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Cutting Edge

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Engagement of tumor cell surface MHC class I chain-related molecule A (MICA) to NKG2D stimulates NK and T cell antitumor immunity. Shedding of MICA by tumor cells facilitates tumor immune evasion, which may in part contribute to tumor progression. Thus, elucidating the mechanisms by which tumors shed MIC is of great importance for therapy to reinforce NK and T cell antitumor immunity. In this study, we report that the membrane type matrix metalloproteinase (MMP)14 mediates MICA shedding. Suppression of MMP14 expression blocks MICA shedding. Concomitantly, overexpression of MMP14 enhances MICA shedding. The regulation of MICA shedding by MMP14 is independent of the activity of a disintegrin and metalloproteinases, which have been reported to mediate MICA shedding. Finally, MMP14 expression in MICA-positive tumor cells regulates the sensitivity of tumor cells to NK cell killing. These findings suggest that MMP14 may be a new target for tumor immune therapy. ***The Journal of Immunology*****, 2010, 184: 3346–3350.**

The MHC class I chain-related molecule A (MICA) is a surface transmembrane protein expressed in most human epithelial tumors but is generally absent in normal tissues (1). Engagement of MICA by NKG2D triggers NK cells and costimulates Ag-specific CTL antitumor responses (2–4). Thus, MICA is proposed to mark nascent tumor cells for NKG2D immune surveillance (5). However, tumors can evade the NKG2D-mediated immunity by the shedding of MICA (6–11), which resulted in impaired NKG2D function on NK cells and CTLs (6–11) and expansion of immunosuppressive NKG2D$^+$ CD4 T cells (12).

Because of the apparent negative effects of tumor shedding of MICA on host antitumor immunity, understanding the mechanisms of MIC shedding may be of significance for targeting MIC shedding to reinforce host tumor immunity. The thiol isomerase ERp5 was shown to be required for MICA shedding, presumably through disulphide-bond exchange that renders MIC susceptible for proteolytic cleavage (13). Inhibitors to the broad-spectrum metalloproteinases, including the matrix metalloproteinases (MMPs), a disintegrin and metalloproteinases (ADAMs), and ADAM with thrombospondin repeats, have been shown to interfere with MIC shedding (14–16). Specific enzymes, such as ADAM10 and ADAM17, have been shown to contribute to MICA shedding (15). ADAM17 was found also contributing to the shedding of MICB, a MICA closely related molecule (16). In this study, we demonstrate evidence that the membrane type MMP (MT-MMP)14 directly mediates MICA shedding independent of ADAMs and that expression of MMP14 in tumor cells undermines tumor cell susceptibility to NK cell cytotoxicity.

Materials and Methods

Cell culture and flow cytometry analysis

The mouse prostate tumor cell lines TRAMP-C2 and MyC-CaP, the human prostate tumor cell line M12 and PC-3, and breast tumor cell line MCF-7 were all maintained in DMEM supplemented with 10% FCS, 25 μg/ml bovine pituitary extract, 5 μg/ml insulin, and 6 ng/ml recombinant human epidermal growth factor (Sigma-Aldrich, St. Louis, MO). Human MICA*01 allele was expressed in mouse tumor cells using the pBMN retroviral vector as previously described (17). MICA expression in tumor cell lines was assessed by flow cytometry poststaining with the anti-MIC (A/B) mAb 6D4.6 (Biolegend, San Diego, CA) or Western blotting with anti-MICA Ab H-300 (Santa Cruz Biotechnology, Santa Cruz, CA). NK-92 cells (American Type Culture Collection) were maintained in MEM-α media supplemented with 12.5% FCS, 12.5% horse serum, and 1000 U/ml of IL-2.

