The serrated neoplasia pathway: investigating the role of serrated polyps in colorectal cancer development

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Chapter 1

General introduction and outline of the thesis
GENERAL INTRODUCTION

Colorectal cancer causes

Colorectal cancer (CRC) ranks as the second most common cause of cancer-related death in the western world.\textsuperscript{1} In general, CRC can be subdivided into sporadic and familial cases. The overall majority of individuals with CRC (~80%) are sporadic cases, lacking any indication of an underlying inherited disorder. Approximately 20% of cases are attributable to a familial (hereditary) disorder. However, in only 5% of these patients the causative genetic defect is identified such as in familial adenomatous polyposis syndrome (FAP), Lynch syndrome or other rare CRC syndromes such as MUTYH-associated polyposis (MAP), Peutz-Jeghers and juvenile polyposis (figure 1). Recently a relatively new condition, called hyperplastic polyposis syndrome (HPS), has been identified which is characterised by the presence of multiple colorectal serrated polyps. This presumed hereditary condition, has been shown to harbour a significantly increased CRC risk.\textsuperscript{2-6}

Although rare, the above mentioned hereditary disorders have proven to be important models to study colorectal carcinogenesis in general and based on these models major advances have occurred regarding our understanding of the molecular events leading to CRC and which potential pathways involving different polyp types are implicated.
Histological polyp types
Traditionally, colorectal polyps were divided into two separate groups, the adenoma which can be divided into tubular, tubulovillous and villous subtypes, and the hyperplastic polyp (HP), which displays serration in the upper one-half to one-third of the crypt. Adenomas have long been known to represent neoplastic precursor lesions of most CRCs, especially those adenomas harbouring a villous component. HPs on the other hand, which constitute the most common occurring serrated polyps, were regarded until recently as innocuous lesions lacking any premalignant potential. Until very recently, clinical guidelines did not recommend surveillance colonoscopies for individuals with HPs because the associated CRC risk was not believed to be higher than the risk in individuals without polyps.
Recent morphological reappraisals of polyps revealed however that serrated polyps can be further subdivided into different histological entities with possibly different premalignant potential.

Hyperplastic polyps (HPs)
Colorectal HPs are typically diminutive polyps (<5mm in diameter) which have a predilection for the sigmoid colon and rectum although larger sized HPs with a proximal localization have also been described. Besides being generally small these lesions are often flat or sessile in shape, unremarkable in colour and often covered with mucus. Serration, which is caused by infolding of the crypt epithelium leading to a saw-toothed appearance in longitudinal section and a star-shaped appearance on cross-section, is limited to the upper half to one-third of the crypt. Crypts are generally narrow and straight, with a normal distribution of the proliferative zone in the base of the crypts. HPs can be further subdivided into microvesicular, goblet cell-rich and mucin-poor subtypes on the basis of mucin content of the epithelial cells although the reproducibility hereof among pathologists is doubtful and the clinical relevance questionable.

Sessile serrated lesion (SSL)
Torlakovic and Snover identified a new hyperplastic polyp variant, formerly known as the sessile serrated adenoma. These polyps are currently referred to as sessile serrated lesions. These authors showed that a subset of lesions that were originally diagnosed as HPs in patients with Hyperplastic polyposis syndrome demonstrated a different morphology with abnormal proliferation and concluded that these SSLs were distinct lesions which should be differentiated from HPs. SSLs are defined by predominantly architectural distortion with
irregular dilated crypts, including dilatation of the base of the crypts that often have a boot, L or inverted T shape. Serration can also be identified at the base of the crypts as well as abnormal proliferation and maturation with mature goblet or foveolar cells. Cytological dysplasia is not usually demonstrated. However, if an area of dysplasia is present, this should be specified. In contrast, HPs have narrow crypt bases and serration is restricted to the upper half of the crypt.

Previous studies have shown that SSLs and HPs are histologically hard to distinguish, leading to confusion with regard to categorization of these serrated polyps. Indeed, it has recently been shown that at re-evaluation the interobserver agreement for the differentiation of serrated polyps remains only moderate. Molecular analysis for the differentiation of these serrated polyps has thus far shown that right-sided SSLs have increased MLH-1 and p16 methylation compared to left-sided SSLs and HPs. Some studies have also shown that SSLs are more often BRAF mutated than HPs. Nevertheless, unambiguous molecular differentiation between these two lesions has as yet not been achieved.

