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MHC Class I–Related Antigen-Processing Machinery Component Defects in Feline Mammary Carcinoma[1]

Alessandra Favole*, Paolo Cascio†, Fulvia Cerruti†, Alessandra Sereno*, Massimiliano Tursi*, Alessandro Tomatis*, Cristina Della Beffa*, Soldano Ferrone‡,§,¶,#, and Enrico Bollo***

*Department of Animal Pathology, University of Turin, Grugliasco, Turin, Italy; †Department of Veterinary Morphophysiology, University of Turin, Grugliasco, Turin, Italy; ‡University of Pittsburgh Cancer Institute, University of Pittsburgh, Pittsburgh, PA, USA; §Department of Surgery, School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA; ¶Department of Immunology, School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA; #Department of Pathology, School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA; **Cancer Immunology Program, School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA

Abstract

Defects in HLA class I antigen-processing machinery (APM) component expression and/or function are frequent in human tumors. These defects may provide tumor cells with a mechanism to escape from recognition and destruction by HLA class I antigen-restricted, tumor antigen–specific cytotoxic T cells. However, expression and functional properties of MHC class I antigens and APM components in malignant cells in other animal species have been investigated to a limited extent. However, this information can contribute to our understanding of the mechanisms underlying the association of MHC class I antigen and APM component defects with malignant transformation of cells and to identify animal models to validate targeted therapies to correct these defects. To overcome this limitation in the present study, we have investigated the expression of the catalytic subunits of proteasome (Y, X, and Z) and of immunoproteasome (LMP2, LMP7, and LMP10) as well as of MHC class I heavy chain (HC) in 25 primary feline mammary carcinomas (FMCs) and in 23 matched healthy mammary tissues. We found a reduced expression of MHC class I HC and of LMP2 and LMP7 in tumors compared with normal tissues. Concordantly, proteasomal cleavage specificities in extracts from FMCs were different from those in healthy tissues. In addition, correlation analysis showed that LMP2 and LMP7 were concordantly expressed in FMCs, and their expression was significantly correlated with that of MHC class I HC. The abnormalities we have found in the APM in FMCs may cause a defective processing of some tumor antigens.

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Introduction

The recognition of tumor cells by MHC class I antigen-restricted, tumor antigen (TA)–specific cytotoxic T lymphocytes is mediated by β_2-microglobulin (β_2-m)-MHC class I heavy chains (HCs)–TA-derived peptide complexes. The generation and expression of these trimolecular complexes on the cell membrane requires the integrity of three essential pathways: 1) the degradation of proteins into peptides in the cytoplasm, 2) the transport of the peptides into the endoplasmic reticulum, and 3) the peptide loading on nascent MHC class I molecules as well as their transport to the cell surface [1]. The peptides

Address all correspondence to: Alessandra Favole, BS, Department of Animal Pathology, School of Veterinary Medicine, University of Turin, Via Leonardo da Vinci 44, 10095 Grugliasco, Turin, Italy. E-mail: alessandra.favole@unito.it

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presented by MHC class I antigens are generated by the degradation of ubiquitin-marked intracellular proteins by the proteasome, a multimeric proteolytic complex; its β-subunits delta (Y), MB1 (X), and Z are responsible for its catalytic activity [2,3]. When cells are incubated with interferon-γ (IFN-γ), the three catalytic subunits Y, X, and Z of the proteasome are replaced by the low-molecular-weight proteins LMP2, LMP7, and LMP10, respectively, leading to the replacement of constitutive proteasome with the so-called immunoproteasome [4]. It is well established that the presence of these IFN-γ–induced subunits changes catalytic activity against model peptide substrates. They enhance cleavages after hydrophobic, basic, and branched chain residues but suppress cleavages after acidic residues. Therefore, immunoproteasomes generate a different spectrum of oligopeptides compared with proteasomes. This type of peptides has been proposed to enhance antigen presentation [5] because the transporter associated with antigen processing (TAP) and MHC class I molecules preferentially bind peptides with carboxyl-terminal hydrophobic and basic residues over those with acidic residues.

