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Immune evasion of microsatellite unstable colorectal cancers

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Mini Review

Colorectal cancers (CRC) develop through 2 major pathways of genetic instability. In contrast to the majority of CRCs, which are characterized by chromosomal instability, high-level microsatellite unstable (MSI-H) CRCs arise as a consequence of the loss of DNA mismatch repair (MMR) functions and show accumulation of insertion and deletion mutations particularly in microsatellite sequences. MSI-H occurs in about 15% of CRCs, and virtually all CRCs occurring in the context of the hereditary cancer-predisposing Lynch syndrome. These tumors are characterized by a comparably good prognosis and a low frequency of distant metastases. Because of the expression of a defined set of tumor-specific antigens, MSI-H CRCs elicit a strong local and systemic antitumoral immune response of the host and therefore use different strategies to evade the control of the immune system. In this review, we will summarize novel molecular mechanisms that at the same time drive pathogenesis, immunogenicity and immune evasion during the development and progression of MSI-H CRCs. We will focus on the current knowledge about alterations in human leukocyte antigen (HLA) antigen presentation and discuss how immune evasion—while offering protection against local antitumoral immune responses—paradoxically might interfere with the ability of the tumor to form distant organ metastases.

What Is Microsatellite Instability?

Colorectal cancer (CRC) is a pathogenetically heterogeneous disease. Although the majority of CRCs shows chromosomal instability, about 15% of CRCs occur because of defects in the DNA mismatch repair (MMR) system.[1–3] MMR deficient CRC may develop sporadically or in the context of the hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome.[4,5] DNA MMR deficiency induces a high number of mutational events mainly consisting of insertion and deletion mutations at microsatellites, because these structures are particularly prone to DNA polymerase slippage during DNA replication.[6] The resulting phenotype is termed high level microsatellite instability (MSI H).

Key words: antigen processing and presentation, beta2 microglobulin, colorectal cancer, human leukocyte antigen, immune evasion, DNA mismatch repair, microsatellite instability, tumor specific antigens

Abbreviations: APM: antigen processing machinery; cMS: coding microsatellite; CRC: colorectal cancer; FasL: Fas ligand; FSP: frameshift peptide; HLA: human leukocyte antigen; HNPCC: hereditary nonpolyposis colorectal cancer; LOH: loss of heterozygosity; MHC: major histocompatibility complex; MMR: mismatch repair; MSI: microsatellite instability; MSI H: high level microsatellite instability; T_{reg} cell: regulatory T cell

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MSI H CRCs are characterized by a distinct pathology that is closely connected to the occurrence of mutations affecting microsatellites in gene encoding regions (coding microsatellites, cMS). Although mutations affecting microsatellites in intergenic or noncoding regions are mostly of unknown functional significance, insertions or deletions of nucleotides in cMS can result in defined translational frame shifts that can interfere with protein function[7,8] (Fig. 1). MSI H CRC cells undergo a continuous Darwinian selection process that results in the outgrowth and persistence of cell clones carrying the most beneficial cMS mutations. Consequently, when cMS mutations inactivate genes that have a tumor promoting effect, these mutations are likely counterselected and are expected to be absent or only occur at a low frequency in MSI H cancers. Conversely, cMS mutations leading to loss of function of tumor suppressor genes will be found at a high frequency in clinical tumor specimens.

The probability of polymerase slippage increases with microsatellite length. However, mutation frequencies observed for different microsatellites of the same length vary considerably, suggesting that tumor promoting frameshift mutations are selected for during MSI H colorectal tumor development and progression.[9] To account for the relation between microsatellite length and likelihood of mutation in an MMR deficient context, a biostatistics model has been developed that, by comparison of expected and observed mutation frequencies, allows predicting cMS mutations that are relevant for tumor development in DNA MMR deficient cells.[12] One of the first identified and most prominent examples of a cMS containing gene with high relevance for MSI H tumor progression is the *TGFBR2* gene that harbors an A_{10} repeat mutated in >90% of MSI H CRCs.[13] Mutations of this repeat lead to an abrogation of *TGFBR2* mediated signaling[14] (Fig. 1) and

