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REVIEW

MHC Class I Antigen Processing and Presenting Machinery: Organization, Function, and Defects in Tumor Cells

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The surface presentation of peptides by major histocompatibility complex (MHC) class I molecules is critical to all CD8+ T-cell adaptive immune responses, including those against tumors. The generation of peptides and their loading on MHC class I molecules is a multistep process involving multiple molecular species that constitute the so-called antigen processing and presenting machinery (APM). The majority of class I peptides begin as proteasome degradation products of cytosolic proteins. Once transported into the endoplasmic reticulum by TAP (transporter associated with antigen processing), peptides are not bound randomly by class I molecules but are chosen by length and sequence, with peptidases editing the raw peptide pool. Aberrations in APM genes and proteins have frequently been observed in human tumors and found to correlate with relevant clinical variables, including tumor grade, tumor stage, disease recurrence, and survival. These findings support the idea that APM defects are immune escape mechanisms that disrupt the tumor cells' ability to be recognized and killed by tumor antigen–specific cytotoxic CD8+ T cells. Detailed knowledge of APM is crucial for the optimization of T cell–based immunotherapy protocols.

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In normal cellular physiology, proteins regularly undergo a process of turnover in which they are degraded and replaced by newly synthesized proteins. The degradation of most cellular proteins occurs by one of two major proteolytic pathways: the lysosomal pathway and the ubiquitin-proteasome pathway (1). The lysosomal pathway degrades proteins taken up by endocytosis (from the extracellular environment) and by autophagy (from the cytosol); in this pathway, bacteria, bacterial antigens, parasites or long-lived bulk proteins, particularly membrane-bound proteins, are delivered to endosomes, which become increasingly acidic as they progress into the interior of the cell, eventually fusing with lysosomes (2). In contrast, the ubiquitin-proteasome pathway is mainly involved in degradation of cytosolic proteins (3), such as regulatory proteins (short-lived proteins that are eliminated soon after completing their functions); misfolded and damaged proteins (4), including defective ribosomal products (ie, newly synthesized proteins degraded within minutes of their synthesis) (5,6); mutated proteins in cancer cells; and virus-derived proteins in infected cells. In both pathways, cellular proteins are cleaved into oligopeptide fragments that are presented to T cells by molecules of the major histocompatibility complex (MHC). Whereas peptides derived from proteins degraded by the lysosomal pathway are primarily presented by MHC class II molecules, peptides generated by the ubiquitin-proteasome pathway are presented by MHC class I molecules (7). A major exception to this rule is cross-presentation, a process specific to professional antigen-presenting cells, whereby peptides derived from proteins that have entered the lysosomal pathway gain access to MHC class I molecules (8).

In humans, there are three main (and several minor) MHC class I molecules, which are also called by their gene name, human leukocyte antigen (HLA). The main function of class I molecules, which are expressed on the plasma membrane of most cell types, is to display these peptides to cytotoxic CD8+ T cells in support of their crucial activity of immune surveillance. Peptides derived from normal cellular (self) proteins are regularly ignored by CD8+ T cells, whereas those from mutated proteins and from the nonself proteins of viruses and other intracellular pathogens are not ignored but trigger an adaptive immune response through binding to the T-cell receptor (TCR). MHC class I molecules also function in the innate immune system by serving as ligands of inhibitory killer cell immunoglobulin-like receptors (KIRs) on natural killer (NK) cells. NK cells have the unique ability to recognize and nonspecifically kill cells lacking self MHC class I molecules. Because all healthy nucleated cells express self MHC class I molecules, inhibitory KIRs ensure that NK cells do not attack normal cells but eliminate infected and tumor cells (which may have reduced MHC class I molecule expression) (9). Because not all infections or cancers reduce MHC class I expression, the role of these proteins in the adaptive immune response is fundamental.

For MHC class I molecules to present self and nonself peptides to CD8+ T cells, the peptides must first be produced by proteolysis in the ubiquitin-proteasome pathway. Proteins are targeted for degradation in this pathway by the covalent attachment of multiple copies of the 76-residue protein ubiquitin to free amino groups (always near the ε-amino group of Lys). Ubiquitination involves a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2), a substrate-specific ubiquitin-protein ligase (E3), and in some

cases an additional conjugation factor (E4) (10). The breakdown of polyubiquitinated proteins and the processing of the resulting peptides until they are presented on the cell surface involve multiple molecular species, including the proteasome in its constitutive and immunoproteasome forms, peptide transporters (TAP1 and TAP2), endoplasmic reticulum chaperones (calnexin, calreticulin, ERp57, and tapasin), and the Golgi apparatus. Acting in concert, these proteins, multimeric protein complexes, and organelles make up what is called the MHC class I antigen processing and presentation machinery (APM) (11–15). Defects in the function or expression of APM components affect the formation of MHC class I peptide complexes and their recognition by $CD8^+$ T cells (and NK cells). This review describes the structure and key functions of the proteasome and immunoproteasome, dissects the four main tasks of antigen processing and presentation, lists APM changes that have been observed in tumors, and explores the possible clinical significance of these defects with a special focus on their potential role in tumor cells' ability to escape immunosurveillance.

Proteasomes and Immunoproteasomes: Structure, Components, and Functions

The proteasome is a multimeric protein complex found in both the cytosol and nucleus (16–18). Structurally, it has a cylindrical shape and contains both a catalytic core and regulatory particles (Figure 1). The catalytic core, called the 20S proteasome, is composed of four stacked heptameric rings that produce a barrel-shaped structure with a central gate. The two outer rings each contain seven α subunits (α1–α7) that interact with regulatory particles and create a physical barrier to regulate access to the gate (17). The two inner rings each contain seven β subunits (β1–β7), three of which (β1 or δ, β2 or Z, and β5 or MB1) have threonine-protease catalytic centers with different cleavage specificities: β1 has caspase-like activity (cleavage after acid residues); β2 has trypsin-like activity (cleavage after basic residues); and β5 has chymotrypsin-like activity (cleavage after hydrophobic residues) (19,20).

The proteasomal gate is normally closed by the N-termini of the seven α subunits to keep the proteasome in a proteolytically inactive state and to prevent unregulated protein degradation. The N-terminus of subunit α3 sticks out the most into the gateway, interacting with every other α subunit (17,21). Cleavage of this N-terminus, which occurs upon conformational rearrangements caused by the attachment of regulatory particles to the α rings, opens the gate, permits the access of substrates, and activates the proteasome (17,21).