Short hairpin RNA construction and lentivirus

A stem-loop structure oligonucleotide containing a specific MMP or ADAM or the mock (clock gene) targeting sequence was designed using the RNAi codex program. Sequences of all the oligonucleotides used are listed in Supplemental Table I. dsDNA of the targeting sequence was synthesized by PCR and cloned under the control of the human U6 promoter in lentiviral vectors (gift of Dr. C. Z. Song, Department of Medical Genetics, University of Washington, Seattle, WA), which contains a GFP reporter. Production of lentiviruses and infection of target cells were as described (18). GFP-positive target cells were isolated by flow cytometry sorting 48 h postinfection.

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Abbreviations used in this paper: ADAM, a disintegrin and metalloproteinase; MIC, MHC class I chain-related molecule; MMP, matrix metalloproteinase; MT-MMP, membrane type matrix metalloproteinase; shRNA, short hairpin RNA; sMICA, soluble MHC class I chain-related molecule A.

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RT-PCR and real-time RT-PCR

RNA extraction, the first strand cDNA synthesis, and PCR reactions were performed using standard protocols. Primers used for amplifying mouse and human MMPs and ADAMs are listed in Supplemental Tables II and III. Real-time RT-PCR was done with the Lightcycler FastStart DNA Master Plus SYBR Green using a Roche Lightcycler following the manufacturer's protocol (Roche Applied Science, Indianapolis, IN). Each sample was assayed in triplicate.

Cloning and expression of MMP14

cDNA encoding the active form of human MMP14 was cloned by RT-PCR from M12 cells using the following primers: (forward) 5′-GCTCACGC-TTGGCACGTATGCCATTC-3′ and (reverse) 5′-GAGGCCCTGAATGG-CATACGTGCCAA-3′. The cDNA was re-expressed in MMP14-negative tumor cells using the pBMN-GFP retroviral system as described (17).

Immuoprecipitation and Western blotting

Detection of soluble MICA (sMICA) in the cell culture supernatant with immunoprecipitation and Western blotting was previously described (17). For detection of MMP14 expression, 50 μg clear cell lysate was resolved in SDS-PAGE and Western blotted with the anti-MMP14 MAB3329 (Millipore, Temecula, CA).

MICA shedding assay

The measurement of the degree of MICA shedding in cells has been previously described (19). Pervanadate was prepared by combining 100 mM sodium vanadate (Calbiochem, San Diego, CA) and 100 mM H_2O_2. At the time of treatment, culture medium was replaced with serum-free medium in the presence or absence of 100 μM freshly prepared pervanadate. After 1 h incubation, culture supernatant and cell lysate were collected for MICA ELISA measurement of MICA shedding.

Cytotoxicity assay

NK-92 cells were used as effector cells. Standard 4 h cytotoxicity assays with ^{51}Cr-labeled target cells were carried out as described (17). A total of 10 μg/ml anti-MIC (A/B) mAb 6D4.6 or 30 μg/ml anti-NKG2D mAb M585 (gift of Amgen, Thousand Oaks, CA) was used for blocking the specific interaction of MICA with NKG2D.

Results and Discussion

MMPs are involved in MICA shedding

Mass spectrometry analysis has identified that MICA was cleaved at multiple sites (13, 15, 17), which suggests that multiple proteases may be involved in MICA shedding. Because most human cancer cells express both MICA and MICB, we overexpressed human MICA in murine prostate tumor cell lines TRAMP-C2 and MyC-CaP to provide a clean system for investigating potential proteases in addition to ADAMs that may be involved in MICA shedding (Fig. 1*A*). ELISA showed that both two-cell lines constitutively shed MICA and that TRAMP-C2 shed MICA at a significantly higher degree than MyC-CaP cells (Fig. 1*B*).

Pervanadate, a tyrosine phosphatase inhibitor that regulates the activity of MT-MMPs and members of the ADAM family, has been shown to accelerate MMP14-mediated shedding of several membrane-anchored proteins (20–24). We showed that pervanadate also enhanced MICA shedding (Fig. 1*C*). In addition, pervanadate had a more pronounced enhancement in MICA shedding in TRAMP-C2-MICA cells than in MyC-CaP-MICA cells (Fig. 1*C*). Neither sodium vanadate nor hydrogen peroxide alone is able to enhance MICA shedding (Fig. 1*C*), confirming the specificity of pervanadate stimulation.

