Molecular genetic alterations in gastrointestinal polyposis syndromes: with emphasis on the Peutz-Jeghers syndrome
Entius, M.M.

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Chapter 1

General Introduction and Outline of the Thesis
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GENERAL INTRODUCTION

Colorectal cancer

Colorectal cancer (CRC) is the second leading cause of cancer related death in the Western world. The disease is most frequently seen in patients between 60 and 70 years with an equal distribution between the two sexes. In the Netherlands about 7300 new CRC cases are diagnosed each year\(^1\) and in the USA of the about 160000 annual new cases 60000 people die\(^2\).

It is now recognized that both genetic and epigenetic factors contribute to the development of colorectal cancer. Specific genetic changes in proto-oncogenes (usually providers of positive growth signals), tumor suppressor genes (antagonize positive growth signals), and DNA mismatch repair (MMR) genes are involved\(^3,4\). The MMR genes are involved in maintaining DNA integrity. A 4\(^{th}\) class of genes, so-called mitotic checkpoint genes or chromosomal maintenance genes, play a role in the proper segregation of chromosomes during cell division, and when inactivated may lead to chromosomal aberration\(^5\); their role in human cancer still needs to be elucidated. The general hypothesis today is that tumors display some form of genomic instability in order to accumulate the somatic mutations leading to CRC, as was first stated by Loeb \textit{et al.}\(^6\). Genomic instability can lead to point mutations, chromosomal deletions and translocations and aneuploidy\(^7\).

In addition to the inherited factors, environmental factors play an important role in CRC development, as is reflected by a relative high incidence of CRC in the Western world when compared with other parts of the world\(^8\).

CRC development follows several histological steps, from normal epithelium to adenoma and eventually invasive carcinoma. This process is called the adenoma-carcinoma sequence\(^9\). Research on the molecular genetic alterations in CRC development shows that this adenoma-carcinoma sequence is closely correlated with a multi-step genetic process involving several subsequent gene mutations in a very specific order. The process from normal epithelium to invasive carcinoma requires years and possibly decades\(^10\).

In most cases CRC occurs sporadically, but in about 15% of the patients there is a positive family history; some 5% have dominantly inherited CRC, while in 10% no clear hereditary pattern can be recognized. Mendelian inherited CRC-related syndromes can be divided into three groups: 1) adenomatous polyposis, 2) non-adenomatous polyposis, and 3) non-polyposis syndromes. Adenomatous polyposis syndromes include familial adenomatous polyposis (FAP) and its expression variants, Gardner syndrome (GS) and Turcot syndrome (TS). Non-adenomatous polyposis syndromes include Peutz-Jeghers syndrome (PJS), Cowden’s disease (CD) and juvenile polyposis syndrome (JP). Non-polyposis syndromes include hereditary non-polyposis colorectal cancer (HNPCC), also known as Lynch syndrome, of which Muir-Torre syndrome (MTS) can be considered as an expression variant.

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Mendelian inherited CRC-related syndromes are caused by a germline mutation in a tumor suppressor gene. Affected individuals inherit one mutant allele of the gene and during life the other allele can become mutated, leading to the phenotypic display of the disease. The tumor suppressor genes that can lead to CRC syndromes can be divided into two groups depending on their function. Vogelstein and Kinzler named the genes 'gatekeepers' and 'caretakers'. Gatekeepers, e.g. APC (adenomatous polyposis coli), have an immediate influence on tumor growth. Caretakers, e.g. the MMR genes, are not directly involved in tumor formation but inactivation in one of these genes leads to an increased mutation accumulation rate in cancer related genes.

Understanding of the genetic events that contribute to CRC enables early diagnosis. Once a specific mutation is identified it can potentially be used as a biomarker in early stages of the disease. In the future an increased insight might facilitate the development of specific drugs complementing the gene defects.

**Adenoma-carcinoma sequence**

The development of CRC follows a sequence of histological steps from normal epithelium to small adenoma, larger adenoma with high grade dysplasia and eventually invasive carcinoma, as was described by Muto *et al* in 1975. This observation was followed by intensive studies addressing the genetic alterations in different stages of CRC development. This work has led to the now widely accepted multi-step ‘adenoma-carcinoma sequence’ model for CRC, as postulated by Fearon and Vogelstein in 1990. In this model, a correlation is made between the histological steps and the genetic mutations that occur during CRC development. The establishment of this model is mostly based on work that has been performed on tumors of FAP patients. FAP is caused by a germline mutation in APC. Inactivation of this gene is a common event and is thought to be necessary for initiating the adenoma-carcinoma sequence.

