Colorectal Cancer
Molecular Diagnostics

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Molecular CRC Testing

- MSI, MMR IHC
- KRAS
- BRAF
- PIK3CA
- PTEN
- APC, SMAD4, BMPRIA, STK11
- Septin 9
MSI Testing

• MSI-H Sporadic (15%)  

• MSI-H Lynch (2-3%)
Lynch Syndrome Cancers

- Colorectal CA 80%
- Endometrial CA 50%
- Pancreatic, gastric, small bowel, sebaceous skin, ovarian, genitourinary, GBM cancers
- Screening: Age 25 or 10 years < than youngest

Annual colonoscopy & endometrial bx, periodic EGD, EUS of pancreas, pelvic exam, brain scans, urine cytology

HUGE & LIFELONG IMPACT ON LYNCH PATIENTS: DX IS CRITICAL
Microsatellite instability (MSI)

Microsatellites: Short, repetitive DNA sequences prone to error during replication

Normally repaired by MMR gene proofreading complex
Microsatellite instability (MSI)

- Mutations in MMR genes lead to accumulation of altered length microsatellites (MSI)
- MLH-1, MSH-2, MSH-6, PMS-2 alterations cause MSI-H cancer
MSI-High Colon Cancer

- **Sporadic:** ~100% MLH1 (methylated)

- **Lynch:** 60% MLH1, 35% MSH2, 5% PMS2, MSH6, other

- **Familial & sporadic path:** identical
Right-sided location
Age < 50 years (Lynch)
Poor differentiation
Absence of dirty necrosis
>2 tumor infiltr lymphs/hpf
Mucinous change
Crohn’s-like lymphoid reaction

Duodenal or Gastric Adenoma

Consider FAP and Lynch Syndrome
Reasons to Diagnose MSI-H CRC

• Hereditary and syndromic components of Lynch
• Prognosis
• Therapy
Lynch Testing

Tumor screening assays (90% sens)
Detect affected patients with tumor
MSI by PCR (paraffin works well)
MMR Immunohistochemistry:
MLH-1, MSH-2, MSH-6, PMS2

Blood germline mutation analysis
Detect affected family members without tumor
MSI/IHC: Other Tumors

- Non-colon tumor tissue
- Endometrial neoplasia
- Other syndromic tumors
- Fresh or fixed (formalin)
MSI Requirements

- Also need constitutional (normal) DNA
  - Non-tumor paraffin tissue
  - Blood
  - Buccal swab
MSI Electropherogram Results

Microsatellite-High (MSI-H)  Instability at ≥ 2 loci
Microsatellite-Low (Indeterminate)  Instability at 1 locus
Microsatellite stable (MSS)  None of 5 loci unstable

Normal

Carcinoma

Unstable  Unstable  Unstable  Unstable  Unstable  Unstable
IHC in Lynch Syndrome
Sporadic vs. Lynch CRC

- Family history
- MSH2, MSH6, PMS2 IHC loss
- Adenoma involvement
- MLH1 promoter methylation
- BRAF point mutation (V600E)
- Germline MMR gene mutation
MSI: Prognosis & Adjuvant Rx

MSI-H in randomized stage-matched sporadic tumors predicts:

• Longer survival
• Chemotherapy

Ribic CM, et al. NEJM 349:247;2003
Consider Lynch syndrome

MSI by:
Immunohistochemical stains or PCR

- Negative for MSI
  - Probably not Lynch syndrome
- Positive for MSI
  - Abnormal MLH1 staining
    - BRAF V600E mutation, MLH1 methylation
      - Alteration absent (wild-type)
        - Seq & Del/Dup MLH1
      - Alteration present
        - Probable sporadic colorectal cancer
  - Normal MLH1 staining and abnormal for MSH2, MSH6, or PMS2
    - Seq & Del/Dup MSH2, MSH6, PMS2
KRAS Testing
The Metastasis Problem

- 50-60% CRC patients present with or develop metastases
- 5-yr survival
  - Stage I + II (N0) → 91%
  - Stage III (N1,2) → 70%
  - Stage IV (M1) → 11%
Search for alternate Rx’s

5 FU/Leucovorin mainstay for decades

After 2000 → new therapies

Oxaliplatin (FOLFOX)

Irinotecan (FOLFIRI)

Anti-VEGF (bevacizumab)

Anti-EGFR (cetuximab, panitumumab)
The diagram illustrates the signaling pathway involving EGFR activation and its impact on cellular processes. EGFR is activated by generating phosphorylation sites (P). This leads to the recruitment of Grb2 and SOS, which in turn activate RAS. RAS then activates RAF and MEK, which further activates AKT. AKT can be inhibited by PTEN. PI3K is activated by AKT, and PI3K can also be inhibited by PTEN. The pathway leads to transcriptional regulation, influencing cell proliferation, growth, survival, and angiogenesis.
KRAS mutation

- <1% response rate to anti-EGFR Rx with codon 12 or 13 or 61 mutations (~40% of CRC)
- ~40% response rate with KRAS WT (~60% of CRC)
- But…. ~ 60% KRAS WT will not respond
- Other markers play a role
Cost (savings)

