Total loss of MHC class I in colorectal tumors can be explained by two molecular pathways: \( \beta_2 \)-microglobulin inactivation in MSI-positive tumors and LMP7/TAP2 downregulation in MSI-negative tumors

**Abstract:** The mechanisms that lead to loss of MHC class I expression in different types of tumors are not yet fully known. Accordingly, we studied colorectal carcinomas to elucidate the specific mechanisms of evasion of the T-cell immune response. We selected tumors with total loss of MHC class I expression and studied 124 colorectal carcinomas with immunohistochemical staining and anti-HLA monoclonal antibodies (mAb). Fourteen of 124 (11%) tumors exhibited a phenotype with HLA class I total loss. Microsatellite instability (MSI) analysis was also carried out in the same tumor samples. The expression of \( \beta_2 \)-microglobulin (\( \beta_2 \)m), HLA-A, B, and C antigens, transporter associated with antigen processing 1 (TAP1), TAP2, low-molecular-weight protein 2 (LMP2), and LMP7 were analyzed using reverse-transcription polymerase chain reaction (RT-PCR) in microdissected tumor samples. Four of 14 microsatellite instability-positive (MSI+) and W6/32 mAb-negative tumors showed biallelic inactivation of \( \beta_2 \)m and accumulation of HLA class I heavy chain in the cytoplasm. MSI-negative (MSI-)/W6/32 mAb-negative tumors presented alterations in the expression of components of the antigen processing machinery (APM). Nine of 10 tumor samples showed LMP7 gene downregulation, and four of 10 presented TAP2 dysregulation. This group apparently expressed normal levels of heavy chain and \( \beta_2 \)m mRNA. Two major mechanisms in colorectal cancer appear to be responsible for the total loss of MHC surface expression (\( \beta_2 \)m mutations and LMP7/TAP2 downregulation) that may contribute to the failure of T lymphocyte recognition during an immune response. The precise identification of the molecular defects that underlie HLA class I abnormalities will have important implications for patients receiving T-cell-based specific immunotherapy.

Tumor cells select specific mechanisms to escape from immune recognition (despite the presence of tumor-reactive T lymphocytes) during neoplastic evolution. Such mechanisms include the secretion of immunosuppressive factors, fas-ligand expression by tumoral cells, deficient T-cell signal transduction, downregulation of costimulatory and adhesion molecules, and the loss or suppression of major histocompatibility complex class I surface expression (1–3). The latter may be due to structural alterations or to the dysregulation of MHC class I antigens and/or deficient expression of different components.
of the MHC class I antigen processing machinery (APM) (4, 5). Abnormalities in MHC class I surface expression and loss of antigen processing function have frequently been identified in murine (6) and human malignant cells (7–9), and have also been associated with tumor progression in melanoma, colorectal cancer and breast carcinoma (7–9). Molecular characterization of the antigen processing machinery complex has provided new tools to elucidate how tumors evade immune recognition. Studies using cultured tumor cell lines, antigen processing mutants and surgically removed malignant lesions demonstrated that loss or dysfunction of the proteasome subunits and the transporter associated with antigen processing (TAP) genes appear to contribute to deficient MHC class I surface expression (10–12). In a panel of human tumors of distinct histology (13, 14), loss or reduced expression and function of TAP and/or low molecular weight protein (LMP) were associated with impaired processing and presentation of antigenic peptides to CD8+ cytotoxic lymphocytes (CTL), resulting in low levels or loss of MHC class I and resistance to CTL-mediated lysis (7). These deficiencies in the antigen processing pathway could be reverted by cytokine treatment, particularly by IFNγ (15, 16).

Likewise, disturbance of the APM and MHC class I antigen dysregulation or loss can result from lack of β2m (β2-microglobulin) expression due to downregulation (9) or mutation in one allele and loss of heterozygosity (LOH) in the second allele (17, 18). Furthermore, around 10–15% of colorectal tumors display MHC class I antigen loss (phenotype I) (19). Within this group the relationship between loss of HLA class I and mutations in the β2m gene is well established. Indeed, these mutations have been found mainly in cell lines and primary tumors that present a mutator phenotype. The microsatellite mutator phenotype (MMP), which is also known as microsatellite instability (MSI), is characteristic of most hereditary non-polyposis colorectal cancers (HNPCC), and about 10–15% of unselected colorectal tumors also display this mutator phenotype (20, 21). In MSI tumors, genetic and epigenetic inactivation of DNA mismatch repair (MMR) genes leads to mutations in cancer genes and hence the development of cancer. The β2m gene mutation is found as an overrepresented mutated gene in tumor cell lines with MSI. Reduced expression correlated with a mutation in one allele of β2m, whereas loss of expression was seen in instances where a line was homozygous for one mutation or heterozygous for two mutations (8, 23).