Traditional serrated adenoma (TSA)
Longacre & Fenoglio Preiser, in a large polyp survey, re-evaluated a group of polyps displaying mixed histological features of both hyperplastic and adenomatous polyps. Rather than representing mixed tumors, these lesions were considered to be adenomas with a serrated configuration similar to HPs and were thus coined serrated adenomas (currently named traditional serrated adenomas). Criteria for TSAs include a pedunculated polyp shape with elongated villiform projections uniformly lined with atypical cells having elongated nuclei and eosinophilic cytoplasm. In the mentioned survey, TSAs
represented less than 1% of all colorectal polyps. Before re-evaluation, approximately one third of these TSAs were originally diagnosed as HP, one third as conventional adenoma and one third as intermediate lesions. This variability underlines the difficulty in diagnosing these rare lesions. TSAs are distinguishable from SSLs by their uniform population of abnormal epithelial cells as well as their pedunculated shape rather than sessile.

**Colorectal carcinogenesis**

**CRC pathways**

Notwithstanding this re-classification of serrated polyps, conventional adenomas are considered to be the main lesions with an undisputable premalignant potential. Clear histological evidence that CRCs develop from adenomas was first established in 1975 by Muto et al. which was further genetically described by Vogelstein et al. who demonstrated in FAP patients that the adenoma-carcinoma sequence is caused by aberrant activation of the Wnt signalling pathway.\(^{23, 24}\) This multi-step process of carcinogenesis is characterised by an initial, bi-allelic inactivation of the adenomatous polyposis coli gene (**APC**) followed by accumulation of genetic mutations in key oncogenes and tumor-suppressor genes including **KRAS**, **DCC** and **p53**. These events, in conjunction with associated chromosomal instability (CIN), result in adenoma initiation and progression to CRC.

Subsequently, an alternative molecular pathway to CRC, the microsatellite instability pathway, was discovered in patients with Lynch syndrome.\(^{25}\) This pathway is initiated by deletion or inactivation of one of the mismatch repair genes (**MLH-1**, **MSH-2**, **MSH-6** or **PMS-2**). This causes defective repair of replication errors in repetitive DNA sequences which are most clearly demonstrated in so called
Microsatellite regions are abundant in non-encoding regions but are also present in encoding regions of growth regulatory genes like tumor suppressor genes such as \textit{TGFBR1, IGF2R} and \textit{BAX}. Alterations in such regions may then cause inactivation of these genes and subsequent CRC formation with microsatellite instability (MSI).\textsuperscript{27-29} MSI-CRCs can be the result of an inherited germline defect of a mismatch-repair gene, as a part of Lynch syndrome in 3\% of all CRC cases, but can also be caused by a somatic deletion or inactivation of a mismatch repair gene in up to 15\% of the sporadic CRCs (figure 1).\textsuperscript{30, 31}

\textbf{Alternative pathway to MSI CRC?}

Both CRCs with CIN and CRCs with MSI from patients with Lynch syndrome have been shown to harbour predominantly \textit{APC, KRAS} and \textit{P53} mutations.\textsuperscript{25, 32-36} In contrast, sporadic MSI CRCs infrequently harbour these mutations which are typically found in conventional adenomas.\textsuperscript{37-39} Instead, sporadic MSI CRCs are shown to have high levels of mutations in the \textit{BRAF} oncogene and extensive CPG- island methylation of multiple genes (CIMP) including \textit{MLH-1}, \textsuperscript{40-44} which are rare findings in sporadic adenomas\textsuperscript{34, 41, 43, 45, 46} but are commonly seen in serrated polyps.\textsuperscript{11, 41, 45, 47} The association between serrated polyps and specifically sporadic MSI-H CRCs is further emphasised by the infrequent presence of \textit{BRAF} mutations and CIMP in Lynch syndrome MSI-H CRCs.\textsuperscript{42, 48} These findings suggested that serrated polyps, instead of conventional adenomas, could represent precursor lesions of sporadic MSI CRCs.

In parallel, clinicohistological evidence arose that serrated polyps may also play a role in colorectal carcinogenesis. These reports involve both sporadic HPs and HPs in the context of hyperplastic
polyposis syndrome and comprise CRCs in close vicinity of large hyperplastic polyps\textsuperscript{49, 50}; CRCs identified in mixed hyperplastic and adenomatous polyps\textsuperscript{51}; increased incidence of HPs and serrated adenomas in patients with sporadic microsatellite-unstable CRCs\textsuperscript{52-54}; and a high prevalence of CRC in HPS patients\textsuperscript{13, 50, 55-57}. More recent cross-sectional association studies also showed a strong and independent association between large (>1 cm) sporadic serrated polyps, i.e. HPs, SSLs and/or TSAs and synchronous advanced CRC.\textsuperscript{58, 59} In another study, 5.8% of CRCs were found to have an adjacent “serrated adenoma” which were more commonly MSI (38%) than in CRCs without an adjacent serrated adenoma (11%).\textsuperscript{53} Currently however, proof of the existence of a serrated CRC pathway data on the natural history of serrated polyps are lacking. Therefore, the appropriate management of serrated polyps is unclear.

