Heredity nonpolyposis colorectal cancer
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Summary
Hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome is a relatively common, dominantly inheriting cancer syndrome characterized by the development of neoplastic lesions in a variety of organs (gastrointestinal, endometrial/ovarian, urinary), most prominently the colorectum. The syndrome is caused by germline mutations in one of the mismatch repair genes (i.e. MLH1, MSH2, MSH6 and PMS2), which are part of a complex DNA repair system responsible for recognition and excision of mismatched nucleotides. The ability to identify subjects at high risk for developing cancer has called for better understanding of the carcinogenesis of the tumors in order to subsequently develop optimal preventive strategies. The present thesis aimed to explore the carcinogenesis of the most common HNPCC-associated tumors, namely colorectal and endometrial cancer, and to evaluate the possible chemopreventive role of the nonsteroidal anti-inflammatory drug sulindac in the tumorigenesis of colorectal cancer in HNPCC.

Clinically, the colorectal neoplastic process in HNPCC appears to follow an adenoma-carcinoma progression similar to that described in familial adenomatous polyposis and sporadic cases, though several of the clinical manifestations, as well as the molecular pathogenesis underlying them, are distinctive. In most studies, HNPCC tumors are described to have a right, i.e. proximal of the splenic flexure, predominance and to develop through an accelerated adenoma-carcinoma sequence. The initiation process of an HNPCC tumor, however, is a subject of controversy; when does mismatch repair dysfunction occur in the process of tumorigenesis? And what is the role of the proximal colon in the carcinogenesis of HNPCC tumors? In chapter 2 one hundred adenomas obtained from patients with HNPCC were studied to elucidate their role in the carcinogenesis of colorectal tumors in HNPCC. HNPCC adenomas in comparison to sporadic ones were more often located in the proximal colon (50% vs. 26%, p=0.018). The proximal propensity was especially evident in highly dysplastic HNPCC adenomas. All large HNPCC adenomas in the proximal colon were highly dysplastic in comparison to only 44% in the distal colon and rectum. Loss of mismatch repair protein expression was observed in all highly dysplastic HNPCC adenomas and in 55% of the low grade dysplastic HNPCC adenomas. HNPCC adenomas have a proximal propensity similar to hereditary nonpolyposis colorectal cancers and more importantly the accelerated adenoma-carcinoma sequence seemed to be site specific in the colon of HNPCC subjects. The results do not indicate that mismatch repair gene malfunction initiates adenoma development but it is present at a very early stage of tumorigenesis and heralds tumor progression.
That loss of function in a mismatch repair gene results in rapid progression to high grade dysplasia in adenomas is also illustrated in chapter 3. Chapter 3 describes an extremely rare clinical scenario in which initiation of adenoma growth and tumor progression is effected through dual inherited germline mutations. Dysfunctional adenomatous polyposis coli tumorsuppressor gene, demonstrated by abnormal expression of β-catenin, triggered early and multiple adenoma formation, while loss of mismatch repair function, illustrated by loss of MLH1 expression, was associated with high grade dysplastic adenomas. The combination of defect genes in two separate phases of the adenoma-carcinoma sequence led clinically to rapid clinical progression and drastic preventive and therapeutic measures, namely proctocolectomy with ileal pouch-anal anastomosis.

Adenomas are indisputably precursor lesions of colorectal cancers in HNPCC. Traditionally, hyperplastic polyps have been regarded as benign lesions, lacking the potential for neoplastic progression. However, recently properties, such as K-ras mutations and chromosome 1p deletions, suggesting potential for neoplastic progression have been demonstrated in hyperplastic polyps. DNA microsatellite instability has also been described in hyperplastic polyps and has been associated with microsatellite instable sporadic colorectal cancers. In chapter 4, the possible role of hyperplastic polyps as precursor lesion in HNPCC was analyzed. Clinical information on the age at colonoscopy and the location of the hyperplastic polyps was collected. MLH1, MSH2, and MLH6 protein expression was evaluated using immunohistochemistry. In our cohort study of 90 hyperplastic polyps none demonstrated loss of mismatch repair function as demonstrated by loss of immunohistochemical expression of mismatch repair proteins. Four polyps demonstrated adenomatous as well as hyperplastic features. A proximal propensity as in HNPCC colorectal cancers and adenomas was not observed for hyperplastic polyps. Actually the majority of hyperplastic polyps were resected from the rectum. Apparently hyperplastic polyps, though frequently found at colonoscopy, do not play a significant role in the carcinogenesis of microsatellite instable tumors in subjects with a germline mismatch repair gene mutation. Our findings do not dictate any changes in clinical practice.

