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**Immune responses and immune evasion in microsatellite unstable colorectal cancer:
Characterization of immune infiltrates and analysis of molecular alterations in cellular
antigen presentation pathways**

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High-level microsatellite instability (MSI-H) in colorectal cancer (CRC) occurs due to defects in the DNA mismatch-repair (MMR) system and is hallmarked by the accumulation of insertion and deletion mutations at microsatellites. Mutations in coding microsatellites (cMS) can result in shifts of the reading frame, the generation of premature termination codons, impaired protein function and translation of highly immunogenic *neopeptides*. MSI-H CRCs provoke pronounced immune responses and T cells specifically recognizing MSI-associated *neopeptides* exist in MSI-H CRC patients. However, despite dense infiltration with T cells, MSI-H CRCs clinically grow out to locally advanced tumors. This suggests that immune evasion mechanisms interfere with the successful elimination of MSI-H CRC cells, either by inhibiting effector T cell function or by impairing antigen presentation by tumor cells. The present thesis aimed to address immune evasion of MSI-H CRC lesions, focusing on mechanisms interfering with pivotal steps of tumor-immune cell interaction, by analysis of T_{reg} cell infiltration and alterations of tumor cell antigen presentation.

Infiltration of MSI-H CRCs with T_{reg} cells was analyzed and compared to microsatellite stable (MSS) CRCs, using nuclear expression of FOXP3 as a marker. Higher numbers of FOXP3⁺ cells in the tumor epithelium (median 8.5 cells/0.25 mm² in MSI-H vs. 3.1 cells/0.25 mm² in MSS; p<0.001, Mann-Whitney test) and stroma (median 181.5 cells/0.25 mm² in MSI-H vs. 137.1 cells/0.25 mm² in MSS; p=0.06) as well as a higher ratio of epithelial to stromal infiltration (0.05 in MSI-H vs. 0.01 in MSS; p<0.001) were detected in MSI-H compared to MSS CRCs. FOXP3⁺ cell counts in MSI-H CRCs were related to CD8⁺ counts (Spearman's rank correlation coefficient $\rho=0.60$; 95% confidence interval 0.43-0.73). The present work

demonstrates that the previously known enhanced infiltration of MSI-H CRCs with TILs also extends to the group of FOXP3⁺ T cells, suggesting that the enhanced infiltration of MSI-H CRCs with T cells of the effector phenotype is paralleled by an enhanced infiltration with T_{reg} cells that may interfere with effector immune cell functions.

Frameshift mutations in cMS of *Beta2-Microglobulin (B2M)*, the HLA class I light chain, are a major mechanism of immune evasion in MSI-H CRC, however their clinical implications were unknown. In a large series of MSI-H colorectal tumors, it was shown that *B2M* mutations are an early event in MSI-H CRC occurring in 15.6% of MSI-H adenomas. The *B2M* mutation frequency increased from adenoma to invasive cancer and reached 43.5% in UICC stage III MSI-H CRCs, pointing towards a tumor-promoting role of *B2M* mutations in local tumor growth and lymphatic spread. In contrast, *B2M* frameshift mutations were absent (mutation frequency 0%) in MSI-H CRCs that had metastasized to distant organs (UICC stage IV), suggesting that the presence of cMS frameshift mutations in *B2M* interferes with distant metastasis formation in MSI-H CRC.

Analysis of HLA class II antigen expression in MSI-H CRCs showed that expression was absent in 23.5% of the analyzed lesions. Frameshift mutations in master regulators of HLA class II antigen expression, *CIITA* and *RFX5* (mutation frequency 2.9% and 26.5%, respectively) were identified. *RFX5* mutations were only detected in lesions with lack of or weak expression of HLA class II antigens (42.9%) and were absent in MSI-H CRCs with strong expression of HLA class II antigen (0%; p=0.006, Fishers' Exact Test). In a *RFX5*-mutated cell line, HLA class II expression could be re-induced with IFN- γ after transfection with wild type *RFX5*, indicating that the *RFX5* mutation caused impaired HLA class II expression. The present study describes for the first time somatic mutations in *RFX5* that may lead to loss of HLA class II antigen expression in tumor cells, potentially as a result of immunoselective pressure.

From the present findings it becomes apparent that multiple mechanisms can interfere with the host's local immune response directed against MSI-H CRC. MSI-H CRCs are densely infiltrated with FOXP3⁺ T cells. These cells may possess regulatory function and may modulate and dampen the anti-tumoral function of cytotoxic effector T cells, thereby counteracting efficient anti-tumoral immune responses. In addition to mutations of the *B2M* gene that represent the major mechanism underlying complete loss of HLA class I antigen presentation, frameshift mutations in *RFX5*, an essential regulator gene of HLA class II antigen expression, were identified as underlying cause for lack of HLA class II antigen expression in a subgroup of MSI-H CRCs.

In summary, the present thesis identified novel mechanisms that may play role in immune evasion of MSI-H CRC. Moreover the results of the thesis demonstrate that immune evasion in MSI-H CRC has profound impact on the metastatic potential of MSI-H CRC and underline the clinical significance of interactions between tumor and immune system for the course of the disease.