What Is New in the Molecular Aspects of Lynch Syndrome?

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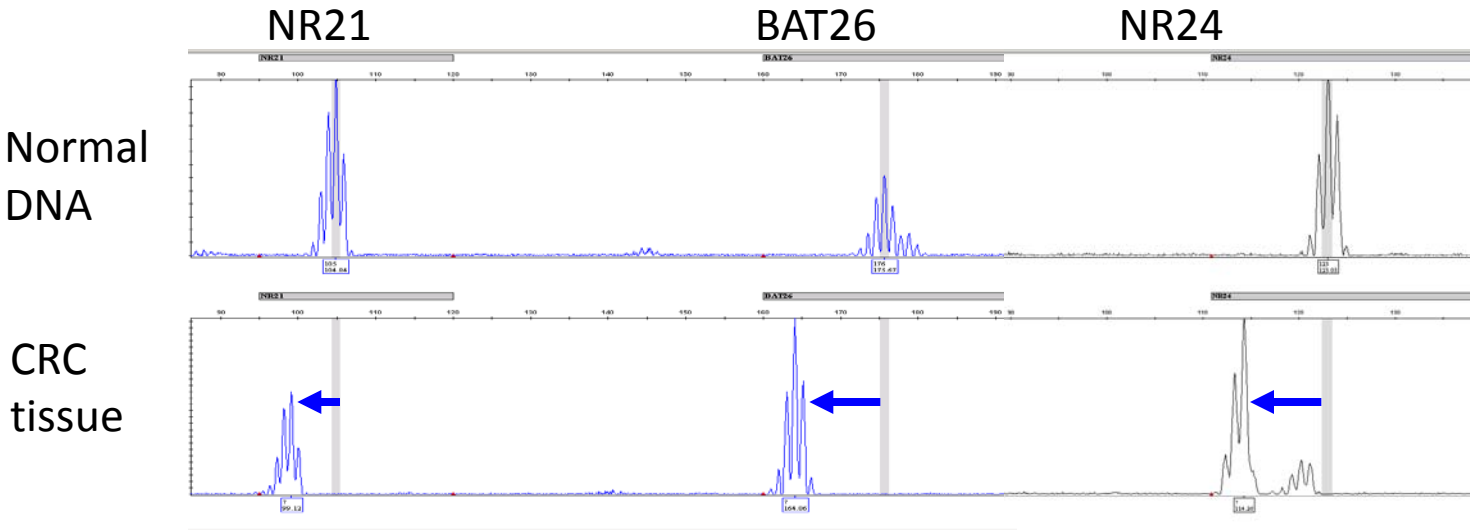
Lynch Syndrome in Israel
June 26, 2012

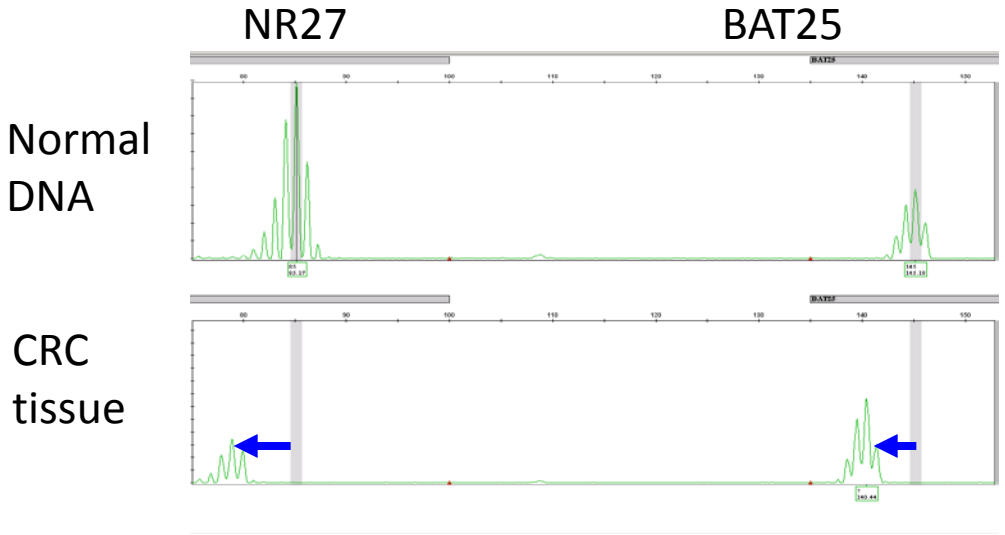
What are microsatellites?

