Molecular pathology of colorectal cancer predisposing syndromes

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Als je nadenkt over het
mysterie van de scheppende
voortgang van de natuur, word
je overstelpt door het besef van
de begrenzingen van het
menselijk intellect.
(A.N. Whitehead)

Aan mijn ouders

Voor Francien en Herman

Contents

Aim and outline of this thesis

Each year, approximately eleven thousand new colorectal cancer (CRC) patients are registered in the Netherlands. Half of these patients will eventually die of this disease, especially those in whom metastasis to regional lymph-nodes or distant organs was present at the time of surgery. Consequently, it is of great importance to identify individuals with an increased risk for CRC. Timely colonoscopic surveillance offered to such individuals could lead to a reduction in the incidence of CRC and a reduction in overall mortality. A way to identify individuals at risk is to look at their family history in terms of the type of cancer and its presence in multiple family members combined with an early age of onset. The majority of families with highly penetrant syndromes will be identified on the basis of their clinical appearance.

Molecular tumor testing can be applied to direct germline gene testing as a cost effective approach in index patients of these families. Subsequently, these patients will be screened for the presence of a germline defect in the known high risk genes (*MLH1, PMS2, MSH2, MSH6,* or *MUTYH*). After identification of the underlying gene defect(s) causing a high risk of CRC, pre-symptomatic testing can be offered to these families, and screening options can be discussed in mutation carriers and individuals at risk who choose not to be tested. CRC families without identified mutations are due to either an undetected defect in known genes or the single high risk gene not yet having been identified as a target for mutations. Alternatively, the high risk for CRC could be the result of a combination of gene variations, with each contributing a low level of risk.

This thesis describes the search for molecular pathology tools that can play a role in identifying individuals with an increased risk for CRC based on their genetic makeup and it provides insight into the tumorigenesis of familial CRC.

The described work can roughly be divided into:

1) The use of reliable methods that are applicable for formalin-fixed paraffin-embedded (FFPE) tissues, which is of utmost importance since the majority of tumor tissue from familial CRC is only available as FFPE tissue.
2) Tumor profiling to guide genetic testing strategies and clinical genetic decision making, to gain insight into the tumorigenesis of familial CRC (including Lynch syndrome and *MUTYH*-associated polyposis), and to study the role of *CHEK2* and *PTPRJ*.

Chapter 1 provides a brief overview of colorectal tumorigenesis and a general introduction of the factors that determine the individual risk of CRC and inheritable CRC syndromes. The contribution of low level genetic risk factors and environmental factors in causing CRC are also discussed.

In **chapter 2** we evaluate the results of microsatellite instability (MSI) analysis in two groups of individuals suspected for Lynch syndrome: one that fulfills the Bethesda cri-

teria and a separate group that does not fulfill those criteria. Furthermore, we compare the results of immunohistochemical (IHC) staining and MSI analysis and assess the additional value of PMS2 staining.

In **chapter 3**, we compare genomic profiles using single nucleotide polymorphism (SNP) arrays in three groups of archival tumors that show a high frequency of microsatellite instability (MSI-high). In one group MSI-high is caused by a pathogenic mutation in one of the mismatch repair (MMR) genes, *MLH1*, *PMS2*, *MSH2,* and *MSH6* (23 patients). A second set of tumors consists of MSI-high carcinomas from patients with an unclassified variant (UV) in one of the MMR genes (8 patients). A third group contains sporadic colon carcinomas with microsatellite instability due to *MLH1* promoter hypermethylation (10 patients).

Chapter 4 describes the value of *KRAS2* somatic mutation analysis for identifying patients with (atypical) *MUTYH*-associated polyposis (MAP). FFPE tumor tissues were studied for *KRAS2* mutations followed by *MUTYH* hotspot analysis in normal FFPE materials.

In **chapter 5**, the patterns of genomic instability in MAP carcinomas are described. Twenty-six carcinomas of MAP patients were studied for ploidy, genome-wide copy number variations, and copy neutral loss of heterozygosity (cnLOH).

Chapter 6 describes a large family in which gene defects of *MUTYH* and *MSH6* cosegregate. In particular, we studied the tumors in a family branch with combinations of defects.

In **chapters 7** and **8**, we studied the individual effect of the cancer susceptibility alleles (*PTPRJ**1176 A>C and *CHEK2**1100delC) in individuals with familial clustering of CRC.

Chapter 9 contains concluding remarks and a discussion of the future implications of this study.

Chapter 10 summarizes the work described in this thesis.

Chapter 11 summarizes the work described in this thesis in Dutch, contains the curriculum vitae and the list of additional publications.

List of abbreviations

AFAP	attenuated FAP
BER	base excision repair
CD	Cowden disease
CIMP	CpG island methylator phenotype
CIN	chromosomal instability
cnLOH	copy neutral loss of heterozygosity
CRC	colorectal cancer
FAP	familial adenomatous polyposis
FFPE	formalin-fixed paraffin-embedded
GWA	genome-wide association
HPPS	hyperplastic polyposis
IHC	immunohistochemistry
JPS	Juvenile polyposis syndrome
LOH	loss of heterozygosity
MAP	*MUTYH*-associated polyposis
MINT	methylated in tumors
MMR	mismatch repair
MSI-high	microsatellite unstable
MSI or MIN	microsatellite instability
MSS	microsatellite stable
MTS	Muir Torre syndrome
PAH	polycyclic aromatic hydrocarbons
PJS	Peutz-Jeghers syndrome
SNP	single nucleotide polymorphism
TS	Turcot syndrome
UV	unclassified variant