Induction of Immunoregulatory CD271⁺ Cells by Metastatic Tumor Cells That Express Human Endogenous Retrovirus H

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Abstract

Human endogenous retroviruses (HERV) are associated with many diseases such as autoimmune diseases and cancer. Although the frequent expression of a variety of HERVs in tumor cells has been demonstrated, their functional contributions in cancer are as yet unclear. Intriguingly, HERVs and other retroviruses include an immunosuppressive domain in their transmembrane envelope proteins, but its mechanism of action and cancer relevance are obscure. In this study, we demonstrate that the human endogenous retrovirus HERV-H has a critical role in tumor metastasis and immune escape. We found that expression of herv-h mRNA was elevated in metastatic tumor cells undergoing epithelial-to-mesenchymal transition (EMT) and in primary tumor tissues from advanced colon cancer. The immunosuppressive peptide H17 derived from HERV-H was sufficient to induce EMT in tumor cells that expressed low levels of HERV-H, and it amplified this event within the tumor microenvironment. H17 also stimulated CCL19 expression in tumor cells, which in turn recruited and expanded a population of pluripotent immunoregulatory CD271⁺ cells, which included mesenchymal stem cells and myeloid-derived suppressor cells. In tumor tissues from patients with advanced colon cancer, we confirmed that CD271⁺ cells were increased in HERV-H⁺ CCL19⁺ tumor tissues. Notably, RNAi-mediated change of HERV-H or CCL19, or depletion of CD271⁺ cells, improved immune responses in vitro and in vivo accompanied by tumor regression. Together, our results argued that HERV-H is a critical determinant of immune escape in cancer, suggesting its candidacy as a promising therapeutic target to treat patients with advanced cancer.

Cancer Res; 74(5); 1361–70. ©2014 AACR.

Introduction

Human endogenous retroviruses (HERV) are derived from ancestral integration of exogenous retroviruses, and are transmitted vertically through the germ line (1, 2). HERVs are associated with autoimmune diseases such as rheumatoid arthritis, multiple sclerosis, and cancer (1, 2). Increase of various HERV expressions such as HERV-H (3, 4), HERV-K (5), HERV-F (6), HERV-R (7), and HERV-S (8) in human tumor cell lines and tumor tissues of patients has been demonstrated so far. However, the meanings of HERV emerge in cancer, or the functional role of HERVs in tumor cells has been rarely pursued. HERVs and other retroviruses have immunosuppressive properties, for which immunosuppressive domain in the transmembrane envelope protein is responsible (9, 10). Stimulation with a 17-mer peptide derived from this domain upregulates immunosuppressive interleukin (IL)-10 production, and downregulates immunostimulator IL-12 production in peripheral blood mononuclear cells (PBMC; refs. 11, 12). However, the precise immunosuppressive mechanism remains unclear. Also, its effect on the tumor side has never been investigated. We assumed that tumor-derived peptide would necessarily modulate both immune cells and tumor cells within tumor microenvironment. In this study, we investigated the role of HERV-H/env60 (designated HERV-H) expressed in tumor cells, and demonstrate a novel molecular mechanism involved in immunoevasion of cancer.

Materials and Methods

Cell lines and mice

Human cell lines (colon cancer Colo320 and HCT116, pancreatic cancer MIAPaca and Panc1, melanoma Hs294T, and normal epithelial cell ARPE19) were purchased from American Type Culture Collection, and were authenticated by short tandem repeat profiling before experiments. Primary tumor cell lines were established using tumor tissues surgically resected from a patient with stage IV melanoma under the protocol approved by the Keio University Ethics Committee. These tumor cells were cultured in 10% fetal calf serum/Dulbecco’s Modified Eagle Medium. Female BALB/c nu/nu mice were purchased from SLC, and were maintained under pathogen-free conditions. These mice were used according to the protocols approved by the Animal Care and Use Committee at Keio University School of Medicine (Tokyo, Japan).
Gene expression analysis

The mRNA expression in tumor cells or tissues was analyzed by semiquantitative reverse transcription PCR (RT-PCR) using extracted RNAs and paired primers (Supplementary Data) specific for open reading frame (ORF) region within the targeted genes as described before (13). The digital images of the bands were quantified using NIH ImageJ software (http://rsb.info.nih.gov/ij/), and the signal intensity was normalized to gapdh expression. The same experiments were repeatedly conducted two to four times, and all data are compiled and averaged in graphs (mean ± SD).