Change in morphology and histology is a result of progressive acquisition of genomic alterations and of a disbalance between proliferation and apoptosis in neoplastic cells. The differences in morphological and histological aspects of adenomas resected from HNPCC
patients (as described in chapter 2) and knowledge concerning the clinical behavior of
HNPCC tumors suggest that tumorigenesis in HNPCC differs from that in sporadic colorectal
cancer at an early stage. In chapter 5 we studied whether this difference could be explained
from disparities in expression of several cell cycle and apoptosis-related proteins in relation to
proliferation and apoptosis in HNPCC (n= 42) and sporadic adenomas (n= 48). Even though
no differences in proliferation and apoptosis indices were detected between HNPCC and
sporadic adenomas, subtle differences in expression of regulating proteins were observed.
Low-grade dysplastic HNPCC adenomas differed from sporadic ones by expressing more
often bcl-2, an anti-apoptotic proto-oncogene, and less often bax, an apoptotic promoter.
Containing a repetitive sequence, the bax gene is a target gene for mutations when mismatch
repair function is impaired. Apparently, change in bax expression occurs early in the
carcinogenesis of HNPCC lesions, before malignant transformation. High grade dysplastic
HNPCC adenomas are different from sporadic ones by expressing less often proliferation
stimulating proteins, cyclin B1, D3 and E. A striking finding in this study is the decreased
expression of p21, not correlating with the proliferation index, in high grade dysplastic
HNPCC adenomas in comparison to sporadic ones (6% vs. 53%, p=0.003). Transforming
growth factor-β type II receptor gene (TGFβRII) has been previously described as target gene
for mutation in cells with dysfunctional mismatch repair genes such as in HNPCC. Mutated
TGFβRII gene may lead to downregulation of p21 through the pRB signaling pathway. The
subtle differences found in adenomas support the concept of alternative carcinogenic
pathways at an early stage. However, the fact that changes in expression of proliferation- and
apoptosis-regulating proteins were not associated with changes in proliferation and apoptosis
suggests that also other regulating proteins or pathways play a (possibly more influential) role
in the carcinogenesis of HNPCC-related colorectal cancer.

The most common extra-colonic tumor in HNPCC-affected persons is endometrial cancer.
Even though in genetically predisposed women the cumulative lifetime risk for endometrial
cancer may exceed that of colorectal cancer, the consequences of mismatch repair dysfunction
on the carcinogenic process of endometrial cancers have received little attention. Chapter 6
describes a study undertaken to explore differences in carcinogenic pathways between
HNPCC and sporadic endometrial cancer by evaluating proliferation and apoptotic indices
and the immunohistochemical expression of proliferation and apoptosis regulating proteins.
Only subtle differences in immunohistochemical expression of proliferation- and apoptosis-
regulating proteins in relation to proliferation and apoptosis were found between HNPCC and sporadic endometrial cancers. A tendency towards an increased proliferation rate was observed in HNPCC in comparison to sporadic endometrial cancers. Cyclin B1 is probably a major cell cycle proliferation regulator in HNPCC endometrial cancers. Similarly to the colorectal adenomas, loss of bax expression was observed more often in HNPCC endometrial cancers than in sporadic ones. However, the altered bax expression apparently had a limited functional role as the apoptotic index was not influenced. Even though bax seems to be a target gene of the microsatellite instable phenotype in endometrial cancers the carcinogenic consequences are disputable and loss of bax function is probably compensated by other, unknown apoptosis inducing pathway(s). Despite the underlying differences in pathogenesis, dysfunctional and functional mismatch repair genes, the carcinogenic pathway of HNPCC endometrial cancers differs only in a subtle manner from that of sporadic cancers. This is in accordance with the minor clinical diversity between HNPCC and sporadic endometrial cancers.

Two aspects - high incidence and early age at diagnosis - of HNPCC endometrial cancer has led to the implementation of a diversity of gynecologic screening programs. The theoretical benefit of such programs is early detection of (pre)malignant lesions, thereby reducing morbidity and mortality due to endometrial (and ovarian) cancer. However, the effectiveness of gynecologic surveillance procedures has not been shown in either prospective or retrospective studies. In chapter 7, the gynecologic screening program at the University Medical Center Groningen was retrospectively analyzed. Despite annual screening and good patient compliance no asymptomatic malignant lesions were detected during 179 appointments, but three asymptomatic premalignant lesions were detected and could be treated appropriately. One interval endometrial cancer was detected as a result of clinical symptoms. No abnormal CA 125 levels were measured and no ovarian cancers were detected. Screening for any type of carcinoma is aimed primarily at the detection of early-stage disease before symptoms occur and should result in a significantly improved overall survival. The 5-year survival rate for endometrial cancer in pre- and postmenopausal HNPCC-women is high (88%) and it remains unclear whether this high survival rate can be improved. Our present study suggests that the potential gain of endometrial surveillance in HNPCC by means of transvaginal ultrasound lies in the possibility of detecting premalignant lesions and thereby possibly preventing malignancies to develop and avoiding extensive treatment i.e.
radiotherapy. Our conclusion for the present clinical practice is that annual gynecologic screening with transvaginal ultrasound as triage for endometrial sampling remains justified for women motivated for it. Regardless of that, patients should be well instructed for early recognition of alarm symptoms and rapid notification should be strongly encouraged.