- Simple repetitive DNA sequences
  - i.e., mononucleotide repeats (MNR), A_n, G_n, etc
  - also, dinucleotide repeats (DNRs), most often $[CA]_n$
  - also, longer repeats, tri- and tetra-nucleotide repeats

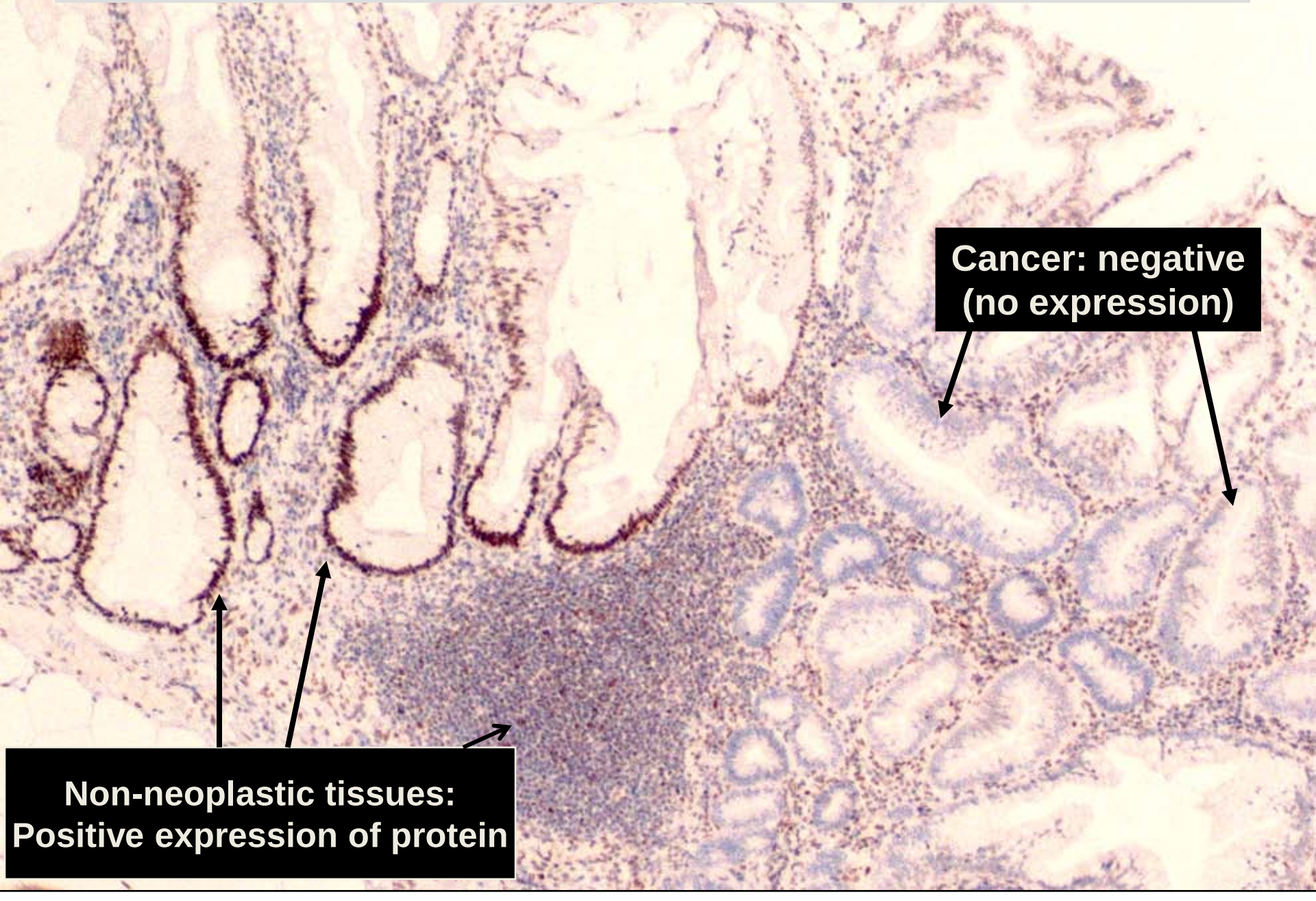
- In the absence of DNA MMR activity, they are very prone to deletion mutations (i.e., A_{10} -> A_9)
  - this is MSI