Inactivation of APC in a colonic epithelium cell results in selective growth advantage compared with surrounding normal cells. An additional somatic mutation, e.g. in K-ras, in one of these cells subsequently increases the growth advantage of a new clone which will overgrow neighbouring cells. This concept of clonality of cancer was first postulated at the end of the 19th century by Virchow and today this concept can be confirmed genetically. As noted, different subsequent histological stages correlate with an accumulation of specific gene mutations. After inactivation of the APC gene, mutation of specific genes occurs. Subsequently a gain-of-function K-ras mutation, followed by loss-of-function of one of the candidate tumor suppressor genes (Smad2, Smad4 or DCC) on chromosome 18q21 is observed.

The APC tumor suppressor gene, located on chromosome 5q21, is mutated in 80% of
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sporadic CRC. *APC* encodes a 312 kD protein and as a complex it participates in the Wnt/APC/β-catenin signalling pathway that regulates cell proliferation\textsuperscript{13,14}. Under physiological circumstances the APC/β-catenin complex is tightly regulated. However, a mutation in APC disturbs this equilibrium and large amounts of free β-catenin become available. This will bind the transcription factor TCF-4 resulting in uncontrolled transcription of TCF-4 target genes\textsuperscript{15}. The *C-myc* oncogene was the first identified transcriptional target of TCF-4\textsuperscript{16} followed by *cyclin D1*\textsuperscript{17} and recently by *PPARδ*\textsuperscript{18}.

The K-*ras* proto-oncogene is activated in 50% of colorectal tumors. K-*ras* encodes a small 21 kD GTPase that is involved in signal transduction\textsuperscript{19}. K-*ras* is known to be oncogenic when mutated or over-expressed. When mutated in codon 12, 13 or 61 the gene encodes a protein with impaired intrinsic GTPase activity, resulting in a constitutive activation of K-*ras* in the signal transduction pathway. Small adenomas show mutations in K-*ras*, indicating that this gene becomes mutated during early stages in the adenoma-carcinoma sequence. Gain-of-function mutations at codon 12 or 13 of K-*ras* are very specific for adenocarcinomas in general; about 85% of the mutations are identified in these codons. K-*ras* is not mutated in the majority of CRC, but it appears to play a role in tumorigenicity\textsuperscript{20}.

Loss of heterozygosity (LOH) on the long arm of chromosome 18 (18q) occurs in about 80% of CRC. It appears as a late event in the sequence of development from adenoma to carcinoma, and this mutation may predict poor prognosis\textsuperscript{21}. Loss of the 18q region leads to inactivation of candidate tumor suppressor genes. Usually the entire chromosome arm 18q is affected by chromosomal loss, which makes a detailed localization of mutated gene(s) difficult. However, up to now three candidate tumor suppressor genes have been identified that might participate in colon cancer progression. These genes are *DCC*, *Smad2* and *Smad4*. The *DCC* (for deleted in colorectal cancer) tumor suppressor gene encodes a large protein with significant sequence similarity to neural cell adhesion molecules and other related cell surface glycoproteins. Mutations in this gene have been found in CRC but only in a small amount of cases\textsuperscript{22}. Alterations of this gene may interfere with normal cell growth and differentiation by disrupting cell-cell or cell-substrate interactions. Recent evidence suggests that the candidate tumor suppressor genes *Smad2* and *Smad4* (also known as *DPC4*) also may be inactivated by allelic loss on chromosome 18q. Both genes participate in the TGFβ-signaling pathway\textsuperscript{23,24}. TGFβ down-regulates the growth of epithelial cells. Mutations in these genes have been identified in a minority of CRC, 7% and 20% respectively\textsuperscript{25}. The relatively low frequency of mutations in the three candidate tumor suppressor genes when compared with the number of chromosomal losses at 18q indicates that the precise role of 18q loss needs further research.

The *p53* tumor suppressor gene on chromosome 17p is the most frequently mutated gene in human cancer, it is mutated in over 70% of the sporadic CRC\textsuperscript{26}. Mutation of *p53* appears to be a late phenomenon in colorectal carcinogenesis presumably occurring before metastasis\textsuperscript{27}. This is an important observation because a *p53* mutation seems to mark the
transition from adenoma to carcinoma. Mutations in p53 have a dominant negative effect but 17p losses are frequently seen in CRC. This mutation may allow the growing tumor with multiple genetic alterations to evade cell cycle arrest and apoptosis. The p53 gene encodes a 53 kD protein and is activated in case of DNA damage and it can promote a G1-cell cycle arrest to allow DNA repair. Further, p53 can promote apoptosis. A point mutation in p53 often results in an altered protein conformation leading to an inhibition of its DNA binding capability and an extended half-life. This latter phenomenon enables immunohistochemical staining for the identification of mutant p53. Sequence analysis showed that mutations in p53 are found throughout the entire gene sequence but exons 5, 6, 7 and 8 are mutated at higher frequency.

