A Novel Role of Metalloproteinase in Cancer-mediated Immunosuppression

A. C. Shu, S. M. Hsu, H. N. Ho, H. C. Lien, S. C. Huang, and R. H. Lin

INTRODUCTION

Escape from immune surveillance prefigures the rapid progression of human cancers (1–3). Various immune escape mechanisms in cancer have been proposed (4, 5). Certain cancer cells may secrete immunosuppressive factors to modify the host immune responses (1–5). The impaired antitumor immunity can also be related to defective immune regulation (4). In a variety of human malignancies, the expression of IL-2Rα on activated TILs was down-regulated (6–9). Because IL-2Rα plays a pivotal role in the development and propagation of functional T cells, its depressed expression may result in poor function of tumor-reactive cytotoxic lymphocytes. For elucidating the mechanism responsible for down-regulation of IL-2Rα, a coculture model of in vitro mixed autologous lymphocytes and tumor cells was established. Kinetic analysis showed that cervical cancer cells down-regulated IL-2Rα expression on encountered T cells. The amount of IL-2Rα mRNA in tumor-infiltrating lymphocytes-derived CD8+ T cells was compatible with that in the corresponding activated CD8+ T cells. Additional evidence showed that cervical cancer cells could induce the release of soluble IL-2Rα expression on encountered T cells. By using protease inhibition assays we demonstrated that tissue inhibitors of metalloproteinase abrogated the cancer-mediated IL-2Rα proteolytic process and restored the T-cell proliferation function. Immunohistochemical stainings further revealed prominent metalloproteinase (MMP) expressions, including MMP-1, MMP-2, and MMP-9, in cervical cancer tissues. Additional in vitro studies showed that MMP-9 mediates cleavage of IL-2Rα and down-regulates the proliferative capability of cancer-encountered T cells. Our findings suggest a new role of MMPs in tumor-mediated immunosuppression and provide a possible therapeutic potential for patients with cervical cancer.

ABSTRACT

Depressed immune responses have been observed frequently in cancer patients. In a variety of human malignancies, the expression of interleukin-2 receptor α (IL-2Rα) on activated tumor-infiltrating lymphocytes was down-regulated. Because IL-2Rα plays a pivotal role in the development and propagation of functional T cells, its depressed expression may result in poor function of tumor-reactive cytotoxic lymphocytes. For elucidating the mechanism responsible for down-regulation of IL-2Rα, a coculture model of in vitro mixed autologous lymphocytes and tumor cells was established. Kinetic analysis showed that cervical cancer cells down-regulated IL-2Rα expression on encountered T cells. The amount of IL-2Rα mRNA in tumor-infiltrating lymphocytes-derived CD8+ T cells was compatible with that in the corresponding activated CD8+ T cells. Additional evidence showed that cervical cancer cells could induce the release of soluble IL-2Rα expression on encountered T cells. By using protease inhibition assays we demonstrated that tissue inhibitors of metalloproteinase abrogated the cancer-mediated IL-2Rα proteolytic process and restored the T-cell proliferation function. Immunohistochemical stainings further revealed prominent metalloproteinase (MMP) expressions, including MMP-1, MMP-2, and MMP-9, in cervical cancer tissues. Additional in vitro studies showed that MMP-9 mediates cleavage of IL-2Rα and down-regulates the proliferative capability of cancer-encountered T cells. Our findings suggest a new role of MMPs in tumor-mediated immunosuppression and provide a possible therapeutic potential for patients with cervical cancer.

MATERIALS AND METHODS

Isolation of TILs, Cancer Cells, and Noncancerous Epithelial and Stromal Cells.

Tissue specimens were aseptically excised immediately after surgery from at least four different tumor sites and two sites of normal cervix. Tissue specimens were cut, minced, and pressed gently through a 380-μm sieve and then a 45.7-μm sieve with RPMI 1640 (Life Technologies, Inc., Grand Island, NY), as described previously (8, 11). PBMCs from the same patient were used as the source of autologous lymphocytes. We demonstrated for the first time that tumor-derived metalloproteinases can induce the proteolytic cleavage of IL-2Rα on activated T cells and suppress the proliferative capability of cancer-encountered T cells.