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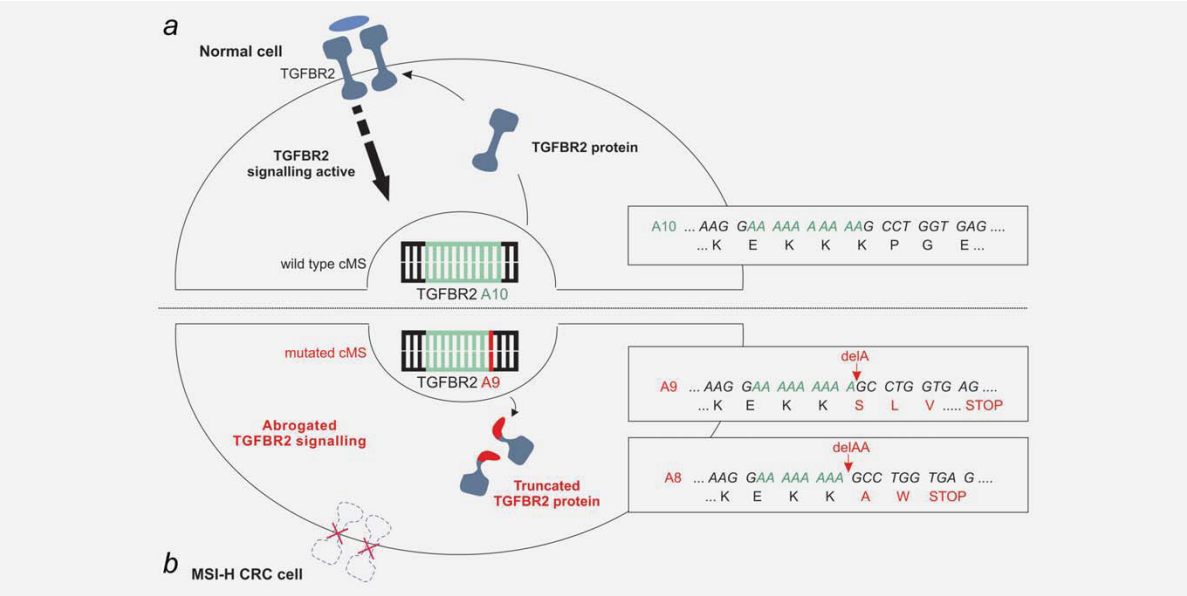

Figure 1. The tumor promoting role of cMS mutations. Schematic illustration of the consequences of cMS mutations on cellular signaling using the example of TGFBR2. (*a*) In a normal, MMR proficient colorectal epithelial cell, TGFBR2 is transcribed and translated from its respective gene locus, which contains an A_{10} microsatellite (schematically displayed by green bars) in the coding region. TGFBR2 is then transferred to the cellular membrane and mediates functional signaling upon binding of its ligand TGFB. (*b*) In a mismatch repair deficient cell, mutations affecting the cMS in the *TGFBR2* gene (*e.g.*, deletion mutation, red bar) can result in abrogation of TGFBR2 signaling, due to expression of truncated TGFBR2 proteins that are nonfunctional, but contain an altered carboxy terminal neopeptide part (displayed in red). Gene sequences surrounding the cMS and the corresponding amino acid sequences of TGFBR2 are displayed in separate boxes on the right. The wild type A_{10} cMS is marked by green letters (upper box). Single nucleotide and dinucleotide deletions result in shifts of the translational reading frame and an altered amino acid sequence (middle and lower panels). Neopeptide sequences and premature termination codons are highlighted in red.

thus induce a higher proliferation rate of affected MSI H tumor cells. Other examples of cMS mutations promoting MSI H tumorigenesis affect the signal transducer *ACVR2* and the proapoptotic *BAX* gene.[15–17]

Immunogenicity of MSI-H CRCs

The strong local immune reaction observed in MSI H CRCs[18] is characterized by peritumoral lymphoid nodules (Crohn's like reaction)[19,20] and a dense overall infiltration of the tumor with lymphocytes,[21] part of which are activated and cytotoxic.[22–26] Whether T cells infiltrating MSI H CRCs are recruited from the periphery or locally expanded due to cytokine or chemokine pattern generated by tumor cells at present is not known. Briefly, after the detection of the MSI H phenotype in CRC, speculation arose that frameshift mutations encountered in DNA MMR deficient CRCs might lead to the generation of tumor specific antigens.[27] *In vitro* experiments with synthetic frameshift peptides derived from cMS sequence data showed that these structures indeed are immunogenic and provoke a strong T cell response.[28–31] However, whether such frameshift peptides are generated *in vivo* has been a subject of debate because several studies demonstrated that frameshift mutations are likely to provoke nonsense mediated RNA decay that interferes with translation of the mutant proteins.[32,33] In addition, it is known that antigens expressed in a noninflamed environment may induce tolerance rather than eliciting an antitumoral immune response.[34]