Functional analysis of tumor cells in vitro and in vivo

Tumor cell functions were evaluated as described before (13). Briefly, cell proliferation was assessed by WST1 assay (Takara). Cell invasion was assessed using a Transwell chamber with a Matrigel-coated membrane (BD Biosciences). Cell adhesion was assessed using Fibronectin-coated 96-well plates (BD Biosciences). Chemokines in the tumor supernatant (culture for 1–2 days) were measured using ELISA kits according to the manufacturer’s instructions (CCL2; Endogen; CCL19; R&D Systems). To knockdown herv-h, snail, or twist expression, several kinds of siRNAs (3 μg; Invitrogen) target- ing a different position of each gene, and its scrambled oligonucleotide as a control were used after making complex with jetPEI (PolyPlus Transfection). For herv-h, four kinds of siRNAs (targeting 5480, 6794, 6965, and 7057) were used. The sequences of these herv-h–specific siRNAs are described in Supplementary Data. Transfection efficacy was validated by RT-PCR as described before (13).

For in vivo study, HERV-H tumor cells transplanted with lentiviral vector encoding GFP gene (Biogenova) were used. These tumor cells were injected both intravenously (for systemic metastasis; 1 × 10⁶ cells) and subcutaneously (for draining lymph node analysis; 1 × 10⁶ cells) into BALB/c nu/nu mice. Three weeks later, lymph node, lung, and spleen were harvested from the mice, and were mechanically dispersed into single-cell suspensions for flow cytometric analysis of GFP+ tumor cells and CD271+ cells.

To evaluate effect of a 17-mer peptide derived from the immunosuppressive domain of HERV-H on tumor cells, HERV-HΔ714–470 peptide (LQNRRGDLTTAEGGGL, designated H17) or another site peptide as a control (HERV-HΔ714–470, LQTDWGTSVPSHLRTS) at 5 μg/mL, or TGF-β at 5 ng/mL (R&D Systems) as an EMT (epithelial-to-mesenchymal transition)-inducer control for 2 to 3 days before assays. These peptides with amiation at C-terminal were synthesized at Invitrogen (purity >98%), and were dissolved in sterile MilliQ water for use. We confirmed by native PAGE that these peptides formed dimers in the preparation for cell culture in comparing with a loading control without peptides (medium only). The fifth glutamin of the HERV-HΔ714–470 peptide was substituted with arginin, because arginin-arginin (RR) has been reported to play a crucial role in the biologic activity of the immunosuppressive domain using carrier-conjugated peptides (12), although we observed no differences of biologic activities between them at least in our study. For signaling pathway analysis, cells were treated with phosphokinase 3-kinase (P3K) inhibitor LY294002 or MAP–ERK kinase (MEK) inhibitor PD98059 at 20 μmol/L before assay. To define EMT induction by H17 peptide, H17-treated cells were permeabilized with cytofix/cytoperm solution (BD Pharmingen), and were stained with monoclonal antibodies (mAb) specific for Twist (Abcam), E-cadherin (BD Biosciences), Fibronectin (R&D Systems), CD44 (BD Biosciences), or the appropriate isotype control Ig, followed by staining with immunofluorescence-conjugated secondary antibodies. The molecular expressions were analyzed using a confocal LSM5 Pascal microscope (Carl Zeiss).

Stimulation and characterization of human PBMCs

Human PBMCs were stimulated with 3-day–cultured tumor supernatant (0.22-μm filtration, 25%), CCL19 (10 ng/mL), and/or H17 peptide (10 μg/mL) for 5 days, and were analyzed by flow cytometry. For tracking CD271+ cell division, CD271+ cells were separated from PBMCs using a MACS system with MicroBeads-conjugated mAbs (Miltenyi Biotec), and were stained with red fluorescent dye PKH26 (Sigma) before stimulation. The immunosuppressive activity of the treated CD271+ cells was evaluated using the T-cell proliferation system. CD271+ cells (inactivation with mitomycin C, MMC) were cocultured (1:10) with autologous CD3+ T cells in the presence of anti-CD3 mAb (1 μg/mL) for 5 days, and cell proliferation was measured by the WST1 assay. The pluripotency of the CD271+ cells was also evaluated as described in Supplementary Data.

