Published in final edited form as:

Semin Colon Rectal Surg. 2011 June 1; 22(2): 65–70. doi:10.1053/j.scrs.2010.12.002.

MOLECULAR BASIS OF HEREDITARY COLORECTAL CANCER

Matthew R. Hughes, MD and Emina H. Huang, MD[Associate Professor]

Department of Surgery, University of Florida, Gainesville, Florida

Abstract

Advances in molecular biology have defined the molecular basis for colorectal cancer (CRC). Though only a fraction of CRC has been determined to have a hereditary component, the discovery of genetic alterations in these clinical syndromes has permitted definition of similar discoveries in sporadic CRC. Here we will delineate the molecular basis for the most common of these defined syndromes, including familial adenomatous polyposis, hereditary non-polyposis colon cancer, *MUTYH* associated polyposis, Juvenile polyposis, Peutz-Jeghers syndrome, and Cowden's syndrome. The newest paradigm with implications for the pathogenesis of sporadic CRC is called the cancer stem cell hypothesis. As this paradigm also implicates aberrations in molecular pathways, a brief discussion of this hypothesis is included.

Keywords

Hereditary; colorectal cancer; genetics; cancer stem cells

Introduction

As the third most common cause of cancer and second most common cause of cancer-related death in the United States, colorectal cancer is a serious health problem. This year there will be an estimated 50,000 deaths in the U.S. alone along with over 140,000 new cases.¹ The World Health Organization estimates that almost 700,000 people died from colorectal cancer (CRC) worldwide in 2008.²

Over the last three decades, research has been able to shed light on the genetics of this disease. Although only about 5% of colorectal cancer cases are attributable to the hereditary syndromes, those patients and their tumors have allowed many questions to be answered in regards to the molecular basis of colorectal cancer. Most sporadic colorectal cancers share some of the genetic mutations seen in the hereditary syndromes.

Along with discussing some of the genetics seen in sporadic colorectal cancer, this review will cover the well known hereditary syndromes: Hereditary Nonpolyposis Colorectal Cancer (HNPCC), Familial Adenomatous Polyposis (FAP), Peutz-Jegher's Syndrome *MUTYH*-Associated Polyposis (MAP), Familial Juvenile Polyposis, and Cowden's syndrome. A new paradigm which implicates alterations in molecular genetics/pathways is known as the "cancer stem cell" hypothesis.³ As these alterations are involved in the

© 2011 Elsevier Inc. All rights reserved.

Mailing Address: Emina H. Huang, MD, Department of Surgery, 1600 SW Archer Road, PO Box 100109, University of Florida, Gainesville, FL 32610, Ph: (352)265-0761 (office), Fax (352)265-1060, Emina.Huang@surgery.ufl.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

maintenance of the protean characteristic of colon cancer stem cells, that is, ‘self-renewal’, this new paradigm is also included.

Hallmarks of Cancer

Most mutations along the pathway to tumorigenesis produce either oncogenes with gain of function or they block tumor suppressor genes resulting in loss of function. Hanahan and Weinberg noted that tumorigenesis is a multistep process and hypothesized that through these many steps tumors gained their abilities to grow and prosper. They posited that six essential alterations in cell physiology dictated malignant cell growth including: Evading apoptosis, self sufficiency in growth signals, insensitivity to antigrowth signals, sustained angiogenesis, limitless replicative potential, and tissue invasion and metastasis.⁴ In colorectal cancer, genetic mutations result in neoplasms with the capacity for this pathophysiology.