Convincing experimental evidence has shown that malignant transformation of cells is frequently associated with defects in the expression of antigen-processing machinery (APM) components and HLA class I antigens in humans [6–8]. These defects may have functional significance because they may provide tumor cells with a mechanism to escape from recognition and destruction by HLA class I antigen-restricted, TA-specific cytotoxic T cells [9–16]. Furthermore, they may have clinical significance because they are often associated with the histopathologic characteristics of the lesions and/or with the clinical course of the disease [17–20]. Nevertheless, the expression and functional properties of MHC class I antigens and APM components in malignant cells in other animal species have been investigated to a limited extent. However, this information can contribute to our understanding of the mechanisms underlying the association of MHC class I antigen and APM component defects with malignant transformation of cells and to identify animal models to validate targeted therapies to correct these defects. To overcome this limitation in the present study, we have investigated the expression of the catalytic subunits of proteasome (Y, X, and Z) and of immunoproteasome (LMP2, LMP7, and LMP10) as well as of MHC class I HC in 25 primary feline mammary carcinomas (FMCs) and in 23 matched healthy mammary tissues. FMC has been selected for our studies because it is the third most common neoplasm in cats and is an informative model for the study of tumor biology in other species, including humans. Furthermore, we have tested the functional properties of proteasome and immunoproteasome in extracts of FMC lesions.

Materials and Methods

Patients and Tissue Samples

Mammary tumors and normal tissues. Twenty-five primary FMCs and 23 matched normal mammary epithelium were collected in total. Paraffin wax blocks of 16 mammary tumor tissues with 14 matched normal mammary epithelium were retrieved from the archives of our laboratory. Each block was reviewed by a pathologist (T.M.) to confirm the diagnosis, and mammary tumors were categorized according to the type of carcinoma (complex, simple solid, simple tubulopapillary, anaplastic, or others). Mammary tumor samples came from primary masses removed for therapeutic purposes at our hospital or other referring hospitals. In the course of the described studies, an additional nine surgical samples of primary mammary tumor and corresponding normal mammary tissue were collected, with the owners' consent. A portion of each tissue sample was fixed in 4% buffered formalin and embedded in paraffin following standard procedures, and a second portion was snap frozen for proteasome activity assay. Samples were stored at −80°C until use.

The mammary tumors collected included 16 simple tubular carcinomas, 4 simple tubulopapillary carcinomas, 3 simple solid carcinomas, and 1 adenocarcinoma. Four of the mammary tumors examined were known to have regional lymph node metastasis. Of the 25 cats with mammary masses, 18 were sexually intact females, aged 8 to 18 years.

Monoclonal Antibodies

The mouse monoclonal antibody (mAb) HC-10, which recognizes a determinant expressed on all of the β_2m-free HLA-B HCs and on β_2m-free HLA-A10, -A28, -A29, -A30, -A31, -A32, and -A33 HCs [21,22]; the LMP2-specific mAb SY-1 [23]; the LMP7-specific mAb HB-2 [23], the LMP10-specific mAb TO-7 [23]; the Y-specific mAb SY-5 [23]; the X-specific mAb SJJ-3 [23]; and the Z-specific mAb NB-1 [23], were developed and characterized as described. mAbs were purified from ascitic fluid by sequential precipitation with ammonium sulfate and caprylic acid [24]. Purity and activity of mAb preparations were monitored by SDS-PAGE and by reactivity with the corresponding antigens in Western blot analysis.

Immunohistochemical Staining

Immunoperoxidase staining of tissue sections was performed using the EnVision+ system (DakoCytomation, Carpinteria, CA). Briefly, 5-μm-thick paraffin-embedded tissue sections were deparaffinized with xylene and rehydrated by passage through decreasing concentrations of ethanol. Tissue sections were incubated for 40 minutes at 98°C in citrate solution, pH 6.0, for antigen retrieval. Then tissue sections were incubated with 3% H_2O_2 in methanol for 15 minutes at room temperature to block endogenous peroxidase activity. After repeated rinsing with phosphate-buffered saline solution (PBS), tissue sections were incubated overnight at 4°C with an optimal amount of mAb. To reduce the background staining, mAb preparations were diluted with PBS supplemented with 10% bovine serum albumin (BSA). After washing, tissue sections were incubated for 30 minutes at room temperature with the EnVision+ reagent. Staining was visualized using the liquid 3,3′-diaminobenzidine substrate-chromogen system (DakoCytomation). Tissue sections were counterstained for 2 minutes with Mayer hematoxylin solution. The percentage of stained tumor cells in each lesion was evaluated independently by two investigators (A.F. and M.T.). Variations in the percentage of stained cells enumerated by the two investigators were within a 10% range. In consideration of the error, we evaluated the percentage of stained tumor cells at 10% levels. Normal lymphocytes and vessel endothelium were used in each specimen as internal positive controls. Negative controls were performed by omitting primary antibodies. Results were scored as (+), (±), and (–) when the percentage of stained cells in the tissue was more than 75%, 25% to 75%, and less than 25%, respectively, according to the criterion established by the HLA and Cancer component of the 12th International Histocompatibility Workshop [25].