Serrated neoplasia pathway: proposed sequence of molecular events

While a single activating point mutation in \textit{BRAF} (V600E) is an oncogenetic hit in the mitogen-activated protein kinase (MAPK) pathway resulting in augmented cell proliferation, survival and inhibition of apoptosis, epigenetic alterations like CPG-island methylation, in which the DNA sequence remains unaltered, can cause inactivation of genes. CPG-islands are seen in approximately 50% of human genes and consist of dense clusters of cytosine-guanosine dinucleotides (CpG) that are susceptible to methylation of the cytosine base resulting in gene silencing. Within the context of CRC, CPG-island methylation can cause transcriptional repression of tumor suppressor genes, like \textit{MLH1} in sporadic MSI CRCs.\textsuperscript{60-63} Despite their different mechanisms of tumor development, \textit{BRAF} mutations and CIMP have been shown to be strongly associated in CRC, with an
odds ratio of 203. This strong association between BRAF and CIMP might be explained by the fact that mutations in the BRAF oncogene alone induce an initial burst of proliferation followed by cell senescence. Cell senescence is regulated by genes such as p16 and p53 which can restrain the initially induced cell proliferation. Further development to CRC may then be dependent on silencing of these regulatory genes so that a stable senescent state is overcome. It has thus been proposed that BRAF-mutant HPs may only progress to CRC, possibly via SSLs, after a second molecular change such as methylation-induced silencing of p16. Interestingly, in a previous study, analyzing the molecular differences between HPs and SSLs, it was shown that p16 was significantly more methylated in SSLs than in HPs, supporting the supposition that SSLs represent more advanced lesions. Similarly, IGFBP7, a mediator of P53-induced senescence has been shown to be methylated and silenced in BRAF mutated CRCs. Thus, it seems that the serrated pathway involves a necessary multi-step process of genetic and epigenetic changes to CRC.

Polyposis syndromes such as FAP and MAP are valuable models for analyzing the molecular mechanisms of the adenoma-carcinoma sequence due to the multitude of conventional adenomas and coincident CRC in these patients. Similarly, HPS patients who harbour multiple serrated polyps and have been shown to have concurrent CRC in up to 50% of cases at clinical presentation, may provide a “scientific workbench” for further analysis of the genetic sequence of events in the serrated neoplasia pathway.
Hyperplastic polyposis syndrome (HPS)

HPS is characterized by the presence of multiple serrated polyps spread throughout the colorectum and has been defined by the World Health Organization as the presence of at least five histologically diagnosed HPs proximal to the sigmoid colon, of which 2 larger than 10mm in diameter, or more than 30 HPs distributed throughout the colon. However, besides multiple HPs, SSLs and traditional adenomas are common findings in this condition as well. HPs and SSLs, both displaying a serrated phenotype, have been shown to be histologically very similar and difficult to differentiate microscopically with only moderate concordance. Consequently, originally diagnosed HPs may represent SSLs and vice versa. It was therefore recently decided to rename the condition ‘serrated polyposis syndrome’ or SPS, with inclusion of all serrated polyp subtypes.

From a practical perspective, considering that most articles in this thesis were published before this new definition was implemented, the term hyperplastic polyposis syndrome (HPS) will be used throughout this thesis.

From a clinical viewpoint, a further reclassification of HPS may be beneficial to identify subtypes that have a higher risk of CRC development. For example, it is assumable that HPS patients with a higher number of polyps or larger polyps also have a higher CRC risk. Also from an etiological viewpoint reclassification of HPS seems prudent. The heterogeneous phenotype of HPS such as (i) the presence of different polyp histologies and (ii) difference in polyp localization and (iii) number of polyps, suggests that HPS represents separate genetic conditions. Also molecular differences are found between HPS phenotypes, such as more CPG-island methylation in
right-sided serrated polyps as compared to left-sided serrated polyps, however these differences are not distinctive.