- ~30,000 new metastatic CRC annually
- KRAS testing = $13 million ($452/pt)
- Cetuximab Rx= $2.1 billion ($71,120/pt)
- Mutated KRAS (~40%) excluded from cetuximab
- Cost savings: ~$750 million annually
- High toxicity; ~2 month added survival
No need for normal tissue for KRAS testing
KRAS Methods

• All methods applicable to formalin-fixed paraffin-embedded tissue

• Tumor microdissection

• Sequencing, Sequenom, allele specific PCR, and melt curve analysis
Future

• Impact of *specific* KRAS 12/13 mutations?
• Other predictors of anti-EGFR response
  • Other KRAS mutations: codon 61, others?
  • BRAF
  • EGFR copy number (FISH, CISH, PCR), specific mutations
  • PTEN
  • PIK3CA
KRAS Summary

- KRAS mutations in 30-40% CRC’s
- Highly predictive of lack of response to anti-EGFR Rx
- Laboratory plays key role determining proper and most cost-effective Rx in stage III-IV CRC
- BRAF, PIK3CA, PTEN downstream markers may be useful in KRAS wild type tumors
- Additional biomarkers expected
BRAF Testing
BRAF

- ~10% colorectal cancers have BRAF mutations
- Predicts anti-EGFR non-response in KRAS WT

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<th>BRAF Wild Type Response</th>
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BRAF mutation
- T to A transversion
- Valine to glutamate at codon 600 (BRAF V600E)

BRAF: Downstream from KRAS

BRAF and KRAS mutations:
- Mutually exclusive

KRAS-WT/BRAF-MUT
- Anti-EGFR therapy non-responders
Chemoradiation Rx: Does it affect GI cancer molecular testing?

• Neoadjuvant Rx common in rectal & esophageal adenocarcinomas

  - Pre and Post neoadjuvant Rx
  - No change in 18 patients for MSI PCR
  - No change in 18 patients for KRAS mutations by Sanger sequencing
Gastrointestinal Polyposes Predisposing to CRC
Case 1

• 18 yo boy currently asymptomatic but strong family history of colon cancer

• Colonoscopy reveals hundreds of colonic polyps

• Colectomy is performed
Familial Adenomatous Polyposis

• Sequencing reveals APC knockout mutation
• Risk of colorectal cancer 100%, average age onset 39 years
• Extra-colonic intestinal manifestations: duodenal & jejunal adenomas/carcinomas, gastric polyps (?)cancer
Case 2

- 45 yo man with 85 adenomas
- No APC mutation in germline
- Family history of colon cancer, recessive inheritance
- MUTYH gene is sequenced; compound Y179C & G396D germline mutation detected
MYH-assc polyposis (MAP)

- Colon polyps usually like attenuated FAP
- Extra-colonic: duodenal ad/ca, cancer of ovary, bladder, skin, sebaceous glands
- Most homozygous or compound heterozygous of Y179C and G396D mutations
Case 3

• 35 yo woman with breast & thyroid cancer

• Sister with ovarian cancer in her 20’s, thyroid nodule at 32, & childhood colon polyps

• FH of endometrial cancer

• Endoscopy reveals colonic, gastric & esophageal polyps
Glycogenic acanthosis in esophagus
Cowden’s syndrome

• Personal medical and FH history implicating Cowden’s syndrome

• PTEN mutation testing to reveal pathogenic germline change (R335X) of Cowden’s

• Emerging literature on colon cancer risk, plus previously known breast, thyroid, endometrial CA risks
Case 4

• 15 yo girl presents with rectal bleeding & small bowel obstruction
• Jejunal intussusception & polyposis
• Bowel resected
Peutz-Jeghers

- Hamartomatous polyps, mostly small bowel (jejunum), also stomach and colon
- Mucocutaneous hyperpigmentation
- Autosomal dominant mutation in STK11 (LKB1) gene (50-90 %)
- Multi-organ cancer syndrome: breast, colon, pancreas, stomach, lung, gyn, testes: 93% lifetime cancer risk
Case 5

• 57 yo man with upper GI bleeding
• Upper GI endoscopy revealed 5x6 cm gastric mass & multiple smaller polyps throughout stomach (biopsied as hyperplastic polyps)
• Mass & few smaller polyps resected
Juvenile Polyposis

- 28% SMAD4, 24% BMPR1A
- SMAD4 also causes hereditary hemorrhagic telangiectasia (HHT)
- SMAD4 mutations may also have severe gastric polyposis & ↑ gastric cancer risk
- JP at high risk for colon (20-70%) & gastric cancer (mostly SMAD4 for gastric)
Summary: CRC Molecular Dx: Current Impact on Practice: 15 Genes

- Metastatic CRC for Anti-EGFR Rx (50-60%): KRAS
- KRAS WT for Anti-EGFR Rx (40%): BRAF, PIK3CA, PTEN
- Sporadic MSI-H CRC (15%): MMR IHC, MSI, MLH1 Methylation, BRAF
- Lynch MSI-H (2-3%): MMR IHC, MSI, MLH1 Methylation, BRAF
- FAP (<1%): APC
- Juvenile Polyposis, Peutz-Jeghers, Cowden’s (<1%): SMAD4, BMPRIA, STK11, PTEN