Microsatellite Instability: all 5 mutated





MLH1 Immunohistochemistry

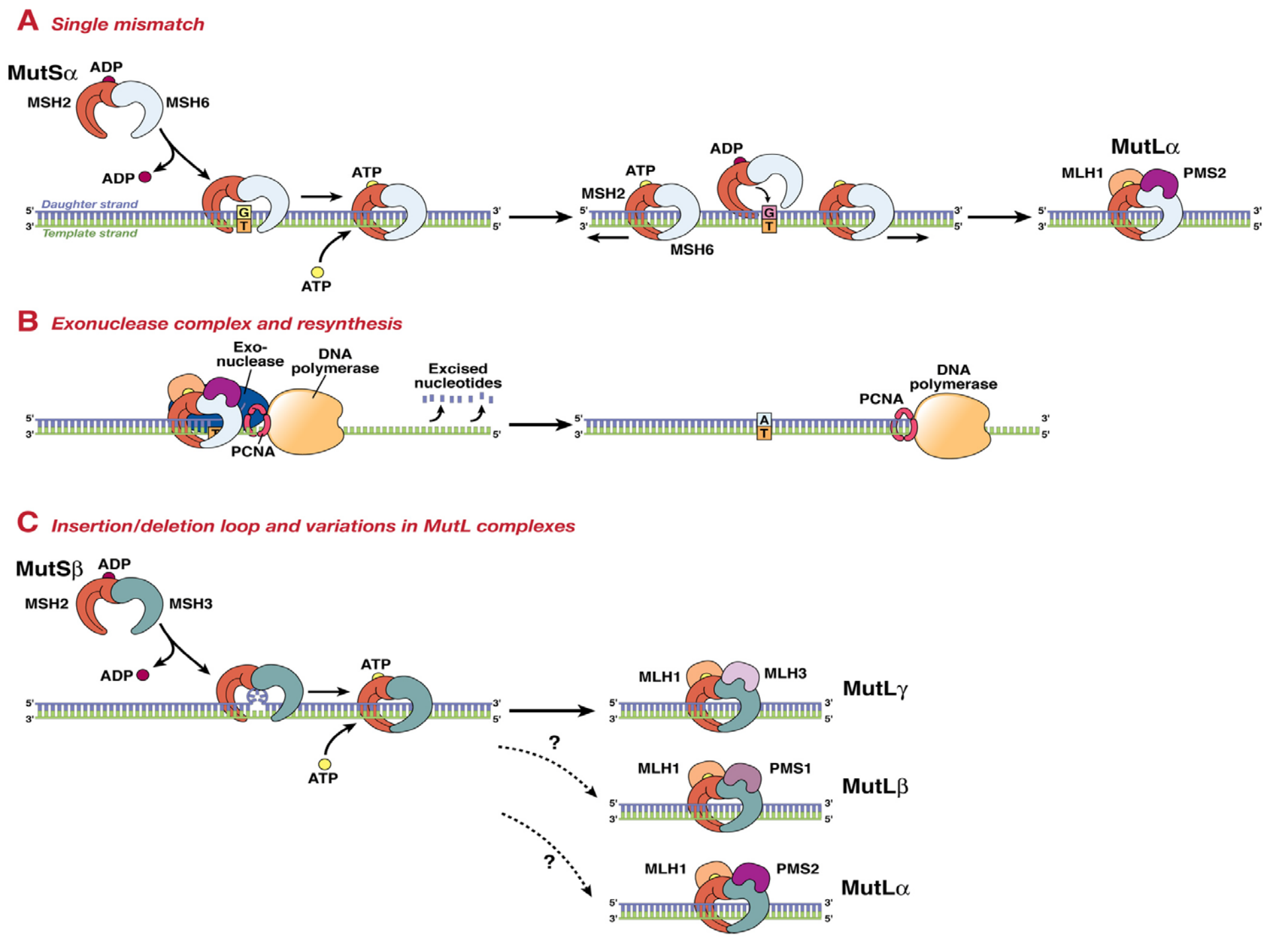


Practical Issues with IHC

- One sees **loss of** expression of the “culprit” gene product
- Occasionally, certain mutations will destroy enzymatic activity of the protein, and preserve immunoreactivity of the protein (i.e., falsely negative)
- Most (75-80%) MSI-H CRCs are due to epigenetic silencing of the hMLH1 gene
  - this is not Lynch syndrome

How to interpret the IHC:

Why are 2 proteins lost when only one gene is mutated?