TILs (12–14). In human CC as well as in other cancers, tumor-specific autologous cytotoxic T cells are easily identified, but the cytotoxic potential of freshly isolated TILs is usually not expressed (10, 13, 14). This suggests the possible existence of as yet unspecified and perhaps complex immunosuppressive factors or cytokines that affect the antitumor capacity of these TILs in the tumor microenvironment. Recently, we and others have demonstrated that the expression of CD25 (IL-2Ra) on TILs derived from various human cancers was down-regulated (6–9). Because IL-2Rα is an essential receptor for the proliferation of T cells, its depressed expression in vivo may result in poor clonogenicity of cytotoxic lymphocytes (15–18) and cause immune suppression. Possible explanations for down-regulation of IL-2Rα on TILs are as follows: (a) intrinsic defects of IL-2Ra biosynthesis in the T cells of patients with cancer; (b) inhibition of IL-2Ra synthesis in TILs by exogenous mediators derived from cancer cells (7, 9); and/or (c) rapid cleavage of IL-2Rα by intrinsic or extrinsic means within the tumor milieu.

In this study, we used a MLTC model to evaluate the kinetic expression of IL-2Rα on in vitro-activated T cells and to infer the mechanism responsible for down-regulation of IL-2Rα on TILs in human CC. A coculture of MLNC from the same patient was used as control. TILs, cancer cells, and noncancerous cells were isolated by a mechanical dispersal technique as described previously (8, 11). PBMCs from the same patient were used as control. TILs, cancer cells, and noncancerous cells were isolated by a mechanical dispersal technique as described previously (8, 11). PBMCs from the same patient were used as control. TILs, cancer cells, and noncancerous cells were isolated by a mechanical dispersal technique as described previously (8, 11). PBMCs from the same patient were used as control.
was performed on a FACScalibur (Becton Dickinson, Inc.). Leukogate was used to measure the proportion of lymphocytes in the sample being studied without any scatter gates.

**Purification of CD8⁺ CTLs.** An indirect magnetic labeling system with a MACS (Miltenyi Biotec, Gladbach, Germany) was used for purification of CD8⁺ T cells. In brief, the cell suspension was incubated in a cold room (6°C to 12°C) for 10 min with 20 μl of Anti-CD8-MACS cocktail added (containing CD4, CD11b, CD16, CD19, CD36, and CD56 antibodies; Miltenyi Biotec). After repeated washing, 20 μl of MACS anti-Hepten microbeads/10⁶ total cells were added. The magnetically labeled cells were passed through a MACS separator in the magnetic field. The effluent was collected as a negative fraction representing the enriched CD8⁺ T-cell fraction. The cell purity was checked by flow cytometry after labeling with anti-CD3-PE. 

**Competitive RT-PCR Analysis.** Total cellular RNA of MACS-purified CD8⁺ T cells was extracted and reverse-transcribed (0.1–0.5 mg of RNA) in the presence of IL-2Rα oligonucleotide primers (Clontech Laboratories, Inc., Palo Alto, CA). First-strand cDNA was synthesized in a DNA thermal cycler (GeneAmp DNA thermal cycler 480; Perkin-Elmer Corp., Norwalk, CT), and the resulting cDNA was amplified by PCR. For competitive analysis of IL-2Rα, another set of synthetic competitors (IL-2Rα mimic primers) with a different length for amplification was used (Clontech Laboratories Inc.). Human β-actin primers and β-actin mimic primers were used as positive controls.