Total Proteins

Samples of mammary carcinoma and healthy mammary gland were collected from the same animal during surgery, frozen, homogenized in

ice-cold extraction buffer (50 mM Tris-HCl pH 7.5, 1 mM dithiothreitol, 250 mM sucrose, 5 mM $MgCl_2$, 0.5 mM EDTA, 2 mM ATP) using an ultraturax DIAX900 homogenizer (Heidolph Instruments, Kelheim, Germany) and centrifuged at 3616*g* for 20 minutes at 4°C. The protein concentration in supernatants was determined using the QUICK START Bradford Dye Reagent 1× (Biorad, Hercules, CA) using a standard curve constructed with BSA. Samples were stored at −80°C until use.

Immunoblot Analyses

Immunoblot analyses of the immunoproteasomal catalytic β subunits, LMP2, LMP7, and LMP10 in both carcinomas and healthy mammary tissues extracts were performed as already described [26]. Briefly, 60 μg of total proteins was separated on a 12% SDS-PAGE gel, and proteins were transferred on a Hybond-P membrane (Amersham Pharmacia, Buckinghamshire, United Kingdom). Membranes were stained with Ponceau Red before incubating with the primary antibody to confirm that similar amounts of proteins had been transferred. The membrane was then incubated in a blocking buffer (2% BSA in 1× PBS; 0.1% Tween-20), followed by incubation with rabbit antisera against LMP2 and LMP7 (a kind gift from Dr K. Tanaka, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan) and with mouse monoclonal anti-LMP10 Ab TO-7 (a kind gift from Prof Ferrone).