Regulatory particles bind to one or both ends of the 20S proteasome. The major regulatory particle, called the 19S regulator (or PA700), binds to the 20S proteasome to form the 26S proteasome (22). The 19S regulator consists of 17 distinct subunits, 9 in a "base: subcomplex and 8 in a "lid" subcomplex (23). The lid contains binding sites for both polyubiquitinated proteins and enzymes that disassemble and recycle ubiquitin chains, called deubiquitinating enzymes. The base interacts with the α rings of the 20S proteasome; it triggers gate opening, unfolds

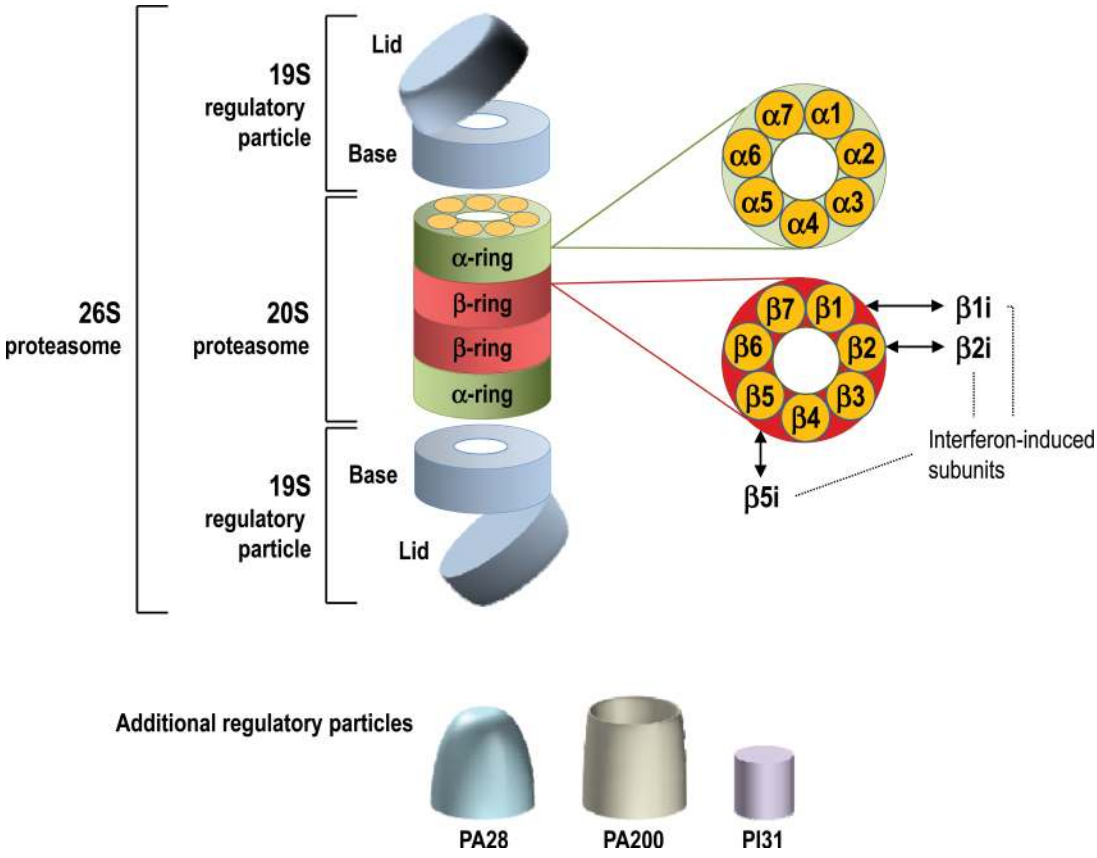

Figure 1. Proteasome composition

protein substrates, and catalyzes protein translocation into the 20S proteasome (24,25). These functions require metabolic energy, and, indeed, the base contains six ATPase subunits.

In addition to the 19S regulator, other regulatory particles, named PA28αβ (26), PA200 (27), and PI31 (28;29) can bind the 20S proteasome and form proteasomal isoforms. These alternative regulators bind the α rings of the 20S proteasome just as the 19S regulator does, but in an ATP-independent way. PA28αβ is a heptameric complex composed of α and β subunits; upon binding with the 20S proteasome, it increases the catalytic activity of the cleavage sites and facilitates the opening of the proteasome gate (30). PA28αβ is particularly abundant in immune tissues, and it is induced by interferon (IFN) γ and infection. PA200 is the most recent proteasome activator to be discovered (27). The original description of this proteasome activator proposed it to be involved in DNA repair, possibly by recruiting proteasomes to double-strand breaks. Following gamma irradiation, PA200 forms hybrid proteasomes with 19S regulator–20S proteasome–PA200 that accumulate on chromatin, leading to an increase in proteolytic activity (27). PI31 is a cellular regulator with inhibitory function that competes with PA28αβ and PA200 for binding with the 20S proteasome (28,29).

Three of the 20S proteasome's β subunits (δ, Z, and MB1) may be replaced by functionally different counterparts named low molecular weight protein (LMP) 2 (also called β1i), LMP7 (β5i), and LMP10 (β2i), respectively (31–33). Proteasomes incorporating LMP2, LMP7, and LMP10 are called immunoproteasomes because they develop under conditions of intensified immune response. Indeed, they are induced in the majority of cells by stimulation with type I (α and β) IFN (34;35) and type II (γ) IFN (34,35) (Figure 1). Cells exposed to IFN in the context of an inflammatory process are not the only cells to contain immunoproteasomes. These are also expressed in a constitutive manner in lymphoid organs such as the spleen, lymph nodes, and thymus (36). Interestingly, dendritic cells were recently found to have approximately equal amounts of proteasomes and immunoproteasomes when immature and only immunoproteasomes when mature (37).

Compared with 20S proteasomes, immunoproteasomes display a weaker ability to cleave peptides after acidic residues but a better ability to cleave after basic and hydrophobic residues (38,39). Immunoproteasomes also serve functions besides antigen processing. They generate biologically active proteins (such as cytokines) that are involved in inflammatory processes (40) and in T-cell differentiation, survival, and function during thymocyte development (41). In addition, immunoproteasomes are thought to have a role in cell differentiation because they are constitutively expressed in mouse ocular lens and brain (42), which are immune-privileged sites with no apparent need to generate class I peptide ligands.

Four Main Tasks of MHC Class I Antigen Processing and Presentation

When polyubiquitinated proteins reach the proteasome (or immunoproteasome), a complex cellular process begins that prepares antigens for presentation on MHC class I molecules. This process consists of four main tasks: 1) peptide generation and trimming; 2) peptide transport; 3) assembly of the MHC class I loading complex; and 4) antigen presentation (11–15) (Figure 2).

Peptide Generation and Trimming

When the proteasome is activated, ubiquitinated proteins pass through the gate, unfold, spread along it, and lose the polyubiquitin chain (deubiquitination) through the action of deubiquitinating enzymes. Proteins are then broken down into peptides ranging from 2 to 25 residues, which are released to the cytosol. The particular peptide repertoire that is generated, in terms of amino acid sequence, length, and quantity (39), varies depending on whether the proteasome or immunoproteasome is involved. Both are able to generate MHC class I epitopes, but dramatic differences exist in the efficiency at which a given epitope is generated. For instance, immunodominant epitopes of infectious organisms have recently been found to be more effectively produced by the immunoproteasome. In particular, in experiments in which HeLa cells were infected with vaccinia virus expressing the hepatitis B virus core antigen, generation of the hepatitis B virus core antigen$_{141–151}$ epitope required the immunoproteasome with subunit LMP7 (43). Moreover, in knock-out mice lacking the three immunoproteasome subunits, dendritic cells could not present several major MHC class I epitopes, and the epitope repertoire was 50% different from that of wild-type mice (44).