Adenoma-Carcinoma Pathway

The Adenoma-Carcinoma pathway for sporadic colorectal cancer was first hypothesized by Fearon and Vogelstein (Fig 1).⁵ Their elegant model described a process by which normal colonic cells could transform to tumor cells. Along with describing a combination of mutated proto-oncogenes and tumor-suppressor genes, they noted that four to five mutations were required to create a malignant cancer. *APC*, *ras*, *DCC*, and *p53* were all implicated. They also noted that the total accumulation of mutations was more responsible than the order of appearance for phenotype. Moreover, some mutant tumor suppressor genes have a biologic effect even in the heterozygous state⁵. Further, this accumulation of mutations is likely to take 15–17 years.⁶ The time needed for this accumulation coupled with an average age at the discovery of CRC in this country of 65 years, has resulted in the recommendation to start sporadic colorectal cancer screening at the age of 50 years. The effect of *APC* mutations and the WNT signaling pathway are delineated below in regards to FAP. While the contribution of *DCC* is now thought to most likely reflect alterations in the TGF β pathway, alterations in *ras* and *p53* contribute to the pathogenesis of sporadic CRC.

KRAS

KRAS is a proto-oncogene that encodes a guanine nucleotide binding protein. Upwards of 25% of all human cancers along with 35–50% of all colorectal cancers have a mutation in this gene.⁷ *KRAS* is a member of the RAS family of proteins and is involved in activating the RAF/MEK/ERK signaling cascade. This cascade mediates cell growth and entry into the cell cycle. Mutations in *KRAS* cause the protein to have reduced GTPase activity, thereby resulting in a protein with a persistently active conformation. Colorectal cancers with *KRAS* mutations are resistant to anti-EGFR agents such as cetuximab.⁸

DCC

DCC is a gene located on 18q21 that is felt to act as a tumor suppressor gene in colorectal cancer. The *DCC* protein is a netrin-dependent receptor that helps regulate apoptosis and may play a role in cell motility. *DCC* can induce apoptosis through caspase mediated cleavage of the addiction dependence domain (ADD), a pro-apoptotic domain. *DCC* mutations are only found in 10–15% of sporadic colorectal carcinomas and are felt to occur late in the tumorigenesis pathway.⁹

p53

While there exists a plethora of literature and indeed, entire careers are devoted to the study of this gatekeeper molecule, this protein is well-known for its role as a tumor suppressor

gene. Located on 17p12, it is the most commonly mutated gene in multiple different kinds of malignant tumors.¹⁰ *p53* produces a protein that normally induces cell cycle arrest to allow for DNA repair. Conversely, if there is an excess amount of DNA damage, it will induce apoptosis. About 80% of *p53* mutations are due to missense mutations between exons 5–8. Mutations in this gene are seen in 40–50% of sporadic colon cancers and are also felt to occur late in the tumorigenesis pathway.^{10–12}

Familial Adenomatous Polyposis

FAP is an autosomal dominantly inherited syndrome caused by a genetic mutation in the adenomatous polyposis coli (*APC*) gene. FAP patients typically develop hundreds to thousands of adenomatous polyps usually during the second to third decades of life. These polyps inexorably progress to CRC if not removed. *APC* gene mutation is also seen in 60–80% of sporadic CRC.¹³ In FAP, a germline allelic mutation is usually followed by loss of heterozygosity. There is a mutation cluster region (MCR) between codons 1286 and 1585 of the *APC* gene.¹² Mutations outside of this MCR are associated with a milder phenotype, referred to as attenuated-Familial Adenomatous Polyposis (aFAP). This subset of patients develops fewer polyps, at a later age, and with a lower risk of CRC (69%).¹⁴

Positional cloning identified the *APC* gene as the causative element for FAP.^{15, 16} The *APC* gene is found on chromosome 5q21 and its gene product is a 310kDa protein. The gene contains 15 exons, all of which can be affected by germline mutations; however, the majority of the mutations are present in exon 15. This protein binds to the axin/ β -catenin/GSK3B complex which leads to phosphorylation of β -catenin. This triggers it to bind to slimb/ β -TrCP which leads to the degradation of the proteasome that contains the phosphorylated β -catenin, thus preventing β -catenin from migrating to the nucleus. Mutations in the *APC* gene interfere with this function and lead to an increased cytoplasmic pool of β -Catenin. This allows β -catenin to migrate into the nucleus, where it activates downstream targets via binding to the Tcf-Lef promoter. Such targets include c-myc, a protooncogene, and cyclin D1, resulting in cell proliferation (Fig 2A). Binding of Tcf-Lef among others also leads to a down regulation of E-cadherin transcription (CDH1). E-cadherin is normally present at cell interfaces where it mediates adhesion. Activation of the Tcf-Lef promoter via β -catenin results in a reduction of E-cadherin mediated cell-cell adhesion and leads to proliferation of cells.