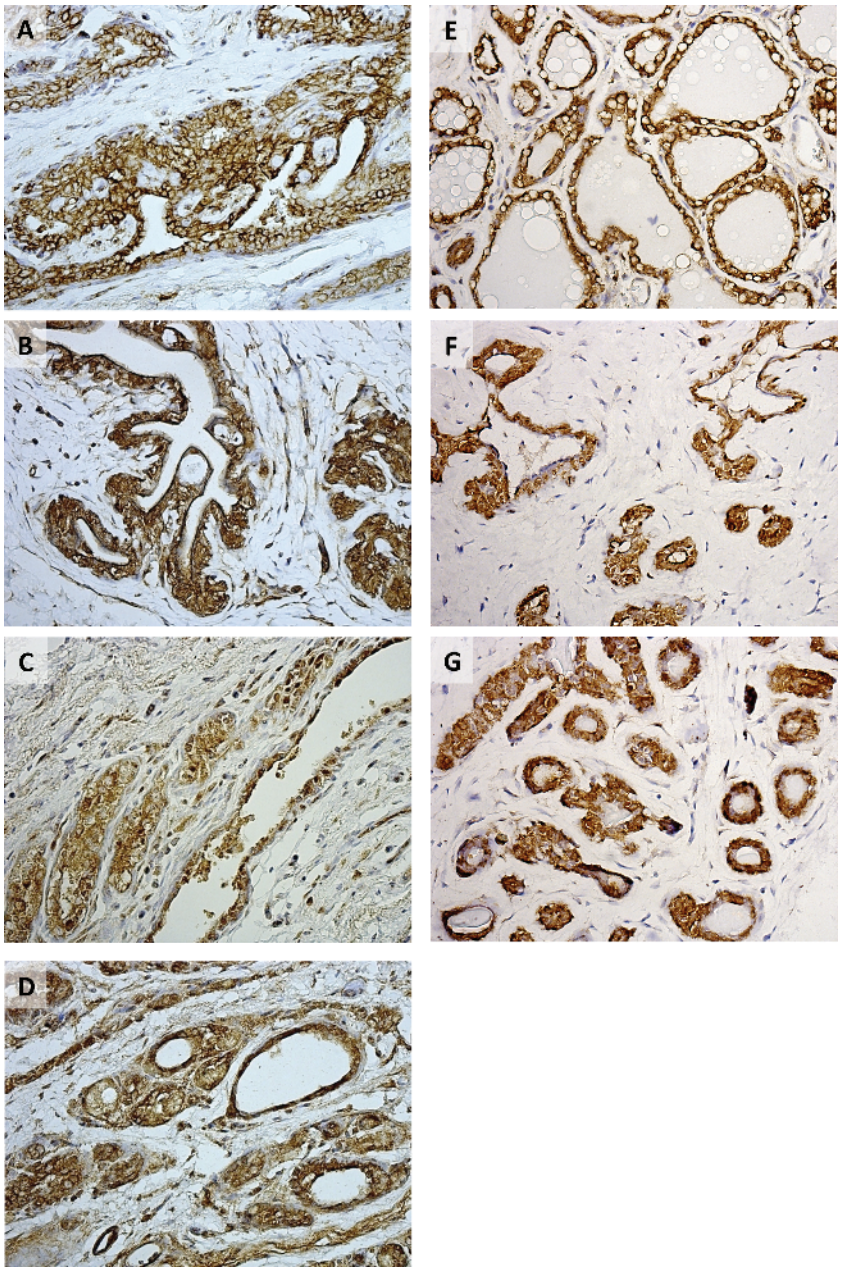

Figure 1. Immunohistochemical staining of MHC class I HCs and antigen-processing machinery (APM) components in normal mammary gland tissue. Sections of formalin-fixed, paraffin-embedded, healthy mammary gland tissue were stained in immunoperoxidase reaction with mouse anti–MHC class I HC (A), anti-LMP2- (B), anti-LMP7- (C), anti-LMP10- (D), anti-Y- (E), anti-X- (F), and anti-Z- (G) specific antibodies. The sections were then counterstained with Mayer hematoxylin solution. Surface epithelia are homogeneously stained by the seven antibodies. Whereas anti-MHC class I was localized at the cell surface, antiproteasome and immunoproteasome catalytic subunits staining was localized in the cytoplasm and/or nuclei. Original magnification, ×400.

Bound antibodies were visualized using the ECL technique (ECLPlus Western Blotting Detection Reagents; Amersham Pharmacia).

Proteasome Activity Assays

Peptidase activities of proteasomes were assayed by monitoring the production of 7-amino-4-methylcoumarin (amc) from fluorogenic peptides as previously described [27]. Briefly, Suc-LLVY-amc (for the chymotrypsin-like activity) and Z-YVAD-amc (for the caspase-like activity) (BACHEM, Bubendorf, Switzerland) were used at a final concentration of 100 μM in 20 mM Tris-HCl pH 7.5, 1 mM ATP, 2 mM $MgCl_2$ and 0.2% BSA. Reactions were started by adding an aliquot of tissue extract and the fluorescence of released amc (excitation, 380 nm; emission, 460 nm) was monitored continuously at 37°C with a Carry Eclipse spectrofluorimeter (VARIAN, Palo Alto, CA). Background activity (caused by nonproteasomal degradation) was determined by adding the proteasome inhibitor MG132 (BACHEM) at a final concentration of 10 μM. Specific proteasomal activity was therefore calculated as the fraction of the total activity that could be inhibited by MG132.

Statistical Analysis

Correlation of APM component expression among themselves and with MHC class I HC expression was analyzed using the Spearman rank correlation coefficient. Furthermore, the Wilcoxon test was used to establish if the chymotrypsin-like and caspase-like activities differed significantly between mammary carcinoma and healthy mammary gland. Data were graphically visualized using box plots.

$P < .05$ was considered statistically significant.

Results

Constitutive Expression of MHC Class I APM Components in Normal Mammary Epithelium

Because the constitutive expression profile of the different APM components in normal mammary epithelium is not known, all the antibodies were first tested on formalin-fixed, paraffin-embedded, healthy mammary gland tissues. As shown in Figure 1, mammary gland epithelium was homogeneously stained by all the antibodies tested. The proteasome subunits and IFN-γ–inducible proteasome subunits LMP2, LMP7, and LMP10 were strongly expressed in the cytoplasm and nuclei of normal mammary cells, whereas immunohistochemical staining with the MHC class I HC-specific mAb HC-10 resulted in a cytoplasmic/membranous staining. Table 1 summarizes the results obtained by immunoperoxidase staining of 23 healthy mammary gland samples with the panel of APM component-specific mAb tested.

Differential Expression of Proteasome and Immunoproteasome Subunits in FMC Lesions

Twenty-five primary FMCs, including 16 simple tubular carcinomas, 4 simple tubulopapillary carcinomas, 3 simple solid carcinomas, and 1 adenocarcinoma, were immunohistochemically stained with X-, Y-, Z-, LMP2-, LMP7-, and LMP10-specific mAb and with MHC class I HC-specific mAb. Normal lymphocytes and vessel endothelia were used in each specimen as internal controls. This analysis showed that the expression of X, Y, Z, and LMP10 in the neoplastic component of the lesion was similar to that in the corresponding healthy mammary tissue in 96% and in 92% of the cases evaluated for proteasome subunits and for the immunoproteasome subunit LMP10, respectively

Table 1. Expression of MHC Class I Antigen-Processing Machinery Components in Healthy Mammary Epithelia and Matched Primary FMCs.

