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Mismatch repair-deficient crypt foci in Lynch syndrome patients – Association with clinical parameters and molecular alterations

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Lynch syndrome is one of the most common inherited cancer syndromes. Tumorigenesis in Lynch syndrome-associated malignancies is caused by deficiency of one of the DNA mismatch repair (MMR) genes, such as *MLH1*, *MSH2*, *PMS2* and *MSH6*. Carriers of a germ line mutation in one allele of the MMR gene acquire a second somatic mutation, following the classical two-hit hypothesis, which inactivates the remaining functional allele of the respective MMR gene. Inactivation of MMR genes consequently leads to the loss of MMR protein expression in tumour cells and to the high level microsatellite-instability (MSI-H) phenotype.

Mismatch repair-deficient crypt foci represent a phenotypic correlate of MMR gene inactivation by second genetic hits and occur abundantly in the normal mucosa of Lynch syndrome patients. At present, it is unclear why MMR-deficient crypt foci occur at high rates in the normal mucosa of Lynch syndrome patients, but only very rarely progress to cancer. In the present thesis, the aim was to characterise essential molecular alterations within MMR-deficient crypt foci and to identify potential correlations with clinical parameters such as tumour localisation, age and gender of the patient.
Resection specimens from Lynch syndrome patients, patients with sporadic MSI-H colorectal cancer, as demonstrated by *MLH1* promoter methylation, and microsatellite-stable (MSS) colorectal cancer were analysed for the expression of MMR protein.
In 714 cm mucosa from Lynch syndrome mutation carriers, 21 MMR-deficient crypt foci were detected, corresponding to a prevalence of 0.84 foci per 1 cm^2 mucosa in the colorectum. No MMR-deficient crypt foci were detectable in patients with sporadic MSI-H cancer or in patients with no MMR gene germ line mutation. The prevalence of these lesions increased by a factor of 1.75 for a change of age at diagnosis of 10 years and they were more frequent in patients with distal compared to proximal colorectal cancer.
On a molecular level, the examination of microsatellite mutation frequency revealed a significantly higher number of mutation events in MMR-deficient crypt foci compared to MMR-proficient crypts. Microsatellite instability is detectable in almost every MMR-deficient crypt focus (89%), including monocryptic lesions. With regard to non-coding microsatellite markers, mutation analysis revealed microsatellite instability in at least one tested marker in 87% of the MMR-deficient crypt foci examined. Among coding microsatellites, mutation frequency was highest for the *HT001* microsatellite (33%) followed by *AIM2* (17%) and *BAX* (10%). These three coding microsatellite-bearing genes likely are among the first target genes to be mutated after MMR gene inactivation.

To summarise, it was demonstrated for the first time that the occurrence of MMR-deficient crypt foci, a lesion abundant in Lynch syndrome, is correlated to patient age and that the vast majority of MMR-deficient crypt foci present with microsatellite instability. Moreover, it was shown that distal tumour localisation is associated with a higher number of MMR-deficient crypt foci at the time point of tumour resection. Mismatch repair deficiency appears to be insufficient for malignant transformation. However, the data of this thesis suggest that certain combinations of coding microsatellite mutations including mutations of the *HT001*, *AIM2* and *BAX* gene, and *TGFBR2* gene mutations in larger MMR-deficient crypt foci may contribute to the progression of MMR-deficient lesions into MMR-deficient cancers.