The serrated neoplasia pathway: investigating the role of serrated polyps in colorectal cancer development
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Summary & Future Perspectives
SUMMARY

Colorectal cancer (CRC) is a condition associated with a high mortality rate. Previous molecular research performed in patients with FAP and Lynch syndrome has revealed that conventional adenomas are premalignant lesions that can progress to CRC. It has been shown that cancer development in these adenomas can follow different molecular pathways involving separate oncogenes and tumor-suppressor genes. Recently, a novel CRC pathway has been suggested involving histologically different polyps namely serrated polyps. In these serrated polyps, alternative molecular processes are suggested to lead to CRC. In this thesis we investigated a proposed ‘serrated neoplasia pathway’. To this aim, patients with hyperplastic polyposis syndrome (HPS) harboring multiple serrated polyps but also conventional adenomas represented a unique demographic opportunity for clinical and molecular analysis of serrated polyps and their association with CRC.

Clinical analyses – Colorectal cancer risk

In chapter 2 of this thesis we describe the clinical and pathological features of a large multi-centre HPS cohort during multiple years of unprotocollized endoscopic surveillance. We showed that one third of HPS patients presented with co-existent CRC and in an additional 7% of patients CRCs were identified despite endoscopic surveillance (interval-CRCs). These findings are substantial considering that the lifetime risk of developing CRC in the general population is estimated to be only 6%. Interestingly, at multivariate logistic regression, serrated polyps (i.e. HPs and SSLs) and not conventional adenomas were significantly associated with CRC presence thus supporting the existence of a predominant ‘serrated pathway’ to CRC in these...
patients. Considering that CRCs were detected in polyps as small as 4mm during endoscopic surveillance, all polyps in HPS seem at risk of representing advanced lesions warranting removal of all polyps (at least polyps ≥3mm) at annual surveillance endoscopies. If this is not feasible, surgical resection should be considered.

Although an increased CRC risk for HPS patients has been established based on previous cohort studies, it was yet unknown whether first-degree relatives (FDRs) have an increased risk of CRC and/or HPS. Consequently, the need for preventive measures, like screening colonoscopies, in this group was doubtful. In chapter 3 we performed a retrospective study involving, at the time, the largest described cohort of FDRs in which we analyzed the incidence rate of CRC and HPS. This incidence we subsequently compared with the general population through person-year analysis. This way we were able to quantify the suggested increased CRC incidence in the literature by means of a relative risk. Our results showed that FDRs of HPS patients have an increased risk for both CRC (RR: 3.7-7.8) and HPS (RR: 13-121) compared to the general population warranting screening colonoscopies for this whole group as long as no genetic substrate is identified which can help us indentify FDRs with a high risk.

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 we investigated the presence of serrated polyps in MYH-associated polyposis (MAP) and analysed whether these polyps were causally related to MAP.
Phenotypically, MAP polyps encompass conventional adenomas and very rarely serrated polyps. Conversely, at re-evaluation of all polyps in our cohort of MAP patients we found that almost half of all patients had at least one serrated polyp and 18% had ≥ 10 serrated polyps. At subsequent molecular analysis, we found serrated polyps to be causally related to MYH-deficiency, reflected by the presence of almost exclusively G:C→T:A transversions in the KRAS gene of these lesions. This implies that distinct pathways, i.e. APC-gene related in adenomas and non-related in serrated polyps, appear to be operational in MAP.

Similar to patients with MAP, detected polyps in patients with Lynch syndrome, who have a germline defect in one of the MMR genes, invariably represent conventional adenomas. However, recent studies have also described serrated polyps in Lynch patients. Subsequent molecular studies evaluating whether these polyps are causally related to Lynch syndrome by means of immunohistochemistry have however been inconclusive. In chapter 5 the presence of serrated polyps in a large cohort of genetically proven Lynch patients was analysed. To examine whether serrated polyps are causally related to Lynch we utilized an alternative molecular approach involving mutation analysis (APC, KRAS and BRAF) in adjunct to defective MMR testing. The possible finding of an association between Lynch and serrated polyps could imply that these lesions follow an accelerated route to CRC. Based on the principle that Lynch CRCs are not BRAF mutated, BRAF analysis is method to distinguish Lynch-associated CRCs from sporadic CRCs. We hypothesised that a high frequency of BRAF mutations in serrated polyps, comparable to sporadic control group serrated polyps, would indicate that these polyps are not Lynch-associated. In this study, we demonstrated that
serrated polyps in Lynch-HPS patients (>10 serrated polyps) are not associated with Lynch due to the high frequency of *BRAF* mutations in these polyps. Alternatively, in Lynch patients with occasional serrated polyps (<10), serrated polyps had significantly lower levels of *BRAF* mutations than control group polyps. It can be surmised that considering this low frequency of *BRAF* mutations in serrated polyps of non-HPS Lynch patients, a causal relationship with the MSI-pathway can not be excluded despite lack of defective MMR. These polyps associated with the accelerated MSI-pathway may represent precursor lesions of MSI-CRC which evolve even faster than in the serrated CRC pathway alone.