Concerning the prevalence of HPS in the general population, a previous screening sigmoidoscopy study in asymptomatic people aged 55 to 64 years estimated the prevalence to be 1:3000. However, in this screening study only the rectosigmoid was investigated without visualization of the remaining proximal colon. In addition, considering that HPs are often diminutive in size, flat in shape and unremarkable in colour, it is also possible that polyps were left undetected. This suggests that the actual prevalence of HPS is probably higher than currently estimated and in any case more frequent in occurrence than other polyposis syndromes such as FAP (1:13,000). Concordantly, a recent large prospective colonoscopy screening study involving 50,148 asymptomatic volunteers in Poland revealed 28 HPS patients leading to an estimated incidence of 1:1800.

**HPS and CRC risk**

HPS is associated with an increased CRC-risk. Previously published case series report CRC at clinical presentation in up to 50% of HPS patients. However, because these findings could also be interpreted as two-co-existent unrelated abnormalities, a causal relationship between HPS and the development of CRC can not by default be made but does seem presumable. Ideally, a large cohort of asymptomatic HPS patients, derived from a population based colonoscopy screening study, that is subsequently surveyed for multiple years would be useful to adequately determine the risk of CRC in these patients. Until such a study is performed, clinical evidence for an increased CRC risk after HPS diagnosis is thus far based on cohort studies with associated ascertainment bias. One such
study describes a case series of 13 HPS patients who were prospectively followed and who received regular endoscopies. Three of 13 (23%) patients developed CRC during follow-up, suggesting that patients with HPS have an increased risk of malignant progression of serrated polyps. Molecular supporting evidence include studies showing a higher number of *BRAF* mutations and CIMP in serrated polyps of HPS patients compared to sporadic serrated polyps. Moreover, HPS patients have far more serrated polyps than people in the general population.

Nevertheless, considering that conventional adenomas, which are traditionally considered premalignant polyps, are also common in HPS, these polyps and the associated Wnt-pathway can not be disregarded. Thus, based on the mixture of different precursor lesion types, HPS patients represent a unique model to evaluate which CRC pathways play a role in HPS and consequently which polyps are clinically relevant and to obtain new evidence supporting the existence of a serrated CRC pathway in humans.

**Endoscopic diagnostics of HPS**

Considering the presumed increased risk of malignant progression of polyps in HPS, detection and removal of polyps seems necessary to prevent CRC development in these patients. In HPS patients however, serrated polyps, which are the overall majority, are generally small, flat in shape and unremarkable in colour. These features are associated with polyp miss-rates of up to 26% using standard colonoscopy. Improved detection of these polyps seems therefore desirable which can be achieved by improving bowel preparation; clearing of fluid and debris; implementing a minimally accepted withdrawal time of 6 minutes; visualizing the proximal sides of folds,
flexures, and valves; and adequate colon distension. In addition, chro-
owendoscopy has been shown to improve the detection of small and flat lesions, specifically HPs, in patients undergoing surveillance colonoscopy. Novel advanced endoscopic techniques, such as narrow-band imaging (NBI) may also be an attractive alternative to improve the detection of polyps in HPS. In contrast with chro-
owendoscopy, which is a labour-intensive and time-consuming technique, NBI ('electronic chro-
owendoscopy') is an easier push-of-a-button technique that enhances mucosal and vascular detail without the use of dyes. It has proven to be superior to high-resolution endoscopy (HRE) for the detection of sporadic HPs but has not been evaluated in HPS. In addition, although removal of all larger polyps seems necessary in any case, accurate differentiation of diminutive polyps may aid the endoscopist in only removing advanced lesions (e.g. conventional adenomas, SSLs and TSAs) and leaving ‘innocuous’ HPs, which display relatively lower levels of BRAF mutations and CIMP, in situ. Since HPS patients have many diminutive polyps, this may save time and considerably prevent complications from unnecessary polypectomies of HPs. Whereas chro-
owendoscopy and NBI may also aid in the differentiation of HPs and adenomas, it is unknown whether differentiation between HPs and SSLs is possible as well. Finally, autofluorescence imaging (AFI), which facilitates differentiation of adenomas from non-adenomatous polyps based on different fluorescence emission spectra, may also be of value for the differentiation of polyps in HPS.
Treatment of HPS
Due to this risk of malignant transformation of polyps, HPS patients are either advised to undergo endoscopic surveillance with removal of polyps or a surgical colonic resection. Recent expert opinions recommend surveillance intervals ranging from one to three years. Concerning the removal of polyps, advice varies from removal of only proximally located polyps to complete removal of all polyps >5mm. As a consequence, lack of clarity exists regarding the recommended surveillance interval and which polyps should be removed. Therefore it seems possible that a proportion of HPS patients may be insufficiently treated and consequently be at risk of developing CRC under surveillance (interval CRC). Conversely, over-treatment with unnecessary removal of possibly harmless lesions which may result in complications should also be avoided.