The mechanisms underlying HLA class I antigen loss have been predominantly studied in cultured tumor cell lines, in which molecular genetic analyzes are easier to accomplish, or in non-microdissected human tumors with contaminating DNA from the tissue surrounding the tumor cell (14, 22). To date, little information is available regarding the underlying molecular mechanisms in colorectal tumors with no expression of HLA-A, B or C antigens. The selection of β2m gene mutations is evident in the MSI-positive (MSI+) group of tumors. However, little is known about the large group of MSI-negative (MSI−) tumors with loss of MHC class I. In this study we examined the precise molecular defects involved in the down-regulation of MHC class I antigens in microdissected samples of both types of human colorectal carcinoma. Our results revealed two independent mechanisms of HLA class I loss that may allow tumor evasion and thus may have important implications for tumor immunotherapy.

Materials and methods

Selection of patients and tumor samples by immunohistochemistry

A total of 124 patients diagnosed with colorectal carcinoma were selected to study loss of HLA. Samples of tumor tissues were provided by the Department of Surgery, Virgen de las Nieves University Hospital in Granada.

Sections of frozen tumor tissues 5–8μm thick were cut on a microtome-cryostat (Bright), allowed to dry at room temperature for 4–18 h, fixed in acetone at 4°C for 10 min, and stored at −40°C until staining. Mouse monoclonal antibodies (mAb) used were: GRH1 recognizing free and HLA class I heavy chain-associated β2m chain; W6/32 against HLA-A, B and C heavy chain/β2m complex; and HC-10 against free heavy chain of HLA-A, B and C molecules. Tissue samples were classified as HLA-A, B or C-negative when at least 75% of the tumor cells were not stained with W6/32 and GRH1 mAbs.

MSI analysis

DNA obtained from tumor samples and normal mucosa was analyzed for MSI using four mononucleotide repeat sequences. BAT-26 and BAT-40 are both polyadenine mononucleotide repeats; BAT-RII is a 10-bp polyadenine repeat within the coding region of the transforming growth factor-β type II receptor (TGFβRII). We also used a tract of eight consecutive guanosines in the third exon of the BAX gene (26). To amplify these microsatellite markers, the 5′ end of one primer of each primer set was tagged with a fluorescent label (Applied Biosystems). Microsatellite instability was determined using the ABI-PRISM 310 DNA sequencer and Genescan 3.1 software (Applied Biosystems). The tumors that were positive in this initial step were later studied using DNA from microdissected tissue samples.
The primers used to amplify microsatellite markers were as follows:

- BAT26-Fw 5’-TGACCTTTGGACTCTGGAGGC;
- BAT26-Bw 5’-AACATTTTAGCTCTCTGGAGGC; D15S209 Bw 5’-ATTAACCTTCTACACCACAAC; BAT40-Bw 5’-GTAAGGAAGACACCACTTG;
- BAX-Fw 5’-ATCCAGGATGCAGGGCG; BAX-Bw 5’-ACTCGCTCACGTCTCTGGTG; BATRII-Fw 5’-AAGCTCTCACCAGTACTG;
- BATRII-Bw 5’TGCACCTCATCAGGCTGAC.

**Microdissection, DNA isolation and loss of heterozygosity**

Cryopreserved tissue sections between 4 and 8 μm thick, stained with a 0.05% wt/vol solution of toluidine blue, were used for microdissection. DNA from microdissected fragments (tumor and stroma) was obtained using the Quiagen DNA isolation kit (Qiamp Tissue Kit, Wetsburg, Leusden, the Netherlands). To study LOH in the β2m gene, we selected the microsatellites D15S209 (15pter-15qter Tel), D15S126 (15q21 next to the 2m gene), and D15S209 (15pter-15qter Tel). We used the following sequences of primers:

- Ramal et al (24, 25). We used the following sequences of primers:
  - D15S126 Fw 5’-GCCAGCAATAATGGGAAGTT; D15S209 Fw 5’-GCTGGAAGGTGACACGCA; β2m Fw 5’-GGCATTCTCTGCTGCTGACTGAC;
  - D15S209 Bw 5’-TGAGGGAAGACACCACTTG; HLA Fw 5’-GACGACTGTGATGTACCTGCT; HLA Bw 5’-CTCGAGGGAGAGGGAGA; HLA 2m Fw 5’-CTCTCGAGGGAGAGGGAGA; HLA 2m Bw 5’-CTCTCGAGGGAGAGGGAGA.
  - BAX-Fw 5’-GAGACATGATGTTACCTGCTG; TAP2 Fw 5’-CTCCTCGTGGCAGGCTCT; TAP2 Bw 5’-TCCTCGTGGCAGGCTCT.
  - BAT26-Fw 5’-TGACTCTTTAGCTCTGGAGGC; HLA Bw 5’-GGCAGCTGCTTTGGAGGC; HLA 2m Fw 5’-GACGACTGTGATGTACCTGCT; HLA 2m Bw 5’-CTCGAGGGAGAGGGAGA.
  - BAX-Bw 5’-GACGACTGTGATGTACCTGCT; HLA 2m Fw 5’-GACGACTGTGATGTACCTTCT; HLA 2m Bw 5’-CTCGAGGGAGAGGGAGA.

The PCR conditions were: 40 cycles at 95°C/1 min, 60°C/1.5 min and 72°C/2 min for 10 min extension after the last cycle.

**Sequencing of β2m genomic DNA and cDNA**

Sequencing was performed using the Big Dye Terminator Cycle sequencing kit and the ABI-PRISM 5700 automated capillary sequencer. The purified cDNA product was cloned in the PCR 4-TOPO vector using the TOPO TA Cloning Kit for sequencing (Invitrogen, Groningen, the Netherlands). The β2m cDNA was sequenced using the same primers as in the reverse transcription-polymerase chain reaction (RT-PCR) analysis. The β2m genomic DNA was amplified using the following primer pairs:

- Exon 1 B2M-100Fw 5’-GTCAGGTGTCCTCTGGGCGCT; Exon 1 B2–1Bw 5’-GAGACATGATGTTACCTGCTG; Exon 2 31555Bw 5’-GAGACATGATGTTACCTGCTG; Exon 2 31555-Fw 5’-CTCCTCGTGGCAGGCTCT; TAP2 Fw 5’-CTCCTCGTGGCAGGCTCT; TAP2 Bw 5’-CTCCTCGTGGCAGGCTCT.

**Analysis of β2m, HLA-HC, TAP-1, TAP-2, LMP2, and LMP-7 mRNA expression from microdissected samples**

Recovery and analysis of RNA from frozen tissue sections was conducted using slightly modified standard methods. We obtained total RNA from microdissected samples using the Micro RNA isolation kit (Stratagene, La Jolla, CA), which uses a guanidine isothiocyanate-phenol chloroform extraction procedure. The RNA was precipitated after organic extraction with an equal volume of isopropanol in the presence of a glycogen carrier at 20 mg/mL. The RNA pellet was washed with 75% ethanol-25% diethylpyrocarbamide (DEPC)-treated water and dissolved in 20 μL of nuclease free-water. The first strand cDNA was synthesized with 10 μL of total RNA, using Sensiscript reverse transcriptase kits (Quiagen) with 40 U/μL of RNAse (Promega, Madison, WI) and random primers (Promega) in a final volume of 20 μL. The samples were incubated at 42°C for 60 min and the reaction was stopped at 95°C for 5 min. Actin was amplified as a positive control. To analyze mRNA expression we used the following primers:

- Actin Fw 5’GGCATCCTGATGGGACTCCG; Actin Bw 5’GCTGGAGGTGGACACGCGA; β2m Fw 5’-GGCATTCTCTGCTGCTGACTGAC;
- Actin Bw 5’-GTAGGGAAGACACCACTTG; HLA Fw 5’-CTCCTCGTGGCAGGCTCT; HLA Bw 5’-CTCGAGGGAGAGGGAGA.