**Preparation of Protease Inhibitors.** Protease inhibitors (Boehringer Mannheim Biochemicals GmbH, Mannheim, Germany, and Calbiochem, La Jolla, CA) for a broad spectrum of inhibition were prepared. The selected protease inhibitors included antipain-dihydrochloride (papain, trypsin, cathepsin A- and B-inhibitor), bestatin (aminopeptidase inhibitor), chymostatin (ε-, β-, γ-, and δ-cathymotrypsin-inhibitor), E-64 (cysteine protease inhibitor), leupeptin (serine and cysteine protease, plasmin, trypsin, papain, and cathepsin B-inhibitor), pepstatin (aspartate protease), aprotinin (serine protease inhibitor, specific for plasmin, kallikrein, trypsin, and chymotrypsin), TIMP-2 (Mr, 72,000 and Mr, 92,000 gelatinase inhibitors), and MMP-inhibitor I (a broad spectrum gelatinase inhibitor). All reagents were prepared in azide-free deionized H₂O. Titrated concentrations of individual protease inhibitors were prepared according to the recommended working formula of the reagent supplier (Boehringer Mannheim Biochemicals). Drug toxicity was tested by the addition of titrated concentrations of reagents in lymphocyte culture for 24 h with >90% viable cells by the trypan-blue staining method.

**Protease Inhibition Assay.** Different protease inhibitors were added to the established MLTC. For maximal expression of IL-2Rα on the surface of T cells, PBMCs were fully activated with 10 μg/ml PHA for 24 h before MLTC. Autologous cancer cells (1 × 10⁶) were first cultured in 1 ml of culture medium containing protease inhibitors. The final concentrations of protease inhibitors as recommended in each tube were as follows: (a) antipain-dihydrochloride, 5 and 50 μg/ml; (b) aprotinin, 1 and 10 μg/ml; (c) bestatin, 4 and 40 μg/ml; (d) chymostatin, 6 and 60 μg/ml; (e) E-64, 1 and 10 μg/ml; (f) leupeptin, 1 and 10 μg/ml; (g) pepstatin, 1 and 10 μg/ml; (h) TIMP-2, 0.1 and 1 μg/ml; and (i) MMP-inhibitor I, 100 and 1,000 μg/ml (Calbiochem). Constant numbers (5 × 10⁵ cells) of PHA-activated T cells were then added to the MLTC. In the control group, which included only activated T cells and coculture of activated T cells with cancer cells, no protease inhibitor was added. The supernatant of each culture tube was collected on the third day of coculture, and the concentration of sIL-2Rα was determined by ELISA.

**Effect of MMP-inhibitors on T-cell proliferation.** PBMCs were fully activated with 10 μg/ml PHA for 24 h before MLTC. Autologous cancer cells (1 × 10⁶) were first cultured in 1 ml of culture medium containing 1 μg/ml TIMP-2 or 100 μg/ml MMP-inhibitor I for 2 h and cocultured with PHA-activated T cells (5 × 10⁵ cells). In the control group, no TIMP-2 was added. Two days after MLTC, T cells were purified by MACS as described previously and cultured at constant numbers (2 × 10⁵ cells/well) in the presence of titrated IL-2 concentrations (1 IU/ml, 3 IU/ml, and 10 IU/ml). Titrated thymidine ([³H]thymidine) was added during the last 18 h of culture. Cells were harvested, and thymidine incorporation was counted by liquid scintillation. All assays were performed in triplicate.

**Expression of MMPs (3), MT-MMPs, and TIMPs in CC Tissue.** An avidin-biotin-peroxidase complex immunohistochemical staining method was performed for examination of the expression patterns of MMP-1, 2, 3, 7, 8, 9, -13, MT1-MMP, MT2-MMP, TIMP-1, TIMP-2, and TIMP-3 in formalin-fixed and paraffin-embedded tissue sections. The antibodies were obtained from Chemicon, Inc. (Temecula, CA), and their specificities were provided by the manufacturer.

**Analysis of MMP-9 Activity by Gelatin Zymography.** Tumor cell nests were carefully separated and scrapped from underlying stromas by a microdissection method. The scraped tissues were homogenized with lysis buffer containing glycerol (10%), Triton X-100 (1%), sodium PP (1 mM), NaCl (137 mm), EDTA (5 mM), sodium orthovanadate (1 mM), NaF (10 mM), and Tris (pH 7.9; 20 mM). T cells isolated from PBMCs were activated and cultured as described previously.