A minority of intracellular proteins are cleaved in proteasome-independent pathways that also generate peptides for MHC class I presentation (45–47). For instance, peptides with a C-terminal lysine are generated by proteasomes with very low efficiency given that lysine is not a preferred proteasomal cleavage site (48). These peptides, which may represent important T-cell epitopes, may, however, be generated by additional proteases such as tripeptidyl peptidase II (TPPII), a cytosolic aminopeptidase with endoproteolytic activity able to cleave after lysine residues (49). This protease is essential for the generation of the immunodominant HLA-A3– and HLA-A11–restricted HIV-1 Nef$_{73-82}$ epitope (50). Another cytosolic protease involved in the direct production of MHC class I peptides is insulin-degrading enzyme, a metallopeptidase that generates epitopes from the melanoma-associated antigen A3 (51), an immunogenic protein highly expressed by several human tumors (52). Nardilysin and thimet oligopeptidase (TOP) are two additional cytosolic endopeptidases that are required, either together or alone, for the generation of T-cell epitopes from the Epstein–Barr virus nuclear antigen 3C, the melanoma antigen recognized by T cells 1 (MART-1), and the preferentially expressed antigen of melanoma (PRAME). TOP operates as a C-terminal trimming peptidase, whereas nardilysin contributes to both the C-terminal and N-terminal generation of the epitopes (53).

Peptides produced in the cytosol are further trimmed by enzymes within the endoplasmic reticulum (ER) (45,54,55) to fit into the groove of the MHC class I molecules. One of these enzymes, an ER aminopeptidase called ERAP1 (ER aminopeptidase associated with Ag processing 1), is considered a "molecular ruler" because of its substrate preference (56): ERAP1 preferentially trims peptides of 9 to 16 residues but spares those of 8 to 9 residues, the typical length for MHC class I binding (56). ERAP1 prefers peptides with hydrophobic C-termini and is induced by type I and II IFNs (57). Recent work has also shown that the ER dipeptidase angiotensin-converting enzyme can make the final C-terminus peptide cut (58).

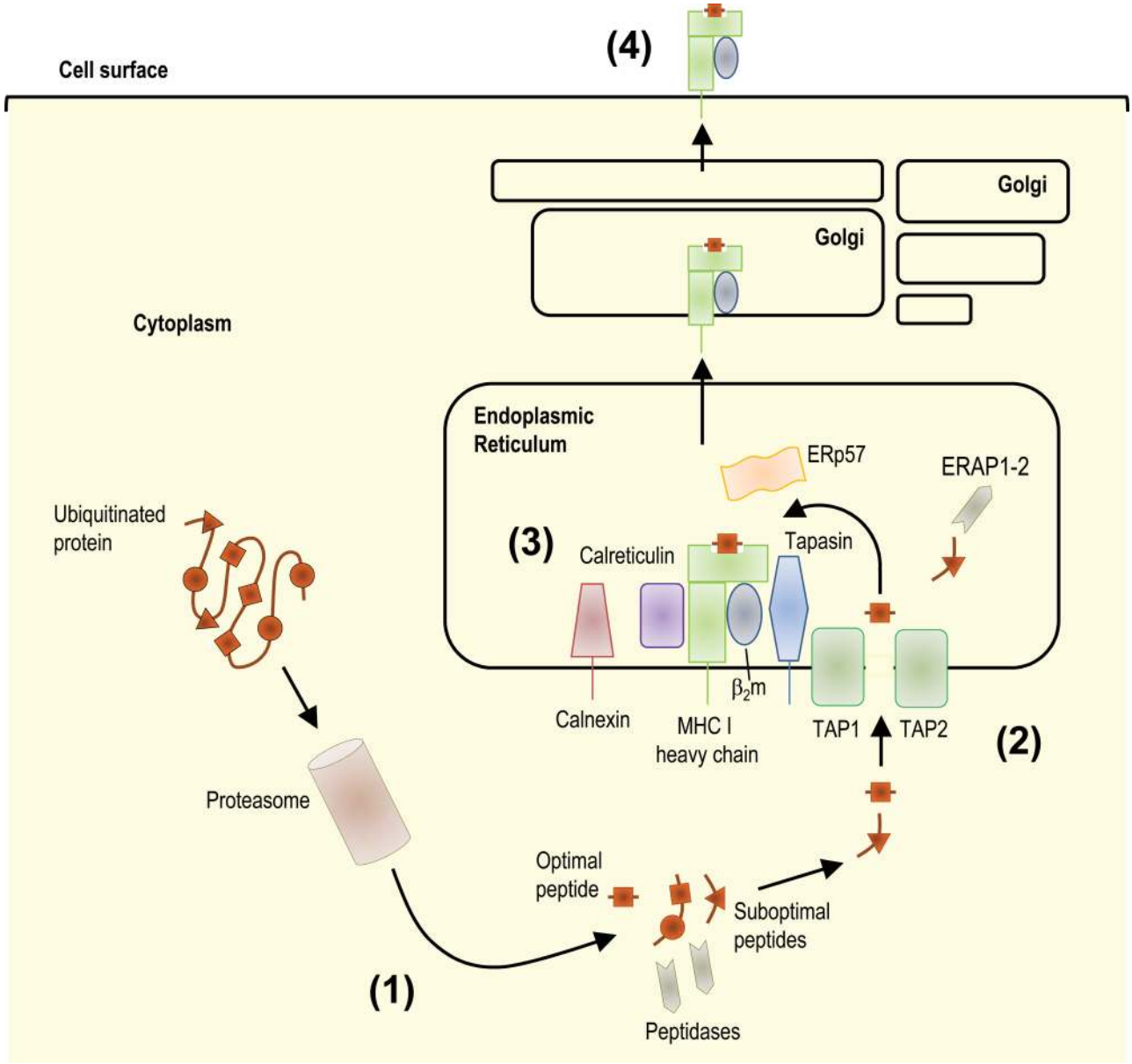

Figure 2. The four main tasks of major histocompatibility complex (MHC) class I antigen processing and presentation: 1) Peptide generation and trimming; 2) peptide transport; 3) assembly of the MHC class loading complex; 4) antigen presentation.

Peptide Transport

Peptides generated by proteasome-dependent and -independent pathways are actively transported from the cytosol into the ER by the transporter associated with antigen processing (TAP). This heterodimeric complex is composed of the two half-transporters, TAP1 and TAP2, that are members of the ATP-binding cassette transporter family. Both TAP1 and TAP2 contain a hydrophobic transmembrane domain and a cytosolic nucleotide-binding domain (59). TAP forms a transmembrane pore in the ER membrane whose opening and closing depend on ATP binding and hydrolysis, respectively (ATP switch model) (60–62).