Two variants of FAP are Gardner's syndrome and Turcot's syndrome. In Gardner's syndrome, the colonic polyposis leading to colorectal cancer is combined with skin tumors, epidermoid cysts, congenital hypertrophy of the retinal epithelium, desmoids tumors, duodenal carcinoma around the ampulla of Vater, hepatoblastoma, papillary and follicular thyroid cancer, and adrenal adenomas.¹⁷ It is unknown what causes certain individuals to display this phenotype versus FAP. Turcot's syndrome is another rare variant associated with *APC* mutation. These patients develop polyposis and CRC along with central nervous system tumors. Turcot's syndrome has also been found in the context of mismatch repair mutations involving *MLH1* and *MSH2* (See below¹⁸).

Hereditary Nonpolyposis Colorectal Cancer (HNPCC)

HNPCC, also known as Lynch syndrome, is an autosomal dominant syndrome that accounts for 3–4% of colorectal cancers. Patients with the syndrome have an 80% lifetime risk of cancer. Originally described in the 1900's by the pathologist, Warthin, and elaborated on by Henry Lynch in 1974,¹⁹ the clinical features seen in HNPCC are due to genetic changes in mismatch repair (MMR) genes. The defects resulting from defective mismatch repair genes were originally defined in yeast by Fishel et al,²⁰ and correlated to human pathogenesis that year.²⁰ MMR gene products help preserve genomic integrity by recognizing and correcting

base pair mismatches and small nucleotide insertion/deletion mutations that occur during DNA replication (Fig 2B).²¹ Cells with MMR deficiency have a mutation rate that is 100–1000-fold greater than normal cells.²² At least four MMR genes that have been linked to HNPCC: *MLH1*, *MSH2*, *MSH6*, and *PMS2*. Germline mutations of these genes have been identified in up to 80% of affected families. *MLH1* and *MSH2* account for 90% of the mutations. Study of the non-encoding microsatellite regions of HNPCC tumor DNA show genetic instability secondary to multiple mutations specifically targeting repetitive sequences. This is referred to as microsatellite instability (MSI) and delineated via alterations in microsatellite sequences including: BAT25, BAT26, D5S346, D2S123, and D17S250. Tumors are classified as MSI-high (≥ 2 markers), MSI-low (< 2 markers), or MSI-stable based on the frequency of mutations detected.²² Most HNPCC tumors are MSI-high and CpG island methylator phenotype (CIMP) negative. 15% of sporadic CRC tumors show MSI, although most of these tumors are CIMP positive and have BRAF mutations, and the MSI occurs late in the tumorigenesis.

Phenotypically, patients with HNPCC have a predilection for right sided CRC tumors. They are also at risk of developing tumors in the endometrium, stomach, ovary, hepatobiliary tract, upper urinary tract, pancreas, small bowel, and central nervous system. A subset of this syndrome is referred to as Muir-Torre syndrome and includes those with skin tumors consisting of sebaceous adenomas, basal cell cancers, and keratoacanthomas.