These observations indicate that TRAMP-C2 and MyC-CaP cells may express different proteases that are involved in MICA shedding. We thus analyzed the expression profiles of MMPs and ADAMs in the two-cell lines by RT-PCR. TRAMP-C2 and MyC-CaP have similar expression of

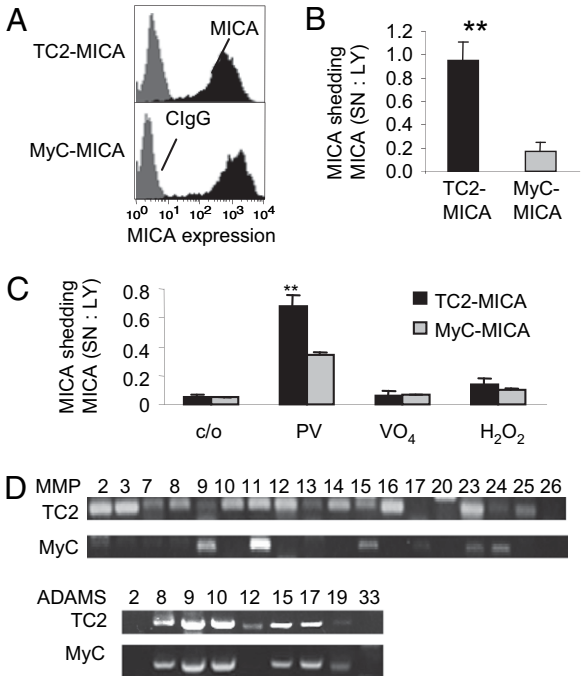

FIGURE 1. MICA shedding is mediated by MT-MMPs and is sensitive to pervanadate stimulation. *A*, Histogram of flow cytometry showing the surface MICA expression in TRAMP-C2 (TC2-MICA) and MyC-Cap (MyC-MICA) cells. *B*, Significantly elevated MICA shedding in TC2-MICA cells in comparison with MyC-MICA cells. *C*, Pervanadate stimulates MICA shedding in both TC2-MICA and MyC-MICA cells; however, with significantly elevated MICA shedding in TC2-MICA cells. *D*, Expression profiles of MMPs and ADAMs in TRAMP-C2 and MyC-CaP cells. Data represent results from three independent experiments. $^{**}p < 0.01$ when compared with MyC-MICA cells.

ADAMs, including ADAM8, 9, 10, 15, 16, and 17 (Fig. 1*D*). However, TRAMP-C2 expresses a diverse range of MMPs, most of which are not expressed by MyC-CaP cells (Fig. 1*D*). Together, these results suggest that the more pronounced MICA shedding by TRAMP-C2 cells than Myc-CaP cells is likely due to the activity of MMPs rather than ADAMs.

Short hairpin RNA suppression of MMP14 expression inhibits MICA shedding independent of ADAMs

To identify specific MMPs that are involved in MICA shedding, we constructed short hairpin RNA (shRNA) to specific mouse MMPs in a lentiviral expression vector that bears GFP as a reporter. We also constructed shRNA to ADAM10 and ADAM17, which have been shown to regulate MICA shedding (15). The expression of MMPs and ADAMs was suppressed in target cells by 70–90% with specific shRNA as evaluated by real-time RT-PCR (Fig. 2*A*). ELISA for MICA shedding showed that, except for MMP14, silencing of other MMPs did not significantly affect MICA shedding in TRAMP-C2-MICA cells (Fig. 2*B*) or MyC-CaP-MICA cells (Supplemental Fig. 1). With an 83% suppression of MMP14 expression in TRAMP-C2 cells (Fig. 2*A*, 2*C*), MICA shedding was inhibited by 65% (Fig. 2*B*). Consistent with other studies (15), silencing of ADAM10 or ADAM 17 inhibited MICA shedding (Fig. 2*B*, Supplemental Fig. 1). The efficiency of silencing MMP14 and the reduction in MICA shedding were also confirmed by Western blot analyses (Fig. 2*C*, 2*D*). These results suggest that MMP14 is involved in MICA shedding in TRAMP-C2-MICA cells. MMP14 can