Staining Score	MHC I HC		LMP2		LMP7		LMP10		Y		X		Z	
	Healthy	Tumor	Healthy	Tumor	Healthy	Tumor	Healthy	Tumor	Healthy	Tumor	Healthy	Tumor	Healthy	Tumor
+	91.3%* (21/23)†	24% (6/25)	91.3% (21/23)	32% (8/25)	87% (20/23)	28% (7/25)	95.7% (22/23)	88% (22/25)	95.7% (22/23)	92% (23/25)	100% (23/23)	96% (24/25)	95.7% (22/23)	92% (23/25)
±	8.7% (2/23)	44% (11/25)	8.7% (2/23)	48% (12/25)	13% (3/23)	32% (8/25)	4.3% (1/23)	8% (2/25)	4.3% (1/23)	8% (2/25)	0 (0/23)	4% (1/25)	4.3% (1/23)	8% (2/25)
–	0 (0/23)	32% (8/25)	0 (0/23)	20% (5/25)	0 (0/23)	40% (10/25)	0 (0/23)	4% (1/25)	0 (0/23)	0 (0/25)	0 (0/23)	0 (0/25)	0 (0/23)	0 (0/25)

Samples were scored as positive (+), heterogeneous (±), and negative (–), when the percentage of stained cells in the entire sample was greater than 75%, between 75% and 25% inclusive, and less than 25%, respectively.
*Percent of samples with the indicated staining score.
†Number of samples with the indicated staining score in relation to the total number of cases tested.

(Table 1). On the contrary, tumor lesions were found to have a highly variable expression profile ranging from a total loss, to heterogeneous but decreased expression levels, to normal expression for LMP2, LMP7, and MHC class I HC (Table 1 and Figure 2). The expression of MHC class I HC and IFN-γ–induced catalytic subunits LMP2 and LMP7 was reduced in tumor lesions compared with matched healthy tissues. As shown in Table 1, among the seven proteins examined, MHC class I HC and LMP7 showed the greatest reduction in expression, being not detectable in 32% and 40% of the lesions, respectively, and down-regulated in 44% and 32% of the lesions, respectively. X showed the smallest reduction, being positive in 96% and downregulated in only 4% of the lesions. LMP2 was not detectable in 20% and downregulated in 48% of the FMC lesions. Y, Z, and LMP10 were positive in 92% and downregulated in 8% of the lesions. Taken together, these results clearly demonstrate that, compared with healthy mammary tissues, mammary carcinomas did not differ in the expression levels of the proteasome subunits X, Y, and Z but displayed a down-regulation of the two immunoproteasome subunits LMP2 and LMP7 and of MHC class I HC.

Additional analyses tested whether the expression level of APM components is correlated to that of MHC class I HC. Analysis by the Spearman rank correlation coefficient showed that LMP2 and LMP7 expression was significantly correlated with that of MHC class I HC