Nevertheless, the existence of T cell responses directed against a plethora of MMR deficiency specific antigens in individuals with MSI H CRC has been demonstrated in patients with respective tumors.[26,35] This strongly suggests that antigenic structures are generated from coding DNA sequences carrying frameshift mutations in sufficient amounts to trigger antigen specific T cell responses. Moreover, the cytokine milieu encountered at the MSI H tumor site is proinflammatory and favoring Th1 response[19,36] rather than contributing to tolerance induction. Intriguingly, this implies that while promoting tumor development cMS frameshift mutations inherently also render the tumor vulnerable to recognition and attack by the host's immune system (Fig. 2).

Immune Evasion in MSI-H CRC

The outgrowth and progression of MSI H CRC cells in the presence of a strong local antitumoral immune reaction

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require adaptation of the tumor and selection of tumor cells that are able to defy immunological control. The purpose of this review is to summarize different mechanisms that con tribute to immune evasion of MSI H CRCs.

Alterations of HLA class I antigens

HLA class I antigens serve for the presentation of cellular peptides to the immune system. When antigenic structures like tumor specific antigens are presented on the cell surface, these can be recognized by CD8 positive T cells that subse quently react by release of cytotoxic factors. Abrogation of antigen presentation by HLA class I antigens therefore repre sents a potent mechanism protecting tumor cells from attack by cytotoxic T cells.[37,38]

Molecular alterations interfering with HLA class I medi ated antigen presentation either directly affect the HLA class I light or heavy chains or components involved in antigen processing and formation of HLA class I complexes. Briefly, for the assembly of HLA class I antigen complexes, peptides degraded from cellular proteins in the proteasome are trans ported into the endoplasmatic reticulum by a heterodimeric complex of transporters associated with antigen processing, TAP1 and TAP2. In the endoplasmatic reticulum, the HLA class I complex is assembled from the HLA class I heavy chain, the light chain (beta2 microglobulin, B2M) and a pep tide fragment in a stepwise process that involves the chaper ones Tapasin, Calnexin, Calreticulin and ERp57.[39]

Alterations of HLA class I antigen presentation in MSI H CRC have first been described by Bicknell *et al.*[40,41] who reported mutations in the *B2M* gene detected in surgical MSI H CRC specimens and established MSI H CRC cell lines. The *B2M* gene harbors 4 cMS in its coding region, 1 dinucleotide $(CT)_4$ repeat in exon 1 and 3 mononucleotide (A_5, C_5 and A_5) repeats in exon 2. Truncating mutations affecting cMS of the *B2M* gene have been established as the major mechanism mediating impairment of HLA class I anti gen presentation in MSI H CRC, affecting about 30 60% of lesions.[42–44] This underlines the wide ranging significance of DNA MMR deficiency for the immunological characteristics of MSI H CRCs. Although MMR deficiency provokes a strong immune response by inducing cMS mutations that give rise to the generation of frameshift peptide antigens, it contributes at the same time to immune evasion of the tumor following cMS mutation induced inactivation of B2M (Fig. 2).