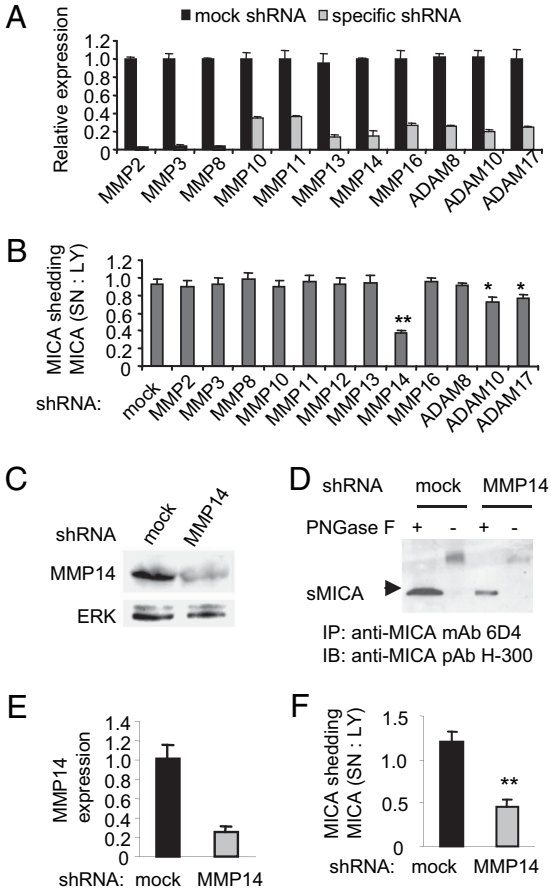

FIGURE 2. Inhibition of MMP14 expression or activity blocks MICA shedding. *A*, Quantitative RT-PCR showing shRNA suppression of MMP and ADAM expression in TC2-MICA cells. *B*, Degree of MICA shedding in TC2-MICA cells with the suppression of indicated MMP or ADAM expression. *C*, Western blots confirm the inhibition of MMP14 expression by shRNA in TC2-MICA cells at protein level. *D*, Western blots demonstrating that suppression of MMP-14 expression inhibits TC2-MICA cells releasing sMICA to the culture supernatant. *E* and *F*, Suppression of MMP14 expression inhibits MICA shedding in human prostate cancer M12 cells. *E*, Real-time RT-PCR showing shRNA suppression of MMP14 expression in M12 cells. *F*, Degree of MICA shedding by M12 cells with the suppression of MMP14 expression. Data represent results from five independent experiments. $^{*}p < 0.05$; $^{**}p < 0.01$ when compared with cells expressing mock shRNA.

activate pro-MMP2 and pro-MMP13 (25); however, direct inhibition of MMP2 or MMP13 expression by specific shRNA did not significant affect MICA shedding (Fig. 2*A*, 2*B*). Together, these results demonstrate that MMP14 directly regulates MICA shedding not by activating pro-MMP2 or pro-MMP13.

Suppression of MMP14 expression had no significant effect on ADAM10 or ADAM17 expression at the mRNA or protein level and vice versa (Supplemental Fig. 2). However, cosilencing of MMP14 and ADAM10 or ADAM17 generated an additive effect in inhibiting constitutive MICA shedding (Supplemental Fig. 2). These results suggest that MMP14 regulates MICA shedding in tumor cells independent of ADAM10 or ADAM17.

Human MMP14 shares 97% amino acid identity to mouse MMP14 (Supplemental Fig. 3). The shRNA to mouse MMP14 we have constructed is also specific to human MMP14. Similarly, with effective suppression of MMP14 expression in the human prostate cancer M12 cells, which express a comparable level of MMP14 to TRAMP-C2 cells (Supplemental Fig. 4), MICA shedding was also significantly reduced (Fig. 2*E*).