There are two sets of criteria to support the diagnosis of HNPCC: the family history based Amsterdam II criteria and the Revised Bethesda criteria.²³ The revised Bethesda criteria serve as guidance for initiating genetic testing in absence of a striking family history. They combine patient and tumor features and take the MSI status of the tumor into account. In particular, features that should alert for possible HNPCC are include CRC with MSI-high histology, the presence of tumor infiltrating lymphocytes, Crohn's-like lymphocytic reaction, mucinous/signet-ring differentiation, or medullary growth pattern, and patient age of less than 50 years.²⁴

MUTYH Associated Polyposis

MUTYH associated polyposis (MAP, also known as MYH-associated polyposis) is an inherited polyposis syndrome with a phenotype similar to FAP but with an autosomal recessive mode of inheritance. The *MUTYH* gene is located on chromosome 1p34. Its gene product, *MUTYH* glycosylase, is a 535 amino acid base excision repair protein involved in the repair of guanine oxidation, one of the most common forms of oxidative DNA damage^{25, 26}. Guanine oxidation results in the appearance of G:C to T:A transversions. *MUTYH* is required under oxidative stress for normal cell-cycle progression and nuclear division. This suggests a role in the maintenance of genome stability and tumor prevention. Mutations in *MUTYH* can cause severely hampered or completely absent DNA binding and repair activity. Molecular profiles of tumors from MAP patients show G:C to T:A transversions in the *APC* gene and K-ras, a proto-oncogene. MAP tumors are usually MSI-low.

Due to the autosomal recessive inheritance pattern, patients with MAP have biallelic mutations in *MUTYH*. They typically develop 10–100 adenomas, often with a more proximal distribution pattern. On average, *MUTYH* patients are diagnosed with CRC in their late 40's. *MUTYH* heterozygotes are at marginally increased risk of developing CRC.²⁵

Juvenile Polyposis syndrome

Juvenile polyposis syndrome²⁷ is a syndrome with a predisposition to the development of hamartomatous polyps in the stomach, small intestine, colon, and rectum. Most polyps are

benign, but some polyps have the potential for malignant transformation. The incidence of CRC in JPS patients is 68% by the age of 60, and the average age when CRC is diagnosed in this syndrome is 42.²⁷

The polyps develop due to a germline mutation in *SMAD4* (homologs of the *C. elegans* protein *SMA* and the drosophila protein *mothers against decapentaplegic*) or *BMPRIA* (Bone Morphogenic Protein) (Fig 2D).

SMAD4 is a tumor suppressor gene that produces a 552 amino acid protein encoded by 1656 nucleotides. It is a critical cytoplasmic mediator of the TGF- β superfamily regulating pathway. The MH1 domain of the *SMAD4* protein directly binds DNA in response to TGF- β signaling. Pathological allelic variants involving that N-terminal binding site significantly reduce DNA binding activity. Mutations in the MH2 domain appear to cause problems with nuclear localization and interaction with MAD proteins and transcriptional activation.

The *BMPRIA* gene product is a 533 amino acid protein that is a type 1 receptor of the TGF- β superfamily that mediates BMP intracellular signaling through *SMAD4*. Abnormal *BMPRIA* proteins result from pathologic DNA variants in the protein kinase domain, occasionally by variants in the cysteine rich region of the extracellular domain.