The gelatinolytic activity of MMP-9 was determined by gelatin-substrate gel electrophoresis. Aliquots of supernatants of coculture or cell lysates (15 μg protein/lane) were applied, without heating or reducing, to a 10% SDS-polyacrylamide gel containing 1 mg/ml gelatin. After electrophoresis, gels were washed for 1 h at room temperature in a 2% Triton X-100 solution before being transferred to a reaction buffer containing 50 mM Tris-HCl (pH 7.4) 0.15 M NaCl, and 10 mM CaCl₂, and incubated at 37°C overnight. The gel was stained with 0.1% Coomassie Blue in 50% methanol:10% acetic acid and destained in 20% methanol:10% acetic acid. Clear zones of gelatin lysis against a blue background stain indicated the presence of enzyme. Quantitative analysis of gelatinolytic activity was achieved by scanning densitometry of the zymographs (IS-1000 Digital Imaging System; Alpha Innotech Corp., San Leandro, CA).

**Effects of Recombinant MMPs on the Function of Activated T Cells.** PBMCs were fully activated with 10 μg/ml PHA for 24 h. Recombinant MMP-1, MMP-2, and MMP-9 proteins (Calbiochem, Inc., Cambridge, MA) were added to the cultures at a final concentration of 1 μg/ml, and the PBMCs were incubated for an additional 6 h. The activated cells were labeled with anti-CD25-PE and checked by flow cytometry (8, 11). In addition, MMP-treated T cells were purified and cultured at constant numbers (2 × 10⁵ cells/well) in the presence of titrated IL-2 concentrations (1 IU/ml, 3 IU/ml, 10 IU/ml, and 100 IU/ml), and the T-cell proliferation assays were conducted as described above. Studies were performed in triplicate.

**Statistical Analysis of Data.** All results were expressed as mean ± SE unless stated otherwise. Data analysis was performed with SAS (Statistical Analysis System, R.6.12; SAS Institute, Inc., Cary, NC). Kinetic data were analyzed by a generalized estimation equation for correlated data of repeated measurements. Statistical significance was defined by a P of <0.05.

**RESULTS AND DISCUSSION**

**CC Cells Can Decrease IL-2Rα Expression on Encountered T Cells.** In the first experiment, PHA-activated PBMCs were cocultured with autologous CC cells or noncancerous cells. The expression of IL-2Rα and HLA-DR on activated T cells was measured by flow cytometry (8, 11). The kinetic expression of IL-2Rα on activated T cells in the MLNC was compatible with the known physiological pattern (16, 17), being highest on day 3 and having progressively decreased by day 5. Thus, the noncancerous cells had no influence on the level of IL-2Rα expression in MLNC. In contrast, CC cells seemed to affect the expression of IL-2Rα by T cells in MLTC. On day 1 post-coculture, the MFI of IL-2Rα expression on activated CD4⁺ cells was similar in both MLTC and MLNC. It was noteworthy that the MFI of IL-2Rα on CD4⁺ cells was significantly lower in MLTC than in MLNC on day 3 (170.7 ± 17.0 versus 360.1 ± 14.7) and day 5 (94.1 ± 6.1 versus 174.3 ± 23.6). A similar finding was obtained with CD8⁺ cells (139.0 ± 8.9 versus 340.0 ± 28.9 on day 3 and 64.4 ± 10.7 versus 196.3 ± 28.7 on day 5 for MLTC and MLNC, respectively; Fig. 1, A and B). However, there was no difference in the kinetic expression patterns of HLA-DR on activated T cells (CD4⁺ or CD8⁺) between MLTC and MLNC (Fig. 1, C and D). The expression of HLA-DR on activated T cells of MLTC and MLNC was low on day 1 and elevated on day 3, and remained persistently high on day 5. Our previous study had shown that the expression of HLA-DR, and CD69 as well, on TILs isolated from patients with CC was not altered (8). Together, these findings indicate that CC cells...
Can reduce IL-2Rα expression on encountered T cells in a specific manner.