CD271+ cell depletion studies

To evaluate effect of CD271+ cells on dendritic cells (DC), purified CD14+ cells before/after CD271+ cell depletion were cultured with granulocyte macrophage colony–stimulating factor (GM-CSF; 10 ng/mL; PeproTech) in the presence of CCL19 and H17 for 6 days, and were analyzed by C11c+ HLA-DR+ cells by flow cytometry. For evaluating effect of CD271+ cells on CTLs, bulk PBMCs obtained from HLA-A24+ healthy volunteers were stimulated with a tumor antigen SAGE CTL epitope peptide (LYKPDSNFEF; Invitrogen) at 10 μg/mL for 6 days, and the separated CD8+ cells (1 × 10⁶) were restimulated with the peptide in the presence of IL-2 (100 IU/mL) and antigen-presenting cells (APC; 5 × 10⁶ of MMC-inactivated autologous PBMCs) before/depletion of CD271+ cells. After two to three cycles of the restimulation, the CD8+ cells were tested for IFN-γ production in response to the peptide (24 hours) using the Cytometric Bead Array Kit (BD Biosciences), and for HLA-A24+ SAGE+ Colo320 tumor-killing activity (6 hours) using the Annexin V system (MBL) according to the manufacturer’s instructions.

Flow cytometric analysis

After Fc blocking, cells were stained with immunofluorescence [fluorescein isothiocyanate (FITC), phycoerythrin (PE), or CyChrome]-conjugated mAbs specific for human CCR7, CD11b, CD16, and CD271 (Abcam), CD33 (R&D Systems), CD45, CD56, CD80, CD86, HLA-DR, and IDO (AbD Serotec), PDL1 (eBiosciences), or the appropriate isotype control IgG. The immunofluorescence-conjugated secondary antibodies were used if necessary. The mAbs except designated ones were purchased.
from BD Pharmingen. For intracellular staining, cells were permeabilized with cytofix/cytoperm before staining. The immunofluorescence was analyzed in comparing with the isotype control using a FACSCalibur cytometer (Cellquest software; BD Biosciences).

**Immunohistochemical analysis of clinical tissues**

Tissue sections, including normal colon tissues and tumor tissues (primary lesions, its adjacent normal portions and the metastatic lesions), derived from patients with stage I–IV colon were purchased from MBL and SuperBioChips, and were analyzed for CCL19 and CD271 expressions immunohistochemically. After deparaffinization, tissue sections were treated with 20% Immunoblock (DS Pharma) for 30 minutes, 10% goat serum for 30 minutes, and then FITC-conjugated anti-CCL19 mAb (Abcam), anti-CCL19 mAb (R&D Systems), or isotype IgG as a control at 4°C overnight. PE-conjugated anti-goat IgG secondary antibody was used for CCL19. After 4',6-diamidino-2-phenylindole (DAPI) or hematoxylin staining followed by fixation with ethanol and xylene, immunofluorescence intensity of the mounted sections was automatically measured as pixel counts at two fields under the same level of DAPI pixel using a LSM700 Laser Scanning Microscope (Carl Zeiss), and the data were averaged in graphs.

**Statistical analysis**

Significant differences ($P < 0.05$) were evaluated using the unpaired two-tailed Student $t$ test. The significance of the data was confirmed by analyzing the cumulative data of the repeated experiments using the nonparametric Mann–Whitney $U$ test, when the number of $n$ was small in an experiment. For graphical representation of data, $y$-axis error bars indicate the SD of the data. Correlation between two gene/molecular expressions in clinical samples was evaluated by the nonparametric Spearman rank test.