**Results**

**Analysis of HLA class I antigen expression in colorectal tumors**

Fourteen of 124 colorectal tumors did not express HLA-A, B or C antigens by immunohistochemical staining with the mAbs that recognize a structural epitope of the heavy chain/β2m complex and β2m. We also used HC-10 mAb, which detects free heavy chain in the cytoplasm of tumoral cells. Five of 14 tumors stained positive with the HC-10 mAb. The patterns of staining in cryostatic sections of colon cancer are shown in Fig.1. Table 1 shows the HLA-A, B and C-negative tumors detected. The frequency of total loss was similar to that described in a previous study (19).
**β₂m mutations correlate with tumors that exhibit the microsatellite instability phenotype**

Our first approach was to analyze the presence or absence of MSI in colon tumor samples obtained from the surgery service at our hospital. Tumors that are MSI⁺ have a 40% probability of having β₂m mutations (26). However in MSI⁻ tumors, spontaneous mutations in the β₂m gene are infrequent or non-existent (22). Accordingly, we analyzed the DNA obtained from tumor tissue for mutations in the BAT-26, BAT-40 microsatellites, the microsatellite-like (A)₁₀ repeats within the transforming growth factor-β type II receptor (TGF-β-RII), and the (G)₈ repeats within BAX. Six of 124 tumors were classified as MSI⁺ (5%), of which 4 tumor samples (CO86, CO117, CO132 and CO135) were negative with mAbs against HLA-A, B and C/β₂m complex and β₂m antigen. The results of this study are presented in Table 2. Four loci containing mononucleotide repeat sequences on different chromosomes were analyzed, and were classified as positive if they exhibited mutations (contractions) in any of the mononucleotide microsatellite loci. Tumor samples CO86, CO117 and CO132 were graded as HNPPC according to the Amsterdam criteria, and CO135 as sporadic MMP (27).

The four HLA-A, B and C-negative and MSI⁺ tumors were studied for the presence of β₂m mutations, which were found in all cases. The results of this study are summarized in Table 3. Using RT-PCR analysis of microdissected tumor tissue samples, we found expression of β₂m mRNA in CO86 and CO117. In contrast, CO132 and CO135 were negative for β₂m mRNA (Table 1). Sequencing of DNA β₂m showed that both CO132 and CO135 tumors presented the same mutation: a frameshift deletion of CT in the CTCTCTCT tract at the leader sequence. To investigate the existence of LOH in the second allele, we used the short tandem repeats (STR) D15S209 and D15S126, which flank the β₂m gene at 15q21-q22. The dinucleotide repeats D15S209 and D15S126 showed MSI in both tumors (data not shown). This result did not indicate the presence or absence of LOH

![Fig. 1. Immunohistochemical staining in cryostatic sections of colorectal tumors. (A) Tumor no. 135 showing a negative reaction with the W6/32 mAb and positive staining with the HC-10 mAb. (B) Tumor no. 14 showing a negative reaction with both W6/32 and HC-10 mAbs. Stromal cells show a positive staining. Magnification: × 400.](image_url)
in the remaining \(\beta_2m\) gene. Although we did not detect \(\beta_2m\) mRNA expression, we assumed that the remaining allele was probably lost.

CO86 presented two different mutations in both alleles of the \(\beta_2m\) gene. The first was found in cDNA \(\beta_2m\): a frameshift deletion of CA at codon 25. Sequencing the genomic DNA for \(\beta_2m\) showed a second mutation: a frameshift deletion of A in the AAAAA tract at exon 2. In tumor CO117 we also found two different mutations: a frameshift deletion of C at codon 91 in the CCCCC tract at cDNA \(\beta_2m\), and a frameshift deletion of CCGTG at the end of exon 2 \(\beta_2m\).

In the group of MSI negative tumors, 10 of 14 expressed \(\beta_2m\) mRNA from microdissected tumor samples; no \(\beta_2m\) mutations were found. More importantly, all four cases with biallelic inactivation of \(\beta_2m\) showed a positive staining pattern with HC-10, an anti-class I reagent that reacts with free (unfolded) class I heavy chains (Fig. 1).

**LMP7 downregulation is a common event in MSI negative/HLA-A, B and C⁻ colorectal tumors**

RT-PCR analysis of HLA-heavy chain, TAP1, TAP2, LMP2 and LMP7 was also used in the group of tumors that exhibited an HLA-A, B or C-negative phenotype.

Table 1 summarizes the results. We found expression of HLA-HC and \(\beta_2m\) in all tumors. Interestingly, MSI negative tumors did not react with the HC-10 mAb (the only exception being tumor CO5), in contrast with the pattern obtained in MSI⁺ tumors.

We found specific downregulation of the components of the APM. Whereas TAP1 expression was detected in 100% of colorectal tumors, TAP2 was not detected in 40% of cases. TAP genes are involved in the transport of peptides into the reticulum, and their downregulation is responsible for the lack of expression of HLA class I on the membrane surface (28). The mechanism involved in these cases may explain the lack of expression in four of 10 tumor samples. Additionally, transcription of the LMP7 gene was absent in nine of 10 tumor tissue samples compared with their normal mucosa counterpart (Fig. 2). This finding reveals the importance of this protein in producing antigenic peptides that stabilize the structure of the heavy chain and permit its surface expression (29).