Decreased Expression of IL-2Rα on TILs Is Not Mediated at the Transcriptional Level. Next, we studied whether the decreased expression of IL-2Rα on TILs in CC was mediated at the transcriptional level. CD8⁺ T cells were isolated with a purity of >97% from TILs, unstimulated PBMCs, and PHA-activated PBMCs. Expression of IL-2Rα mRNA was measured by competitive RT-PCR. The amount of IL-2Rα mRNA in TIL-derived CD8⁺ T cells was comparable with that in the activated CD8⁺ T cells. In both TILs and activated CD8⁺ T cells, the amount of IL-2Rα mRNA was about 100-fold of that expressed in nonstimulated PBMC-derived CD8⁺ cells (Fig. 1, E and F). A deficiency in IL-2 protein and IL-2R expression despite adequate levels of IL-2 mRNA has also been shown in TILs from patients with breast cancer (7). Because the IL-2Rα mRNA was abundant in TILs, cancer-mediated suppression did not occur at the transcriptional level.

CC Cells Enhance the Release of sIL-2Rα from Activated T Cells. The possibility exists that the CC-associated decreased expression of IL-2Rα results from excessive shedding of IL-2Rα from T cells. To clarify this possibility, we analyzed the amounts of sIL-2Rα in the supernatants of MLTC and MLNC. The mean concentration of sIL-2Rα in MLNC supernatants was 1045.1 ± 173 pg/ml and increased about 2- and 3-fold by day 3 and day 5, respectively. The cumulative concentration of sIL-2Rα in the supernatants of MLNC was compatible with the pattern of natural shedding of IL-2Rα (16, 17). However, the mean concentration of sIL-2Rα in the supernatants of MLTC was significantly higher than that in MLNC in day-3 and day-5 cocultures (increased more than 3- and 4-fold, respectively; P < 0.001; Fig. 2A). It became evident that CC cells could enhance the release into the surroundings of sIL-2Rα from activated T cells, and that a proteolytic cleavage might mediate the process (17, 18).

![Image](331x160 to 538x558)

**Fig. 1.** Cancer cells down-regulated IL-2Rα expression on encountered activated T cells. MFIs of IL-2Rα and HLA-DR expression on activated T cells when cocultured with cancer cells (○, MLTC) and normal stroma/epithelial cells (□, MLNC). The data are representative of six independent experiments of cocultures in triplicate. A, MFI of IL-2Rα expression on activated CD4⁺ T cells. B, MFI of IL-2Rα expression on activated CD8⁺ T cells. C, MFI of HLA-DR expression on activated CD4⁺ T cells. D, MFI of HLA-DR expression on activated CD8⁺ T cells. The expression of IL-2Rα on activated CD4⁺ and CD8⁺ T cells was significantly lower in MLTC than that in MLNC on days 3 and 5 of coculture (P < 0.001). E, competitive RT-PCR analysis of IL-2Rα mRNA expression in purified CD8⁺ T cells. The level of IL-2Rα mRNA expression in CD8⁺ TILs was >100-fold the expression in corresponding unstimulated CD8⁺ T cells. F, the level of IL-2Rα mRNA expression in CD8⁺ TILs is comparable to the expression in corresponding activated CD8⁺ T cells.

![Image](60x374 to 281x742)

**Fig. 2.** Cancer-induced IL-2Rα cleavage can be reversed by MMP inhibitors. A, cancer induces IL-2Rα cleavage. The levels of sIL-2Rα in the supernatants of MLTC (○) and MLNC (□) were analyzed by ELISA. B, MMP inhibitors block cancer-induced IL-2Rα cleavage. The mean concentration of sIL-2Rα in the MLTC group (T+C, upper dashed line) was much higher than that in the T-cells-only group (T only, lower dashed line). Two final concentrations indicated in “Materials and Methods” (×10, solid bar; and ×10, open bar) for each protease inhibitor were used in the MLTC. IMP, MMP inhibitors; ANT, antipain-dihydrochloride; APR, aprostim; BES, bestatin; CHY, chymostatin; E64, E-64; LEU, leupeptin; PEP, pepstatin. C, MMP inhibitors restore cancer-induced T-cell nonresponsiveness. Various amounts of IL-2 were added to the cultures of purified activated T cells from PBMCs (○), MLTC (□), and MLTC in the presence of MMP-inhibitors (△). The IL-2-dependent [3H]TdR proliferation was analyzed. Data from six independent experiments are presented as means ± SE.
MMP Inhibitors Block Cancer-induced Proteolytic Cleavage of IL-2Rα. Using the kinetic experiments of MLTC described above, we conducted a protease inhibition assay to determine whether specific inhibitors could reverse or limit the cancer-induced proteolytic cleavage of IL-2Rα. For achieving maximal expression of IL-2Rα, PBMCs were fully activated with 10 μg/ml PHA for 24 h before MLTC. After 3 days of coculture, the concentration of sIL-2Rα in the MLTC group was ~3-fold that in the T-cells-only group (6001.0 ± 679.6 pg/ml versus 2241.3 ± 197.3 pg/ml; n = 6, P < 0.001). Protease inhibitors such as E-64, aprotinin, bestatin, leupeptin, pepstatin, chymostatin, and antipain-dihydrochloride had no apparent activity in suppressing CC cell-mediated proteolytic cleavage of IL-2Rα (Fig. 2B).