**Results**

**HERV-H is involved in cancer metastasis**

As previously shown elsewhere (3, 4), herv-h mRNA expression was observed in various human cancer cell lines, but not in normal tissues except testis, placenta, and pancreas (Supplementary Fig. S1A). Also, herv-h mRNA was more frequently expressed in tumor lesions compared with the adjacent normal portion of the identical patients with colon cancer, particularly of more advanced stage III/IV patients having metastasis (Supplementary Fig. S1B). We assumed that HERV-H expression in tumor cells might be associated with tumor progression and metastasis. Then, we evaluated herv-h knockdown effect on tumor functions using human HERV-H+ tumor cell lines (colon cancer Colo320 as the same type of cancers as the clinical tissues used, and pancreatic cancer MIAPaca as another cancer). When HERV-H+ tumor cells were transfected with herv-h-specific siRNAs, the Matrigel-invasive ability was significantly reduced ($P < 0.01$), and Fibronectin-adhesive ability was significantly elevated ($P < 0.05$), although cell proliferation was not influenced (Fig. 1A). This was accompanied by decreased expression of mesenchymal markers Snail, Twist, and Fibronectin, and increased expression of an epithelial marker E-cadherin ($P < 0.01$ vs. control siRNA; Supplementary Fig. S2A). HERV-H−/low cells were not influenced by siRNA-herv-h transfection (data not shown). Tumor metastasis to lymph nodes and lung in vivo was also significantly inhibited in immunodeicient mice when siRNA-herv-h was transfected into HERV-H+ tumor cells injected ($P < 0.04$; Fig. 1B). When HERV-H−/low cells (colon cancer HCT116, pancreatic cancer Panc1, melanoma Hs294T as another cancer, and noncancerous epithelial cell ARPE19) were stimulated with TGF-β, which is a potent inducer of EMT, for 2 days, herv-h mRNA expression significantly increased ($P < 0.01$; Fig. 1C). Interestingly, no increase of snail and twist was seen under herv-h knockdown even after TGF-β stimulation (Supplementary Fig. S2B). This implies that HERV-H may be required for Snail and Twist induction in tumor cells undergoing TGF-β-induced EMT. By RT-PCR using primers specific for ORF of each HERV envelope protein, we confirmed that mRNA expression of other HERVs (HERV-E, -F, -K, -R, and -W) was not affected by siRNA-herv-h transfection or TGF-β stimulation (data not shown). These results suggest that HERV-H is involved in EMT, which is a cellular event to acquire high motility and invasive ability leading to metastasis (14).

HERV-H encodes a transmembrane envelope protein with an immunosuppressive domain of 17 amino acids. A synthetic peptide corresponding to this domain is designated H17. We next evaluated how the H17 peptide would affect tumor invasion. When HERV-H−/low cells were stimulated with H17 peptide for 2 days, tumor invasive ability was significantly elevated ($P < 0.05$ vs. control peptide; Fig. 1D), and Twist, Fibronectin, and CD44 increased, whereas Snail and E-cadherin only slightly changed (Fig. 1E and Supplementary Fig. S2C). This effect was abrogated by PI3K inhibitor LY294002, but not by MEK inhibitor PD98059. This suggests that PI3K is involved in the signaling pathway, although both MEK/ERK (extracellular signal–regulated kinase) and PI3K/Akt have been reported in the HERV-induced immunosuppressive mechanism (12). Twist has been reported to promote tumor metastasis via PI3K activation (15). These suggest that the H17 peptide has a unique property to induce EMT possibly via the Twist–PI3K pathway in tumor cells.

**HERV-H is required for CCL19 production in tumor cells**

Chemokines are closely associated with cancer metastasis, and these expressions in tumor tissues are correlated with a metastasis incidence and a poor prognosis of patients with cancer (16, 17). We next investigated the relationship between chemokines and HERV-H expression in tumor cells. In the siRNA-herv-h–transfected HERV-H+ tumor cells, ccl2 and ccl19 expressions were almost completely abrogated, although no impact on other chemokines tested (Supplementary Fig. S2D). However, when HERV-H−/low cell lines were stimulated with TGF-β following siRNA transfection, CCL19 induction was significantly inhibited only by siRNA-herv-h, although CCL2 induction was inhibited by either siRNA specific for herv-h, snail, or twist ($P < 0.001$; Fig. 2A and B). This indicates that HERV-H expression is necessary for CCL19 production in metastatic tumor cells. Interestingly, H17 peptide rather than TGF-β strongly induced CCL19 production in tumor cells dose-
HERV-H+ tumor cells recruits and expands pluripotent and immunoregulatory CD271+ cells utilizing H17 and CCL19