Overexpression of MMP14 enhances constitutive and pervanadate-stimulated MICA shedding

To provide further evidence that MMP14 is directly involved in MICA shedding, we overexpressed the active form of human MMP14 in the MMP14^{-} MyC-CaP cells (Fig. 3*A*). The results showed that overexpression of MMP14 significantly increased MICA shedding (Fig. 3*B*) and that the shedding was further enhanced by pervanadate stimulation (Fig. 3*C*). Further, flow cytometry analyses revealed that overexpression of MMP14 significantly reduced surface expression of MICA in MyC-CaP-MICA cells (Fig. 3*D*). We obtained similar results when MMP14 was overexpressed in the MMP14^{-} MCF-7 cells that express MICA but no MICB (Supplemental Figs. 4–6).

Expression of MMP14 regulates the susceptibility of tumor cells to NK cell killing

As shown in Fig. 4*A*, suppression of MMP14 expression significantly increased the susceptibility of TRAMP-C2-MICA and the M12 cells that express MICA but not MICB (Supplemental Fig. 6) to NK-92 cell killing. Concomitantly, overexpression of MMP14 in the MyC-CaP-MICA and the MICA-expressing MCF-7 cells markedly decreased the sensitivity of target cells to NK-92 cell killing (Fig. 4*B*). Masking tumor cell surface MICA with the mAb 6D4.6 (Fig. 4*A*, 4*B*) or NK cell surface NKG2D with the mAb M585 (Fig. 4*C*, 4*D*) completely diminished the sensitivity of MICA-expressing tumor cells to NK-92 cell killing independent of MMP14 expression. These results suggest that MICA is the sole target on tumor cells to be regulated by MMP14 to modulate tumor cell sensitivity to NK cells. Our previous studies have shown that sMIC resulting from shedding does not reach the

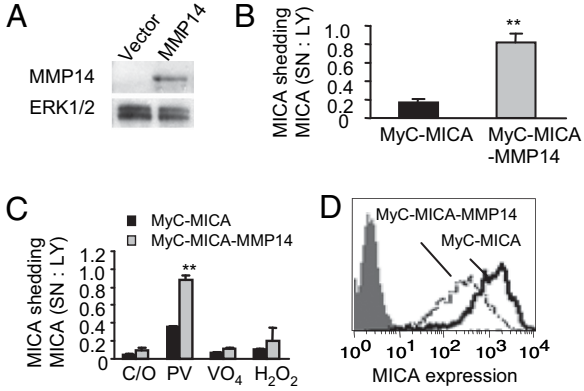

FIGURE 3. Overexpression of MMP14 increases constitutive and pervanadate-stimulated MICA shedding. *A*, Western blots showing MMP14 expression in MyC-MICA cells. *B*, Overexpression of MMP14 significantly increased constitutive MICA shedding in MyC-MICA cells. *C*, Overexpression MMP14 significantly increased pervanadate-stimulated MICA shedding in MyC-MICA cells. *D*, Flow cytometry histogram demonstrating that overexpression of MMP14 reduces surface MICA expression in MyC-MICA cells. Filled profile represents negative control MyC cells stained with the anti-MIC mAb 6D4.6. Data represent results from three independent experiments. $^{**}p < 0.01$ when compared with MyC-MICA cells.