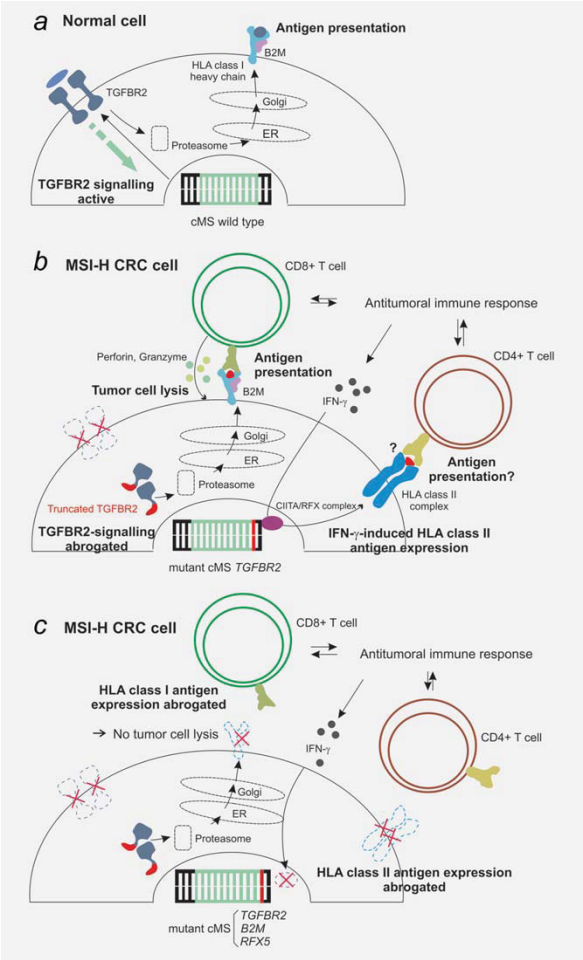

Figure 2. Immunogenicity and immune evasion provoked by cMS mutations. (*a*) Schematic display of a normal mismatch repair proficient cell with functional antigen presentation machinery. The immune system is tolerant toward normal peptides presented on HLA class I complexes. (*b*) Schematic display of an MSI H CRC cell with mutant *TGFBR2*, but intact cellular antigen presentation machinery. The resulting frameshift derived neopeptides (red) are degraded in the proteasome and presented to the immune system on HLA class I complexes. The generated neoantigens have immunogenic potential and can elicit an antitumoral immune response of the host. Secondarily, release of IFN γ induces synthesis of *CIITA* and subsequent upregulation of HLA class II antigen expression. CD8+ effector T cells may recognize epitopes derived from frameshift neoantigens presented on HLA class I antigens as foreign and may thus induce tumor cell killing by releasing cytototoxic granula. Similarly, the immune system may directly or indirectly target tumor cells, which express HLA class II antigens. (C) MSI H CRC cell with mutant *TGFBR2* and altered cellular antigen presentation machinery. As a consequence of the immunoselective pressure resulting from the presentation of tumor antigen derived epitopes bound to HLA complexes on tumor cell surface, MSI H CRC cells that have acquired defects in HLA mediated antigen presentation are frequently selected for during MSI H CRC development. Frameshift mutations affecting cMS located in the genes *B2M*, *CIITA* or *RFX5* can result in the complete abrogation of antigen presentation in the context of HLA class I and class II, respectively. As a result, recognition and killing of tumor cells by cells of the adaptive immune system are impaired.

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Along with the pronounced lymphocyte infiltration observed in MSI H adenomas,[45] *B2M* mutations occur already at early stages of colorectal MSI H tumorigenesis (mutation frequency in MSI H adenomas 16%[46]), suggesting an early onset of immunoselective pressure. *B2M* mutation frequency increases with tumor progression and reaches about 40% in lymph node metastasis positive MSI H CRCs.[46] The frequency of mutations observed at mononucleotide repeats in the coding region of the *B2M* gene is much higher than expected to occur by chance, as the predicted frequency of mutations observed at a microsatellite with a length of 5 nucleotides in MSI H CRC is lower than 1%.[12] This observation further argues in favor of a high relevance of B2M inactivating cMS mutations for local tumor development and expansion that is most likely mediated by protection of the tumor against cytotoxic T cell attack following a breakdown of HLA class I antigen presentation.

Biallelic truncating mutations affecting the *B2M* gene or monoallelic mutations combined with LOH lead to a lack of functional B2M and subsequently result in the complete loss of assembled HLA class I complexes.[41,47,48] Heterozygous *B2M* mutations may result in reduced levels of *B2M* transcripts[40] but also complete loss of the protein.[43] Although the mechanisms underlying this observation are unclear, mutations or epigenetic alterations affecting noncoding regions of the *B2M* gene or potentially a dominant negative effect[49] of truncated B2M might play a role. However, DNA isolated from cancer tissues is frequently contaminated by DNA from nonmalignant cells; this may result in substantial underestimation of the frequency of homozygous mutations in the tumor tissue.

