Molecular Basis for E-cadherin Recognition by Killer Cell Lectin-like Receptor G1 (KLRG1)*

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The killer cell lectin-like receptor G1, KLRG1, is a cell surface receptor expressed on subsets of natural killer (NK) cells and T cells. KLRG1 was recently found to recognize E-cadherin and thus inhibit immune responses by regulating the effector function and the developmental processes of NK and T cells. E-cadherin is expressed on epithelial cells and exhibits Ca2+-dependent homophilic interactions that contribute to cell-cell junctions. However, the mechanism underlying the molecular recognition of KLRG1 by E-cadherin remains unclear. Here, we report structural, binding, and functional analyses of this interaction using multiple methods. Surface plasmon resonance demonstrated that KLRG1 binds the E-cadherin N-terminal domains 1 and 2 with low affinity (Kd ~ 7–12 μM), typical of cell-cell recognition receptors. NMR binding studies showed that only a limited N-terminal region of E-cadherin, comprising the homodimeric interface, exhibited spectrum perturbation upon KLRG1 complex formation. It was confirmed by binding studies using a series of E-cadherin mutants. Furthermore, killing assays using KLRG1+ NK cells and reporter cell assays demonstrated the functional significance of the N-terminal region of E-cadherin. These results suggest that KLRG1 recognizes the N-terminal homodimeric interface of domain 1 of E-cadherin and binds only the monomeric form of E-cadherin to inhibit the immune response. This raises the possibility that KLRG1 detects monomeric E-cadherin at exposed cell surfaces to control the activation threshold of NK and T cells.

Natural killer (NK)3 cells play a critical role in the innate immune system because of their ability to kill other cells. For example, NK cells can kill virus-infected cells and tumor cells without presensitization to a specific antigen, and they produce various cytokines, including interferon-γ and tumor necrosis factor-α (1). NK cells are controlled by both inhibitory and activating receptors that are expressed on their surfaces (2). The killer cell Ig-like receptor, Ly49, CD94/NKG2, and paired Ig-like type 2 receptor families include both inhibitory and activating members and thus are designated as paired receptor families. On the other hand, some inhibitory receptors, including KLRG1 (killer cell lectin-like receptor G1), and activating receptors, such as NKG2D, also exist. The integration of the signals from these receptors determines the final functional outcome of NK cells.

These inhibitory and activating receptors can also be divided into two structurally different groups, the Ig-like receptors and the C-type lectin-like receptors, based on the structural aspects of their extracellular regions. The Ig-like receptors include killer cell Ig-like receptors and the leukocyte Ig-like receptors, and the C-type lectin-like receptors include CD94/NKG2, Ly49, NKG2D, Ly49(LKRA), NKR-P1(LKRB), and KLRG1. Many of these immune receptors recognize major histocompatibility complex class I molecules or their relatives (2–4), but there are still many orphan receptors expressed on NK cells. KLRG1 was one such orphan receptor; however, E-cadherin was recently found to be a ligand of KLRG1 (5, 6). Although major histocompatibility complex-recipient interactions have been extensively examined, the molecular basis of non-major histocompatibility complex ligand-recipient recognition is poorly understood.

KLRG1 is a type II membrane protein, with one C-type lectin domain in the extracellular region, one transmembrane region, and one immunoreceptor tyrosine-based inhibitory motif. KLRG1 is expressed on a subset of mature NK cells in spleen, lungs, and peripheral blood during normal development. KLRG1 expression is induced on the surface of NK cells during viral responses (7, 8). NK cells expressing KLRG1 produce low levels of interferon-γ and cytokines and have a slow in vivo turnover rate and low proliferative responsiveness to interleukin-15 (9). Furthermore, KLRG1 is recognized as a marker of some T cell subsets, as follows. KLRG1 defines a subset of T cells, short lived effector CD8 T cells (SLECs), which are mature effector cells that express high levels of KLRG1 and cannot be differentiated into long lived memory CD8 T cells. In addition, memory precursor

1 The abbreviations used are: NK, natural killer; SLEC, short lived effector CD8 T cell; MES, 4-morpholinooctanesulfonic acid; SPR, surface plasmon resonance; AUC, analytical ultracentrifugation; HSQC, heteronuclear sequential quantum correlation; PE, phycoerythrin.