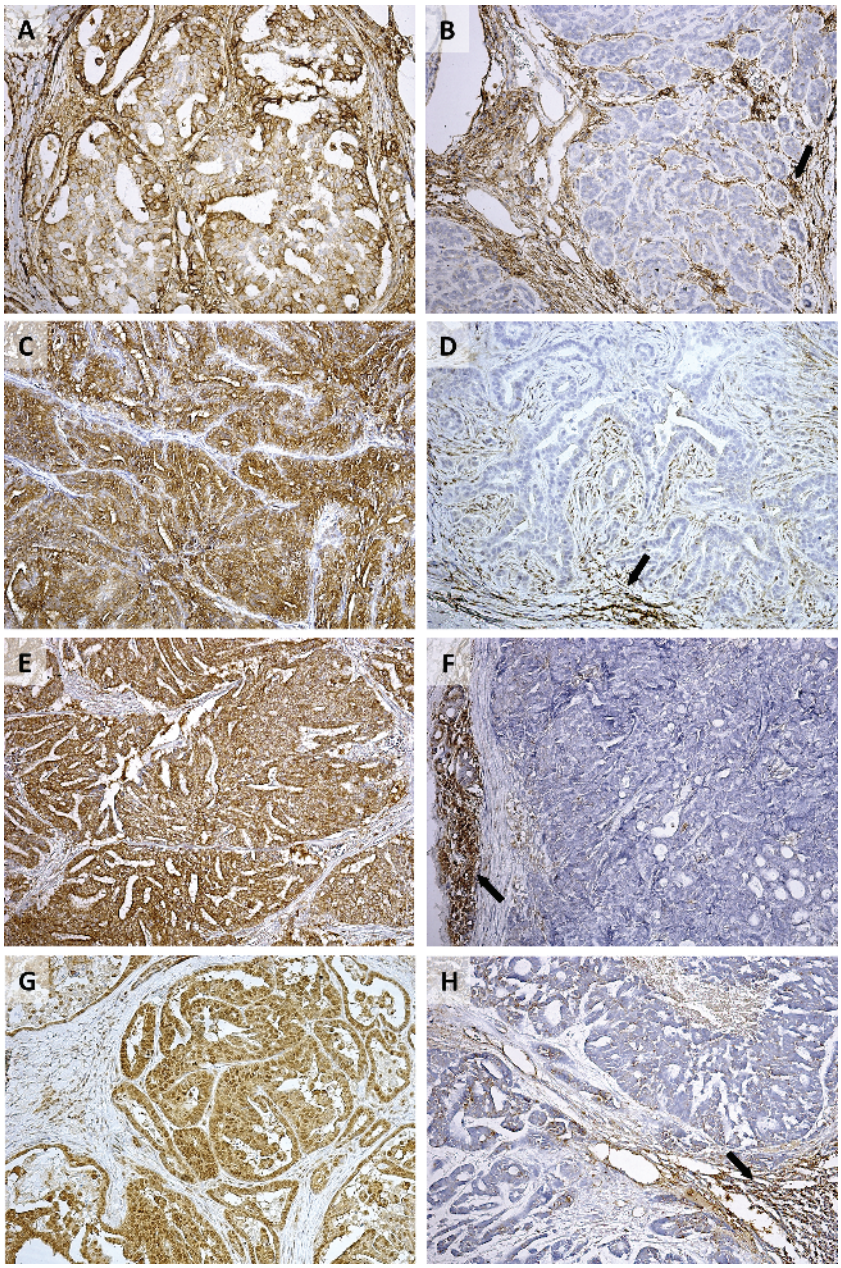

Figure 2. Immunohistochemical staining patterns of formalin-fixed, paraffin-embedded primary FMC lesions with MHC class I antigen and APM component-specific mAbs. The staining with MHC class I HC-specific mAb (A and B), with LMP2-specific mAb (C and D), with LMP7-specific mAb (E and F) and with LMP10-specific mAb (G and H) was scored as positive (A, C, E, and G) and as negative (B, D, F, and H), respectively. Normal lymphocytes, which serve as the internal control, are stained as well (arrow). Original magnification, ×200.

Table 2. Chymotrypsin-like and Caspase-like Activities in Carcinoma Lesions and Related Healthy Tissue.

	Healthy Mammary Tissue	Feline Mammary Carcinoma
Chymotrypsin-like activity (nmol Suc-LLVY-AMC cleaved/mg min)	0.139 ± 0.013 (0.141)	0.398 ± 0.116 (0.273)
Caspase-like activity (nmol Z-YVAS-AMC cleaved/mg min)	0.003 ± 0.0005 (0.0025)	0.011 ± 0.004 (0.0055)

Data are presented as the mean ± SEM (median) of the specific activities of all animals analyzed.
SEM, standard error of the mean.

($r = 0.43$, $P = .017$ and $r = 0.70$, $P < .0001$, respectively). Interestingly, LMP2 and LMP7 are concordantly expressed in FMCs ($r = 0.58$, $P = .001$), suggesting that they are regulated by similar mechanisms.

Enhanced Proteasomal Activity in Feline Mammary Carcinoma

To investigate whether the down-regulation of IFN-γ–induced catalytic subunits, LMP2 and LMP7, detected by immunohistochemistry had functional relevance, we next analyzed the cleavage specificity of proteasomes in homogenates of FMCs and related healthy mammary tissues by means of specific fluorogenic substrates. By this approach, we demonstrated that chymotrypsin-like activity, the main proteasomal proteolytic activity which is rate limiting in protein degradation, is nearly three-fold higher in FMCs than in healthy mammary tissues (Table 2 and Figure 3*A*; $Z = -2.521$ $P = .012$, Wilcoxon test). In addition, caspase-like activity was significantly enhanced in FMCs albeit to a lesser extent (Table 2 and Figure 3*B*; $Z = -2.536$ $P = .011$, Wilcoxon test). Together, these results demonstrate that chymotrypsin and caspase-like activities of proteasomes are increased in extracts from FMCs.