Early detection and treatment of (pre)malignant lesions in the colon as well as the endometrium is at present the primary mode of care of members of HNPCC families. Primary prevention of these potentially harmful and mortal lesions would be much better. Increasing evidence from cell line, animal and human studies reveals that the administration of nonsteroidal anti-inflammatory drugs (NSAIDs) represents a viable option for chemoprevention of familial adenomatous polyposis-associated and sporadic colorectal cancer. The molecular basis for the chemoprotective action of NSAIDs has not yet been fully elucidated. However, the use of NSAIDs is associated with a lower risk of colorectal adenoma and cancer development, and a lower risk of recurrent colorectal adenomas and carcinomas. These effects are independent of gender, age and site in the colorectum. The efficacy of chemoprevention remains largely unexplored in HNPCC. In search of possibilities for chemoprevention in HNPCC, the effect of the NSAID sulindac on the epithelium of normal appearing colon of HNPCC patients is explored in chapter 8. Twenty-two subjects were included in the randomized double-blind cross-over study and biopsies were taken from four locations in the colon after the use of sulindac (150 mg twice daily) or placebo during two separate four weeks periods. To identify possible mechanisms of action of chemoprevention with sulindac proliferation and apoptosis regulating proteins were identified from previous studies and were evaluated using immunohistochemistry. Proliferation labeling index (using MIB-1 antibodies) was higher during sulindac treatment than during placebo use in both ascending and transverse colon, but not in the sigmoid and the rectum. The apoptotic index did not alter after sulindac treatment. Except for an increase in cyclin D3 upon sulindac treatment, no further differences were found in expression of regulating proteins. Sulindac proved to induce an increase of epithelial cell proliferative activity, both in the whole crypt and in the upper crypt compartment, in the proximal colon, without affecting this activity in the sigmoid and rectum, and without affecting apoptosis in any of the regions of the colorectum. A limitation of all previous studies is that only the effects on the epithelium of rectum and sigmoid were studied. It is thus unknown whether similar effects occur in the proximal colon of subjects without a predisposition for HNPCC, but the finding of it in an
HNPCC population has implications by its own. The preference for the development of cancers in the proximal colon in HNPCC makes one reluctant to use agents that affect proliferation in that part of the colon in a way that is generally considered to mark an increased cancer risk. Although the clinical value of the biomarkers used is disputable, the results cast doubts on the chemopreventive effects of sulindac, and NSAIDs in general, in HNPCC.

**Future perspectives**

At the present, care and treatment of patients with HNPCC are mainly concentrated on identifying those at risk, identifying germline mutations and, subsequently, entering these subjects in standard screening programs for early detection of (pre)malignant lesions. Although screening programs for HNPCC subjects are well-established they do not offer complete protection. Further understanding of the carcinogenesis of HNPCC tumors and the development of novel chemoprevention strategies could lead to a reduction of neoplastic lesions and better care of HNPCC subjects.

In the present thesis, adenomas as well as hyperplastic polyps were studied as (possible) precursor lesion of HNPCC colorectal cancer. Adenomas are undisputable precursor lesions which can not be said from hyperplastic polyps. Sessile serrated polyps, containing hyperplastic and adenomatous features, have been proposed as precursor lesions for sporadic microsatellite unstable colorectal cancers. The microsatellite instability in these lesions seems to be a result of inactivation of the MLH1 gene through promoter hypermethylation and, not of a mutation in a gene as in HNPCC. In the study, described in this thesis, neither hyperplastic nor serrated adenomas exhibited loss of mismatch repair function, thus suggesting that these lesions are not precancerous in HNPCC-subjects. However, a role for serrated adenomas in carcinogenesis of HNPCC can not be fully excluded as the number of adenomas studied was small. The possibility of multiple precursor lesions for HNPCC colorectal cancer necessitates resection of all macroscopic tumors seen during screening colonoscopy.