A Single mismatch
MutSα
ADP
MSH2
MSH6
ADP
ATP
Daughter strand
Template strand
5'
3'
G
T
ATP
ADP
ATP
MSH2
MSH6
MutLα
MLH1
PMS2
B Exonuclease complex and resynthesis
Exo-
nuclease
DNA
polymerase
Excised
nucleotides
PCNA
A
T
DNA
polymerase
PCNA
C Insertion/deletion loop and variations in MutL complexes
MutSβ
ADP
MSH2
MSH3
ADP
ATP
ATP
MLH1
MLH3
MutLγ
?
MLH1
PMS1
MutLβ
?
MLH1
PMS2
MutLα

EARLY-ONSET CRC WITHOUT A FAMILY HISTORY SUGGESTING LYNCH SYNDROME

3 studies

Unexpected Lynch Syndrome among young CRC patients

- 75 CRC patients with no more than one relative with CRC, all <50 years old (mean 34.5), no FAP/UC, all from BUMC
- MSI (pentaplex PCR of 5 mononucleotide repeats)
- IHC testing of tumor tissue; MSH2, MSH6, MLH1, PMS2
- (No germline testing)

- 72% in the distal sigmoid colon or rectum
- MSI in 21%; abnormal IHC in 21% (n=16)
    - MSH2: 3: all MSI
    - MLH1: 3: all MSI
    - PMS2: 5: all MSI
    - MSH6: 5 (and only 2/5 had MSI)
- KRAS mutations in 22% if MMR defective; 78% if MMR normal
- No BRAF mutations in any young CRC patient

Goel et al, Clin Gastro & Hepatol 8:966, 2010

Epicolon Collaboration: Early-Onset CRC

- 140 CRC patients ≤50 years old, Spanish consortium (Epicolon); MSI, IHC, germline mutations in MMR genes, and MUTYH germline mutations
- Positive family histories not excluded
  - 26% had a + FH of CRC; 5.8% Amsterdam+
- 11.4% had MSI (5 MNRs), 14.3% had abnormal IHC
- 75% of the CRCs were in the distal colon
- Identified MMR germline mutations in 11 (7.8%)
- Somatic methylation of MLH1 in 1 (0.7%)
- KRAS mutations in 28%, BRAF mutations in 3.6%

Giraldez et al, Clin Can Res 16:5402, 2010

Epicolon Study of Early-Onset CRC

- Germline mutations
  - MLH1: 4 (2.8%)
  - MSH2: 1 (0.7%)
  - MSH6: 6 (4.3%) – 2/6 were MSS
  - Biallelic MUTYH: 4 (2.8%)

- About 15% have DNA MMR defects
- Underscores the role of MSH6 and MUTYH in young CRC patients

Giraldez et al, Clin Can Res 16:5402, 2010

Summary: early-onset CRC without a Lynch Syndrome family history

- Tend to be distal lesions (rectum, sigmoid)
- 14-21% are “cryptic” Lynch Syndrome
- Prominent involvement of MSH6 and PMS2
  - MSH6 may not show MSI
- Most are still a mystery

Epigenetics

- Alterations in gene expression that do not involve a change in the nucleotide sequences
- Includes DNA methylation (which can silence gene expression)
- Changes in miRNA expression
  - master controllers of gene expression