Cowden's Syndrome

Cowden's syndrome is an autosomal dominant condition associated with multiple hamartomas in multiple areas of the body. The lifetime risk of CRC approaches 10 %.²⁸ Germline mutations of *PTEN* (phosphatase and tensin homologue deleted on chromosome 10) are found in 80% of Cowden's patients. *PTEN* is a tumor suppressor gene that encodes a 403 amino acid protein which acts as a lipid phosphatase to negatively regulate the PI3K/AKT pathway (Fig 2C).²⁸ Mutations in *PTEN* have been shown to cause increased nuclear β -catenin leading to increased expression of c-Myc and cyclin D1 (see FAP above). Interestingly, though both *PTEN* loss and aberrations in the Wnt signaling pathway lead to increased nuclear β -catenin, and both alterations result in a polyposis phenotype, the types of polyps present are different. In the former, the polyps are hamartomatous representing both epithelial and stromal proliferation, while in FAP, the polyps are adenomas, with a dominantly epithelial histology. Though both pathways engage β -catenin as a transcription factor at the minimum, the differences in phenotype suggest the potential for overlap in signaling pathways, differential levels of expression, or influences of the stroma on the ultimate phenotype. Regarding CRC, specifically, *PTEN* mutations have been shown to lead to an increase in the invasion and migration of colorectal cancer cells. Low *PTEN* expression correlates with CRC tumor size, depth of invasion, lymphatic invasion, lymph node metastasis, and higher TNM staging.^{28, 29} Patients with CRCs without *PTEN* expression have shorter survival *PTEN* suppression or loss is believed to contribute to tumor invasion and metastasis in advanced CRC disease.²⁹ One of the functions of *PTEN* is to inhibit the PI3K/AKT pathway. Activation of the PI3K/AKT pathway is common in several cancers. AKT has been shown to help regulate apoptosis and PI3K activation may play a role in chemotherapy resistance.

Cowden's syndrome typically involves mucocutaneous lesions of the face and mouth, along with tumors of the breast, thyroid gland, genitourinary tract, gastrointestinal tract, nervous system, and skeletal system. Breast cancer is the most common malignancy. Gastrointestinal involvement with polyps is seen in up to 85% of patients, and these almost always involve the sigmoid colon and the rectum. Hamartomas tend to be the predominant histological type, but lipomatous, fibromatous, hyperplastic, inflammatory, and adenomatous lesions may also be present.²⁸

Peutz-Jeghers Syndrome

Peutz-Jeghers Syndrome is an autosomal dominant syndrome in which affected patients develop gastrointestinal hamartomas and mucocutaneous hyperpigmentation. Most patients with PJS have a germline mutation of the gene *STK11* (*LKB1*), a tumor suppressor gene located on chromosome 19p13. *STK11* encodes a serine-threonine kinase that modulates cell polarity and cell proliferation.²⁷ It also helps in responding to low cellular energy levels. *STK11* inhibits the AMP-activated protein kinase which in turn regulates the gene product of tuberlin. Tuberlin facilitates the generation of Rheb-GDP from Rheb-GTP which activates mTOR. Germline *STK11* mutations result in the down-regulation of tuberlin, upregulation Rheb-GTP, and induction of mTOR. This inhibition is dysregulated in PJS patients.³⁰

Gastrointestinal cancers are frequent in PJS. The overall incidence of carcinomas ranges from 20–50%. Specifically, colon polyps are present in 53% while rectal polyps are seen in 32% of affected subjects³⁰. PJS patients have a 39% chance of developing colon cancer over their lifetime²⁷.

Stem Cells in CRC

Stem cells are defined by two properties: self renewal and multipotency. Self renewal is the cell's ability to perpetuate itself for an extended amount of time. Multipotency is the ability to generate all the differentiated cells of the tissue of origin. Normal colon stem cells can be found at the bottom of the epithelial crypts.³¹ The terminally differentiated cells at the luminal surface of the crypt are derived from these stem cells.

A new paradigm regarding tumorigenesis is termed the “cancer stem cell hypothesis”. This paradigm suggests that tumors are generated and maintained by a small subset of undifferentiated cells able to self renew and differentiate into the bulk tumor population.³ This paradigm was originally substantiated in leukemia, but now appears to be validated in colorectal cancer. Three recent studies appear to have found that colon cancer stem cells can be identified using certain cell markers: CD44, CD133, EpCAM, CD166, and aldehyde dehydrogenase (Fig 3).^{32–35}

Both normal stem cell self-renewal and cancer stem cell self-renewal are regulated by many pathways. However, for both leukemias and colon cancer the WNT pathway, for example, is likely to play a role.^{36, 37} Other pathways with implications for CRC include the Notch, Hedgehog, *PTEN/AKT*, *Bmi*, and *p53* molecular signaling pathways. The cancer stem cell theory has important implications for prevention and therapy. The fact that most cytotoxic agents used to treat colon cancer are designed to kill actively proliferating cells means that the cancer-causing stem cells are left behind and contribute to selecting resistance.³⁸ In the future, the use of cancer stem cell enrichment and evaluation will be invaluable to seek these rare cells as biomarkers for diagnosis and prognosis, and to identify targets for therapy.