Next, to examine how CCL19 produced by HERV-H+ tumor cells would modulate immune responses, we firstly conducted chemotaxis assay using human PBMCs and Transwell chambers. When the migrated cells were analyzed by flow cytometry, CD4+ cells and CD56- cells were found to be the majority of the cells recruited by CCL19 or HERV-H+ tumor supernatant (Supplementary Fig. S3). Only CD4lowCD56low cells decreased when CCL19 or its receptor CCR7 (data not shown) was blocked by the specific mAb, suggesting that CCL19–CCR7 axis is, at least in part, responsible for the migration of the CD4lowCD56low cells. The phenotype (CD45+/CD56+/CD271+CCR7+) of these cells was similar to that of mesenchymal stem cells (MSC) reported in human PBMCs and bone marrow cells (Supplementary Fig. S3C, refs. 18–20). Particularly, one of the potential MSC markers CD271 (21, 22) was distinguishably expressed in the migrant cells. It is inferred that these cells may be one of the MSC subpopulations, although evaluation of the MSC activities was unable due to a small number of cells in the assay. Interestingly, CD271+ cells also significantly increased in number when PBMCs were stimulated with HERV-H+ tumor supernatant for 5 days (P = 0.001 between MIAPaca-treated cells vs. Panc1-treated cells), and such increase was not seen using siRNA-transfected tumor supernatant (Fig. 3A and Supplementary Fig. S4A). This suggests that HERV-H+/herv-h–transfected tumor supernatant (Fig. 3A and Supplementary Fig. S4A). This suggests that HERV-H–derived soluble factors could be involved in the mechanism. Indeed, purified CD271+ cells proliferated in response to CCL19 and H17, particularly in the presence of other CD271+ cells (Fig. 3B), resulting in significant increase of CD271+ cells in the PBMC culture (P = 0.019; Fig. 3A). This suggests that a small number of CD271+ cells are resident in human PBMCs, and are recruited and expanded by CCL19 cooperatively with H17 and other cells within the HERV-H+ tumor microenvironment. CD271+ cells sorted from the culture with CCL19 and H17 frequently differentiated into mesenchymal lineages such as adipocytes, osteocytes, and chondrocytes (Supplementary Fig. S4B), and...
significantly suppressed autologous T-cell proliferation \((P = 0.0002; \text{Fig. 3C})\), suggesting a sort of pluripotent and immunoregulatory MSCs. CD271\(^+\) cells treated with no stimulants also showed suppressive activity on T cells (Fig. 3C).

As previously shown (22), there were two subpopulations, CD271\(^-\)CD56\(^-\) cells and CD271\(^+\)CD56\(^-\) cells in the CD271\(^+\) cells, and both subpopulations significantly and similarly suppressed T-cell proliferation (Fig. 3D). However, many differences were found in phenotypic molecular expressions (Fig. 3E). The CD271\(^+\)CD56\(^-\) cells highly expressed T-cell costimulatory molecules, immunosuppressive molecules such as PD-L1 and IDO, and myeloid markers such as CD11b and CD33, indicating a sort of myeloid-derived suppressor cells (MDSC; 23). In contrast, the CD271\(^-\)CD56\(^-\) cells expressed none of these molecules, indicating a sort of MSCs although having weak expression of CD45. Probably, pluripotency of CD271\(^-\) cells may be attributed to this subpopulation. These data suggest that CD271\(^+\) cells, including two types of immunoregulatory cells, are the significant effector cells for immunosuppression caused by HERV-H\(^+\) tumor cells.

A close connection between HERV-H/CCL19 expression in tumor cells and increase of CD271\(^+\) cells in clinical tissues

To evaluate the clinical relevance of the HERV-H–induced mechanism, first, primary tumor cell lines derived from a patient with stage IV melanoma (available for us) were tested similarly to the above. HERV-H knockdown by siRNA-herv-h transfection significantly inhibited tumor invasion \((P < 0.03\) vs. control siRNA), CCL19 production \((P < 0.01)\), and CD271\(^+\) cell induction in PBMCs \((P < 0.02; \text{Fig. 4A})\). This suggests a possibility that HERV-H could play a critical role in immunoevasion of tumor cells in patients with cancer.