Familial risk
As opposed to FAP which has an autosomal dominant inheritance pattern, in HPS the mode of inheritance is yet unknown. However, although no genetic substrate has yet been identified, families have previously been reported from which both an autosomal recessive and autosomal dominant inheritance could be considered. For this reason, screening colonoscopies of first-degree relatives is currently advised by some authorities.

The possible increased risk of developing CRC for first-degree relatives is however unclear. In first-degree relatives CRC has been reported, ranging in frequency from 0 to 50%. In one retrospective case series the incidence of polyps and CRC in first degree relatives was studied. Of 17 first degree relatives who underwent screening colonoscopy, 10/17 (59%) had polyps. In three patients HPs were
detected, predominantly in the rectum and one patient (6%) was diagnosed with HPS. This patient had multiple concomitant adenomas as well. In the other 6 patients adenomas were discovered proximal to the sigmoid colon. Of these patients, seven were under the age of 45 and the youngest was 32 years of age. No carcinoma was found. However, these data were not prospectively acquired and instead of performing a screening colonoscopy on all first-degree relatives of patients with HPS, screening was primarily performed because of CRC occurrence in the index-patient or because of an established family history of CRC. To make an adequate estimate of the incidence of polyps and HPS in first-degree relatives and the CRC-risk in this group due to HPS, prospective screening of larger series of first-degree relatives should be performed.
OUTLINE OF THE THESIS

The foundation of this research project was laid at the endoscopy unit and out-patient clinic of the Academic Medical Center where an increasing number of patients with hyperplastic polyposis syndrome (HPS) have been diagnosed. HPS is characterized by the presence of multiple colorectal hyperplastic polyps which are traditionally considered to be benign lesions. The surprisingly common occurrence of colorectal cancer (CRC) in these patients prompted further research into the clinical and molecular characteristics of HPS patients and the associated endoscopic management.

Part I: Clinical analyses – Colorectal cancer risk

The first part of the thesis focuses on the clinicopathological features of HPS patients and their first-degree relatives. Previously published case series report CRC at clinical presentation in up to 50% of HPS patients. Chapter 2 evaluates the risk of developing CRC after HPS diagnosis and which variables are associated with CRC in a large multi-centre cohort of HPS patients undergoing endoscopic surveillance. In chapter 3 the prevalence of polyps and CRC in first-degree relatives is described and compared with the background prevalence in the general population.

Part II: Molecular analyses – Etiology and colorectal cancer pathways

Although an underlying genetic disorder seems likely, thus far no genetic causes of HPS have been identified. However, previous case series of patients with a known genetic disorder have reported individuals with multiple serrated polyps. In chapter 4 and 5 we aimed to molecularly analyze whether serrated polyps identified in the setting of MYH-associated polyposis and Lynch syndrome are causally related to these respective disorders.

Besides hyperplastic polyps (HPs), sessile serrated lesions (SSLs) and conventional adenomas are also common findings in HPS. Due to this heterogeneity, it is unknown which polyps eventually lead to CRC in HPS and thus are clinically relevant. In chapter 6 we aimed to analyze which polyps lead to CRC in HPS patients by performing combined immunohistological and molecular analyses.
Part III: Endoscopic imaging – Detection and differentiation of polyps

Considering the presumed increased risk of malignant progression of polyps in HPS, detection and removal of polyps seems necessary to prevent CRC development in these patients. Besides following general quality guidelines of colonoscopy, novel advanced endoscopic techniques, such as narrow-band imaging (NBI) may improve the detection of polyps in HPS. Chapter 7 involves a randomized cross-over study comparing NBI and high-resolution endoscopy with regard to their miss-rates of polyps. In addition to improved detection of polyps in HPS, accurate differentiation of HPs and SSLs, which appear endoscopically very similar, may aid the endoscopist in only removing SSLs and leaving HPs, which display comparatively lower levels of genetic mutations, in situ. In chapter 8 we evaluated high resolution white-light endoscopy, NBI and autofluorescence imaging for the endoscopic differentiation of polyps in HPS.
REFERENCES


Chapter I