Conclusion

Although the hereditary syndromes account for only 10–15% of cases, the study of their mutations has shed a great amount of light on the genetic mutations and molecular basis of colorectal cancer. As research continues in these areas, we may be able to target specific mutations with new therapies. Identification of certain mutations in tumors may assist us in better predicting prognosis and defining treatment strategies. The new cancer stem cell hypothesis for colorectal cancer lends an explanation for those patients who develop late recurrences or who develop asynchronous metastases. Further, additional research will result in focused treatment and eradication of these tumor initiating and propagating cells.

Acknowledgments

Supported in part by NIH R01 CA142808 (to EHH)

References

1. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. *CA Cancer J Clin.* Sep–Oct; 2010 60(5): 277–300. [PubMed: 20610543]
2. Saif MW, Chu E. Biology of colorectal cancer. *Cancer J.* May–Jun; 2010 16(3):196–201. [PubMed: 20526096]
3. Wicha MS, Liu S, Dontu G. Cancer stem cells: an old idea--a paradigm shift. *Cancer Res.* Feb 15; 2006 66(4):1883–1890. discussion 1895–1886. [PubMed: 16488983]
4. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell.* Jan 7; 2000 100(1):57–70. [PubMed: 10647931]
5. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell.* Jun 1; 1990 61(5): 759–767. [PubMed: 2188735]
6. Wood LD, Parsons DW, Jones S, et al. The genomic landscapes of human breast and colorectal cancers. *Science.* Nov 16; 2007 318(5853):1108–1113. [PubMed: 17932254]
7. Bos JL, Fearon ER, Hamilton SR, et al. Prevalence of ras gene mutations in human colorectal cancers. *Nature.* May 28–Jun 3; 1987 327(6120):293–297. [PubMed: 3587348]
8. Kranenburg O. The KRAS oncogene: past, present, and future. *Biochim Biophys Acta.* Nov 25; 2005 1756(2):81–82. [PubMed: 16269215]
9. Grady WM. Making the case for DCC and UNC5C as tumor-suppressor genes in the colon. *Gastroenterology.* Dec; 2007 133(6):2045–2049. [PubMed: 18054576]
10. Baker SJ, Fearon ER, Nigro JM, et al. Chromosome 17 deletions and p53 gene mutations in colorectal carcinomas. *Science.* Apr 14; 1989 244(4901):217–221. [PubMed: 2649981]
11. Takayama T, Miyanishi K, Hayashi T, Sato Y, Niitsu Y. Colorectal cancer: genetics of development and metastasis. *J Gastroenterol.* Mar; 2006 41(3):185–192. [PubMed: 16699851]
12. Baker SJ, Preisinger AC, Jessup JM, et al. p53 gene mutations occur in combination with 17p allelic deletions as late events in colorectal tumorigenesis. *Cancer Res.* Dec 1; 1990 50(23):7717–7722. [PubMed: 2253215]
13. Powell SM, Zilz N, Beazer-Barclay Y, et al. APC mutations occur early during colorectal tumorigenesis. *Nature.* Sep 17; 1992 359(6392):235–237. [PubMed: 1528264]
14. Knudsen AL, Bulow S, Tomlinson I, Moslein G, Heinemann K, Christensen IJ. Attenuated Familial Adenomatous Polyposis (AFAP) Results from an international collaborative study. *Colorectal Dis.* Jan 22; 2010
15. Groden J, Thliveris A, Samowitz W, et al. Identification and characterization of the familial adenomatous polyposis coli gene. *Cell.* Aug 9; 1991 66(3):589–600. [PubMed: 1651174]
16. Kinzler KW, Nilbert MC, Su LK, et al. Identification of FAP locus genes from chromosome 5q21. *Science.* Aug 9; 1991 253(5020):661–665. [PubMed: 1651562]
17. Juhn E, Khachemoune A. Gardner syndrome: skin manifestations, differential diagnosis and management. *Am J Clin Dermatol.* 2010; 11(2):117–122. [PubMed: 20141232]
18. Lebrun C, Olschwang S, Jeannin S, Vandenbos F, Sobol H, Frenay M. Turcot syndrome confirmed with molecular analysis. *Eur J Neurol.* Apr; 2007 14(4):470–472. [PubMed: 17389002]
19. Lynch HT, Smyrk T, Lynch JF. Molecular genetics and clinical-pathology features of hereditary nonpolyposis colorectal carcinoma (Lynch syndrome): historical journey from pedigree anecdote to molecular genetic confirmation. *Oncology.* Mar–Apr; 1998 55(2):103–108. [PubMed: 9499183]
20. Fishel R, Lescoe MK, Rao MR, et al. The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. *Cell.* Dec 3; 1993 75(5):1027–1038. [PubMed: 8252616]
21. Fishel R, Wilson T. MutS homologs in mammalian cells. *Curr Opin Genet Dev.* Feb; 1997 7(1): 105–113. [PubMed: 9024626]