In line with the findings of our study, it is generally assumed that inflammatory cells constitutively express immunoproteasome catalytic subunits (Figure 2) [2]. Therefore, to determine whether the enhanced proteasomal activity detected in FMCs resulted from the constitutively expression of LMP2, LMP7, and LMP10 in inflammatory cells infiltrating the tumor, we next analyzed the expression of immunoproteasomes catalytic β subunits in total homogenates of FMCs and related healthy mammary tissues. To this purpose, tissue extracts from two surgically removed mammary gland lesions (scored as negative in immunohistochemistry analysis for LMP2 and LMP7 expression in tumoral cells) and matched healthy tissues were analyzed by Western blot analysis using specific antibodies for immunoproteasomal catalytic β subunits. This analysis showed (Figure 4) an enhanced expression of LMP2, LMP7, and LMP10 in the neoplastic component compared with corresponding healthy tissue in all evaluated cases. Taken together, these results clearly demonstrate that three important components of the APM strongly induced by γ-interferon are expressed at significantly higher levels in total homogenates of FMCs than in healthy mammary gland epithelium. These data corroborate the hypothesis that the enhanced proteasomal proteolytic activity observed in homogenates from FMCs may be due to tumor infiltration by inflammatory cells, which constitutively express immunoproteasome catalytic subunits.

Discussion

Immunohistochemical staining of surgically removed FMCs and surrounding autologous normal mammary tissue with mAb has shown defects in the expression of MHC class I HC and of the immunoproteasome subunits LMP2 and LMP7 in at least 32% of the lesions analyzed. In contrast, the expression of the proteasome subunits X, Y, and Z and of the immunoproteasome subunit LMP10 in FMCs was similar to that in normal mammary tissues in most lesions tested. To the best of our knowledge, this is the first report to analyze the expression of MHC class I HC and proteasome and immunoproteasome subunits in FMCs. Furthermore, the present study is the first one to show that changes in LMP2 and LMP7 expression have functional relevance because proteasomal cleavage specificities in extracts from FMCs were different from those in the autologous healthy tissues. The up-regulation of the proteasomal chymotrypsin- and to a lesser

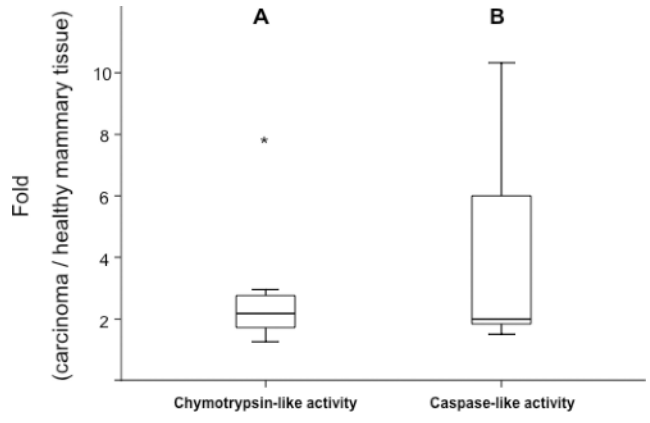

Figure 3. Increase in proteasomal chymotrypsin-like activity and caspase-like activity in tissue extracts from mammary carcinomas compared with healthy mammary epithelium. Chymotrypsin-like activity (A) is nearly three-fold higher in FMCs than in healthy mammary tissues. In addition, caspase-like activity (B) was significantly enhanced in FMCs albeit to a lesser extent.

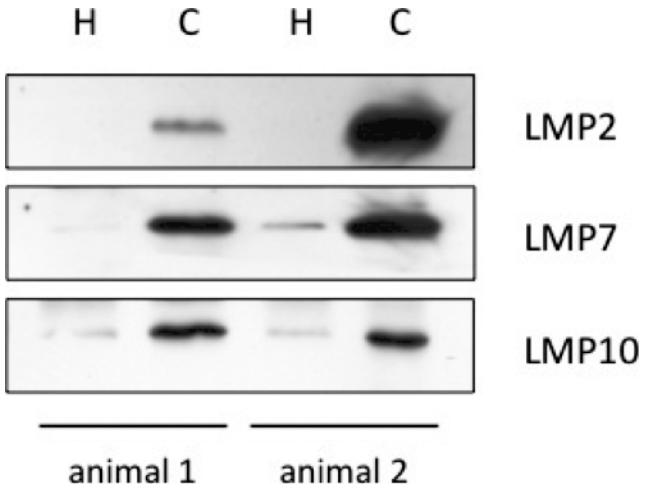

Figure 4. Enhanced APM component expression in surgically removed FMC lesions compared to matched healthy tissues. Two representative Western blot analyses of LMP2, LMP7, and LMP10 expression. C indicates carcinomas; H, corresponding healthy mammary tissues.

