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Consequences of mismatch repair deficiency in normal and transformed epithelial cells

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In the present thesis, the molecular status of tumors at different, selected time points in the evolution of MSI-H colorectal cancer was examined to characterize the consequences of MMR deficiency in epithelial cells during tumor initiation and progression.

The results of the present thesis show that advanced stages of MSI-H CRC are characterized by pronounced intratumoral, intermetastatic and intrametastatic heterogeneity with regards to both coding and non-coding microsatellite instability. Especially the intratumoral heterogeneity observed for coding MSI should be considered in future studies and for the design of novel therapeutic approaches.

The observation of a mutation frequency of TGFBR2, C4orf56, PCNXL2, SEC63, and PTHLH significantly above what was previously reported in literature demonstrates, that regional microdissection and separate MSI analysis of several regions of a tumor is more sensitive to determine the mutation status of a tumor with respect to a certain coding microsatellite than whole-tumor analysis and underlines the wide variety of pathway inactivation that commonly takes place during MSI-H cancer progression.

Microsatellite instability does not affect different regions of a tumor to the same extent for all coding microsatellites. Rather, patterns of coding microsatellite instability and the expression of wild type or mutated alleles in a tumor vary for the different coding microsatellites. This observation suggests, that mutations in certain coding microsatellites might only be beneficial in certain regions of a tumor or at a specific time during tumorigenesis.

In that line, the observation of significantly higher mutation frequencies of ELAVL3 and ASTE1 in lymph node metastases may point towards their significance especially in the process of lymphatic metastasis, possibly endowing cells harboring mutations with

metastatic properties or a growth advantage in a different environment than that of the primary tumor.

Detection of intrametastatic heterogeneity demonstrates ongoing, independent clonal evolution after metastasis formation, indicating that the lymph node metastasis itself is a site of continuous mutational changes. These results suggest that lymph node metastases are molecularly dynamic sites of tumor progression, which should be taken into consideration in the debate about the clinical significance of lymph node resection during oncologic surgery of the colorectum.

Moreover, the results of the present thesis could establish coding microsatellite instability profiling as a kind of MMR deficiency-related molecular fingerprint which is suited to identify individual cancer patients, at least in a subset of patients. These results provide the basis for further development of a refined coding microsatellite instability typing tool.

One of the most important findings of the present thesis is the identification of an entirely novel lesion occurring in the intestinal mucosa from Lynch syndrome mutation carriers. The "mismatch repair-deficient crypt focus" (MMR-deficient crypt focus) in the non-tumorous mucosa of Lynch syndrome mutation carriers presents a previously unrecognized morphologic correlate of biallelic MMR gene inactivation in Lynch syndrome.

The high abundance of these foci in the intestinal mucosa of Lynch syndrome mutation carriers shows for the first time, that biallelic MMR gene inactivation is a very frequent event in the context of Lynch syndrome.

The detection of microsatellite instability in some MMR-deficient crypt foci demonstrates, that MMR deficiency can already have functional consequences in the epithelial cells composing the crypts. Early alterations like these may then pave the way for the accumulation of a number of additional mutations that may eventually lead to the initiation of cancer.

However, the abundance of mismatch MMR-deficient crypt foci in the non-tumorous mucosa

of Lynch syndrome mutation carriers is in sharp contrast to the actual frequency of colorectal and small bowel carcinomas observed in Lynch syndrome patients and the documented incomplete penetrance of the disease. One may hypothesize, that the majority of MMR-deficient crypt foci are eliminated by the immune system while only a small number of cells, that have acquired molecular changes allowing them to escape detection and elimination by immune surveillance mechanisms, persists and enters malignant transformation. Immunologic elimination of MMR-deficient crypt foci would be in line with the previous observation of frameshift peptide antigen-specific immune responses in clinically healthy Lynch syndrome mutation carriers, which can now be explained by the findings of the present work.

In summary, the results of the present thesis shed new light on MMR-deficient cancer initiation and progression, and provide researchers with unique novel tools for studies on tumor initiation and also tumor elimination in general.