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Regulation of the Local Antigen-Specific Immune Response in Microsatellite-Unstable Colorectal Cancers

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High-level microsatellite unstable (MSI-H) colorectal carcinomas (CRC), accounting for approximately 10-15% of CRC, arise due to deficiency in the MMR machinery, leading to numerous deletion or insertion mutations in microsatellites. MSI-H CRC are characterized by a dense lymphocyte infiltration in the malignant tissue, most likely resulting from the generation of FSP antigens as a consequence of frameshift mutations within gene-encoding regions. FSP are neo-peptides and can elicit an FSP-specific cellular and humoral immune response. Strikingly, FSP-specific T cell responses have also been identified in the peripheral blood of healthy Lynch syndrome mutation carriers, suggesting a potential protective effect of the immune system. However, MSI-H tumors obviously develop mechanisms to avoid immune control and grow out to locally advanced lesions. The pronounced infiltration with Treg cells in MSI-H compared to MSS CRC suggests that Treg cell recruitment and differentiation might play a role in tumoral immune evasion.

The present thesis aimed to characterize in detail the host's local immune response against MSI-H CRC-related antigens. S100-positive DC and CD163-positive macrophages represent professional APC that play a crucial role in the induction and regulation of immune responses. Both cell types were significantly more prevalent in MSI-H (n = 33) compared to MSS CRC (n = 36) (S100: epithelium median 6.0 in MSI-H vs. 3.0 in MSS, $p = 0.018$; stroma 152.5 in MSI-H vs. 104.4 in MSS, $p = 0.042$; CD163: epithelium 3.7 in MSI-H vs. 1.1 in MSS, $p < 0.001$; stroma 111.1 in MSI-H vs. 96.9 in MSS, $p = 0.046$; Mann-Whitney test). Additionally, CD208-positive mature DC were elevated in the epithelial compartment of MSI-H CRC (1.3 in MSI-H vs. 0.8 in MSS, $p = 0.047$). A low proportion of intraepithelial CD208-positive mature DC among the total number of S100-positive DC was significantly associated with high numbers of intraepithelial FOXP3-positive Treg cells ($p = 0.004$, Spearman's rank correlation). These results demonstrate that the number of APC like DC and macrophages is elevated in MSI-H compared to MSS CRC, suggesting that these cells are involved in the uptake of MSI-H induced antigens, like FSP antigens, and might activate antigen-specific lymphocytes. The observation that a low proportion of mature DC is linked with a high number of Treg cells suggests that impaired DC maturation might contribute to the enhanced infiltration with Treg cells in MSI-H CRC.
In order to analyze mechanisms potentially underlying Treg cell induction, mRNA expression of TGFbeta was compared between MSI-H and MSS CRC. TGFbeta is one potential factor that induces Treg cell differentiation from naïve CD4-positive T cells. TGFbeta mRNA expression was increased in MSI-H (n = 16) lesions with at least one mutated allele of TGFBR2 compared to MSS CRC (n = 14) (median ratio 0.221 in MSI-H vs. 0.037 in MSS, $p = 0.046$). Moreover, the proportion of apoptotic DC was significantly higher in MSI-H CRC (n = 62 vs. n = 48 MSS) (0.556 in MSI-H vs. 0.357 in MSS, $p = 0.002$) and was correlated to the infiltration with FOXP3-positive Treg cells ($p < 0.001$). The uptake of apoptotic DC by immature DC has previously been identified to inhibit DC maturation and elicit TGFbeta

expression in the immature DC. The results of the present thesis reveal that the proportion of apoptotic DC and the TGFbeta mRNA expression is higher in MSI-H than MSS CRC and represent potential factors contributing to Treg cell induction in these tumors.
Although MSI-H CRC display a high density of Treg cells, the influence of Treg cell suppression on the recognition of FSP antigens by effector T cells has not been analyzed so far. In the present study, Treg cell impact on T cell responses was compared between selected MMR-deficiency induced FSP antigens and peptides derived from the classical CRC-associated antigens MUC1 and CEA, which are all currently evaluated as vaccination targets in clinical trials. Pre-existing FSP-specific T cell responses were detected in the majority of tumor infiltrating and peripheral T cell samples from MSI-H CRC patients ($n = 4$ and $n = 14$, respectively) and were more frequent than T cell responses against the peptides from MUC1 and CEA ($p = 0.049$). Both tumor infiltrating and peripheral T cell samples from MSS CRC patients ($n = 26$ and $n = 17$) were only rarely detected with FSP-specific T cell responses and T cell reactivity against none of the analyzed peptides was detected in samples from healthy donors ($n = 7$). Depletion of Treg cells resulted in a pronounced increase of T cell responses against the MUC1- and CEA-derived peptides, but only slightly increased the frequency of T cell responses against FSP antigens.
These data suggest that the selected FSP antigens are able to elicit effector T cell responses that may only rarely be suppressed by Treg cells and might therefore represent a relevant pool of target antigens for immunotherapeutic approaches. The findings of this thesis support the hypothesis that impaired DC maturation, TGFbeta expression, and induction of apoptosis in DC are involved in the establishment of an immunosuppressive milieu and the activation of Treg cells in MSI-H CRC. The influence of Treg cells on T cell responses is variable for different antigens and not all tumor antigen-specific T cell responses are equally subjected to Treg cell suppression. In summary, the present thesis revealed additional insights into the immune cell composition of MSI-H cancers and offered potential factors that might contribute to local immune evasion in these lesions. FSP antigens have been evaluated as promising vaccination candidates that are currently being tested for their potential to induce an effective anti-tumoral immune response in MSI-H CRC patients.