We have no explanation for the mechanism that underlies HLA class I antigen loss in tumor CO5. As CO5 was MSI negative and HC-10 mAb-positive, we surmise that it has no alterations in the \(\beta_2m\) gene and that transcription of LMP7 is accurate. If the accumulation of HLA class I heavy chain in the cytoplasm is related to

### Table 1

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Exon</th>
<th>Homozygous/heterozygous</th>
<th>Mutation</th>
<th>Stop codon localization</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO86</td>
<td>Exon 2</td>
<td>Homozygous</td>
<td>CA deletion, codon 25</td>
<td>56</td>
</tr>
<tr>
<td>CO117</td>
<td>Exon 2</td>
<td>Heterozygous</td>
<td>C deletion, codon 91</td>
<td>102</td>
</tr>
<tr>
<td>CO132</td>
<td>Exon 1</td>
<td>ND</td>
<td>CT deletion, codons</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>13-14</td>
</tr>
<tr>
<td>CO135</td>
<td>Exon 1</td>
<td>ND</td>
<td>CT deletion, codons</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>13-14</td>
</tr>
</tbody>
</table>

* ND — not determined

### Table 2

<table>
<thead>
<tr>
<th>Tumor</th>
<th>BAT26</th>
<th>BAT40</th>
<th>TGFβ-RII</th>
<th>BAX</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO86</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CO117</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>+*</td>
</tr>
<tr>
<td>CO132</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CO135</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

+ Presence of MSI
ND = Absence of MSI
*Microdissection studies showed that the wild-type allele coexisted with the mutated allele.
the lack of β2m due to mutations in MSI+ tumors (see above), then other alterations are implicated, such as defects in chaperon proteins involved in the stabilization of heavy chain before binding to β2m on the endoplasmic reticulum membrane or TAP or LMP mutations (30, 32).

**Discussion**

In this study we illustrate two major mechanisms that explain the lack of expression of HLA-A, B and C molecules in colorectal tumors: β2m mutations in MSI+ tumors and LMP7/TAP2 downregulation in MSI-negative tumors. Moreover, the two types of HLA-A, B and C-negative tumors differ, as revealed by mAbs that recognize unfolded heavy chain proteins. These two different patterns of immunostaining can be explained by separate defects in the MHC class I assembly pathway. The assembly process begins with the association of newly synthesized heavy chain with a membrane-bound chaperon known as calnexin (30). The complete lack of β2m appears to cause an early misfolding event and transfers the misfolded heavy chain proteins from the endoplasmic reticulum (ER) to the cytosol through the translocon Sec61 complex (31). Calnexin has also been implicated in this process (32). The HC-10 mAb can recognize these translocated heavy chain free proteins before degradation. In contrast with the other HLA-A, B and C-negative tumors, the presence of β2m may lead to incompletely assembled proteins being retained in the ER and complexed to luminal chaperons, even in the absence of peptides with TAP or LMP defects. The dislocation of heavy chain proteins does not require the complete unfolding of these molecules (33). However, binding of β2m delays cytoplasm dislocation and the appearance of the class I heavy chain in the free fraction, probably as a result of different dislocation kinetics from those that exist in the cytoplasm (34). Furthermore, the absence of reactivity of mAbs against β2m in HLA-A, B and C-negative tumors with no mutated β2m genes indicates that most of the β2m must be retained in the ER, where they are not accessible to react with mAbs. Likewise, it is possible that the epitope recognized by the mAbs leads to steric hindrance because the chaperons bind to the HC/β2m complex.
The functional consequences of these molecular defects may also differ in both groups of tumors. In fact, whereas TAP or LMP downregulation is often restored by IFN-γ treatment (14), structural defects cannot be overcome and can have a negative impact on the outcome of T cell-based immunotherapy (14, 18). We cannot rule out however, the existence of structural mutations that inhibit the protein expression in the group of LMP positive tumors, as our LMP7 expression data are presented at the mRNA level. However this possibility is unlikely as it must involve a biallelic inactivation. In contrast, it is clear that biallelic inactivation of the β2m gene is responsible for the lack of HLA class I antigens. As a result of the high frequency of this defect (four of four) found in MSI tumors in this study, we assume that β2m mutations are selected during tumorigenesis of the mutator pathway, and thus evade the immune response of CD8+ T cells. In this context, although β2m is not directly involved in cell growth, it should be considered a target of inactivation in MSI tumors, and as a suppressor gene (26). In this study, we found frameshift mutations in both copies of β2m gene. This contrasts with the findings for other genes that play a suppressor role in colorectal carcinogenesis via a p53-independent pathway (mutator pathway), BAX or TGF-βRII (35, 36). This fact may be congruent with the unfavorable prognosis of colorectal tumors of the microsatellite mutator phenotype with inactivation of β2m (37). Although MSI tumors may have a greater potential for the efficient presentation of antigenic peptides linked to enhanced immunogenicity, the β2m inactivation is associated with a poor prognosis (38, 39).