TAP transports most efficiently peptides of a certain length (8–12 residues) and with hydrophobic or basic C-termini that bind to MHC class I molecules (63–68). Transport of longer peptides occurs with reduced efficiency (64–66,68). These longer peptides, which do not fit the class I binding groove, can be further trimmed in the ER lumen or, alternatively, can be transported back to the cytosol where they are trimmed by cytosolic peptidases and recycle back to the ER in a TAP-dependent fashion for association with MHC class I molecules (69).

Assembly of the MHC Class I Loading Complex

Peptides transported into the ER by TAP are loaded onto nascent MHC class I molecules with the assistance of four chaperone proteins: calnexin (70), the thiol oxidoreductase ERp57 (71,72), calreticulin (73), and tapasin (74;75). These proteins, along with MHC class I molecules and TAP, form the MHC class I loading complex that combines the activities of peptide transport and loading onto MHC molecules (70–78). Specifically, a newly synthesized MHC class I heavy chain, translocated into the ER, acquires a $Glc_1Man_9GlcNAc_2$ glycan moiety that serves as a recognition element for the membrane-bound chaperones calnexin and

calreticulin (79,80). The MHC class I heavy chain interacts with calnexin., which both facilitates its complete folding and, by acting in concert with ERp57 (81), ensures its correct oxidation (82). At this point, the heavy chain is conformationally recognizable by $\beta_2$microglobulin (83). Their binding triggers the release of calnexin (83,84). The resulting conformational changes give the heavy chain/$\beta_2$microglobulin heterodimer an "open" form that interacts with calreticulin (73). Peptide binding requires the additional participation of TAP, which assures a pool of free peptides, and tapasin. This chaperone bridges class I molecules to TAP, allowing peptides to gain access to the ER for class I binding (73,85). It also stabilizes heavy chain/$\beta_2$microglobulin heterodimers (75,86) and optimizes the class I peptide load (87).

Antigen Presentation

After the binding of a peptide, the ER-resident chaperones are released and the peptide-MHC class I complex leaves the ER within vesicles that traverse the Golgi apparatus, migrate to the cell membrane, and fuse with it, so that the bound peptide in the MHC class I groove is exposed extracellularly. Here the peptide-MHC class I complex may be recognized by and interact with the cognate TCR on $CD8^+$ T cells. Peptides derived from unmutated (self) proteins are normally ignored by $CD8^+$ T cells, whereas those derived from mutated or pathogen-derived (nonself) proteins are not. Using this system of intracellular surveillance, $CD8^+$ T cells play a crucial role in eradicating viruses and other intracellular pathogens and also exert potent antitumor activity.

Defects in Class I APM in Human Tumors

Defects in the expression and function of APM components have been found in various solid and hematologic tumors. They occur individually or in combination, and the frequency and nature of the defect vary substantially according to tumor type (Table 1). The molecular mechanisms underlying these defects have been partly identified for some components only and seem to take place at the genetic and epigenetic levels (Table 2). There is also some evidence that transcriptional and post-transcriptional defects may occur.

Defects in Proteasome Subunit Expression Patterns

Alterations of proteasome subunits have been identified only recently, thanks to the availability of monoclonal antibodies that permit semiquantitative analyses. Downregulation of one or more of the 20S proteasome's β subunits δ, MB1, and Z is characteristic of colorectal (88), bladder (89), and ovarian (90) carcinomas, as well as medulloblastoma (91). Similarly, downregulation of one or more of the inducible subunits (LMP2, LMP7, and LMP10) is prominent in acute myeloid leukemia (92), in carcinoma of the head and neck (93,94), esophagus (95–97), stomach (98), colorectum (88,99,100), kidney (101,102), bladder (89,103), prostate (104), cervix (105,106), ovary (90), and breast (107;108), and in astrocytoma (109), medulloblastoma (91), neuroblastoma (110), and melanoma (111,112). We have also found that the constitutive subunits δ, Z, and MB1 and the immunoproteasome subunits LMP2 and LMP10 are progressively lost in premalignant and malignant plasma cells from bone marrow of patients with monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma (MM) (113). It should be noted that interpretation of data related to the expression of immunoproteasome subunits is quite complex, given that they are likely unexpressed under basal conditions but are induced after exposure of cells to IFN-γ. Furthermore, no data are available regarding the expression of immunoproteasome subunits in most normal tissues. Therefore, in several cases it is not possible to establish whether the expression of an immunoproteasome subunit in malignant cells is a normal phenotype and its lack of expression is a downregulation process or whether such lack is a normal phenotype and its expression reflects regulatory abnormalities.

Little is known about the molecular basis of the defects in proteasome components. Mutations at coding microsatellites of genes encoding LMP7 have been detected in gastric cancer (98). Single nucleotide polymorphisms in the *LMP2* and *LMP7* genes have been identified in cervical carcinoma (105,114). In preliminary experiments, we found that treatment of myeloma cells with decitabine, a potent DNA methyltransferase inhibitor, restored the expression of several proteasome subunits, suggesting that promoter methylation alterations and epigenetic regulation were involved (113). Loss of IFN-γ–mediated upregulation of LMP2 in one renal cell carcinoma cell line has been associated with the lack of IFN regulatory factor 1 and signal transducer and activator of transcription 1 (STAT1) binding activities, as well as of Janus associated kinase (JAK)1, JAK2, and STAT1 phosphorylation (115). More recently, loss of IFN-γ–mediated upregulation of LMP2 and LMP10 in melanoma cell lines has been found to be caused by a deletion of the *JAK2* gene on chromosome 9 (116).

Defects in TAP1 and TAP2

Low to undetectable levels of TAP1 and/or TAP2 mRNA and/or protein have been reported in primary cells and cell lines from several tumors, including carcinomas of the head and neck (93,94,117–119), esophagus (95–97,120), stomach (98), pancreas (121), colorectum (88,99,100,122–124), breast (108,125–127), and cervix (105,106,128–130); renal cell (101,102), prostate (104) and bladder (89,103) cancer; and melanoma (111,112,131–133), astrocytoma (109,134), medulloblastoma (91), neuroblastoma (110), acute myeloid leukemia (92), and multiple myeloma (113). In several tumor cell lines in which TAP was downregulated, its levels were restored by IFN-γ treatment (135,136). At the genetic level, mutations in *TAP* genes that resulted in loss of expression or in expression of a nonfunctional protein have been observed in colorectal (88,100,123), cervical (114,129,137), gastric (98), and lung (138) carcinomas. Methylation of the *TAP1* gene promoter has also been found in cervical carcinoma (105). A post-translational downregulation of TAP2 has been observed in a melanoma cell line that does not express TAP1 because of a frameshift mutation that generates a stop codon in the *TAP1* gene (139). In this cell line, transcription of *TAP1* and *TAP2* genes proceeds normally, but, in the absence of TAP1 translation, the TAP2 protein is unstable and is lost from the cell; these results suggest that TAP expression is regulated through a mechanism of coordinated stabilization of the TAP heterodimeric complex. Moreover, as for immunoproteasome subunits, loss of JAK2 has been shown to impair the IFN-γ inducibility of TAP1 and TAP2 in melanoma cell lines. (116).

