methylation
  - CIMP+/MSI+
Loss of MSH2/MSH6 expression
<table>
<thead>
<tr>
<th>IHC</th>
<th>MLH1</th>
<th>PMS2</th>
<th>MSH2</th>
<th>MSH6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MLH1</strong> Mutation</td>
<td>Loss</td>
<td>Loss</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td><strong>PMS2</strong> Mutation</td>
<td>Positive</td>
<td>Loss</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td><strong>MSH2</strong> Mutation</td>
<td>Positive</td>
<td>Positive</td>
<td>Loss</td>
<td>Loss</td>
</tr>
<tr>
<td><strong>MSH6</strong> Mutation</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Loss</td>
</tr>
</tbody>
</table>

- Cleveland Clinic
  (J Clin Oncol 31: 1336-40, 2013)

- Only to surgeons (2004-2007)
  - Genetic counseling (GC) for 32% of possible LS
  - Gene sequencing (GT) for 26%

- Surgeons & genetic counselor (2007-2008)
  - GC for 64% & GT for 45%

- Direct contact by genetic counselor (July 2008-)
  - GC for 71% & GT for 66%
Ohio State University

- Excellent Cancer Genetics department
- No good infra structures until 4-5 years ago
- Screening Algorithm
  (Am J Clin Pathol 2013: 140: 177-183)
Old practice at UK

- Only highly suspicious cases
- Genetic counseling first
  - Amsterdam Criteria
  - Bethesda guideline
- MSI test $\rightarrow$ IHC $\rightarrow$ genetic testing
Our new protocol
(all new colon resections: 200 cases/yr)
Our new protocol

- Liz Reilly, Genetic Counselor

- Biopsies by clinician’s or GC’s request
  - e.g.: pre-chemoradiation biopsy for rectal cancer

- MSI testing for certain cases including equivocal IHC cases

- Other tests by GC’s request
  - large deletions of EPCAM gene (lies next to MSH2)
Take home message

- Very common, preventable cancers
- Colonoscopic examination/screening
  - early diagnosis of cancer
  - removal of adenomas
- Lynch syndrome
  - diagnosis
  - screening for family members
  - ? joint efforts in future
MSI Testing

- PCR
- Dissection or micro-dissection
- 2002 Revised Bethesda Panel
  - 2 mononucleotide and 3 dinucleotide repeats
- MSI-High: at least 2 of 5 markers are abnormal
- MSI-Low: 1 of 5 markers is abnormal (unknown clinical significance)
IHC vs MSI

- Similar sensitivities and specificities
- Pros & cons
  - Convenience (e.g.: no micro-dissection)
  - Candidate gene for GT
Newly diagnosed CRC: IHC for MLH1, MSH2, MSH6, PMS2

- All proteins present
- Absent MLH1 and PMS2

  BRAF mutation analysis

- BRAF mutation present
- No BRAF mutation

  - Gene testing of MLH1/PMS2
  - Gene testing of whichever has absent staining

  If germline mutation, then LS; if no germline mutation, correlate with family history, MSI analysis, MLH1 methylation, etc.