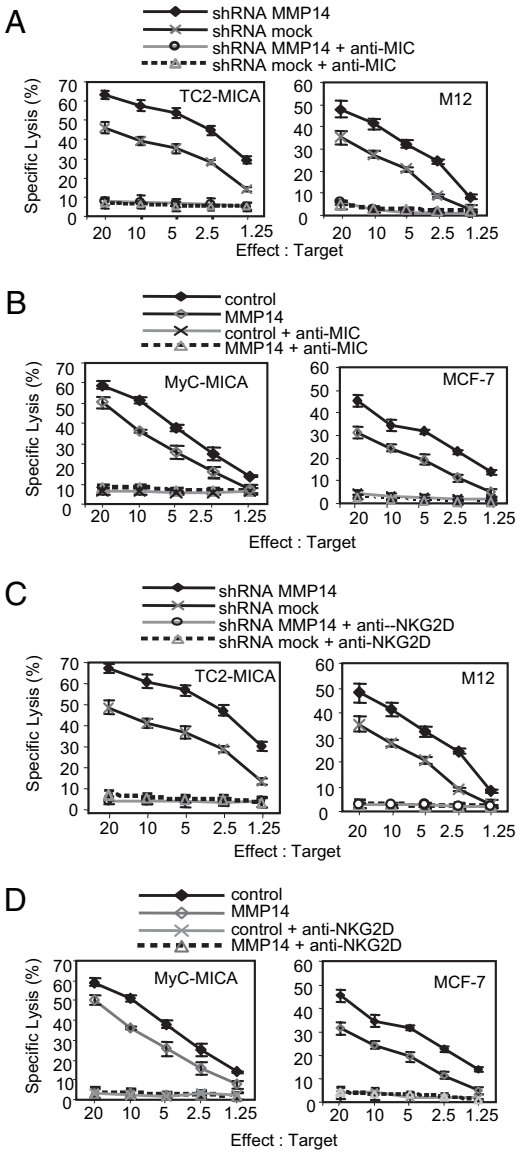

FIGURE 4. MMP14 regulates sensitivity of MICA-positive tumor cells to NK-92 cell cytotoxicity. *A*, Inhibition of MMP14 expression by shRNA significantly increased the susceptibility of tumor cells to NK-92 cell killing. *B*, Overexpression of MMP14 markedly decreased the sensitivity of tumor cells to NK-92 cell killing. Masking tumor cell surface MICA with anti-MIC mAb 6D4.6 (*A*, *B*) or masking NK-92 surface NKG2D with anti-NKG2D mAb M585 (*C*, *D*) completely diminished tumor cell sensitivity to NK-92 cell killing independent of MMP14 expression in tumor cells. Data represent results of three independent assays.

threshold level of downregulating NKG2D expression on effector NK cells in the 4-h in vitro cytotoxicity assay (17, 19). Thus, the impaired sensitivity of MMP14-positive tumor cells to NK cell killing in the 4-h in vitro assay is likely due to reduction in surface MICA expression. These data suggest that the activity of MMP14 in tumor cells may subvert host protective immunity in patients with cancer through reducing tumor cell surface MICA expression in addition to causing accumulation of sMICA, which impairs NKG2D function on effector cells (6–11). MMP14 expression has been shown to be upregulated by TGFβ (26). Thus, enhancing MICA shedding by MMP14 may be accounted for by one of the mechanisms by which TGFβ downregulates NKG2D function in patients with cancer (27).

Conclusion

Ectodomain shedding of cell surface transmembrane protein is commonly mediated by several proteases (23, 28). In this study, we demonstrated that shedding of the NKG2D ligand MICA is mediated by MMP14 independent of ADAMs. MMP14 is often overexpressed in malignant tumor tissues and plays an important role in cancer progression (29). In this study, we present the first evidence that MMP14 can undermine host immune response through shedding of tumor cell surface NKG2D ligand. Together with the evidence that obstruction of MIC shedding can prevent tumor growth in vivo (19), the current findings may endorse MMP14 as an important target for cancer immune therapy.

Disclosures

The authors have no financial conflicts of interest.

