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Immune infiltration of normal colonic mucosa from colorectal cancer patients – association with hereditary predisposition and implications on tumor phenotype

Fach/Einrichtung: Pathologie
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High level microsatellite instability (MSI-H) is a hallmark of 15% of colorectal cancers (CRCs) and caused by a defective DNA mismatch repair (MMR) machinery. A part of these MSI-H CRCs develops in patients with Lynch syndrome. MSI-H CRCs result in the formation of immunogenic frameshift peptide (FSP)-derived neoantigens which provoke strong anti-tumoral immune responses that are greatly elevated compared to sporadic, microsatellite stable (MSS) CRCs. However, FSP-specific T cell and humoral immune responses have also been reported in Lynch syndrome mutations carriers prior to cancer development. Recently, mismatch repair-deficient crypt foci (MMR-DCFs) have been discovered in normal colonic mucosa of Lynch syndrome carriers, representing frequent sites with biallelic inactivation of the respective MMR protein and evidence of MSI-H. These findings imply that MMR-deficient cells and the immune system may already interact closely within the healthy colonic mucosa and thus before the development of clinically overt cancer. However, the immune infiltration in normal colonic mucosa has not been assessed so far. Therefore, this thesis analyzed the immune infiltration in normal colonic mucosa from Lynch syndrome-associated CRC patients and sporadic, MSS CRC patients. Four different immune parameters (CD3, CD8, FoxP3 and lymph follicles) were assessed in both tumor-adjacent and tumor-distant normal mucosa.

In tumor-adjacent normal mucosa, CD3+, CD8+, FoxP3+ T cell infiltration as well as lymph follicle count were all found to be significantly increased in Lynch syndrome-associated CRCs compared to MSS CRCs ($p_1<0.0001$; $p_2=0.017$; $p_3<0.002$; $p_4=0.011$). In tumor-distant normal mucosa, CD3+ and FoxP3+ T cell infiltration were enhanced in Lynch syndrome-associated CRC patients whereas lymph follicle count was increased in MSS CRC patients but all differences did not quite reach statistical significance ($p_1=0.064$; $p_2=0.115$; $p_3=0.058$). No difference was observed for CD8+ T cell infiltration ($p=0.362$).

This is the first study to demonstrate that there are significant differences in immune infiltration between MSS CRC patients and Lynch syndrome-associated CRC patients affecting normal colonic mucosa and thus areas outside of the tumor. The findings from tumor-adjacent normal mucosa resemble the intratumoral situation and imply that the immunostimulatory effects associated with the generation of MSI-H-derived FSPs in Lynch syndrome-associated CRCs appear to spread also to the tumor-adjacent mucosa thus promoting immune infiltration there. Accordingly, the observed higher FoxP3+ T cell infiltration in tumor-adjacent mucosa may represent an immune evasive mechanism of Lynch syndrome-associated CRCs to allow tumor outgrowth despite the high density of cytotoxic T

cells. Moreover, the reported differences in tumor-distant mucosa suggest that Lynch syndrome may already be characterized by systemic immune alterations in normal colonic mucosa independent of local, tumor-mediated effects. These alterations potentially occur before and thus possibly affect cancer development in Lynch syndrome and might be a feature of hereditary cancer patients in general. The changes may be due to the early sensitization of the immune system towards FSP-derived neoantigens generated in MMR-DCFs. These results contribute to a better understanding of early Lynch syndrome-related changes and may also help to explain the low penetrance of Lynch syndrome-associated CRCs.

The second part of this thesis was focused on a potential correlation between immune infiltration in normal mucosa of Lynch syndrome-associated CRCs and the presence of *Beta2-microglobulin (B2M)* mutations within the tumor. *B2M* mutations represent a major, potentially immune evasive alteration in MSI-H CRCs as they result in a complete abrogation of antigen presentation via HLA class I which protects tumor cells from cytotoxic T cells. However, for reasons that have thus far been unknown, *B2M* mutations only occur in a minority of 30-40% of Lynch syndrome-associated CRCs. To shed more light on this issue, this thesis was the first to analyze if the occurrence of *B2M* mutations may be related to a stronger immune selection milieu during carcinogenesis. To that end, *B2M* mutation status was determined and correlated to the immune infiltration data from normal colonic mucosa.

It was shown that *B2M*-mt Lynch syndrome-associated CRCs presented with a significantly lower FoxP3+ T cell infiltration in tumor-adjacent normal colonic mucosa than *B2M*-wt CRCs ($p=0.023$). This finding demonstrates for the first time that the molecular phenotype of CRCs is related to the immune cell infiltration in normal mucosa. A low density of FoxP3+ T cells may be indicative of a more active local immune milieu that is able to apply a stronger immunoselective pressure and therefore more capable of eliminating emerging dysplastic cells. Conversely, this may eventually result in the outgrowth of poorly immunogenic tumor cells that no longer present antigens via HLA class I due to the acquisition of *B2M* mutations. This observation strongly supports the concept that *B2M* mutations in MSI-H CRCs represent immune evasive alterations that develop as a result of immune selection. This validates the immunoediting hypothesis in human carcinogenesis, which postulates the selection of poorly immunogenic tumor cells due to continuous immune surveillance. In light of the good prognosis of *B2M*-mt MSI-H CRCs, the correlation between *B2M* mutations and low FoxP3+ T cell levels even suggests that the latter may also be an indicator of good prognosis in CRC.

In summary, this thesis demonstrates for the first time that Lynch syndrome-associated CRC patients and MSS CRC patients are characterized by significant differences in the immune cell infiltration of normal colonic mucosa, and thus areas outside of the tumor. Moreover, it was shown that the immune cell infiltration of colonic mucosa is related to the molecular phenotype of Lynch syndrome-associated CRCs thus supporting the role of the immuno-editing hypothesis in human cancer development. Additionally, the immune milieu in tumor-adjacent normal mucosa was identified as a possible prognostic indicator in MSI-H CRCs.