Table 1. Expression defects in antigen processing and presenting machinery components, by tumor type*

Body region	Tumor type	δ	MB1	Z	LMP2	LMP7	LMP10	TAP1	TAP2	ERAP1-2	CLX	CLT	TPN	ERp57	HLA-I	β_2m	Reference
Head and neck	Head and neck squamous cell carcinoma [P, M]							■	■						■	■	118
	Head and neck squamous cell carcinoma [P]		.		■	■		■	■					.	■		93
	Head and neck squamous cell carcinoma [P, M]							■	■				■		■		117
	Maxillary sinus squamous cell carcinoma [P]										■	■	■	■	■	■	142
	Laryngeal squamous cell carcinoma [P]				■			■			■	■	■	■	■	■	94
	Oral squamous cell carcinoma [P]														■	■	170
	Oral squamous cell carcinoma [P]												■				144
Lung	Lung carcinoma [P]									■							140
Gastrointestinal system	Esophagus squamous cell carcinoma [P]				■	■		■	■						■	■	95
	Esophagus squamous cell carcinoma [P, M]							■	■								120
	Esophagus squamous cell carcinoma [P]				■			■							■		96
	Esophagus squamous cell carcinoma [P]					■		■		■	■		■	■	■		97
	Gastric adenocarcinoma [P, M]													■			146
	Gastric adenocarcinoma [P]				■	■	■	■	■						■		98
	Pancreatic carcinoma [P]							■							■	■	121
	Pancreatic carcinoma [P]									■							140
	Colorectal carcinoma [P]							■							■	■	123
	Colorectal carcinoma [P, M]				■	■		■					■		■	■	99
	Colorectal carcinoma [P]	■	■	■	■	■	■	■	■		■	■	■	■	■	■	88
	Colorectal carcinoma [P]				■	■		■	■		■	■	■	■	■	■	100
	Colorectal carcinoma [P, M]							■	■						■	■	122
	Colorectal carcinoma [P]							■	■						■		124
Genitourinary system	Renal carcinoma [P]				■	■		■					■		■	■	102
	Renal carcinoma [P, M]				■	■		■							■	■	101
	Bladder carcinoma [P]				■	■		■	■				■		■	■	103
	Bladder carcinoma [P]			■			■	■			■		■	■	■	■	89
	Prostate carcinoma [P]				■	■		■	■		■	■	■	■	■	■	104
	Cervical squamous cell carcinoma [P]				■	■		■	■	■	■	■	■	■	■	■	105
	Cervical squamous cell carcinoma [P]				■	■		■	■	■			■	■	■	■	106
	Cervical adenocarcinoma [P]				■	■		■	■	■			■	■	■	■	106
	Ovarian carcinoma [M]														■		192
	Ovarian carcinoma [P]												■				145
	Ovarian carcinoma [P]									■							140
	Ovarian carcinoma [P]		■			■		■	■	■				■			90

(Table continues)

Table 1 (Continued).

Body region	Tumor type	δ	MB1	Z	LMP2	LMP7	LMP10	TAP1	TAP2	ERAP1-2	CLX	CLT	TPN	ERp57	HLA-I	β_2m	Reference
Breast	Breast cancer[P,M]							■							■	■	126
	Breast cancer[P]							■	■						■		127
	Breast cancer[P,M]				■		■								■	■	107
	Breast cancer[P,M]				■			■	■		■		■		■	■	108
	Breast cancer[P]									■							140
Skin	Malignant melanoma[P,M]				■	■		■	■						■		111
	Malignant melanoma[P]				■	■		■	■						■	■	112
Nervous system	Astrocytoma[P]							■	■				■		■	■	134
	Astrocytoma[P]				■			■								■	109
	Astrocytoma[P]											■	■		■		91
	Medulloblastoma[P]	■	■	■	■	■	■		■		■	■	■		■	■	91
	Neuroblastoma[P]			■	■	■			■				■		■	■	110
Hematopoietic system	Acute myeloid leukaemia[P]				■	■		■	■						■	■	92
	Multiple myeloma[P]	■	■	■	■	■	■	■	■								113

* Only papers in which APM component expression was scored on a numerical scale were considered. Data refer to protein and mRNA expression in primary tissue samples only, with the exclusion of cell lines. Defects were recognized by comparing the tumoral tissue with the apparently normal surrounding tissue and/or the corresponding tissue from non-neoplastic patients. CLT = calreticulin; CLX = calnexin; M, metastases; P = primary lesions; TPN = tapasin.

Defects in ERAP1 and ERAP2

A comparison of the distributions of ERAP1 and ERAP2 in normal and neoplastic samples from the same tissues revealed changes that are thought to occur during malignant transformation (140): 1) low expression of ERAP1, ERAP2, and MHC class I as the most frequent phenotype observed in tumors, regardless of histotype; 2) downregulation of ERAP1 and/or ERAP2 as the most frequent condition in breast, ovary, and lung carcinomas that derive from tissues coexpressing ERAP1 and ERAP2; 3) upregulation in colon and thyroid carcinomas that derive from tissues lacking detectable levels of both ERAP1 and ERAP2; and 4) ERAP1/ERAP2 imbalance in essentially all the tested tumor histotypes. In addition, low expression of either ERAP1 or ERAP2 results in low levels of functional trimming activities. The heterogeneity of expression found at the protein level matches that found at the genetic level and is associated with the haplotypes for several single nucleotide polymorphisms found in the *ERAP1* gene in cervical carcinoma (137), as well as in the *ERAP1* and *ERAP2* genes in a large series of melanoma cell lines (141). These latter cell lines also display considerable diversity in their *ERAP* gene promoter activities, and, in some cases, there is discordance between this activity and mRNA levels or between mRNA and protein levels (141). Thus, genetic, transcriptional, and post-transcriptional control mechanisms are likely to be involved in the regulation of the ERAP expression.