A genetic variant that is caused by mutations in MMR genes can also lead to the formation of CRC. In some 10-15% of all CRC a defect in the MMR machinery is seen. The genes that are involved in the mismatch repair process are hMLH1, hMSH2, hMSH3, hMSH6, PMS1 and PMS2. During DNA replication, a protein complex made of these genes ensures the integrity of the DNA. A mutated MMR gene can lead to a defect MMR mechanism and the accumulation of random DNA errors, especially in repeat sequences, leading to a phenomenon called microsatellite instability (MSI). Tumors with MSI display a replication error positive (RER+) phenotype. The RER+ positive tumors are somewhat indolent and thus have a better prognosis than RER- tumors. In MMR-related tumorigenesis mutations also occur in cancer related genes, e.g. TGFβRII and BAX. Another discrepancy with conventional CRC is seen in the expression of cyclooxygenase-2 (COX-2). Tumors from HNPCC patients show lower expression of COX-2. Finally, colorectal cancer cell lines with MSI have a normal mitotic checkpoint response contrasting with cell lines without MSI.

Colorectal cancer syndromes

Polyposis syndromes:

Familial Adenomatous Polyposis: Familial adenomatous polyposis (FAP) is an autosomal dominant polyposis syndrome, caused by a germline mutation in the APC gene and it is characterized by the development of more than hundred colorectal adenomatous polyps. At least some of these progress to cancer and consequently, due to the high number of polyps, these patients have a virtually 100% chance of developing CRC at relatively young age. Besides colorectal cancer, FAP patients have an increased risk at developing malignancies at other sites e.g. stomach, small bowel, thyroid and brain. APC mutation variants regarding the site of mutation in the gene correlate with phenotype expression variants. Expression variants of FAP, Gardner and Turcot syndrome, are caused by APC mutations as well, and are characterized by desmoid tumors or brain tumors respectively. An other variant of FAP is
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attenuated adenomatous polyposis coli (AAPC). This variant has a relatively low number of polyps, and a less penetrant pattern of disease expression in affected individuals. A genetic polymorphism of APC found in Ashkenazi Jews leads to a hypermutable region and consequently an increased CRC risk.

Peutz-Jeghers Syndrome: Peutz-Jeghers syndrome (PJS) is an autosomal dominantly inherited syndrome characterized by intestinal hamartomatous polyposis and melanin pigmentation of the skin and mucous membranes. In addition, PJS is associated with an increased risk for various forms of cancer. The polyps can be found throughout the entire gastrointestinal tract with the highest frequency in the small bowel. The most common malignancies are colorectal, small intestinal, stomach and pancreatic adenocarcinomas. Other cancer types that probably occur in excess in PJS families include breast and cervical cancer, as well as testicular and ovarian sex cord tumors. The relative risk of cancer may be as high as 18 times that of the general population, and the carcinomas in PJS carry a poor prognosis. Recently, the predisposing locus was mapped to 19p13. Subsequently, the causative gene was identified as STK11 (for serine/threonine kinase-11) that is ubiquitously expressed in human tissues. The gene is also known as LKB1. Although the protein function of STK11 is largely unknown, it might be involved in G1-cell cycle arrest. A number of studies demonstrate that STK11 mutations in sporadic cancer can occur albeit at relatively low frequency.

Cowden’s Disease: Cowden disease (CD), or multiple hamartoma syndrome, is a rare autosomal dominant syndrome characterized by a mixture of hamartomatous lesions of ectodermal and mesodermal tissue, and there is an elevated risk for breast, thyroid, and skin cancers. The syndrome is caused by mutations in PTEN (for phosphatase and tensin homolog) located on chromosome 10q22, although there is evidence for genetic heterogeneity. Allelic to CD is Bannayan-Riley-Ruvalcaba syndrome that also can be caused by PTEN mutations. Because of the large spectrum of tumors that occur in CD the disease can be mistaken for another syndrome.