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effector cells express low levels of KLRG1 and harbor the potential to become long lived memory CD8 T cells (10). Since SLECs exhibit stronger effector function than memory precursor effector cells, it is potentially beneficial, in terms of preventing harmful excess cytotoxicity, that SLECs express KLRG1 at a higher level to inhibit the immune response. Taken together, the expression of KLRG1 during the viral response and normal development might confer the inhibition of effector function and the regulation of NK and T cell proliferation (9).

E-cadherin plays a pivotal role in Ca\(^{2+}\)-dependent cell-cell adhesion and also contributes to tissue organization and development (11–14). E-cadherin is primarily expressed on epithelial cells, and its extracellular region consists of several domains that include cadherin motifs (15, 16). These domains mediate Ca\(^{2+}\)-dependent homophilic interactions to facilitate cell adhesion. When E-cadherins form cis- or trans-homodimers, they utilize their N-terminal regions as an interface, which can dock with domain 1 of another E-cadherin to form strand exchange (17). Therefore, the N-terminal region plays important roles in homophilic binding and cell adhesion.

KLRG1 recognizes E-cadherins (and other class I cadherins), which are widely expressed in tissues and form tight adhesive cell-cell junctions, and Itó et al. (5) demonstrated that E-cadherin binding by KLRG1 inhibits NK cytotoxicity. Further, Grünbermann et al. (6) showed that the E-cadherin-KLRG1 interaction inhibits the antigen-induced proliferation and induction of the cytolytic activity of CD8 T cells. Therefore, it is plausible that E-cadherin recognition by KLRG1, expressed on the surfaces of NK cells and T cells, may raise their activation thresholds by transducing inhibitory signals. Such an inhibition would prevent the excess injury of normal cells, which might result in inflammatory autoimmune diseases. KLRG1 may also have an important role in monitoring and removing cancer cells that lose E-cadherin expression. A recent report demonstrated that N-terminal domains 1 and 2 of E-cadherin are critical for KLRG1 recognition (18); however, despite accumulating evidence supporting the functional importance of the E-cadherin-KLRG1 interaction, the molecular basis of this interaction is poorly understood. Here, we report that the N-terminal region of E-cadherin, comprising the dimer interface, is the binding site for KLRG1. This suggests that KLRG1 does not recognize the dimeric form of E-cadherin but rather recognizes the monomeric form, which is exposed on the cell surfaces of disrupted or infected cells. This may suppress excess immune responses.

**EXPERIMENTAL PROCEDURES**

Preparation of Recombinant Proteins—The plasmid pET15bEC-D1D2(His10Xa), encoding domains 1 and 2 of E-cadherin, with a His\(_{10}\) tag, a spacer sequence, and a Factor Xa recognition site at the N terminus (EC-D1D2(His10Xa)), and pET15bEC-D2D3(His10) encoding domains 2 and 3 (residues 109–332) with an additional His\(_{10}\) tag (EC-D2D3(His10)) were used to express recombinant proteins in *Escherichia coli* strain BL21 (DE3) pLysS. Soluble EC-D1D2(His10Xa) was subjected to Ni\(^{2+}\)-nitrilotriacetic acid affinity chromatography (His-TrapFF, 5 ml; GE Healthcare), or the inclusion bodies of EC-D1D2(His10Xa) were dissolved in guanidine buffer (6 M guanidine HCl, 50 mM MES-NaOH, pH 6.5, 100 mM NaCl, 10 mM EDTA). To refold the recombinant protein, 10–20 mg of solubilized inclusion bodies were gradually diluted by the addition of refolding buffer (20 mM Tris-HCl, pH 7.9, 300 mM NaCl, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride) at 4 °C, into a final volume of 1 liter. The resulting solution was stirred for 2 days and was concentrated to 5–10 ml by a VIVA FLOW system and an Amicon Ultra filter (Millipore). The protein was purified by gel filtration chromatography (HiLoad26/60 Superdex 75 pg; GE Healthcare). After purification, EC-D1D2(His10Xa) was treated with Factor Xa to cleave the extra amino acids. The resulting EC-D1D2 protein was designated as EC-D1D2. EC-D2D3(His10) was prepared by the method of the refolded EC-D1D2.

