Molecular pathology of colorectal cancer predisposing syndromes

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CHAPTER 10

Summary

In this thesis, molecular tools were applied to tumor tissues to identify individuals burdened with a genetic risk for colorectal cancer (CRC) and to generate insight into the tumorigenesis of familial CRC.

Chapter 1 gives a general introduction about the factors that determine the individual risk of CRC in the general population. A brief overview on colorectal tumorigenesis is given. Inheritable CRC syndromes and the contribution of low level genetic risk factors and environmental factors to CRC risk are also described.

Tumors from individuals with an early onset in addition to clustering of CRC in the family are analyzed for microsatellite instability and expression of the mismatch repair (MMR) genes (*MLH1*, *PMS2*, *MSH2*, *MSH6*) to identify Lynch syndrome and to give direction to possible additional germline mutation analysis. When a pathogenic mutation in one of the MMR genes is found, all family members can undergo testing for the presence of the identified germline defect. In **chapter 2**, the yield of microsatellite instability (MSI) analysis in families suspected for Lynch syndrome, for a group fulfilling the Bethesda criteria and a group that does not, was evaluated. We found that it would be better to include late onset families (three or more cases of CRC diagnosed at age >50 years) in the testing schemes and to raise the age at diagnosis of CRC from 45 to 50 years. In addition, we compared the results of immunohistochemical (IHC) staining and MSI analysis and assessed the additional value of PMS2 staining. Based on that part of the study, we recommend the inclusion of PMS2 staining in the panel of antibodies (MLH1, MSH2, and MSH6) to identify families eligible for mutation analysis.

In **chapter 3**, the patterns of genomic abnormalities of microsatellite unstable (MSI-high) CRC tumors from carriers of pathogenic germline mutations or unclassified variants (UVs) in MMR genes and tumors with methylation of the *MLH1* gene were studied. We identified different chromosomal aberrations in terms of frequency and distribution in the three MSI-high carcinoma groups, although these differences were subtle. Of interest was the increased number of chromosomal aberrations in colon carcinomas from MMR-UV carriers compared to pathogenic MMR mutation carriers and carcinomas with *MLH1* promoter hypermethylation. Apparently, chromosomal instability (CIN) was added to microsatellite instability in these MMR-UV cases during tumorigenesis.

To identify *MUTYH*-associated polyposis (MAP) families that do not fulfill the clinical criteria for *MUTYH* germline mutation screening, we studied the feasibility of implementing a *KRAS2* c.34 G>T pre-screening method followed by an *MUTYH* hotspot mutation analysis in **chapter 4**. *KRAS2* c.34 G>T is found in 60% of MAP carcinomas but is infrequent in consecutive series of CRC. We tested formalin-fixed paraffin-embedded (FFPE) tumor tissues from individuals who presented with <10 adenomas or familial mismatch repair proficient colorectal carcinomas with <10 concomitant adenomas for somatic *KRAS2* mutations and for three Dutch hotspot *MUTYH* germline mutations (p.Tyr165Cys, p.Gly382Asp and p.Pro391Leu). We identified bi-allelic mutation carriers

with this approach. Therefore, we recommend performing the *KRAS2* c.34G>T somatic pre-screening and, if the result is positive, a subsequent *MUTYH* hotspot mutation analysis. When heterozygous hotspot *MUTYH* mutations are identified, a complete germline *MUTYH* mutation screening should be carried out if possible. Immediate *MUTYH* hotspot mutation analysis was a practical alternative in patients with >10 adenomas or in cases of multiple CRCs in one generation for which only FFPE tissue was available.

In CRC, there are two classical pathways that direct tumorigenesis: microsatellite instability (MSI or MIN) with near-diploidy and CIN. In MAP, the pathway involved in tumorigenesis remains unclear; both aneuploidy in adenomas as well as near-diploidy in carcinomas have been reported. In **chapter 5**, we analyzed 26 MAP carcinomas using SNP arrays. The high prevalence of copy neutral loss of heterozygosity (cnLOH) detected in those MAP carcinomas suggests a relationship between mitotic recombination and base excision repair (BER) deficiency, although further research into this possible relationship is required.

In the inherited MAP and Lynch syndrome, somatic mutations occur due to a loss of the caretaker functions that BER and MMR genes have, respectively. In **chapter 6**, a branch of a Lynch syndrome family in which *MSH6* and *MUTYH* germline mutations co-segregate was studied. One patient carried three mutations (1x *MSH6*, 2x *MUTYH*) and had an extremely mild clinical phenotype with only a few adenomas so far. We concluded that our data support the notion that abrogation of both MSH*6* DNA mismatch repair and base repair might be mutually exclusive in humans.

It is essential that candidate CRC predisposing genes appearing in the literature are verified in well-defined familial CRC cohorts and unexplained familial CRC cohorts. To improve efficiency, we studied the use of two high-throughput methods to analyze candidate CRC genes (chapters 7 and 8). In **chapter 7**, we describe the importance of the newly identified *PTPRJ**1176 A>C allele that appears to be limited to familial CRC. We concluded that MassEXTEND LOH analysis (using Sequenom's MassARRAY RT software) was a sensitive, high-throughput, and cost-effective method to screen SNP loci for LOH in FFPE tissues. In **chapter 8**, we concluded that homozygosity for the *CHEK2**1100delC mutation is not lethal in humans and does not lead to a severe clinical phenotype and that the loss of CHEK2 protein expression observed in familial CRC is not caused by the *CHEK2* germline variants, R117G, R137Q, R145W, I157T, and R180H. Furthermore, we concluded that immunohistochemistry on tissue microarrays is a valuable pre-screening method. The disadvantage of this technique is that the genetic alterations in the tumors must by definition lead to protein abrogation, and an antibody against the target of interest must also be available.

Chapter 9 contains concluding remarks and implication for the future. Molecular pathology has a high potential for playing an active role in identifying individuals with CRC

predisposing syndromes in a diagnostic setting as well as in studying tumorigenesis of CRC in a research setting.

Tests such as MSI, additional MMR IHC (chapter 2), and *KRAS2* mutation analyses (chapter 4), which are readily applicable and straightforward, are now extensively used in our daily molecular pathology diagnostics.

In the research setting, molecular pathology will be an important player in study the contribution to an increased CRC risk of the susceptibility alleles that are being identified. Furthermore, we now argue that the distinct tumor profiles that are found (chapters 3 and 5) are convincing examples that molecular pathology approaches are also crucial in the characterization and elucidation of unresolved familial causes of CRC.