The microsatellite mutator phenotype (MMP) pathway in colon cancer is characterized by genomic instability, leading to the accumulation of deletion and insertion mutations at simple repeat sequences. The fixation of these slippage-induced replication errors as mutations is associated with defects in DNA mismatch repair (35). The MMP tumors represent a distinct mutational tumorigenic pathway (the mutator pathway) because gastrointestinal cancers exhibit many differences in genotype and phenotype compared with tumors without MSI. The differences in genotype can be explained by MMR deficiency leading to the rapid accumulation of mutations in mononucleotide tracts present in some cancers genes such as the transforming growth factor (TGF)-β receptor type II (TGFβRII) and BAX. Both genes are often inactivated by frameshift mutations in cancers with MMP+, but rarely in MMP- tumors (36). BAX mutations have been detected in 51% of primary MMP+ colorectal carcinomas (35), but not in other human tumors. The mutations that affect the BAX gene have been found in both alleles. This biallelic inactivation of BAX gene may explain why colon tumors of the mutator pathway do not contain p53 mutations (35). We found one case of biallelic inactivation in BAX and another in TGFβ-RII genes (Table 2). In this context, biallelic heterozygous inactivation of the β2m gene must also be under strong selective pressure during tumorigenesis in this type of tumors. β2m mutations were absent in the 10 MSI negative tumors analyzed. In contrast, abnormalities in the expression or function of components of the APM may be related to the marked downregulation of HLA class I on the cell surface (nine of 10 tumors). Defects in the APM that have been identified in several types of malignant lesion may impair the processing of tumor-associated antigen (TAA) and in the presentation of TAA-derived peptides to CD8+ T cells, thereby providing tumor cells with an immune escape mechanism (14, 40).

Downregulation of TAP1, TAP2, LMP2 and LMP7 genes has been demonstrated in different cell lines and surgically removed tumor lesions (4, 14). Defects in the expression of LMP2, TAP1 and TAP2 have been found in human cell lines of distinct histology, but only those with LMP7 downregulation showed correlation with the level of expression of MHC class I (41, 42), but not with TAP1, TAP2 and LMP2 downregulation. These results probably indicate a direct relationship between levels of MHC class I expression and LMP7 gene downregulation. In this regard, LMP7 is responsible for altering the proteasome complex specificity to increase the production of hydrophobic immunogenic peptides. Interestingly, LMP7 downregulation, found in nine of 10 microdissected colon tumors in this study, suggests an important role for the LMP7 gene in producing antigenic peptides that are presented by HLA molecules on the cell surface to CD8+ lymphocytes. We also found TAP2 downregulation (but not TAP1 downregulation) in four of 10 tumors in this group of colon cancers. Remarkably, no cases of MSI+ colon cancer exhibited LMP7 downregulation.

On the other hand, different human and murine cell lines have shown coordinated downregulation of TAP1 and LMP2 genes, as both are controlled by the same bidirectional promoter (10, 43). Although downregulation of the APM components TAP2 and LMP7 has been found in human and murine cell lines, and this argues for a common regulatory mechanism (6), we found simultaneous downregulation in only four of 10 cases of colon cancer. TAP2 and LMP7 may have a different regulatory mechanism, and therefore downregulation in colorectal tumors LMP7 and TAP2 may be simultaneous rather than coordinated.

Understanding the mechanisms that operate in MMP+ and MMP- pathways in colorectal tumors is crucial for developing strategies to improve the efficacy of immunotherapy. For instance, the β2m biallelic mutation found in colorectal tumors of the MMP+ group could be included, as the molecular alteration is reverted by IFNγ. Finally, we would also emphasize the usefulness of immunostaining with HC-10 mAb in identification of tumors with possible structural mutations in the β2m protein.
References