The DNA encoding the extracellular region (residues 73–188) of *Mus musculus* KLRG1 was ligated into pET3c, to create the plasmid pET3cKLRG1. The recombinant protein was expressed as inclusion bodies and was refolded, in a similar manner as EC-D1D2 and EC-D2D3. The refolded protein was purified by gel filtration chromatography (Fig. 1). For surface plasmon resonance (SPR) analysis, a biotinylated version of KLRG1 was prepared, as described previously (5).

SPR—Recombinant cadherins, including the Ala-scanning mutants, were dissolved in HBS-P buffer (10 mM HEPES, pH 7.4, 150 mM NaCl, 0.005% Surfactant P20) (BIAcore AB). SPR experiments were performed with a BIAcore2000 (Biacore AB). Biotinylated KLRG1 was immobilized on the CM5 sensor chip (Biacore AB), onto which streptavidin had been covalently coupled. Biotinylated bovine serum albumin was used as a negative control protein. All cadherin samples were injected over the immobilized KLRG1 protein, at a flow rate of 10 μl/min, in HBS-P buffer with 10 mM CaCl\(_2\) or 3 mM EDTA (for the Ca\(^{2+}\)-free conditions). The binding response at each concentration was calculated by subtracting the equilibrium response measured in the control flow cell from the response in each sample flow cell. The data were analyzed using the BIA evaluation version 4.1 (Biacore AB) and ORIGIN version 7 software (Microcal Inc.). Affinity constants (K\(_{\text{d}}\)) were derived by nonlinear curve fitting of the standard Langmuir binding isotherm.

Analytical Ultracentrifugation (AUC)—AUC was carried out using a Beckman Optima XL-I analytical ultracentrifuge with absorption optics, an An-50 Ti rotor, and standard double-sector centerpiece cells. Sedimentation equilibrium measurements were performed at 4 °C, and concentration profiles were recorded at 0, 20, 23, 26, and 29 h after the velocity of the rotor reached 9,000, 14,000, and 20,000 rpm. At each time and velocity, one scan was collected. Data were analyzed using the standard Optima XL-I data analysis software. All of the protein samples were in 20 mM Tris-Cl buffer, pH 7.4, with 100 mM NaCl. Monomeric EC-D1D2 and monomeric KLRG1 were mixed at a molar ratio of 1:1 without calcium.

**NMR Analysis of E-cadherin Binding to KLRG1**—Uniformly \(^{15}\)N-labeled EC-D1D2 was expressed in *E. coli* BL21(DE3) pLysS bearing the plasmid pET15bEC-D1D2(His10Xa), grown in M9 minimal medium containing 1 g/liter \(^{15}\)NH\(_4\)Cl, and was prepared by the same method used for the unlabeled EC-D1D2. KLRG1 was prepared as described previously. The KLRG1 and EC-D1D2 proteins were both dissolved in the HBS-E buffer (10 mM HEPES, pH 7.4, 150 mM NaCl, 3 mM EDTA). For the direct
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RESULTS

Preparation of Recombinant Proteins—N-terminal domains 1 and 2 of *M. musculus* E-cadherin (residues 1–221) with a His10 tag, a spacer sequence, and a Factor Xa recognition site at the N terminus were expressed in *E. coli* either as a soluble protein or inclusion bodies. The soluble protein was purified using Ni2+-nitrilotriacetic acid chromatography, and the inclusion bodies were refolded by the standard dilution method. For both proteins, the extra N-terminal amino acids were removed with Factor Xa, resulting in the authentic N-terminal sequence (Asp1-Trp2-Val3-), designated as EC-D1D2. EC-D1D2 was further purified by ion exchange chromatography. A final yield of 2–4 mg of purified EC-D1D2 was obtained from 1 liter of culture. N-terminal domains 2 and 3 of *M. musculus* E-cadherin (designated as EC-D2D3; residues 109–332) was also produced as inclusion bodies in *E. coli* and refolded by the standard dilution method. The refolded protein was purified by gel filtration chromatography.

Next, the extracellular region of *M. musculus* KLRG1 (residues 73–188) was expressed in *E. coli* as inclusion bodies. KLRG1 was refolded by the dilution method, in a similar manner as E-cadherin. The refolded protein was purified by gel filtration chromatography (Fig. 1, A and B), and the final yield was 1 mg of KLRG1 from 1 liter of culture.