22. Narayan S, Roy D. Role of *APC* and DNA mismatch repair genes in the development of colorectal cancers. *Mol Cancer*. Dec 12.2003 2:41. [PubMed: 14672538]
23. (NCCN) NCCN. Clinical practice guidelines in oncology: colorectal cancer screening. Vol. 1. 2010.
24. Gruber SB. New developments in Lynch syndrome (hereditary nonpolyposis colorectal cancer) and mismatch repair gene testing. *Gastroenterology*. Feb; 2006 130(2):577–587. [PubMed: 16472609]
25. Nielsen M, Morreau H, Vasen HF, Hes FJ. *MUTYH*-associated polyposis (MAP). *Crit Rev Oncol Hematol*. Jul 19.2010
26. Poulsen ML, Bisgaard ML. *MUTYH* Associated Polyposis (MAP). *Curr Genomics*. Sep; 2008 9(6): 420–435. [PubMed: 19506731]
27. Giardiello FM, Brensinger JD, Tersmette AC, et al. Very high risk of cancer in familial Peutz-Jeghers syndrome. *Gastroenterology*. Dec; 2000 119(6):1447–1453. [PubMed: 11113065]
28. Farooq A, Walker LJ, Bowling J, Audisio RA. Cowden syndrome. *Cancer Treat Rev*. Dec; 2010 36(8):577–583. [PubMed: 20580873]
29. Bowen KA, Doan HQ, Zhou BP, et al. *PTEN* loss induces epithelial--mesenchymal transition in human colon cancer cells. *Anticancer Res*. Nov; 2009 29(11):4439–4449. [PubMed: 20032390]
30. Chen HM, Fang JY. Genetics of the hamartomatous polyposis syndromes: a molecular review. *Int J Colorectal Dis*. Aug; 2009 24(8):865–874. [PubMed: 19381654]
31. Barker N, Clevers H. Leucine-rich repeat-containing G-protein-coupled receptors as markers of adult stem cells. *Gastroenterology*. May; 2010 138(5):1681–1696. [PubMed: 20417836]
32. Dalerba P, Dylla SJ, Park IK, et al. Phenotypic characterization of human colorectal cancer stem cells. *Proc Natl Acad Sci U S A*. Jun 12; 2007 104(24):10158–10163. [PubMed: 17548814]
33. Huang EH, Hynes MJ, Zhang T, et al. Aldehyde dehydrogenase 1 is a marker for normal and malignant human colonic stem cells (SC) and tracks SC overpopulation during colon tumorigenesis. *Cancer Res*. Apr 15; 2009 69(8):3382–3389. [PubMed: 19336570]
34. O'Brien CA, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature*. Jan 4; 2007 445(7123):106–110. [PubMed: 17122772]
35. Wang Q, Chen ZG, Du CZ, Wang HW, Yan L, Gu J. Cancer stem cell marker CD133+ tumour cells and clinical outcome in rectal cancer. *Histopathology*. Sep; 2009 55(3):284–293. [PubMed: 19723143]
36. Vermeulen L, De Sousa EMF, van der Heijden M, et al. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. *Nat Cell Biol*. May; 2010 12(5):468–476. [PubMed: 20418870]
37. Wang Y, Krivtsov AV, Sinha AU, et al. The Wnt/beta-catenin pathway is required for the development of leukemia stem cells in AML. *Science*. Mar 26; 2010 327(5973):1650–1653. [PubMed: 20339075]
38. Dylla SJ, Beviglia L, Park IK, et al. Colorectal cancer stem cells are enriched in xenogeneic tumors following chemotherapy. *PLoS One*. 2008; 3(6):e2428. [PubMed: 18560594]