Juvenile Polyposis: Juvenile polyposis (JP) is an autosomal dominant condition characterized by multiple hamartomatous juvenile polyps seen predominantly in the colon but also at other sites in the gastrointestinal tract. JP is seen predominantly in the first and second decade of life. The polyps show at cut section dilated glands lined by mucus secreting epithelium. The surface may be ulcerated and the lamina propria inflamed. JP is associated with an increased risk for GI-cancer in 9 to 68% of JP patients. Linkage and sequence analysis demonstrates that JP can be caused by mutations in either PTEN on chromosome 10q22 or Smad4 on 18q21 indicating genetic heterogeneity. As described earlier, Smad4 (DPC4) participates in the TGFβ signaling pathway. Since Cowden’s disease (CD) can also
be caused by mutations in \textit{PTEN} this indicates that JP and CD are expression variants. In addition to these expression variants, another possible variant of JP has been identified and termed hereditary mixed polyposis syndrome (HMPS) characterized by atypical juvenile polyps, with mixed features of hamartomas and adenomas. The MPSH locus maps to 6q16\textsuperscript{67}.

\textbf{Non-polyposis syndromes:}

\textbf{HNPCC (Lynch syndrome):} HNPCC (or Lynch syndrome) is an autosomal dominantly inherited trait associated with the development of CRC at relatively young age\textsuperscript{68}. Contrasting with FAP, patients with HNPCC do not have numerous adenomas. Common extra-intestinal carcinomas in HNPCC are found in the endometrium, ovary and stomach. HNPCC is defined by the so-called Amsterdam criteria, which were recently redefined at the genetic level\textsuperscript{69-71}. Genetic analysis shows that almost all patients who meet these criteria have a germline mutation in a MMR gene. HNPCC can be caused by a mutation of one of the DNA MMR genes leading to microsatellite instability (MSI)\textsuperscript{72,73} and to a replication error positive (RER+) tumor phenotype as described above. The vast majority of carcinomas in HNPCC patients are RER++; in contrast only 10\% to 15\% of sporadic colon cancers are RER+.\textsuperscript{7}

\textbf{Muir-Torre syndrome:} MTS can be a variant of HNPCC and is defined by the occurrence of intestinal carcinomas in combination with a sebaceous glands tumor (adenoma, epithelioma, or carcinomas), keratoacanthoma, basell cell carcinoma or squamous cell carcinoma\textsuperscript{74,75}.

\textbf{REFERENCES}


Introduction


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Introduction


Chapter 1


Chapter 1

OUTLINE OF THE THESIS

A better understanding of the molecular basis of genetic diseases is beneficial for patients and individuals at risk for the disease. The studies in this thesis focus on genetic alterations that underlie gastrointestinal tumors and related tumors. In chapters 2 and 3 a recent review is given on the genetic and phenotypic specifications in both adenomatous and hamartomatous gastrointestinal polyposis syndromes. In chapter 4 we describe the possible clonal potential of multiple hyperplastic polyps in the stomach by analysing alteration in the K-ras oncogene, and the p53, p21 and MDM2 tumor suppressor genes. The following chapters 5-10 describe the various molecular analyses performed on the genetic basis of PJS syndromes and PJS tumorigenesis. These chapters describe our studies on both the germline mutations that lead to the PJS and on the possible somatic mutations that might be involved in PJS related tumorigenesis. The germline analysis described in chapters 6 and 9, describes the mutation spectrum in the STK11 gene in several families, including an analysis performed in the family originally described by dr Peutz in 1921. The somatic analysis as described in chapters 5, 8 and 10 was performed on tissue from polyps and carcinomas. We focused on possible alterations in STK11 and in the genes known from the adenoma-carcinoma sequence in PJS related tumorigenesis. In addition to these somatic studies we analysed both familial and sporadic pancreatic tumor for mutations in STK11 as is described in chapter 7. In summary, the current challenge is to understand the functional role of these genes in normal cellular physiology and make the connections between pathways that knit together integrated cellular homeostasis. A complete understanding of the regulatory pathways, and the synthesis and modifications of the proteins involved, will provide novel targets for therapeutic agents. Combining our knowledge of the genetic and epigenetic events implicated in this disease may allow a broader understanding of the pathogenesis of colorectal cancer and hence the design of better anti-tumor interventions. In addition, insight in the genetic pathophysiology of PJS can reveal the presence of a distinct pathway towards colorectal carcinogenesis, which can be considered as the hamartoma-carcinoma sequence. Finally, in chapter 11 we describe the potential use of a molecular marker to identify MSI in sebaceous gland carcinomas (SGC) to confirm the diagnosis of Muir-Torre syndrome (MTS). An early diagnosis can be of vital importance for the patient and molecular markers can play a central role in this process. Because of the difficulty in diagnosing HNPCC or MTS on histological grounds, analysis for MSI can produce an important indication for the presence of either of the two syndromes.