Surface Plasmon Resonance Analysis of KLRG1-E-cadherin Binding—To clarify the molecular interaction between KLRG1 and E-cadherin, we performed SPR analysis, using recombinant E-cadherins EC-D1D2 and EC-D2D3. Recombinant E-cadherin variants EC-D1D2 and EC-D2D3 were injected over the flow cells, in which biotinylated KLRG1 had been immobilized at a level of 1,000 response units. As a negative control, biotinylated bovine serum albumin was immobilized on one of the flow cells, at a level similar to that of KLRG1. The response derived from the negative control was subtracted from each response derived from the recombinant E-cadherins. Fig. 1, C and D, shows the conventional plots of these binding data. In accordance with recent results showing that the deletion of either domain 1 or 2 abolished reactivity in KLRG1 reporter cell assays (18), Fig. 1C indicates that KLRG1 can bind to domains 1 and 2 of E-cadherin (EC-D1D2) but not to domains 2 and 3 (EC-D2D3). The KLRG1-EC-D1D2 interaction conforms to a simple 1:1 (Langmuir) binding model, and its dissociation con-
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FIGURE 1. Binding analysis of KLRG1 to E-cadherin using SPR and AUC. A, gel filtration chromatogram of KLRG1 on HiLoad26/60 Superdex 75 pg (GE Healthcare). The bars indicate the elution positions of the molecular mass markers (kDa). KLRG1 was eluted at the peak indicated by the black arrow. B, this peak was analyzed by SDS-PAGE, which indicated that it contained KLRG1 (indicated by the arrow on the right of the gel). Lane M contained protein markers with standard molecular masses. C and D, binding of E-cadherin variants to KLRG1 in the presence of 10 mM calcium chloride (D) or in the absence of calcium chloride (D). Black squares and black circles indicate EC-D1 and EC-D2D3, respectively. KLRG1 was immobilized on research grade CM5 chips (BIAcore) at 1,000 response units (RU). E, analytical ultracentrifugation indicated that the molecular mass of the EC-D1D2-KLRG1 complex is 36 kDa and the binding affinity is 9 μM. In the lower panel, the open circles show the actual values obtained in this experiment, and the solid line indicates the fitted data based on the monomer-monomer binding model. In the upper panel, the residuals derived from the fitted data are shown.

FIGURE 2. NMR and mutagenesis analyses of E-cadherin binding to KLRG1. A and B, a map of the amino acids with HSQC peaks that shifted or disappeared upon complex formation with KLRG1 is shown (red, disappeared; purple (Tyr36, small open circle to the right of A), 0.06 ppm chemical shift change; green, < 0.06 ppm chemical shift change; white, unassigned). The dotted line indicates the putative KLRG1 binding area. The surfaces of EC-D1D2 (A) and EC-D1 (B) are shown. C, mapping of the significant residues that showed no or reduced binding affinity on the structure of E-cadherin domain 1. Trp2, colored orange, did not bind to KLRG1. Light orange coloring of the residues (Val3 and Pro6) indicates that these mutations reduced the KLRG1 binding by 5–6-fold, relative to the wild type. Yellow coloring of the residues (Ile4 and Pro5) indicates that these mutations abolished KLRG1 tetramer binding. All mutations of orange, light orange, and yellow residues abrogated KLRG1-mediated inhibition of NK cell cytotoxic activity. D, amino acid sequence alignment of E-cadherin and other type I classical cadherins. The amino acid numbers above the alignment are derived from E-cadherin. The red characters show the positions of the Ala mutations that reduced KLRG1 binding. Residues that showed large chemical shift changes or disappeared upon complex formation in the HSQC spectra are boxed.
Mutagenesis Mapping of the KLRG1 Binding Site on E-cadherin by Tetramer Staining and Reporter Cell Assays—To confirm the results of the NMR binding study, alanine-scanning mutagenesis was performed. The mutants of EC-D1D2, shown in Table 1, were prepared as described under “Experimental Procedures.” SPR analysis using these mutants showed that the W2A mutant cannot bind to KLRG1. In addition, both the V3A and P6A mutants showed significantly reduced KLRG1 binding. All of these amino acids are in the N-terminal region of E-cadherin (Fig. 2, C and D), consistent with the results of the NMR analysis. The other mutants, including the D90K mutant, bound to KLRG1 at levels comparable that of the wild-type protein. The mutagenesis data are summarized in Table 1.