TIMP-2 and MMP-inhibitor I significantly inhibited the CC cell-induced IL-2Rα cleavage in a dose-dependent pattern (Fig. 2B). At a concentration of 1 μg/ml of TIMP-2, cancer-induced IL-2Rα cleavage was totally inhibited. The concentration of sIL-2Rα in the TIMP-2-MLTC group was 1953.1 ± 81.2 pg/ml, compared with a concentration of 6001.0 ± 679.6 pg/ml in the MLTC group without inhibitor (n = 6; P = 0.004). At a concentration of 0.1 μg/ml TIMP-2, partial inhibition existed (3136.2 ± 327.7 pg/ml versus 6001.0 ± 679.6 pg/ml; P = 0.011). At a concentration of 100 μM/ml MMP-inhibitor I, IL-2Rα cleavage was also totally inhibited. The concentration of sIL-2Rα in the MMP-inhibitor I-MLTC group was 2114.7 ± 165.7 pg/ml, compared with a concentration of 6600.1 ± 640.5 pg/ml in the MLTC group without inhibitor (n = 6; P = 0.001). At a concentration of 10 μM/ml of MMP-inhibitor I, partial inhibition existed (3264.2 ± 226.2 pg/ml versus 6600.1 ± 640.5 pg/ml; P = 0.006). The finding indicates that an MMP-mediated proteolytic process is likely to be responsible for the IL-2Rα cleavage, and that MMP inhibitors block this process.

MMP Inhibitors Restore IL-2-promoted Proliferation Function of TILs. To examine whether TIMP-2 or MMP-inhibitor I added to cultures could restore the proliferative function of cancer-encountered T cells, we also performed an IL-2-promoted T-cell proliferation assay. In the MLTC experiments illustrated in Fig. 2C, cancer-encountered T cells proliferated poorly in the presence of IL-2. However, at a concentration of 1 μg/ml TIMP-2 or 100 μM/ml MMP-inhibitor I, the proliferative ability of cancer-encountered T cells could be restored in an IL-2 dose-dependent manner, which indicated that MMP inhibitors are functionally capable of restoration of the T-cell proliferation function by blocking the cancer-induced IL-2Rα cleavage.

MMP-1, -2, and -9 Are Abundantly Expressed by CC Cells. We then studied the expression of various types of MMPs, including MMP-1, -2, -3, -7, -8, -9, and -13, two membrane-type MMPs (MT1 and MT2), and three types of inhibitors (TIMP-1, -2, and -3) by immunohistochemical staining in 30 cases of CC tissues. MMP-1, MMP-2, and MMP-9 are abundantly expressed in most, if not all cancer cells, but are not expressed or are only very weakly expressed in normal cervical epithelial or stromal cells (Fig. 3, A–D, data not shown). The expression of other types of MMPs and two types of membranous MMPs was not detected or was only weakly expressed. Diffusely weak stainings of TIMP-1, TIMP-2, and TIMP-3 were observed in selected cases of CC (weakly expressed in 10–20% of cases). Low TIMP expression and/or increased MMP:TIMP ratio in tissues were reported to associate with a poor prognosis for CC (19, 20). In the gelatin zymographic study (Fig. 3E), the gelatinolytic activities of MMP-9 (M̄, 92,000) were strongly expressed in all CC cells but varied widely in supernatants of MLTC (data not shown). The enzyme activities of MMP-9 in activated T cells were about 1–8% of CC cells.