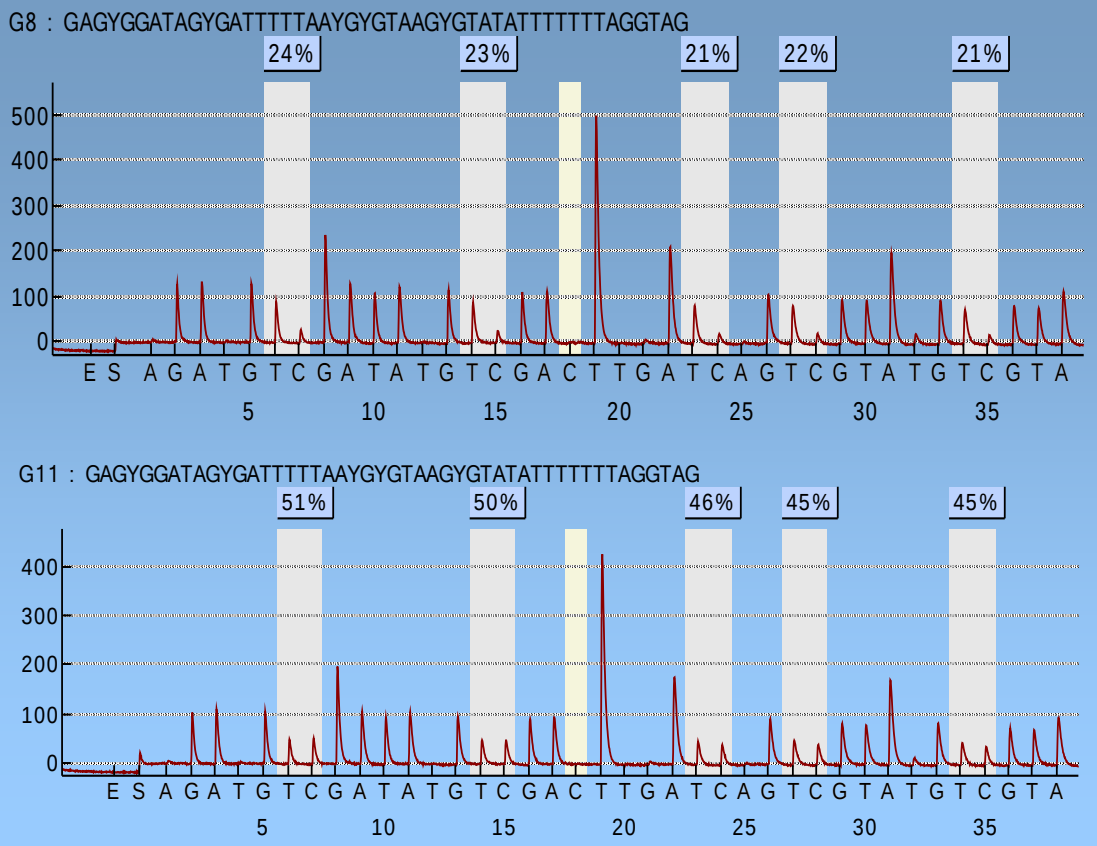
DNA **Hypo**methylation in Cancer

- Global hypomethylation:
  - Feinberg + Vogelstein (Nature, 1983)
  - Widespread through the genome in CRC
    - Reduced methyl-cytosine content by 8-10% in colorectal cancers and adenomas compared with normal colon
  - Loss of methylation at CpG sites in repetitive elements (LINEs, etc)
    - function and mechanisms are uncertain
    - associated with chromosomal instability

DNA **Hyper**methylation in CRC

- CpG Island Methylator Phenotype (CIMP)
- Baylin + Issa (1999)
  - Silences promoters at CpG sites (about half our genes)
- Promoter methylation associated with ageing
  - also with cancer
  - distinction between these is unknown
- CRCs may evolve principally through CIMP
- Also, methylation-induced silencing of MLH1 causes acquired (non-Lynch syndrome) MSI in CRC

Buccal mucosa and hair follicle tissues also show MLH1 hypermethylation



Buccal Mucosa

Average meth. = 22%

Hair Follicle

Average meth. = 48%

Goel, Hitchins, et al, Int J Cancer 128:869, 2010

Soma-wide Hypermethylation of MLH1 in a young patient with CRC

- 20 year old woman developed a 3 cm cancer in the descending colon, mucinous, Stage II
- MSI-H, loss of expression of MLH1, PMS2 and MSH6
- Negative family history
- No germline mutations in MLH1, MSH2 or MSH6
- MLH1 methylated in the tumor tissue (pyrosequencing, Deng-C)
  - Buccal mucosa DNA - 22% methylated
  - Hair follicle DNA - 48% methylated
  - PBL-LCL DNA - methylated 14% and 8%, respectively
    - PBL methylation rose to 22% after 12 cycles of FOLFOX chemo
    - No methylation in either parent or brother
- Somatic LOH of the unmethylated allele in the tumor
  - Methylated allele was paternal

Goel et al, Int J Cancer 128:869, 2011

Soma-wide hypermethylation of MLH1 (cont.)