In addition to mutations of the *B2M* gene, selective loss or downregulation of HLA class I heavy chains has been observed in about two thirds of MSI H CRCs.[44] The mechanisms underlying this observation are not yet fully understood. Large allelic losses or rearrangements of the short arm of chromosome 6 have been identified as the mechanism underlying loss of HLA class I antigen expression in other tumor types like melanoma.[50] Such rearrangements are rare in MSI H cancers; however, the analysis of loss of heterozygosity (LOH) may be hampered in MSI H cancers because of the MMR deficiency induced length alterations of polymorphic markers conventionally used for LOH analysis.[2] Nevertheless, individual MSI H gastric cancers showing LOH at the HLA A, B and C loci have been described.[51] Irrespective of the causative mechanism, selective loss of HLA A is closely associated with the MSI H phenotype and, accordingly, an improved prognosis in CRC.[52]

Alterations of the HLA class I antigen processing machinery

In addition to *B2M* mutations that completely abrogate HLA class I mediated antigen presentation, cMS mutations were also identified in genes encoding components of the associated antigen processing machinery (APM). Mutations in *TAP1* and *TAP2*, *Tapasin*, *Calreticulin*, *Calnexin* and *ERp57* have been observed, albeit with reported mutation frequencies of about 10% or less for each gene.[45,53] Transporter proteins TAP1 and TAP2 are essential for the presence of peptide loaded HLA class I complexes on the cell surface,[54] suggesting that cMS mutations affecting *TAP1* and *TAP2* genes are likely to interfere with antigen presentation in the context of HLA class I. The functional relevance of cMS mutations in genes encoding chaperone molecules Tapasin, Calreticulin, Calnexin and ERp57, however, is less clear at present. This is in part related to the fact that it is not known whether all these chaperones are essential APM components. Studies in transgenic mice have shown that peptide loaded MHC class I complexes can still be assembled and presented on the cell surface in the absence of tapasin or calreticulin[55]; however, this does not preclude gradual differences in the efficiency of antigen presentation resulting from cMS mutations in these genes.

Differences between sporadic and hereditary (Lynch syndrome-associated) MSI-H CRC

The MSI H phenotype is observed in about 15% of sporadic CRCs, and virtually all CRCs developing in individuals with the inherited cancer predisposing Lynch syndrome.[56] Lynch syndrome associated MSI H CRCs appear to be more densely infiltrated with TILs in comparison to sporadic MSI H CRCs.[57] One possible explanation for the differential degree of TIL infiltration is related to the different molecular pathogenesis of sporadic and hereditary MSI H CRCs. Sporadic MSI H CRCs develop on the basis of promoter methylation of the DNA MMR gene *MLH1* in somatic cells, whereas Lynch syndrome associated CRCs acquire DNA MMR deficiency as a consequence of a germline mutation in 1 allele of an MMR gene (*MLH1*, *MSH2*, *MSH6* or *PMS2*) and a somatic second hit in the other allele.[5] In Lynch syndrome patients, the genetic predisposition, which is present in all cells of the organism, might contribute to a more pronounced immune response *via* presensitization of the immune system toward MMR deficiency induced frameshift antigens, which might result either from episodes of transient haploinsufficiency or from the repeated occurrence of MMR deficient cells that do not grow out to detectable tumors.[58] This "autovaccination" hypothesis is supported by a substantial prevalence of frameshift peptide specific T cells detected in healthy Lynch syndrome mutation carriers without having developed any detectable tumors.[26] In this context, it is conceivable that Lynch syndrome associated MSI H CRCs usually undergo a long process of immunoselection during tumor development. In line with this concept, mutations of the *B2M* gene are more frequent in hereditary compared to sporadic MSI H CRCs.[46,53] Taken together, these observations suggest that Lynch syndrome associated CRCs represent a subgroup of MSI H CRC where the host's immune surveillance influences tumor development at a very early stage. Conversely, these considerations highlight the importance of

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the immune system for the natural course of Lynch syn drome. The concept of continuous immune surveillance and selection is in good agreement with the clinical observation that 50% or less of affected gene carriers ever develop CRCs during their life.[59] Preventive vaccination with DNA MMR deficiency associated frameshift peptides that are shared among the majority of MSI H cancer cell clones and expressed early in the carcinogenic cascade might thus help to induce an adaptive immune response sufficient to eradi cate MSI H cancer cell clones before they can form clinically overt tumor masses. This approach may protect from MSI H tumor formation and thus represents a promising concept for novel tumor prevention strategies in these individuals.