MMPs Directly Mediate the Proteolytic Cleavage of IL-2Rα. To characterize further the role of MMP in IL-2Rα cleavage, we directly incubated activated T cells with 1 μg/ml recombinant MMP-1, MMP-2, or MMP-9 protease. As demonstrated by flow-cytometric analysis, MMP-1 had no effect on the expression of IL-2Rα on activated T cells (Fig. 4A). MMP-9 and, to a lesser extent, MMP-2, can down-regulate the expression of IL-2Rα on activated T cells (Fig. 4, B and C). In the experiments illustrated in Fig. 4D, MMP-2-treated T cells proliferated well in an IL-2 dose-dependent manner, which was compatible with the pattern of activated T-cell controls. MMP-9-treated T cells proliferated poorly in the presence of low-dose IL-2, but their proliferative ability could be restored by either IL-2 at a higher dose or MMP-inhibitors. Thus, the observed cancer-mediated IL-2Rα down-regulation can be attributed mainly to proteolytic cleavage by MMP-9 from cancer cells. Because IL-2Rα plays a pivotal role in the development and propagation of functional T cells, cleavage of IL-2Rα down-regulates the proliferative capability of T cells (16, 17; Figs. 2C and 4D).

The general roles of extracelluar-matrix enzymes, especially MMPs, and the immunological reaction remain to be investigated (21). The MMPs have different substrate specificities with complicated regulation of cytokines and other inflammatory mediators on immune cells (21–23). Changes in the balance between active MMPs and their inhibitors may result in a number of human disorders, including inflammation, fibrosis, and tumor invasion (21–26). The latent pro-MMPs permit autocatalytic cleavage and MMP-activating proteolytic pathways to generate the fully active MMPs. However,
less is known about the molecular mechanisms that regulate the augmented proteolytic cascades of pro-MMPs in immune cells (21–24). In macrophages and T cells, MMP-2 and MMP-9 can be expressed (22, 23), and the expression of MMP-9 in T cells was dependent on the activation status of these T cells and was regulated by IL-2 (27). Expression of MMP is important for the trans-basement-membrane migration of T cells and for MMP-activating proteolytic cleavage of certain cytokines and receptors (21–26).

MMPs are abundant in a variety of human cancer cells, including those of CC (19, 20, 25). Cancer-derived MMPs may trigger the proteolytic cleavage of cytokines and their receptors, including tumor necrosis factor-R (28, 29), IL-6R (29, 30), and also IL-2Rα, as shown in the present study. A recent study by Wang et al. (31) showing that MMP-9-deficient mice cannot resolve a contact hypersensitivity reaction further implies the down-regulatory role of MMP-9 in immune responses. Moreover, cancer-encountered T cells lacking IL-2Rα signaling may be resistant to Fas-mediated apoptosis (32, 33), which may reciprocally explain the abundance of, but the relatively poor function of, TILs within the tumor milieu.

In conclusion, the mechanism for apparent inhibition of IL-2Rα expression on activated T cells in tumors is governed by a cancer cell-directed and MMP-dependent cleavage. Cancer-derived MMP-9, and other MMPs as well, can inhibit the proliferative function of tumor-encountered T cells, and this may explain the relative anergic state of TILs. Through the MMP-proteolytic pathway, cancer cells may facilitate immunosuppression, cancer invasion, and metastasis. It is noteworthy that TIMP activity was significantly lower in CC tissues than in other gynecological cancer tissues (20), and that a reduction in TIMP expression in tissue is associated with a poor prognosis for CC (19). Reversal of tumor-induced immunosuppression has been a major concern regarding prospects for adoptive T-cell therapy. Our finding should provide important insights into the understanding of the interaction between cancer cells and the immune system, as well as into the development of anticancer strategies.

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