We next analyzed clinical tissues derived from stage I–IV patients with colon cancer for CCL19 and CD271 expressions immunohistochemically. Both CCL19 and CD271 expressions significantly \((P < 0.02\) vs. normal) and correlatively \((P < 0.05)\) increased in tumor tissues of any stages, but not in normal tissues (Fig. 4B and C). No differences were seen among tumor stages. CCL19 was expressed mostly in tumor cells, and CD271\(^+\) cells were colonizing at many sites in the CCL19\(^+\) tumor tissues particularly of stage III-IV patients (Supplementary Fig. S5). The size of CD271\(^+\) colonies in stage IV tumors was larger than that in stage III tumors. In the stage IV tumors, a part of CCL19\(^+\) tumor cells that colocalized with the CD271\(^+\) colonies also expressed CD271, and such CCL19\(^+\)CD271\(^+\) tumor cells also increased in metastatic lesions such as lymph node and lung, and even in the tumor-adjacent normal portion of the identical patient (8 of 10 patients), implying tumor escape to neighboring and distant tissues (Supplementary Fig. S5). The CCL19\(^+\)CD271\(^+\) tumor cells may be cancer stem cells (CSC) because CD271 is one of the representative markers for CSCs in some cancers (24, 25).
Because MSCs have been reported to promote CSC proliferation (26), the colocalizing CD271+ MSC-like cells might have expanded the CCL19+CD271+ CSC-like cells in the stage IV patients. When stage III tumor tissues (rarely having CCL19+CD271+ tumor cells) were analyzed by semiquantitative RT-PCR, both ccl19 and cd271 mRNA expressions increased significantly and correlatively with herv-h mRNA expression \( (P < 0.01; \text{Fig. 4D}) \). This suggests a close connection between HERV-H/CCL19 expression in tumor cells and increase of CD271+ cells in clinical cancer.

Elimination of CCL19 and CD271+ cells ameliorates antitumor immune responses in vitro and in vivo

Finally, to determine whether HERV-H/CCL19/CD271 would be a candidate therapeutic target for treating cancer, we conducted in vitro study using human PBMCs to assess how CD271+ cells would affect DC differentiation with GM-CSF, and CTL induction with tumor antigen peptide SAGE. In the presence of CCL19 and H17, CD14+ DC precursors differentiated into tolerogenic DC-like CD11c+ cells with reduced HLA-DR expression (Fig. 5A). However, predetection of CD271+ cells...
prevented such untoward incidence, and CD271⁺ cell-depleted APCs generated better CTLs having significantly higher activities of IFN-γ production and cytotoxicity (P < 0.05; Fig. 5B). These results suggest that depletion of CD271⁺ cells is a preferred treatment for reprogramming antitumor immunity.

When immunodeficient mice were implanted with siRNA-ccl19–transfected HERV-H⁺ tumor cells, tumor metastasis to lymph nodes and subsequent increase of CD271⁺ cells were inhibited simultaneously (Supplementary Fig. S6). This suggests that HERV-H⁺ tumor-derived CCL19 could be responsible for CD271⁺ increase in vivo as well as in vitro. To evaluate effect of CCL19 blockade accompanied by CD271⁺ elimination on antitumor immune responses in vivo, we in addition used a syngenic tumor model that was implanted with murine colon