Fig 1.

The Adenoma to Carcinoma sequence. Adapted from Fearon and Vogelstein's landmark discoveries,⁵ this schema has been amended to include contemporary modifications in which genetic and epigenetic alterations parallel the neoplastic progression from benign colonic mucosa to the invasive phenotype. *APC*; adenomatous polyposis coli, MSI; microsatellite instability, Cox-2, cyclooxygenase; k-ras; kirsten ras; DCC, deleted in colon cancer.

Fig 2.

Molecular mechanisms in hereditary cancer. **Panel A:** The WNT signaling pathway. The transcription factor, β -catenin, is sequestered by APC in a complex and thus marked for degradation by the proteasome. In familial adenomatous polyposis, mutations in the APC complex result in excessive free β -catenin which can translocate to the nucleus and interact with the promoter, TCF-Lef. This binding facilitates cell proliferation via c-myc and cyclin D1. **Panel B:** Mismatch repair. In hereditary non-polyposis colon cancer, proteins which recognize and repair DNA are mutated. Thus, errors in replication are not recognized. **Panel C:** *PTEN*/Akt. Defects in *PTEN*, a tumor suppressor, result in Cowden's syndrome. *PTEN* normally inhibits the PI3 kinase/Akt complex. If *PTEN* does not inhibit this complex, growth signals progress unchecked. **Panel D:** TGF β /SMAD. Germline mutations in the SMAD transcription factors are responsible for familial juvenile polyposis. These proteins are the dominant mediators of the Transforming Growth Factor (TGF) family. In the absence of inhibition, the SMAD molecules translocate to the nucleus where they promote growth enhancing genes. Wnt; Wingless, APC; adenomatous polyposis coli, GSK3 β ; glycogen synthase kinase, Tcf/Lef; T-cell factor/lymphocyte enhancing factor, *PTEN*; phosphatase and tensin homologue deleted on chromosome 10, Akt; protein kinase B, a serine/threonine protein kinase, mTOR; mammalian target of rapamycin, mdm2; murine double minute, an inhibitor of *p53*, Smad; mothers against decapentaplegic homolog.

Fig 3. Isolating and perpetuation of Colon Cancer Stem Cells. A. Tumor fragments (1–2 mm) isolated from fresh operative specimens are implanted into the subcutaneous space of immunocompromised mice (H&E, 400×). B. Once tumors have grown to 1–1.5 cm, they are harvested, and dissociated. C. Flow cytometry identifies those cells enriched for tumor initiating activity (green). D. As few as 10 cells from this enriched population are capable of recapitulating the parent tumor (H&E, 400×). Note resemblance to the H&E in A.