Gastrointestinal polyposis syndromes

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Summary and concluding remarks
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Patients affected by the autosomal dominant gastrointestinal polyposis syndromes, such as familial adenomatous polyposis (FAP) and Peutz-Jeghers syndrome (PJS), have an increased risk for intestinal and extra-intestinal malignancies. There is also high morbidity/mortality from benign lesions, for example intestinal hamartomas causing intussusception in PJS. Improvement of care for individuals pre-disposed to these syndromes depends on (i) recognition of affected individuals and risk-assessment based on genetic testing, (ii) the development of primary and secondary chemopreventive treatment modalities, (iii) the development of biomarkers for surveillance and of chemopreventive treatment outcome, and (iv) optimization of surveillance guidelines based on estimations of the cancer risk in cohorts of patients. Of note, carcinogenesis in hereditary gastrointestinal cancer syndromes reflects sporadic carcinogenesis. Therefore, investigations of the pathogenesis of these syndromes have importantly contributed to our understanding of colorectal carcinogenesis in the general population. Also, studies of gastrointestinal cancer syndromes will be essential in the future development of large-scale preventive strategies against sporadic colorectal cancer, which is the second leading cause of cancer related death in the Western world.

The studies described in part I of this thesis address the mechanism of sulindac-based chemoprevention of adenomas in FAP, and investigate molecular alterations related to sulindac-resistance. Understanding sulindac-based chemoprevention and sulindac-resistance may help in the development of improved chemopreventive treatment regimens, and of biomarkers to monitor treatment outcome. The studies described in part II of this thesis address molecular alterations in PJS related tumors, and investigate the phenotype and cancer risk of PJS.

Chapter 1 of this thesis (introduction and outline) describes molecular and phenotypic aspects of gastrointestinal polyposis syndromes, in particular FAP and PJS. FAP is caused by a germline mutation in the APC gene, the key tumor-suppressor gene of the colon, resulting in widespread initiation of the adenoma-carcinoma sequence. This is reflected by the development of multiple adenomas and subsequently colorectal carcinoma at young age. Colectomy and close surveillance are required; guidelines for management are listed. PJS is a hamartomatous gastrointestinal polyposis syndrome, caused by a germline mutation in the STK11 tumor-suppressor gene. Although a very high cancer risk in PJS has been established, the pre-malignant potential of hamartomas is still a matter of debate. Screening is required and guidelines are listed.

Part I: Chemoprevention of the Adenoma-Carcinoma Sequence

Chapter 2 reviews molecular and clinical aspects of chemoprevention of the adenoma-carcinoma sequence with NSAIDs or COX-2 inhibitors. All reports of FAP patients treated with those agents are listed, showing that the chemopreventive effect is incomplete. Sulindac-resistant carcinomas appeared as flat ulcerative but infiltrating lesions in a rectum devoid of adenomas, suggesting that sulindac may mask progression of the adenoma-carcinoma sequence. Also, current insights in the mechanisms underlying chemoprevention are discussed. Chapters 3 and 4 show that sulindac affects the number and localization of apoptotic bodies (counted in H&E stained
slides) throughout normal rectal epithelial crypts in FAP patients affected by adenomas who show a clinical response (regression of adenomas) to treatment (chapter 3), but not in normal rectal epithelium of pre-symptomatic FAP patients (chapter 4). In the affected colon, sulindac appears to interfere with the regulation of rectal epithelial cell kinetics, resulting in a relative increase in apoptosis in surface cells compared with deeper crypts. However, this change appears inadequate as a biomarker to monitor treatment efficacy, due to heterogeneity in the “apoptotic response”, and its non-predictive nature in pre-symptomatic FAP patients on sulindac. Furthermore, counting apoptotic bodies in H&E stained slides could not predict the development of adenomas in pre-symptomatic FAP patients, precluding its use as a biomarker in surveillance programs. **Chapter 5** shows that sulindac resistant adenomas from FAP patients (n=34) display less nuclear accumulation of β-catenin and less COX-2 expression when compared to adenomas removed before treatment with sulindac (baseline) from the same FAP patients (n=9) or FAP patients with a complete response to sulindac (n=14). These differences may reflect intrinsic features of resistant adenomas, or point to down-regulation of these factors by sulindac. Furthermore, there were more K-RAS mutations in resistant adenomas compared to baseline adenomas, suggesting that K-RAS contributes to resistance. The effect of sulindac on nuclear β-catenin was further explored in **chapter 6**. Adenomas from FAP patients removed before treatment with sulindac (n=17) had more nuclear β-catenin when compared to adenomas from the same patients removed after up to 6 months sulindac treatment (n=17). Sulindac-resistance appears to develop after 6 months of treatment. Thus, the most likely explanation for our finding is that sulindac decreases nuclear accumulation of β-catenin. This conclusion is supported by in vitro experiments showing diminished β-catenin/TCF mediated transcription in colorectal cancer cell lines (DLD1 and SW480) exposed to sulindac or indomethacin. Also, NSAIDs decreased the expression of non-phosphorylated (oncogenic) β-catenin and the positively regulated TCF-targets Met and cyclin-D1, but increased the expression of the negatively regulated TCF-target CD68. This is consistent with downregulation of nuclear (oncogenic) β-catenin.