In the near future most of the primary care of HNPCC patients will remain the early detection and resection of premalignant lesions. Jarvinen et al have demonstrated that regular
colonoscopy with polypectomy results in a significant survival advantage and a reduction in the incidence of colorectal tumors.\textsuperscript{2} However, there is a need for better endoscopic visualization as the small polyps prone to malignant transformation may be easily missed by standard colonoscopy. Recently, Lecomte \textit{et al} and Hurlstone \textit{et al} demonstrated that relative to conventional colonoscopy, high-resolution colonoscopy with chromoendoscopy markedly improves the detection of adenomas in patients with HNPCC syndrome.\textsuperscript{3,4} Many more, novel endoscopic techniques, e.g. narrow band imaging, fluorescence imaging, and elastic (light) scattering spectroscopy, endocytoscopy and immunoscopy, are currently under investigation but are not yet available for routine use. The most successful clinical method probably will be a combination of techniques, providing wide-area surveillance and point detection methods.\textsuperscript{5} 

The appropriate regimen for reducing the risk of gynecologic cancer in HNPCC remains subject of discussion. Gynecologic screening programs as described in chapter 7 may detect premalignant as well as endometrial cancers. Schmeler \textit{et al} published overwhelming evidence to support prophylactic gynecologic surgery, hysterectomy and bilateral salpingo-oophorectomy, with a prevented fraction of 100\% for both ovarian and endometrial cancer.\textsuperscript{6} However, it remains to be seen whether the costs of the prophylactic surgery (including surgical complications, premature menopause and its sequelae) will outweigh the benefits. In addition, the reduced number of diagnosed gynecologic cancers may not translate into reduced morbidity or mortality. Decision on the most appropriate method for gynecologic risk reduction in HNPCC awaits the results of a prospective trial.

The underlying differences in pathogenesis, dysfunctional and functional mismatch repair genes, and the different clinical behavior between HNPCC and sporadic cancers suggest two distinct carcinogenic pathways. In the present thesis, in which HNPCC cancers were defined as cancers diagnosed in patients fulfilling the Amsterdam criteria and/or having a germline MMR gene mutation, only subtle differences could be identified between HNPCC and sporadic premalignant and malignant lesions. Abdel-Rahman \textit{et al} demonstrated that colorectal cancers from patients belonging to families fulfilling the clinical Amsterdam criteria for HNPCC, but without a germline MMR gene mutation or even without MMR gene dysfunction in the tumors are distinct from colorectal cancers in patients belonging to families linked to MMR gene defects and from sporadic cases.\textsuperscript{7} In the above mentioned study, tumors from MMR gene mutation positive families have significantly more often active
Wingless/Wnt signaling as indicated by aberrant β-catenin localization with or without CTNNB1 mutations compared to tumors from MMR gene mutation negative families. At the same time a possible role of the Wnt pathway and activating β-catenin mutations in the (advanced) tumorigenesis of microsatellite instable colorectal tumors in HNPCC was described by Johnson et al. 8 These studies illustrate the complexity and diversity of HNPCC tumors. ‘HNPCC colorectal cancer’ is a term based on clinical criteria (e.g. Amsterdam criteria) and most probably includes a diversity of colorectal tumors which may be subdivided into separate groups depending on the precise pathogenesis, such as absence or presence and type of microsatellite instability in combination with absence or presence of MMR gene mutations. In the future these subgroups of HNPCC colorectal tumors should be studied individually (when comparing with sporadic colorectal cancer) which most probably will demonstrate involvement of novel predisposition genes and pathways in their carcinogenesis.

Preventive measures, e.g. chemoprevention, for risk reduction would be welcome aspects in the treatment of patients at high inherited risk for cancer as in HNPCC. Although the efficacy of non-steroidal anti-inflammatory drugs has been demonstrated in patients with familial adenomatous polyposis and also in the general population, one should be reluctant to use sulindac in HNPCC. In the present thesis we demonstrated that sulindac affects proliferation in the proximal part of the colon in a way that is generally considered to mark an increased cancer risk. Despite these results chemoprevention with NSAIDs should not be discarded in HNPCC. Firstly, additional markers as surrogate end-points which correlate closely to disease progression should be established to evaluate potential chemopreventive regimens before drawing a definite conclusion concerning NSAIDs in HNPCC. Secondly, the development of combinations of drugs could alter/increase the chemopreventive effect of NSAIDs by targeting specific signaling pathways in (pre-)malignant cells. NSAIDS in combination with other drugs, such as peroxisome proliferators-activated receptor-γ ligands (PPAR-γ) or 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (HRIs), may be efficient in the chemoprevention of colorectal cancer in HNPCC as both drugs for example modulate the expression of β-catenin. 9 In the future, the mechanism of action of the chemopreventive agent sulindac in combination with other drugs should be further clarified and the effectiveness should be examined.
The way forward in the care of HNPCC subjects is to attempt to prevent the evolution of normal epithelium to adenomatous polyps to colorectal cancer through novel combinations of chemopreventive agents and to maximize the detection rate of premalignant lesion, thereby most likely further reducing the incidence of colorectal cancer.
References