Figure 4. CD271⁺ cells increase in HERV-H⁺ CCL19⁺ tumor tissues in advanced patients with cancer. A, primary tumor cell lines derived from patient with a stage IV melanoma (primary lesion and metastatic lymph node and skin) were transfected with siRNA-herv-h or the control siRNA and were tested for cell invasion, CCL19 production, and CD271⁺ cell induction in PBMCs (n = 3 per experiment; mean ± SD). These data are representative of two independent experiments. B–D, normal colon tissues (n = 6) and tumor tissues derived from patients with colon cancer (stage I–II, n = 4; stage III, n = 30; stage IV, n = 10) were analyzed for CCL19 and CD271 expression immunohistochemically. The adjacent normal portion and the metastatic lesion of the identical stage IV patients were also analyzed (n = 19). Using stage III tumor tissues, correlation among ccl19, cd271, and herv-h mRNA expressions was analyzed by semiquantitative RT-PCR (D). P values in scatter plot graphs were analyzed by the nonparametric Spearman rank test. In normal tissues, pixel counts were <68, and mRNA expression was <0.1.
cancer CT26 tumor cells expressing both murine endogenous retrovirus antigen AH1 (27) and CCL19 (28). Intratumoral injection with siRNA-ccl19 simultaneously prevented increase of CD271+ cells and other immunoregulatory cells such as CD4+Foxp3+ regulatory T (Treg) cells and CD11b+Gr1+ MDSCs in the treated tumors (Supplementary Fig. S7). Tumor growth (P = 0.006 vs. control siRNA) and lymph node metastasis (P = 0.035) were significantly suppressed, and splenic CD8+ T cells of these mice showed significantly higher CTL activities (P < 0.02; Supplementary Fig. S7F). These results suggest that targeting CCL19 as well as CD271+ cells in tumor microenvironment is a promising strategy for treating patients with HERV-H+ tumors.

Discussion

The role of HERVs expressed in tumor cells has never been clarified. Our study revealed that HERV-H is profoundly associated with cancer immunoevasion. HERV-H expression increases in tumor cells undergoing EMT, and the HERV-H–derived H17 peptide further amplifies EMT event, possibly leading to escalation of tumor metastasis. H17 stimulation also induces CCL19 production in tumor cells, and the released CCL19 recruits and expands pluripotent and immunoregulatory CD271+ cells in cooperation with H17. CCL19 and CD271 expressions significantly and correlatively increase in HERV-H+ tumor tissues of advanced patients with colon cancer. This indicates a close relationship between HERV-H/CCL19 increase in tumor cells and expansion of CD271+ cells in tumor microenvironment. Elimination of either factor ameliorates antitumor immune responses followed by tumor regression in vitro and in vivo. Thus, HERV-H is an important determinant that regulates cancer progression via tumor metastasis and immunosuppression.

Connections between CCL19 and immune dysfunction have been recently demonstrated. CCL19 directly impairs T cells by suppressing cell proliferation and IL-2 production through CCR7 signaling (29), or by promoting activation-induced cell death (30). As an indirect action, CCL19 recruits immunosuppressive CCR7+CCR10+ Treg cells in tumor microenvironment (31, 32). Also, Treg cells decrease, but mature DCs increase in lymph nodes of CCL19/CCL21-depleted plt mice (33). However, the role of CCL19 in antitumor immune network remains controversial and unclear. In this study, we demonstrated that CCL19 is more than a chemoattractive factor, which is the significant effector molecule for immunoevasion of tumor cells by recruiting and expanding immunoregulatory CD271+ cells. The HERV-H/CCL19–associated CD271+ cells, including MDSC-like CD271+ CD56+ cells and MSC-like CD271+ CD56− cells, may be a better prognostic indicator for immunosuppressive status in patients with cancer than either one population, MDSCs or MSCs. These two subpopulations have been previously shown using human bone marrow–derived MSCs (22). However, only pluripotency was focused in these studies on regenerative medicine.

There are many studies on immunosuppressive properties using purified or recombinant retroviral transmembrane envelope proteins, and inactivated virus particles (9, 10). However, the precise immunosuppressive mechanism has been largely unknown. In addition, the effect of the relevant peptide has never been investigated on tumor properties. Our study
unveiled a novel mechanism induced by HERV-H–derived H17 peptide: promotion of both CD271+ cell-governing immunosuppression and tumor invasion via the Twist–PI3K pathway.

On the basis of a unique strategy analyzing both tumor cells and host simultaneously, we previously demonstrated that Snail-induced EMT simultaneously causes tumor metastasis and immunosuppression in murine (13) and human system (34). However, the entire mechanism of cancer immunoevasion remains to be fully elucidated. The present study focusing on HERV-H in addition untangled one more part of the complicated networks. HERV-H, CCL19, and CD271+ cells may be attractive targets for treating HERV-H+ cancers.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

References


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