**Part II: Molecular and Phenotypic Studies of Peutz-Jeghers Syndrome**

**Chapter 7** is a historical note about Dr. Peutz and Dr. Jeghers and the initial reports about PJS. The Dutch physician Peutz was the first to recognize the combination of characteristic melanin pigmentation and gastrointestinal polyposis as a distinct syndrome in his publication in the “Nederlandsch Maandschrift voor Geneeskunde” in 1921. Jeghers and colleagues provided a detailed description of a series of patients with the disorder, and established the autosomal dominant pattern of inheritance. **Chapters 8 and 9** show that carcinogenesis in PJS follows a different pattern than the usual adenoma-carcinoma sequence. Inactivation of STK11 by LOH of the wild type allele was found in 38% of hamartomas (n=39) and 73% of carcinomas (n=11). Inactivation of APC by mutations or LOH, or activating mutations in β-catenin were not found in hamartomas, and only rarely present in carcinomas. However, nuclear β-catenin was found in 45% of carcinomas, indicating that this pathway still seems involved in PJS-related carcinogenesis. A minority of carcinomas had K-RAS mutations (18%), positive immunostaining for p53 (36%) or LOH at the p53-locus (10%), whereas none of the hamartomas displayed such
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alterations. Some hamartomas showed focal immunohistochemical alterations with nuclear B-catenin (18%), or disturbed topographical expression of Ki67 and p21^\text{waf1/cip1} (20%), possibly indicating neoplastic potential within these benign lesions. Furthermore, over-expression of COX-2 was present in 25% of hamartomas (25%) and 64% of carcinomas, providing a rationale for chemopreventive studies with NSAIDs or COX-2-inhibitors against carcinogenesis in PJS.

Chapter 10 describes inactivation of STK11 in PJS related but not in sporadic nasal polyps, providing molecular-genetic support for the association between nasal polyposis and PJS, as originally reported by Dr. Peutz. Chapter 11 further explores the association between nasal polyposis and PJS. Of 51 well documented Dutch PJS patients, 8 (16%) suffered from nasal polyposis. One of those patients died from a nasopharyngeal carcinoma, possibly indicating a premalignant potential of nasal polyps in PJS. Interestingly, PJS related nasal polyps rarely showed eosinophilia, a hallmark of sporadic nasal polyps. Thus, PJS related nasal polyposis seems a distinct entity with a different pathogenesis compared to sporadic nasal polyposis. COX-2 expression in PJS related nasal polyps may be a potential target for experimental chemopreventive treatment with NSAIDs or COX-2 inhibitors. In chapter 12, the mucocutaneous pigmentation-pattern in PJS patients (n=34) and matched controls (n=110) is studied in detail. A typical PJS-like pigmentation pattern was found in 71% of PJS patients and 15% of controls. The sensitivity and specificity of this finding for the diagnosis PJS was 71% and 86% respectively. In particular, pigmentation on the lips, buccal mucosa, peri-nasal skin and eyelids were associated with PJS. Fading of pigmentation with increasing age occurred in 18% of PJS patients. Missense mutations in exon 4 may predispose to a more intense pigmentation pattern. Chapter 13 describes the tumor spectrum and cancer risk in a cohort of 93 Dutch PJS patients. In addition to gastrointestinal carcinomas, malignancies of the breast, pancreas, ovary, testis, kidney (Wilms tumor), skin (melanoma) and nasal cavity were found. The cumulative risk of developing cancer (intestinal or extra-intestinal) for both sexes was 59.6% until the age of 60. The mean age of cancer diagnosis was 45 years. Ninety-two percent of PJS patients with cancer died from it a mean age of 45.6 years.

Conclusions and future developments:

Although NSAIDs based chemoprevention against the development of colorectal cancer in FAP patients appears promising, clinical use is limited. The chemopreventive efficacy of NSAIDs is incomplete and more powerful chemopreventive treatment regimens are needed to overcome resistance. Combination therapy appears attractive in this regard, potentially increasing efficacy and decreasing toxicity. Wider application of chemopreventive treatment against the development of colorectal cancer depends on the identification of high-risk groups who will benefit from such an approach. Whether NSAIDs-based chemoprevention of cancer is beneficial to patients with PJS deserves further investigation. Studies in patients with hereditary non-polyposis colorectal cancer (HNPCC) are ongoing. Identification of biomarkers as intermediate endpoints for treatment outcome is essential to the development and clinical application of chemopreventive agents. Using currently available methods, cell kinetic parameters are inappropriate biomarkers,
however, more sensitive assays may become available in the nearby future. Also, proteomics-based approaches may lead to the identification of new biomarkers.

Further progress depends on studies addressing the molecular biology of cancer, which will point to novel targets for treatment and chemoprevention. In particular, insights in the role of STK11 and its interactors in PJS related carcinogenesis may provide novel viewpoints for cancer biology, since it is the only known tyrosine-kinase with tumour-suppressive activity. Currently, no treatment modalities other than surveillance can be offered to PJS patients. Appropriate risk assessment and optimal surveillance is however hampered by (i) the absence of STK11 mutations in a (small) subset of PJS patients, (ii) the wide inter- and intra-familial variation in the phenotypic expression of PJS, and (iii) the small numbers of patients reported in the few available risk assessment studies. Also, studies prospectively investigating the value of surveillance strategies in PJS are lacking, precluding an evidence-based approach. The future identification of a second PJS gene, large international phenotype-genotype studies (currently undertaken), and long term follow-up of prospectively initiated surveillance programs are needed to overcome these hurdles.

Finally, major efforts in cancer research should focus on prevention. Lessons from hereditary colorectal cancer regard to surveillance and chemoprevention should be translated to the general population. The identification of individuals at risk for the development of colorectal cancer is essential to increase the power of preventive programs. Therefore, (genetic) epidemiological studies, investigating non-autosomal dominant predisposition, may importantly contribute to successful prevention of colorectal cancer in the general population.