Defects in Chaperone Protein Expression

A substantial downregulation of calnexin has been observed in carcinoma of the maxillary sinus (142), larynx (94), esophagus (97), colorectum (88,100), bladder (89), prostate (104), cervix (105,130), and breast (108), as well as in medulloblastoma (91). Tapasin has been found to be downregulated in cell lines from small cell lung carcinoma, pancreatic carcinoma, colon carcinoma, head and neck squamous cell carcinoma, and renal cell carcinoma (143). It also was reported as being downregulated in primary cells of head and neck (94,117,119,142–144), esophageal (97), colorectal (88,99,100), renal (102), bladder (89,103), prostate (104), cervical (105,106), ovarian (145), and breast (108) cancers, as well as in melanoma (131), astrocytoma (91,134), medulloblastoma (91), and neuroblastoma (110). ERp57 was reported to be downregulated in carcinomas of the maxillary sinus (142), larynx (94), esophagus (97), stomach (146), colorectum (88,100), bladder (89), prostate (104), cervix (105,106), and ovary (90). We found that expression levels of calnexin, calreticulin, tapasin, and ERp57 were higher in plasma cells from MM patients than in either premalignant plasma cells from MGUS patients or normal plasma cells from healthy donors (113). Methylation of the *ERp57* gene promoter has been found in cervical carcinoma (105). Frameshift mutations in calnexin, calreticulin, tapasin, and *ERp57* genes have been reported in colorectal carcinoma (100), whereas in gastric carcinoma mutations at coding microsatellites were seen in calnexin and tapasin genes (98). A transcriptional control of tapasin expression has been described in a cell line overexpressing HER-2/neu (human epidermal growth factor receptor 2). In this cell line, site-directed mutagenesis of the p300 and E2F binding sites within the tapasin promoter restores the HER-2/neu–mediated suppression of tapasin (147). A deregulation of tapasin has also been associated with defective IFN-γ signaling in melanoma cell lines (116) (as has also been observed for immunoproteasome and TAP).

Table 2. Molecular mechanisms underlying changes in major histocompatibility class I antigen processing and presenting machinery components*

Molecular mechanism	Affected molecules	Tumor	Reference
Genetic			
Loss of heterozygosity	HLA-I (6p21.3), β_2m (15q)	Head and neck squamous cell carcinoma	118,184
	HLA-I (6p21.3)	Laryngeal carcinoma	171
	HLA-I (6p21.3)	Colorectal carcinoma	100
Gene mutation			
Point mutation	β_2m	Colorectal carcinoma	162
Frameshift	β_2m	Colorectal carcinoma	162
Frameshift (start codon, splice-site)	β_2m	Colorectal carcinoma	122, 100
Frameshift	TAP1, TAP2	Colorectal carcinoma	88, 100
Frameshift	HLA-I, CLX, CLT, TPN, ERp57	Colorectal carcinoma	100
Frameshift	β_2m	Gastric carcinoma	165
Frameshift (start codon deletion, stop codon generation)	β_2m	Melanoma	166
Coding microsatellite	β_2m	Colorectal carcinoma	88
Coding microsatellite	β_2m, LMP7, TAP1, TAP2, CLX, TPN	Gastric adenocarcinoma	98
Single nucleotide polymorphism	TAP1, TAP2, LMP2, LMP7, ERAP1	Cervical carcinoma	137, 114, 141
Defective allele	TAP1	Lung cancer	138
Defective allele	TAP1, TAP2, HLA-A	Cervical carcinoma	129
Epigenetic, transcriptional, post-transcriptional, post-translational			
Gene promoter methylation	HLA-I	Esophagus squamous cell carcinoma	96
	HLA-I	Gastric adenocarcinoma	98
	TAP1, LMP7, ERp57, TPN	Cervical squamous cell carcinoma	105
Gene promoter mutation	ERAP1	Melanoma (cell lines)	141
E2F1-mediated gene promoter regulation	TPN	HER-2/neu$^+$ fibroblasts (cell line)	147
Frameshift mutation in TAP1 gene	TAP2	Melanoma (cell line)	139
IFN-γ signal transduction pathway defects			
Lack of IRF1 and STAT1 binding to gene promoter	TAP1, LMP2	Renal cell carcinoma (cell line)	115
JAK2 deletion	LMP2, LMP10, TAP1, TAP2, TPN, HLA-I	Melanoma (cell lines)	116

* CLT = calreticulin; CLX = calnexin; E2F1 = E2F transcription factor 1; HER-2/neu = human epidermal growth factor receptor 2; IRF1 = interferon regulatory transcription factor 1; JAK2 = janus associated kinase 2; STAT1 = signal transducer and activator of transcription 1;TPN = tapasin.

Defects in MHC Class I Molecule Expression

MHC class I molecules are integral membrane proteins of 45 kDa, but the full-length gene product can be naturally shed from cells. Moreover, soluble isoforms of 43, 39, and 35 kDa exist in serum and urine (148–151). The 39 kDa truncated isoform lacking the transmembrane domain results from alternative mRNA splicing (152), whereas the 35 kDa isoform also lacking the cytoplasmic domain is the product of metalloprotease cleavage (151,153,154). Soluble MHC class I molecules have been detected in plasma both complexed to and free of β_2 microglobulins (151,153,155–159).

Aberrations in MHC class I molecule expression regard both integral membrane forms and secreted soluble forms. Defects in the surface expression of MHC class I molecules have been demonstrated in a large variety of human tumors. The molecular mechanisms underlying these changes vary according to the tumor type, and different mechanisms can lead to the same alteration in surface expression (160,161). These alterations can be genetic (at the gene or chromosome level) or regulatory (at the transcriptional level) and range from total loss or downregulation of all class I molecules to selective losses of HLA class I haplotypes or alleles.

The total loss of MHC class I expression from the cell surface is associated with mutations in the β_2 microglobulin gene, microsatellite instability, defects in peptide formation and transport (as a result of alterations in other APM components, usually LMPs, TAP, and tapasin), deficient peptide loading of MHC molecules, and hypermethylation of *MHC* gene promoters. Total loss has been described in colorectal carcinoma (100,122,162–164), gastric carcinoma (98,165), melanoma (164,166–169), oral squamous cell carcinoma (170), laryngeal carcinoma (164,171), cervical carcinoma (105,128), esophageal squamous cell carcinoma (96), breast cancer (172), and astrocytoma (134).

Locus-specific downregulation is due to the transcriptional regulation of particular *MHC* genes and may be caused by the loss of DNA binding factors required for optimal promoter activation (173,174). Locus-specific downregulation has been found in colorectal carcinoma (163,175), cervical and laryngeal carcinoma (171,176), and melanoma (177). Total loss or locus-specific downregulation of MHC class I molecules has been described in mos-, myc-, ras-, and HER-2/neu–transformed murine and human cell lines, confirming that multiple signal transduction pathways control MHC class I molecule expression either directly or through the regulation of other APM components (147,178–182).

Allele-specific MHC class I defects result from point mutations in or partial deletions of *MHC* genes, chromosomal breakage, or somatic recombination. These defects have been detected in colon carcinoma (163), melanoma (183), cervical carcinoma (129,183), laryngeal carcinoma (171,176), and astrocytoma (134).

Haplotype-specific MHC class I loss has been associated with loss of heterozygosity on chromosome 6 due to total or partial deletion of the chromosome, chromosomal nondisjunction or mitotic recombination (160,161,164,173). It has been documented in laryngeal carcinoma and colorectal carcinoma (171), head and neck carcinoma (118,184), melanoma (164,183), and pancreatic adenocarcinoma (185).