References

1. Groh, V., R. Rhinehart, H. Secrist, S. Bauer, K. H. Grabstein, and T. Spies. 1999. Broad tumor-associated expression and recognition by tumor-derived gamma delta T cells of MICA and MICB. *Proc. Natl. Acad. Sci. U.S.A.* 96: 6879–6884.
2. Bauer, S., V. Groh, J. Wu, A. Steinle, J. H. Phillips, L. L. Lanier, and T. Spies. 1999. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science* 285: 727–729.
3. Groh, V., R. Rhinehart, J. Randolph-Habecker, M. S. Topp, S. R. Riddell, and T. Spies. 2001. Costimulation of CD8alphabeta T cells by NKG2D via engagement by MIC induced on virus-infected cells. *Nat. Immunol.* 2: 255–260.
4. Wu, J., V. Groh, and T. Spies. 2002. T cell antigen receptor engagement and specificity in the recognition of stress-inducible MHC class I-related chains by human epithelial gamma delta T cells. *J. Immunol.* 169: 1236–1240.
5. Nausch, N., and A. Cerwenka. 2008. NKG2D ligands in tumor immunity. *Oncogene* 27: 5944–5958.
6. Groh, V., J. Wu, C. Yee, and T. Spies. 2002. Tumour-derived soluble MIC ligands impair expression of NKG2D and T-cell activation. *Nature* 419: 734–738.
7. Wu, J. D., L. M. Higgins, A. Steinle, D. Cosman, K. Haugk, and S. R. Plymate. 2004. Prevalent expression of the immunostimulatory MHC class I chain-related molecule is counteracted by shedding in prostate cancer. *J. Clin. Invest.* 114: 560–568.
8. Doubrovina, E. S., M. M. Doubrovin, E. Vider, R. B. Sisson, R. J. O'Reilly, B. Dupont, and Y. M. Vyas. 2003. Evasion from NK cell immunity by MHC class I chain-related molecules expressing colon adenocarcinoma. *J. Immunol.* 171: 6891–6899.
9. Raffaghello, L., I. Prigione, I. Airoldi, M. Camoriano, I. Levreri, C. Gambini, D. Pende, A. Steinle, S. Ferrone, and V. Pistoia. 2004. Downregulation and/or release of NKG2D ligands as immune evasion strategy of human neuroblastoma. *Neoplasia* 6: 558–568.
10. Märten, A., M. von Lilienfeld-Toal, M. W. Büchler, and J. Schmidt. 2006. Soluble MIC is elevated in the serum of patients with pancreatic carcinoma diminishing gammadelta T cell cytotoxicity. *Int. J. Cancer* 119: 2359–2365.
11. Jinushi, M., M. Vanneman, N. C. Munshi, Y. T. Tai, R. H. Prabhala, J. Ritz, D. Neuberg, K. C. Anderson, D. R. Carrasco, and G. Dranoff. 2008. MHC class I chain-related protein A antibodies and shedding are associated with the progression of multiple myeloma. *Proc. Natl. Acad. Sci. U.S.A.* 105: 1285–1290.
12. Groh, V., K. Smythe, Z. Dai, and T. Spies. 2006. Fas-ligand-mediated paracrine T cell regulation by the receptor NKG2D in tumor immunity. *Nat. Immunol.* 7: 755–762.
13. Kaiser, B. K., D. Yim, I. T. Chow, S. Gonzalez, Z. Dai, H. H. Mann, R. K. Strong, V. Groh, and T. Spies. 2007. Disulphide-isomerase-enabled shedding of tumour-associated NKG2D ligands. *Nature* 447: 482–486.
14. Salih, H. R., H. G. Rammensee, and A. Steinle. 2002. Cutting edge: down-regulation of MICA on human tumors by proteolytic shedding. *J. Immunol.* 169: 4098–4102.
15. Waldhauer, I., D. Goehlsdorf, F. Gieseke, T. Weinschenk, M. Wittenbrink, A. Ludwig, S. Stevanovic, H. G. Rammensee, and A. Steinle. 2008. Tumor-associated MICA is shed by ADAM proteases. *Cancer Res.* 68: 6368–6376.
16. Boutet, P., S. Agüera-González, S. Atkinson, C. J. Pennington, D. R. Edwards, G. Murphy, H. T. Reyburn, and M. Valés-Gómez. 2009. Cutting edge: the metalloproteinase ADAM17/TNF-alpha-converting enzyme regulates proteolytic shedding of the MHC class I-related chain B protein. *J. Immunol.* 182: 49–53.
17. Wang, X., A. D. Lundgren, P. Singh, D. R. Goodlett, S. R. Plymate, and J. D. Wu. 2009. An six-amino acid motif in the alpha3 domain of MICA is the cancer therapeutic target to inhibit shedding. *Biochem. Biophys. Res. Commun.* 387: 476–481.
18. Rubinson, D. A., C. P. Dillon, A. V. Kwiatkowski, C. Sievers, L. Yang, J. Kopinja, D. L. Rooney, M. Zhang, M. M. Ihrig, M. T. McManus, et al. 2003. A lentivirus-based system to functionally silence genes in primary mammalian cells, stem cells and transgenic mice by RNA interference. *Nat. Genet.* 33: 401–406.
19. Wu, J. D., C. L. Atteridge, X. Wang, T. Seya, and S. R. Plymate. 2009. Obstructing shedding of the immunostimulatory MHC class I chain-related gene B prevents tumor formation. *Clin. Cancer Res.* 15: 632–640.
20. Vecchi, M., L. A. Rudolph-Owen, C. L. Brown, P. J. Dempsey, and G. Carpenter. 1998. Tyrosine phosphorylation and proteolysis. Pervanadate-induced, metalloprotease-