The importance of immune surveillance in protecting from CRC development might also be reflected by a slight increase in CRC incidence in transplant recipients treated with immunosuppressive drugs (odds ratios between 1.35 and 2.14[60]), however not in HIV/AIDS patients (odds ratios range from 0.45 to 1.41). Although there are no systematic studies on the molecular phenotype of these cancers, it is conceivable that MSI H cancers have a higher incidence in these patients, a hypothesis that is further supported by the observation that HIV related CRCs were predominantly located in the proximal colon.[61]

Alterations of HLA II-mediated antigen presentation

Although the molecular alterations affecting the HLA class I antigen processing and presentation pathway have been well characterized in MSI H CRC, only little information has been gathered about the role of HLA class II antigens in this disease.

In contrast to HLA class I antigens, HLA class II antigens are not constitutively expressed in normal colonic epithe lium,[62] but their expression can be induced upon immune stimulatory factors like IFN γ.[63] Accordingly, HLA class II antigen expression can be observed in tumors that provoke a strong immune response, like malignant melanoma[64,65] and MSI H CRC.[66] However, only up to two thirds of MSI H CRCs express HLA class II antigens.[66] HLA class II antigen expression is tightly regulated by the master regulator CIITA and the essential RFX complex comprising RFX5, RFXAP and RFXANK.[67–69]

First evidence that mechanisms actively abrogating HLA class II antigen expression might exist in MSI H CRC cells was provided by Satoh *et al.*,[70] who showed that *CIITA* pro moter methylation was associated with loss of IFN γ induci ble HLA class II antigen expression in the MSI H CRC cell lines HCT116 and RKO. Recently, frameshift mutations in coding microsatellites of *CIITA* and *RFX5* have been identi fied[71] in MSI H CRC cell lines and tumors. *RFX5* mutations are observed in about 40% in MSI H CRC displaying absent or weak expression of HLA class II antigens. This suggests that DNA MMR deficiency induced cMS mutations affect functionality of HLA class II antigen expression in MSI H CRC, similar to inactivation of HLA class I antigen presenta tion following *B2M* mutations. At present, it is unclear whether tumor specific antigens are presented *via* HLA class II complexes on MSI H CRC cells. Of interest, there is exper imental evidence that presentation of cellular antigens (com prising tumor antigens) can occur through HLA class II anti gen complexes under certain conditions. For example, presentation of intracellular tumor antigens *via* HLA class II complexes has been demonstrated for human breast cancer and uveal melanoma cells lacking the light invariant chain (Ii).[72,73] In a mouse model, HLA class II mediated antigen presentation of cellular antigens was observed also in the presence of Ii.[74] Further functional studies analyzing T cell responses in dependence of HLA class II antigen expression on target cells will help to clarify whether inactivation of HLA class II antigen expression in MSI H CRCs occurs as a consequence of immunoselective pressure directed against HLA class II antigen expressing tumor cells.

Other mechanisms interfering with the host's antitumoral immune response

In addition to tumor cells, certain types of immune cells, *e.g.*, regulatory T (T_{reg}) cells, can interfere with efficient antitu moral immune responses in CRC.[75,76] Although initial studies demonstrated a high density of TILs with cytotoxic potential in MSI H CRC,[22–24] it has been shown more recently that this is paralleled by enhanced numbers of TILs expressing nuclear FOXP3,[77,78] a marker for regulatory T (T_{reg}) cells.[79] T_{reg} cells in the environment of MSI H CRC may well act as local suppressors of the host's immune response against MSI H CRC. The association of high TIL densities with a pro longed overall and disease free survival in CRC highlights the clinical relevance of the local immune response for the course of this disease.[80,81] The contribution of T_{reg} cells and the prognostic impact of T cell infiltration in the subgroup of MSI H CRCs need to be addressed in further studies. How ever, their abundance among the TILs suggests that T_{reg} cell suppression may represent a potential therapeutic option in MSI H CRC patients.

