Concluding remarks and implications for further research
1. TOWARDS MOLECULAR DIAGNOSIS, TREATMENT AND PREVENTION OF COLON CANCER

Tumours arising from various parts of the colon have long been considered and treated as identical pathological entities. However, accumulating studies on (molecular) tumour development have shown that distinct colon tumour subsets differ in important clinical parameters such as frequency of dissemination and response to adjuvant treatment. Although surgical excision will remain inevitably the major step of treatment irrespective of the tumour type, to design (customized) therapeutic strategies it is essential to discriminate distinct tumour subsets by molecular analyses in advance [1]. Insight into the steps of tumorigenesis may eventually lead to cancer prevention by enabling early detection of precursor lesions, eliminating risk factors, or even vaccination [2, 3].

Concerning the latter, the identification of altered HLA class I expression in colon tumours, especially in those with mismatch repair (MMR) deficiency on a sporadic basis or in the context of the Lynch syndrome, has given immune escape processes a definite position on the roadmap to colon cancer. This observation might support the potential of immune-mediated eradication as a preventive or therapeutic measure. The variation in type of HLA alteration among different tumour subtypes may suggest as yet unresolved differences in immune editing. The variation in type of HLA alteration among different tumour subtypes may suggest as yet unresolved differences in immune editing. The variation in type of HLA alteration among different tumour subtypes may suggest as yet unresolved differences in immune editing. The variation in type of HLA alteration among different tumour subtypes may suggest as yet unresolved differences in immune editing.

Another question unanswered is the onset of the ‘danger signal’. This signal is normally evoked at the earliest start of inflammation and is able to define the cascade of following immune response. It may even lead to loss of systemic tolerance [4]. Inflammation is a natural response to any tissue damage and is microscopically already visible in adenomas. Consequently, the danger signal is evoked during the adenoma stage, thus before invasion of the surrounding tissue starts. Interestingly, in Lynch syndrome-adenomas increased tumour-infiltration of lymphocytes was found only in MMR deficient tumours which suggests that this immune response is secondary to the MMR knock-out [6].

Finally, the immune response to metastases needs to be studied. As discussed previously, dissemination is not necessarily a roadblock on the roadmap to cancer. Therefore, metastasizing cancer cells may be challenged by the same type of adaptive immune responses, albeit that they will additionally be confronted more easily by the innate immune system (including natural killer cells) as they migrate through the lymph or bloodstream. Alternately, they might evoke a second danger signal or give positive-feedback to the primal danger signal as a result of tissue damage at the site of metastasis [7].

2. TIMING OF COLON CANCER IMMUNE RESPONSES

The studies presented in this thesis have solely focused on the nature of immune escape mechanisms in primary colon tumours; we did neither study the onset of events during tumour progression, nor did we study tumour metastasis. In other words, the position of immune escape on the roadmap is, to date, unknown. Insight into the position on the timeline may reveal essential information on the type and the effect of both the immune edit and immune escape mechanisms. For instance, the amount and type of displayed tumour-antigens will vary during stages of tumour development, which might determine whether this leads to tumour immune tolerance or attack [4, 5].
3. MISMATCH REPAIR DEFICIENT TUMOUR IMMUNITY

The relation of immune response evocation and tumour antigen display is of particular interest in DNA repair deficient tumours. We identified frequent HLA alterations in both sporadic MMR deficient tumours, and in Lynch syndrome-related tumours; additionally, it has been frequently identified in MUTYH-associated polyposis-related tumours [8]. Due to deficient DNA repair both of these types of tumours accumulate a high amount of DNA errors that potentially could lead to an acceleration of tumour progression compared to DNA repair proficient tumours. Such acceleration has been observed in the adenomatous phase of Lynch syndrome-tumours [9]. In the case of MMR deficiency, proofreading errors result in frame-shift encoded proteins that may be, once processed to peptides, loaded on HLA class I molecules and transported to the cell surface, presented as ‘foreign’ antigens; some have indeed been shown to be potentially immunogenic [10-12]. Additionally, microsatellite mutations are frequently found within the untranslated regions (UTR) of genes [13]. Such mutations would not lead to an altered protein product. Although we did not observe it for the IFNGR1, mutations of the UTR can affect RNA stability leading to functional inactivation as has been shown for the BMPR2 [14].

The frameshift-mutated antigens need to accumulate into a large enough amount of protein in order to lead the necessary cross-presentation of dendritic cells to effector cytotoxic T cells. Newly designed protein accumulation assays and RNA stability assays have been developed to predict such [15-17]. The results of these pre-screening assays will most likely limit the potential pool of MMR tumour-antigens as well as the repertoire of applicable immunogenic frameshift-mutated antigens for vaccination strategies.

The identification of distinct molecular mechanisms of HLA alteration between the sporadic and hereditary mismatch repair deficient colorectal tumour subsets suggests that they are related to distinct anti-tumour immune responses. What would cause that difference? Patients suffering from the Lynch syndrome may display a mild degree of microsatellite instability throughout the body due to their inherited heterozygous mismatch repair gene mutation [18, 19]. The latter would suggest a role of haplo-insufficiency of MMR defects. In contrast to sporadic MMR deficient tumours, in Lynch syndrome cases a low level of frameshift-mutated antigens may have been displayed to the immune system during or even before tumour formation [20]. The latter might result in a different danger signal. Whether it would lead to immune tolerance or, oppositely, to a stronger immune response is not known.

Active adaptive cell-mediated immune response (Th1) as detected by intra-tumour infiltrate phenotype and gene expression profiles have been related to a favourable disease-free survival of sporadic colorectal tumours, irrespective of tumour type, clinical classification, or immune escape mechanisms [21]. The protective property of the elevated immune response may be situated locally in reducing the number of disseminating tumour cells, as well as in the periphery in controlling occult tumour growth from dormant metastatised tumour cells [22, 23]. The acquisition of HLA alterations may lead to opposite effects on clinical behaviour and patient survival [24-26]. These studies however are limited and show somewhat contradictory results. In sporadic colorectal tumours it may be associated with a favourable prognosis, whereas in Lynch syndrome-tumours it has been associated with a poor prognosis. Whether this can
explained by the type of immune escape mechanism itself (e.g. the capacity to escape natural killer cell annihilation or not) or by intrinsic co-existing tumour (or immune-response) features could so far not be concluded [27].

Further research is needed to answer these intriguing questions. It would not only help us to understand why the distinct immune escape mechanisms are employed, but would also clarify the immune potential already present in colon cancer patients. This will lead to important clues in designing appropriate adjuvant immunotherapy or even preventive vaccination of colon cancer.

4. REFERENCES