Aberrations in levels of soluble MHC class I molecules in malignant diseases have been investigated in a few studies. Low levels have been described in gastric cancer (186) and melanoma (187). High levels have been observed in Japanese patients with pancreatic cancer (188). In addition, high levels of soluble MHC class I and β_2 microglobulin have been reported in MM (158,159,189,190), chronic myelogenous leukemia (151), acute myeloid leukemia (153,155), myelodysplastic syndrome (155,190), and non-Hodgkin lymphoma (155) by our group and other groups.

Clinical Meaning of Class I APM Defects

Abnormalities in the expression of APM components, especially TAP and MHC class I, are of particular clinical interest because of their strict link with disease aggressiveness and clinicopathological outcome (Table 3). For instance, downregulation of TAP expression is more frequent in metastatic than in primary melanoma lesions and in nevi. TAP1, in particular, is an independent prognostic factor for melanoma metastases (112), and it is never lost in primary melanoma lesions undergoing spontaneous regression (131). Downregulation of MHC class I expression associates with primary melanoma lesion thickness, advanced stage of disease, and reduced time to disease progression (111). The APM expression profiles of stage III and IV melanoma (as graded according to the American Joint Committee on Cancer) can be used to distinguish patients into two groups that differ in survival (191). Downregulation of MHC class I, TAP1, and TAP2 is also associated with breast cancer lesion grading, given that it is more frequently observed in high-grade (G2 and G3) than in low-grade (G1) lesions (127). In the same tumor, primary lesions with positive estrogen receptor or progesterone receptor status express lower levels of TAP2 than those with negative estrogen receptor or progesterone receptor status (108).

A connection between changes of APM components and clinical course has also been described in astrocytoma (109,134), gastric (146), colorectal (99,124), bladder (89), prostate (104), cervical (106,128), ovarian (90,145,192), head and neck (93,94,117,119,142,144), and esophageal squamous carcinoma (95–97). In particular, high-stage bladder carcinoma displays lower levels of immunoproteasome components than low-stage urothelial carcinoma; higher expression of delta and lower expression of calreticulin are associated with lower survival in urothelial carcinoma and in all types of bladder carcinoma (89). Downregulation or loss of calnexin and MHC class I molecules correlates with higher Gleason grade and early prostate cancer recurrence (104). Partial MHC class I loss is statistically associated with decreased overall survival of patients with cervical carcinoma (106). In the same tumor, TAP1 and ERAP1 loss is associated with decreased overall and disease-free survival, and ERAP1 downregulation is an independent predictor for worse overall and disease-free survival in multivariable analysis (106). LMP2, LMP7, TAP1, TAP2, and MHC class I expression rates in primary head and neck squamous cell carcinoma were found to predict overall survival, and the level of LMP7 expression was inversely associated with disease recurrence at 2 years (93). The loss or downregulation of MHC class I, TAP1, LMP7, calnexin, and ERp57 in esophageal squamous carcinoma was directly associated with tumor grade and lymph node status (95). APM component deficiencies occur more frequently in Ki-ras–mutated colorectal carcinoma lesions, and APM abnormalities in combination with Ki-ras mutations appear to be associated with disease stage (99).

Regarding hematological tumors, a negative correlation between proteasome subunit levels and clinical progression of MGUS to MM has been demonstrated by our group (113). Levels of soluble MHC class I and β_2 microglobulin have been reported to correlate with poor prognosis in MM (158,159,189,190), chronic myelogenous leukemia (151), acute myeloid leukemia (153,155), myelodysplastic syndrome (155,190), and non-Hodgkin lymphoma (155).

Immunological Consequences of APM Defects

The mechanisms underlying the above-mentioned clinical associations are likely to be immunologic. They reflect the negative effect of APM dysfunction, caused by numerous possible defects in the generation and expression of trimolecular class I β_2 microglobulin–peptide complexes, on immune recognition of tumor cells. First, downregulation of proteasome subunit expression can inhibit the processing of antigens in the cytoplasm, thus decreasing the efficiency of epitope generation. Second, variations in proteasome (or immunoproteasome) subunit ratios may modify the characteristics of presented peptides, thus altering the tumor cell antigen repertoire. Third, TAP abnormalities may reduce the translocation of peptides into the ER, resulting in decreased formation of stable MHC class I molecule–peptide complexes or in expression of "peptide-free" MHC class I molecules. Fourth, changes in chaperone protein levels may hamper proper loading and assembly of MHC class I molecules, thus altering their maturation and stability. Finally, loss of cell surface β_2 microglobulin and MHC class I molecules may cause their accumulation in the extracellular milieu as soluble forms. All these events can have profound consequences on $CD8^+$ T-cell and NK cell immune responses against tumors: only mature MHC class I molecules with a peptide in their binding cleft are recognized by T-cell receptors, activating T-cell cytotoxicity (adaptive immune response) (193), and cells lacking MHC class I molecules on their cell surface are unable to bind inhibitory KIR and therefore are subject to NK cell killing (innate immune response) (9).

The impact of MHC class I APM defects on the human immune system can be studied in type I bare lymphocyte syndrome (BLS) (194,195). Type I BLS is a rare immunodeficiency syndrome mostly caused by mutations in TAP (194–196). Similarly to MHC class I K^bD^b-deficient mice, type I BLS patients have reduced plasma membrane levels of MHC class I molecules and low numbers of $CD8^+$ αβ T cells. Their NK cells are cytotoxic upon activation but less cytotoxic than those of normal healthy donors in resting conditions (194,197–199). In line with the type I BLS model, examination of different tumors has revealed that

Table 3. Clinical correlates of changes in major histocompatibility class I antigen processing and presenting machinery components, by tumor type*