extent caspase-like activities found in homogenates from FMCs compared with healthy mammary tissues was unexpected because of the down-regulation of two immunoproteasome subunits found in FMCs. This apparent paradox may reflect an up-regulation of the constitutive proteasome catalytic subunits (X, Y, and Z) in the neoplastic tissue, perhaps to compensate for the down-regulation of the immunoproteasome subunits. Expression of the X and Y genes is reciprocal to that of the LMP genes: X and Y are upregulated in mutant cell lines lacking LMPs [28]. In this regard, it is noteworthy that our immunohistochemical analysis evaluated the number of stained tumor cells in the entire lesion but did not evaluate the staining intensity at the level of single-stained tumoral cells. Therefore, in tumor lesions, an increased expression of constitutive proteasome catalytic subunits cannot be excluded. An additional mechanism contributing to the enhanced proteasomal proteolytic activity observed in homogenates from FMCs may be represented by the infiltration of tumors with inflammatory cells, which constitutively express immunoproteasome catalytic subunits (Figure 2) [2]. Immunoblot analysis supports this hypothesis: tissue extracts from two surgically removed mammary gland lesions, scored in immunohistochemistry analysis as negative for LMP2 and LMP7 expression in tumoral cells, on the contrary by Western blot analysis showed an enhanced expression of immunoproteasome catalytic subunits compared with healthy mammary tissues. In line with our results, defective expression of MHC class I antigens and APM components has been demonstrated in almost all works by immunohistochemistry analysis because this technique allows to discriminate between inflammatory and tumoral cells [31,32,36].

The changes in the proteasomal cleavage specificities found in extracts from FMCs argue in favor of the possibility that LMP2 and LMP7 down-regulation in FMCs affects the repertoire of TA-derived peptides expressed by tumor cells. Whether these changes influence the recognition of mammary tumor cells by MHC class I antigen-restricted, TA-derived peptide-specific T cells remains to be investigated. Should this be the case, cats with mammary tumors represent a useful model to investigate the contribution of immunoproteasome subunit defects to escape mechanisms used by tumor cells to avoid recognition and destruction by MHC class I antigen-restricted, TA-specific cytotoxic T lymphocyte. Furthermore, cats with mammary tumors may represent useful models to develop and test strategies to counteract escape mechanisms caused by changes in the expression and functional properties of immunoproteasome subunits.

The expression of immunoproteasome and proteasome subunits has been investigated in human mammary tumors. The phenotype of FMCs we have described resembles that of human breast carcinoma lesions as Gobbi et al. [29] found a down-regulation of LMP2 and MHC class I HC in 51.4% and 40.0% of the lesions tested, respectively. Whereas all normal tissues and benign lesions were positive for β_2-m and HLA class I HC, total loss of HLA class I antigens was found in 43% of the breast primary tumors and in 70% of the lymph node metastases [30]. To the best of our knowledge, the expression of immunoproteasome subunits has been described in a number of human solid tumors, whereas that of proteasome subunits has been investigated in a much lower number of solid tumors. The frequency of LMP2 and LMP7 down-regulation we have found in FMCs is similar to that described in renal, cervical, and head and neck carcinomas [15,20,31]. In addition, an unbalanced expression of LMP2, LMP7, and LMP10 has been described in many human solid tumors [20,34,35]. Nevertheless, the expression of proteasome subunits has been described only in human brain and bladder carcinomas [32,33]. As we have described in this study, the X, Y, and Z housekeeping proteasomal subunits, the LMP10 immunoproteasomal subunit, were detected in most medulloblastoma and astrocytic tumor lesions [32].

It is generally assumed that immunoproteasome subunits expression is constitutive only in immunologic cells, whereas it is induced in other cells on exposure to cytokines (i.e., IFN-γ), thereby increasing the number of peptides capable of binding MHC class I antigens [5]. At variance with this notion, we observed basal expression of LMP2, LMP7, and LMP10 in normal mammary epithelial cells. In fact, LMP2, LMP7, and LMP10 seem coordinately expressed in all healthy mammary tissues tested in our study. It should be stressed that the expression of immunoproteasome subunits in nonimmune cells under basal conditions is not unique to feline mammary tissue because it has also been described in human mammary, renal, laryngeal, neural, bladder, prostate, and cervical tissues [15,18,20,29,32–36].

From a methodological view point, it is noteworthy that the present study is one of the very few ones that compare the expression of MHC class I HC in malignant cells and autologous surrounding tissues. To the best of our knowledge, a similar comparison has been done only in a study that analyzed the expression of APM components in bladder carcinoma cells and in surrounding normal cells. The conclusions from these studies about changes in the expression of APM components in malignant cells are more meaningful than those derived from most of the published studies, including our own, which assess the expression of these molecules in malignant cells by comparison with the endothelial cells and lymphoid cells present in the tissue section analyzed [36].

In summary, this study provides the first description of changes in the expression and function of MHC class I–related APM components in FMCs. Characterization of the role of these changes in the interactions of FMCs with the host immune system may contribute to our understanding of the molecular mechanisms used by tumor cells and may suggest targeted strategies to counteract these escape mechanisms.

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