Because of the unique mixture of different precursor lesion types in HPS, it was yet unknown which polyps lead to CRC in HPS and thus which polyps are clinically relevant. In chapter 6 we evaluated which pathways are operational in HPS by studying the histological and molecular characteristics of all available CRCs in a large cohort of HPS patients. We found in this comprehensive cohort study of HPS patients a high number of combined serrated polyp-CRC lesions which showed identical *BRAF* mutations in both components, supporting the existence of a serrated CRC pathway. Overall, we demonstrated that both microsatellite-stable and –unstable CRCs in HPS predominantly originate from serrated polyps, reflected by a high percentage of *BRAF* mutations in these CRCs, but also from conventional adenomas. The predominance of a serrated CRC pathway over the classical Wnt-pathway seems due to the numerical prevalence of serrated polyps in HPS patients. From this it is inferred that all polyp types in HPS, should be considered clinically relevant. Furthermore, we showed at molecular analysis of lesions in the same cohort that CRCs evolving through the serrated CRC pathway were
predominantly right-sided. Considering that CRC in HPS can be as small as 4mm, it seems advisable to remove at least all polyps ≥3mm and especially proximally located serrated polyps.

**Endoscopic imaging – Detection and differentiation of polyps**

Due to the increased risk of malignant progression of HPS polyps, optimal endoscopic detection, differentiation and removal of in particular high-risk polyps is necessary to prevent CRC development in these patients. **Chapter 7** showed that NBI significantly reduces polyp miss-rates in HPS. To evaluate for which polyps NBI was particularly of value, we compared miss-rates for different polyp histologies and shapes. NBI was especially of value for the detection of flat serrated polyps as opposed to conventional adenomas. Considering the predominance of a serrated CRC pathway in HPS, our findings are clinically relevant and support the implementation of NBI for the endoscopic surveillance of HPS patients.

**Chapter 8** evaluates the value of ETMI (high-resolution endoscopy, NBI and autofluorescence imaging) for the real-time endoscopic differentiation of polyps in HPS. None of the three modalities rendered a sufficient diagnostic accuracy for the differentiation of SSLs from HPs. Differentiation of adenomas from HPs however was well possible with NBI but not with AFI. Based on these findings, we conclude that ETMI offers insufficient diagnostic tools to differentiate between high-risk SSLs from relatively ‘innocuous’ HPs but is of value for the differentiation of conventional adenomas from HPs.
FUTURE PERSPECTIVES

Research in this thesis provides additional supporting evidence for a serrated CRC pathway in humans. This pathway is particularly predominant in patients with HPS who have a high-risk of malignant progression, even under unprotocolized endoscopic surveillance. Currently, no uniform and adequately substantiated management protocol exists for these patients. Prospective management studies, based on previous clinical and molecular research, will hopefully provide us with data with which we can construct a safe and effective management protocol for these patients. In this light, adequate endoscopic detection as well as real-time differentiation and removal of only high-risk polyps in HPS would strongly increase management efficiency. Regarding polyp detection, future multi-centre studies need to be performed to evaluate whether NBI indeed increases polyp detection in HPS. Concerning real-time differentiation, results from recent studies analyzing polyp differentiation using NBI, AFI and even endomicroscopy have been disappointing, especially regarding SSAs. Future endoscopic techniques with which molecular characteristics such as mutation- and methylation status (“molecular imaging”) can be evaluated real-time during endoscopy may be of value in this respect.

Thus far, HPS patients have been mostly identified when symptomatic (the reason for the endoscopy), and a large proportion of them have a co-incident CRC at the time of diagnosis. With the recent development of whole-genome sequencing, genome-wide association studies are currently being performed in order to unravel the genetic make-up and inheritance pattern of HPS patients. Based on these findings a reclassification of HPS may be possible so as to identify subtypes that have a higher risk of CRC development (e.g. patients with a higher number of polyps or larger polyps) or a more dominant
inheritance pattern. Based on these findings we will hopefully be able to recognize which individuals need to receive (more frequent) screening colonoscopies. In addition, causative target genes may be identified which in turn can be treated with specific gene-inhibitors. Until then, prospective screening studies should be performed in first-degree relatives of HPS patients to adequately assess the risk of HPS in these individuals.