Tumor	Protein	Clinical or histopathological correlate	Correlation	Reference
Head and neck squamous cell carcinoma	LMP7	Disease recurrence at 2 years	Inverse	93
	LMP2, LMP7, TAP2	Overall survival	Direct	
	TAP1, TAP2, TPN, HLA-I	Primary lesions *vs* metastases	Greater down-regulation in metastases	117
	HLA-I†	Disease-free survival	Direct	
Maxillary sinus squamous cell carcinoma	β_2m	T stage, TNM staging system	Direct	142
	TPN	Tumor grade	Direct	
	TPN, HLA-I†	Disease-free survival	Direct	
Laryngeal squamous cell carcinoma	HLA-I	Disease recurrence	Inverse	94
	HLA-I	Disease-specific death	Inverse	
	LMP2, β_2m, HLA-I†	Disease-free survival	Direct	
	β_2m, HLA-I†	Cause-specific survival	Direct	
Oral squamous cell carcinoma	TPN	Tumor grade	Inverse	144
	TPN	5-year survival	Direct	
Esophageal carcinoma	LMP7, TAP1, CLX, TPN, ERp57, HLA-I	Tumor grade	Inverse	97
	LMP7, TAP1, CLX, TPN, ERp57, HLA-I	Depth of tumor invasion	Inverse	
	LMP7, TAP1, CLX, ERp57, HLA-I	Lymph node involvement	Inverse	
	CLX	Tumor vascular invasion	Inverse	
Esophageal squamous cell carcinoma	TAP1, HLA-I	Tumor grade	Inverse	96
	HLA-I	Depth of tumor invasion	Inverse	
	LMP2, TAP1, HLA-I	Lymph node involvement	Inverse	
	LMP2, HLA-I	Tumor stage, I–IV	Inverse	
Gastric adenocarcinoma	ERp57	Depth of tumor invasion	Inverse	146
	ERp57	Tumor stage	Inverse	
	ERp57	Survival, postoperative	Direct	
Colorectal carcinoma	LMP2, LMP7, TAP1, TPN, β_2m, HLA-I	Tumor stage	Inverse	99
	LMP2, LMP7, TAP1, TPN, β_2m, HLA-I	Ki-ras mutations	Inverse	
	TAP1	Lymph node involvement	Inverse	124
	TAP1	Tumor grade	Inverse	
Bladder carcinoma	LMP2, LMP7, LMP10, CLX	Tumor stage	Inverse	89
	Δ	Overall survival	Direct	
	CLX	Overall survival	Inverse	
Prostate carcinoma	CLX	Gleason score ≥7	Inverse	104
	CLX, HLA-I	Early disease recurrence	Inverse	
Cervical carcinoma	LMP2, LMP7, LMP10, TAP1,TAP2, CLX, CLT, TPN, ERp57, ERAP1, HLA-I	Depth of tumor invasion (>15 mm)	Inverse	106
	LMP2, LMP7	Lymph node involvement	Direct	
	TAP1, ERAP1†, HLA-I	Overall survival	Direct	
	TAP1†, ERAP1	Disease-free survival	Direct	
Ovarian carcinoma	HLA-I	Primary lesions vs metastases	Loss in metastases	192
	TAP1, TPN	Tumor stage	Inverse	145
	TAP1, TAP2, TPN, β_2m	Tumor grade	Inverse	
	TAP1, TAP2, TPN	Lymph node involvement	Inverse	
	β_2m	M stage, TNM staging system	Inverse	
	TAP1, TAP2, TPN, β_2m, HLA-I	Survival	Direct	
	MB1†	Disease-specific survival	Inverse	90
	LMP7	Disease-specific survival	Direct	
Breast carcinoma	TAP1, CLX, β_2m	Primary lesions vs metastases	Greater down-regulation in metastases	108
	TAP1, TAP2	Tumor stage, AJCC	Inverse	
	TAP2	Nuclear grade	Inverse	
	TAP2	Estrogen receptor and progesterone receptor	Inverse	
	TAP1, TAP2, HLA-I	Tumor grade	Inverse	127
Melanoma	β_2m	Overall survival	Direct	191
	TAP1†, TAP2	M stage, TNM staging system	Inverse	112

(Table continues)

Table 3 (Continued).

Tumor	Protein	Clinical or histopathological correlate	Correlation	Reference
	TAP1	Spontaneous regression	Positive	131
	LMP2, LMP7, TAP2, HLA-I	Tumor thickness	Inverse	111
	LMP2, LMP7, HLA-I	Tumor stage	Inverse	
	HLA-I	Time to disease progression	Direct	
Astrocytoma	LMP2, TAP1, β_2m	Tumor grade, WHO II–IV	Inverse	109
	HLA-I	Tumor grade, WHO)	Inverse	134

* AJCC = the American Joint Committee on Cancer; CLT = calreticulin; CLX = calnexin; TNM = the TNM Classification of Malignant Tumors; TPN = tapasin; WHO = World Health Organization.

† Independent prognostic factor for that variable

the extent of CD8+ T-cell infiltration directly correlates with the expression of several APM components (94,108,112,124,142,145) and that, in some cases, the lack of cytotoxic CD8+ T-cell recognition is associated with the downregulation of specific APM components (113,119). Indeed, defective generation of MHC class I–peptide complexes (eg, surface expression of peptide-free MHC class I complexes) might impair the activation of CD8+ T cells if this requires direct CD8+ T-cell priming by tumor cells instead of mediation by dendritic cell–dependent cross-priming (200). In the same way, expansion at the tumor site of previously primed CD8+ T cells and successful recognition of tumor cells by effector CD8+ T cells might be weakened by the reduced expression of MHC class I–peptide complexes on the tumor cell membrane. Furthermore, β_2 microglobulin and MHC class I molecules released from the surface of tumor cells may cause apoptosis of activated CD8+ T cells (201), as suggested by in vitro experiments (202–205) and by the finding that injection of appropriate MHC class I–peptide complexes into tumor-bearing mice suppressed T cell–mediated control of tumor growth (206,207). Besides these "quantitative" effects, the strength of the IFN-driven process of proteasome–immunoproteasome replacement might shape the tumor cell antigen profile and compromise ongoing CD8+ T-cell responses against dominant epitopes.

With respect to NK cells, if the expression of MHC class I molecules on the surface of tumor cells is reduced, one might expect an enhancement of NK cell–mediated killing because of a decline in inhibitory KIR-mediated effects. However, there are examples in which NK cell activity against tumors is reduced (208,209). This may occur because soluble MHC class I molecules released from the tumor induce NK cell apoptosis or impair NK cell cytotoxicity by binding CD8 or members of the inhibitory receptor superfamily [reviewed in (201)].

As already mentioned, APM components, in addition to their immunological roles, participate in activities essential for cell survival, cell cycle progression, and inhibition of apoptosis. These include the control of quality of newly synthesized proteins in the ER and the degradation of proteins tagged by ubiquitin. This means that two opposing selection forces shape the APM phenotype of tumor cells. On one hand, for tumor cells to survive, normal APM processes of protein degradation and ER function must be active. On the other hand, the function of these pathways sustains the generation of MHC class I–peptide complexes recognized by CD8+ T cells, thus exposing the tumors to negative immune selection. As a result, the tumor is subject to immunoediting, whereby those tumor cells with selective APM defects (not essential for cell survival) survive but cells with widespread defects in most APM components are eliminated.

Conclusions and Perspectives

Greater knowledge about the molecular mechanisms underlying APM defects may shed light on the mechanisms of tumor progression and ultimately help to develop personalized immunological approaches for cancer treatment. Ideally, a means to upregulate APM components by immunotherapy protocols should be investigated. Pharmacological manipulation of tumor cells may be feasible, although the upregulation of surface MHC class I expression can promote CD8+ T cell–mediated killing and simultaneously hinder lysis by NK cells. Thus, a fine tuning of this pathway is needed to increase the overall level of tumor cell recognition by the host immune system. A method of antigen delivery that bypasses the requirements for both transport and proteolysis may also be considered for targeting APM-deficient tumors. Therefore, further studies should be directed at investigating strategies to modulate in vivo APM expression in tumor cells.

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