- 18 year old male, cancer in ascending colon (T3N1M0), MSI-H, loss of MLH1 and PMS2 in tumor, no FH, no mutation in MLH1
- Dense hemiallelic methylation (36-50%) in tumor, PBLs, buccal DNA, saliva, hair follicles; none in parents; methylated allele was maternal, somatic loss of the non-methylated paternal allele in the tumor.

- Summary of 15 patients with constitutional MLH1 epimutations:
  - ages range from 18-67; 8/15 under age 50
  - Only 1/12 had BRAF mutations; 3/11 had KRAS mutations
  - LOH of non-methylated allele in 8/13; 2 had missense mutation 2nd hit

- Mechanism uncertain, but *acquired methylation* of MLH1 is linked to a SNP in the promoter (-93G/A), and the AA or AG genotype has less affinity for transcription factors and is prone to methylation

Goel + Hitchins, et al, Int J Cancer 128:869, 2011

Clinical Features of Soma-Wide Methylation of MLH1

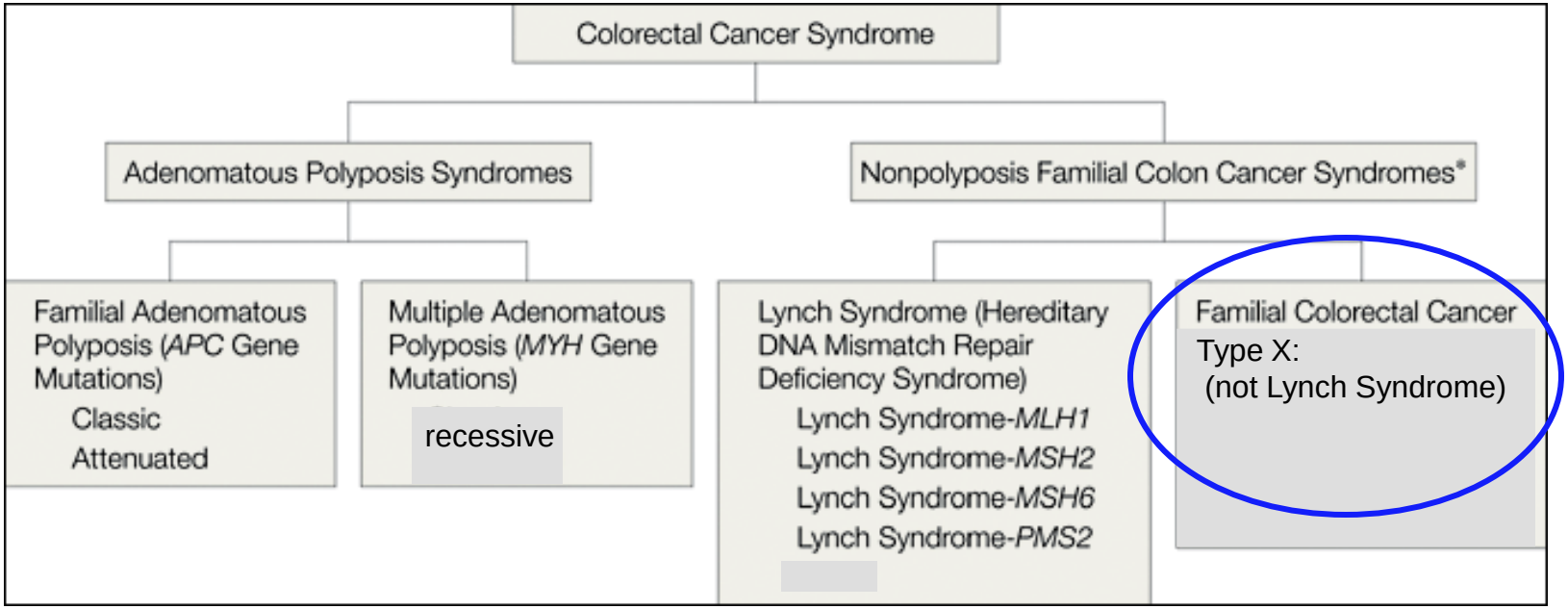
- Uncommon (~1% of MSI tumors)
- May occur in young adults (half <50)
- Methylation of MLH1 promoter in all 3 germ tissues
- May act like Lynch Syndrome
  - produces early-onset CRC
  - full tumor spectrum unknown
- May be acquired on maternal or paternal alleles
- Tumors may occur after LOH or mutation of the unmethylated allele

Possible Mechanism for Familial Soma-wide Methylation

- Single family with dominant inheritance of constitutional epigenetic silencing of MLH1
- Linked to a rare SNP in the MLH1 promoter (c.-27 C>A)
- Soma-wide mosaic MLH1 methylation and transcriptional silencing
- Methylation erased in sperm, but reinstated in somatic cells of the next generation
- Affected haplotype harbors 2 SNPs in tandem: c.-27C>A and c.85G>T, but c.-27C>A reduced transcription in reporter assays
- Not present in our patient

Hitchins Cancer Cell 20:200, Aug 16, 2011

FCC Categories



Lindor, N. M. et al. JAMA 2005;293:1979-1985.