dependent cleavage of the ErbB-4 receptor and amphiregulin. *J. Biol. Chem.* 273: 20589–20595.
21. Kajita, M., Y. Itoh, T. Chiba, H. Mori, A. Okada, H. Kinoh, and M. Seiki. 2001. Membrane-type 1 matrix metalloproteinase cleaves CD44 and promotes cell migration. *J. Cell Biol.* 153: 893–904.
22. Essick, E., S. Sithu, W. Dean, and S. D'Souza. 2008. Pervanadate-induced shedding of the intercellular adhesion molecule (ICAM)-1 ectodomain is mediated by membrane type-1 matrix metalloproteinase (MT1-MMP). *Mol. Cell. Biochem.* 314: 151–159.
23. Thathiah, A., and D. D. Carson. 2004. MT1-MMP mediates MUC1 shedding independent of TACE/ADAM17. *Biochem. J.* 382: 363–373.
24. Velasco-Loyden, G., J. Arribas, and F. López-Casillas. 2004. The shedding of betaglycan is regulated by pervanadate and mediated by membrane type matrix metalloprotease-1. *J. Biol. Chem.* 279: 7721–7733.
25. Osenkowski, P., M. Toth, and R. Fridman. 2004. Processing, shedding, and endocytosis of membrane type 1-matrix metalloproteinase (MT1-MMP). *J. Cell. Physiol.* 200: 2–10.
26. Kuo, Y. C., C. H. Su, C. Y. Liu, T. H. Chen, C. P. Chen, and H. S. Wang. 2009. Transforming growth factor-beta induces CD44 cleavage that promotes migration of MDA-MB-435s cells through the up-regulation of membrane type 1-matrix metalloproteinase. *Int. J. Cancer* 124: 2568–2576.
27. Eisele, G., J. Wischhusen, M. Mittelbronn, R. Meyermann, I. Waldhauer, A. Steinle, M. Weller, and M. A. Friese. 2006. TGF-beta and metalloproteinases differentially suppress NKG2D ligand surface expression on malignant glioma cells. *Brain* 129: 2416–2425.
28. Arribas, J., and A. Borroto. 2002. Protein ectodomain shedding. *Chem. Rev.* 102: 4627–4638.
29. Poincloux, R., F. Lizárraga, and P. Chavrier. 2009. Matrix invasion by tumour cells: a focus on MT1-MMP trafficking to invadopodia. *J. Cell Sci.* 122: 3015–3024.