Besides evading recognition and attack by immune cells, tumor cells can actively suppress the host's immune response. This phenomenon is referred to as immune subversion.[82] Data concerning the impact of immune subversion in the pathogenesis of MSI H CRC are still scarce and basically lim ited to the analysis of counterattack mechanisms by expres sion and secretion of Fas ligand (FasL). FasL can induce apo ptosis in Fas receptor positive T cells[83] and is often expressed by CRC cells.[84,85] Although one study reported expression of FasL to be less frequent in MSI H compared to non MSI H CRCs,[86] another[85] did not confirm this observation. More over, FasL expression does not seem to affect TIL density or the number of apoptotic TILs in MSI H CRC.[84,85] According to our present knowledge, there is no evidence that FasL expression is a major modulator of the host's immune response against MSI H CRC.

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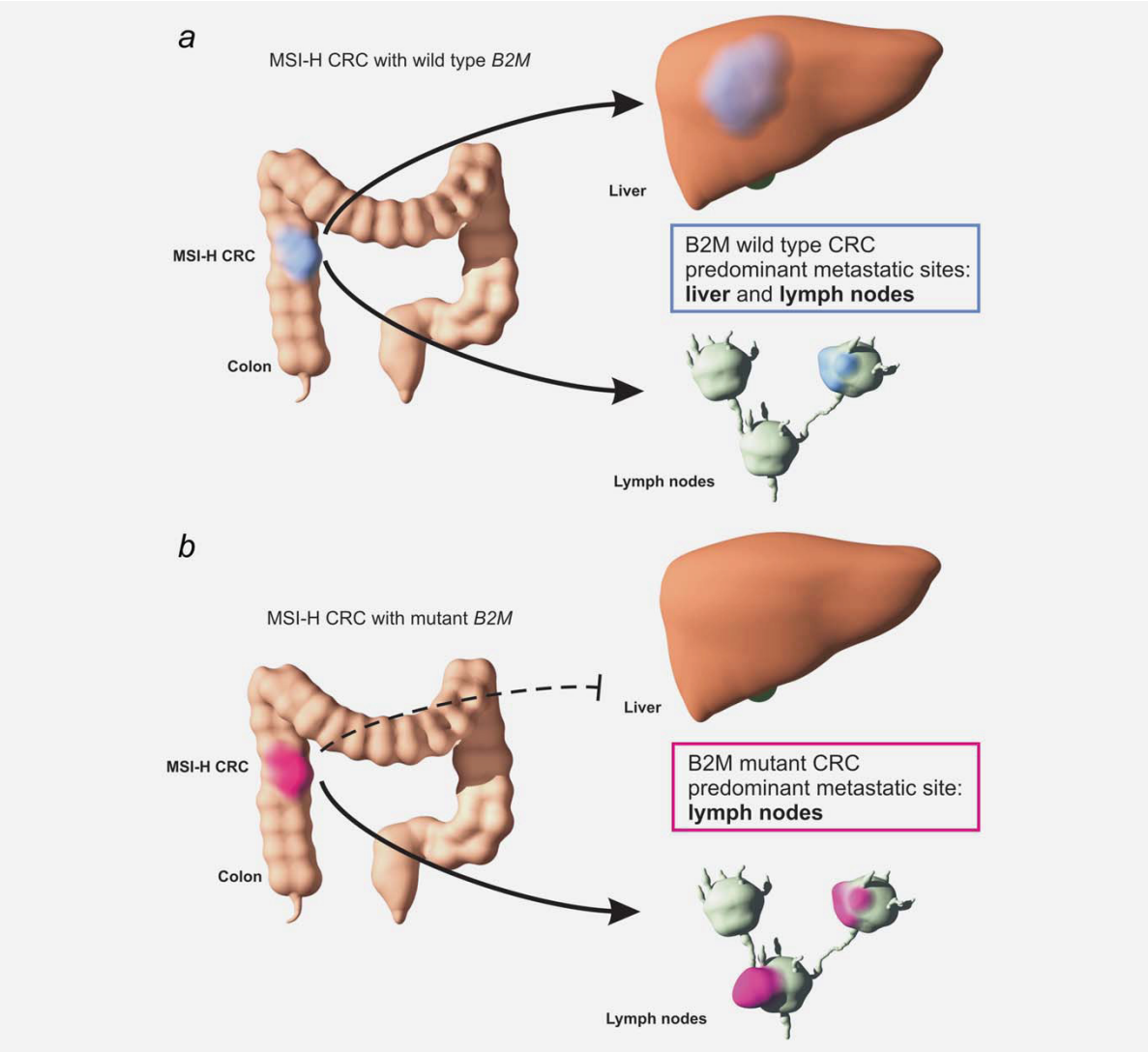