Familial Colorectal Cancer that is not Lynch Syndrome (FCC-type X)

- Collaboration with X. Llor (Univ. Ill, Chi)

- Four groups of colorectal cancers:
  1. Amsterdam +, MSS, n=22
  2. Amsterdam +, MSI-H (Lynch Syndrome), n=21
  3. Sporadic MSS, n=92
  4. Sporadic MSI-H, n=46

- Methylation analyzed at 5 validated promoters (CIMP); LINE-1 methylation; mutations in BRAF and KRAS

- Methylation Index (MI) calculated from the 5 promoters (>5-10% meth)
  - "Low-MI" if 1-3 promoters methylated
  - "High-MI" if 4-5 promoters methylated

Goel et al, Gastroenterology 138:1854, 2010

Methylation Index (MI) in Syndrome X

Tumor Group	Low MI	High MI
MSS HNPCC (Syndrome X)	100% (22)	0
Sporadic MSS (92)	95.6% (87)	4.4% (4)
Lynch Syndrome (21)	90.5% (19)	9.5% (2)
Sporadic MSI (46)	32.6% (15)	67.4% (31)

Goel & Llor, Gastroenterology 138:1854, 2010

Line-1Methylation in Syndrome X

Tumor Group	% Line-1 methylation	Mean Rank (P =)
MSS HNPCC (Syndrome X)	60.08%	56.05 (--)
Lynch Syndrome	66.29%	94.80 (p=.015)
Sporadic MSI	67.27%	105.41 (p=.001)
Sporadic MSS	65.13%	86.22 (p=.009)

Goel & Llor, Gastroenterology 138:1854, 2010

RAS/RAF Mutations in Syndrome X

Tumor Group	BRAF	KRAS
MSS HNPCC (22) (Syndrome X)	0	31.8% (7, all codon 12)
Lynch Syndrome (21)	0	9.5% (2, both codon 12)
Sporadic MSS (92)	2.2% (2)	39.2% (36; 25 codon12, 11 codon 13)
Sporadic MSI (46)	28.3% (13)	4.4% (2 - codons12 + 13)

Goel & Llor, Gastroenterology 138:1854, 2010

Genetic Alterations in Syndrome X

Familial CRC without DNA MMR inactivation:

1. Significantly lower degree of Line-1 methylation (i.e, *less* global methylation) than in all other groups

   - probably reflects global hypomethylation

2. No evidence for promoter methylation (CIMP)

   - not a cryptic form of familial CIMP

3. No BRAF mutations (consistent with absence of CIMP); KRAS mutations similar to sporadic CRC

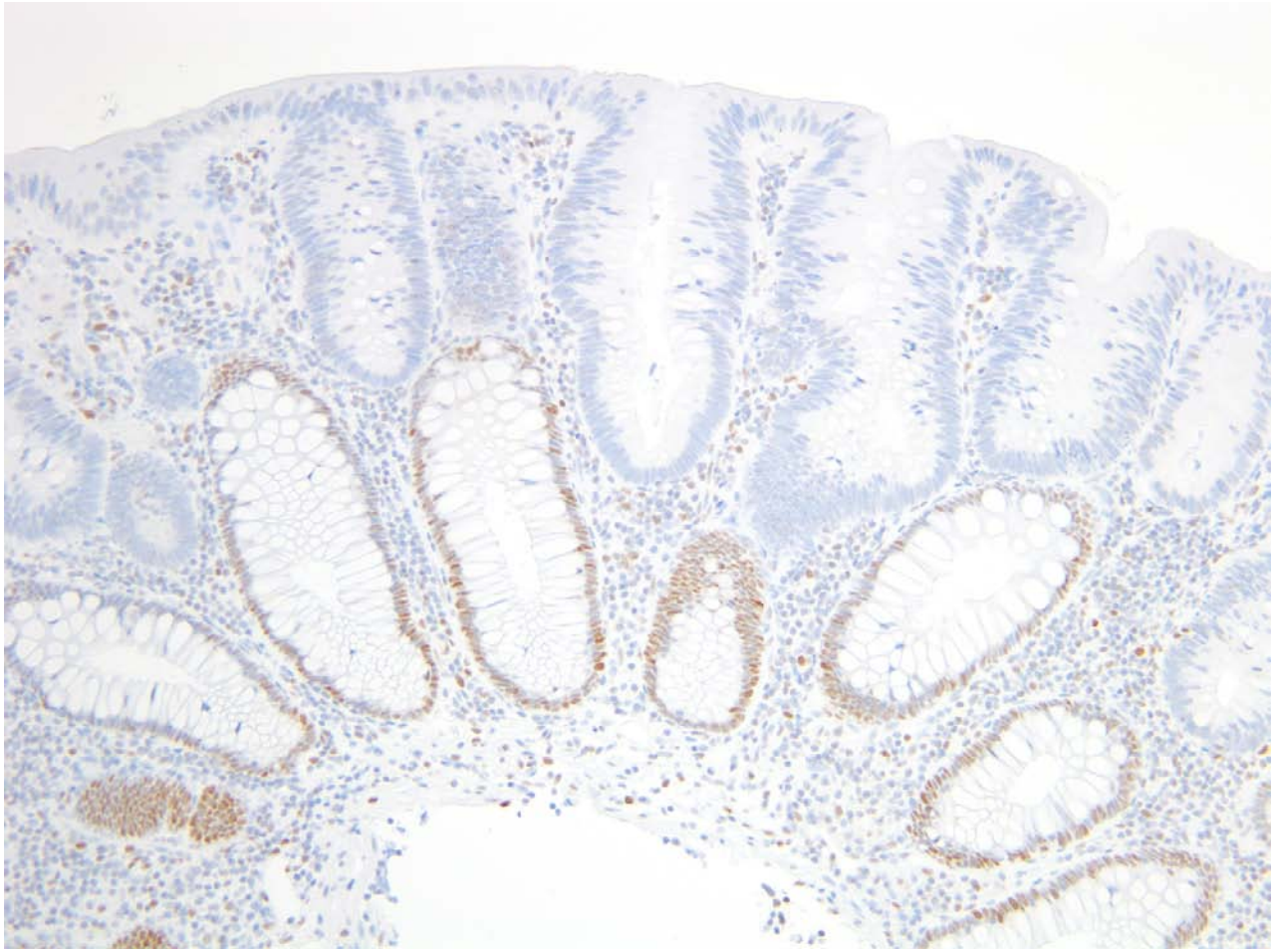
Goel & Llor, Gastroenterology 138:1854, 2010

When do loss of MMR proteins and MSI occur in Lynch Syndrome neoplasms?

- IHC and MSI testing on colorectal polyps from 34 Lynch Syndrome patients
  - 62 colorectal polyps (37 adenomas, 23 hyperplastic, 2 SSPs)
- MSI-H seen in 15/37 (41%) of adenomas, MSI-L in 8%;
- MSI in 0/21 hyperplastic polyps, 1/2 SSPs
- Abnormal IHC seen in 18/36 (50%) of adenomas, 0/21 hyperplastic polyps
- MMR defects tend to occur in larger polyps
  - present in 6/6 >10 mm, 2/7 if 5-9 mm, 7/22 if < 5 mm
  - MSI in 48% if MSH2, 33% if MLH1, 25% if MSH6

M.B. Yurgelun et al, MSI and DNA MMR Protein Deficiency in Lynch Syndrome Colorectal Polyps. Cancer Prevention Research:574-82, 2012

Loss of MMR Protein in Adenoma



Yurgelun et al, CPR, 2012

Conclusions from Kloor & Yurgelun

- DNA MMR-deficient loci are common in LS
  - Not necessarily the precursors of the neoplasms
    - Very frequent; most probably do not survive
    - Do not look neoplastic
    - Distinct from aberrant crypt foci morphologically
- Adenomas seem to evolve in the same fashion as sporadic polyps, and the “second hit” occurs during evolution of the adenoma
    - These lesions appear to grow very quickly
    - Positive tests are helpful; one cannot interpret negatives

Thank you.
Questions?