Figure 3. Metastatic pathways observed in MSI H CRC patients. Metastasis formation in MSI H CRC apparently depends on the presence or absence of *B2M* mutations in the tumor. (*a*) MSI H CRCs that do not harbor truncating mutations of the *B2M* gene (blue) can spread to lymph nodes and liver and can grow out to clinically manifest metastases. (*b*) MSI H CRCs that harbor truncating cMS mutations in the *B2M* gene (pink) frequently metastasize to local lymph nodes, whereas liver metastases are extremely rare.

Prognostic implications of immune evasion

Information about the prognostic consequences of immune evasion in MSI H CRCs is sparse. Interpretation and comparison of studies examining HLA class I antigen expression are generally very difficult, because methods used for the analysis of HLA class I antigen expression vary substantially. However, there is consensus that in tumor types like cutaneous melanoma, bladder cancer or small cell lung cancer, loss of HLA class I antigens is associated with poor prognosis and survival.[48]

For CRC, early studies did not reveal an association of HLA class I antigen expression with patients' prognosis or survival.[87,88] Later studies identified downregulation, but not complete loss of HLA class I antigen expression as a factor adversely affecting patients' prognosis in CRC.[89,90] A likely explanation for this observation is that complete loss of HLA class I antigen expression renders cells susceptible toward NK cell mediated killing, whereas downregulation of HLA class I antigens might protect the tumor cell from both, T cell and NK cell mediated immune response.[52,91]

Infiltration with NK cells is sparse in primary MSI H CRCs[22] and CRCs in general.[89,92] However, activity of NK cells in the peripheral blood or target sites of metastasis like the liver might protect from distant metastasis formation, as

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it has been shown in a mouse model for MHC class I antigen negative uveal melanoma, where peripheral or liver NK cells mediate protection against formation of liver metastasis.[93] Indeed, uveal melanoma patients frequently present with liver metastasis, but only if HLA class I antigen expression is retained on the tumor cell surface.[94]

A reduced metastatic potential of HLA class I antigen negative CRCs would be in line with the observation that distant metastases are virtually absent in patients with HLA class I antigen negative MSI H CRCs that harbor truncating *B2M* mutations[46,95] (Fig. 3). This suggests that loss of B2M or HLA class I antigen expression might interfere with the formation of distant organ metastasis, or, conversely, distant metastasis formation requires HLA class I antigen expression by CRC cells. A study by Menon *et al.*[96] reporting that HLA class I antigens were detectable in the vast majority (>95%) of liver metastases and 100% of paired primary tumors supports this hypothesis.

Based on these observations, it is tempting to speculate that the innate and the adaptive immune responses coordinately work together in the host's defense against tumors at different stages of tumor growth and metastasis. The adaptive immune system supposedly plays a major role in controlling local tumor growth and lymph node metastasis, whereas the innate immune system may interfere with metastasis formation in distant organs like the liver. It is conceivable that the environment surrounding primary CRC is immunologically very different from that encountered in target organs of metastasis formation. As a consequence, alterations that allow highly immunogenic MSI H CRCs to evade T cell responses locally at the primary tumor site (loss of HLA class I antigen expression by mutation of the *B2M* gene) might at the same time prove disadvantageous for metastasis formation to distant organs like the liver where NK cell response is thought to play an important role. Mutations of the *B2M* gene that are encountered in about one third of MSI H CRC might thus be one causal factor underlying the low frequency of distant metastases in patients with MSI H CRC. Accordingly, development of MSI H CRC under the pressure of local immunoselection might be an example of dead end evolution in terms of distant metastasis formation.

Conclusive Remarks

Since the discovery of DNA MMR deficiency and MSI in CRC, much progress has been made in understanding the particular immunological phenotype of MSI H tumors. With the large repertoire of DNA MMR deficiency induced neoantigens, that are predictable *in silico* on the basis of the human genome databases, MSI H tumors represent an ideal system for studying the interaction between tumor cells and the host's immune system. This research is likely to contribute to a substantially better understanding of antitumoral immune response and tumor immune evasion in cancer.

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