To further examine the KLRG1 binding site on the N-terminal region of E-cadherin, KLRG1 tetramer staining to bind wild type or a series of mutant E-cadherins (D1A, W2A, V3A, I4A, and P5A) expressed on the cell surface was examined by flow cytometry (Fig. 3A). BW5147 cells expressing wild-type or mutant E-cadherin were generated by retroviral transduction, and E-cadherin expression was confirmed by anti-E-cadherin staining with monoclonal antibody, ECCD2, as shown in supplemental Fig. S2. PE-conjugated KLRG1 tetramer was prepared as described under “Experimental Procedures.” The W2A, V3A, I4A, and P5A mutants failed to bind the KLRG1 tetramer. These data indicate that KLRG1 recognizes a relatively large area of the N-terminal region of E-cadherin that is responsible for homophilic association, supporting the idea that KLRG1 can only bind to monomeric E-cadherin. Interestingly, the D1A mutant E-cadherin bound to the KLRG1 tetramer at levels comparable with that of wild type (Fig. 3A).

We next performed KLRG1 reporter cell assays using the KLRG1-expressing cell line BWZ.muKLRG1 and BW5147 cells expressing either wild-type or mutant E-cadherins. The BWZ.muKLRG1 cells express a chimeric receptor that has the extracellular and transmembrane regions of KLRG1 and the cytoplasmic region of mouse T cell receptor ζ chain, which can mediate the activating signal to induce interleukin-2 gene expression. This cell line also harbors a β-galactosidase reporter gene under the control of the interleukin-2 promoter. Thus, the E-cadherin binding to the chimera KLRG1 can stimulate the β-galactosidase expression. The W2A, V3A, I4A, and P5A E-cadherin mutants failed to induce β-galactosidase expression (Fig. 3B). In contrast, the wild-type and D1A E-cadherins induced the expression of β-galactosidase (Fig. 3B). These results are consistent with the SPR and tetramer binding experiments and indicate that the N-terminal region of E-cadherin plays an essential role in KLRG1-mediated cellular signaling.

**Table 1**

| Analytes | $K_d$ (25°C) | Analytes | $K_d$ (25°C) |
|----------|--------------|----------|--------------|
| Wild type | 12 ± 3.0     | K73A     | 5.5 ± 0.7    |
| D1A      | 19 ± 0.0     | I75A     | 8.0 ± 0.0    |
| W2A      | NB           | Y77A     | 22.0 ± 0.0   |
| V3A      | 66 ± 2.1     | E89A     | 30.0 ± 0.7   |
| P6A      | 86 ± 9.2     | D90K     | 20 ± 2.1     |
| N20A     | 14 ± 0.7     | P91A     | 8.6 ± 0.2    |
| F35A     | 78 ± 0.1     | E93A     | 24 ± 2.8     |
| S37A     | 62 ± 0.3     | V95A     | 22 ± 0.0     |
| T39A     | 88 ± 0.3     | T97A     | 19 ± 0.7     |
| Q64A     | 13 ± 0.7     |          |              |

This result indicates that KLRG1 can recognize the N-terminal region of EC-D1D2. The opposite face of the N-terminal region did not have any residues that showed large chemical shift changes (Fig. 2A).

**Discussion**

E-cadherin is expressed mainly by epithelial cells and mediates Ca$^{2+}$-dependent cell-cell adhesion. The N-terminal regions of cadherins are known to play an important role in homotypic adhesion (14, 17). The N-terminal region of E-cadherin can adopt two conformations. In the intramolecular conformation, Trp2 interacts with Glu89 and Met92. In the intermolecular conformation, the N-terminal region can dock with domain 1 of another cadherin to form an adhesive dimer, in which strand exchange occurs (Fig. 4, left) (21). This study shows that KLRG1 recognizes a relatively broad area of the N-terminal region of E-cadherin (Trp$^2$–Pro$^6$), which largely overlaps with the strand-exchanged homodimer interface. In addition, cells expressing E-cadherin mutants (W2A, V3A, I4A, and P5A) could not interact with KLRG1 tetramer or induce intracellular signaling of KLRG1 reporter cell lines. Further, the expression of the N-terminal mutant E-cadherins (W2A, V3A, I4A) on target cells did not inhibit the cytotoxic activity of KLRG1-expressing NK cells. These results demonstrate that KLRG1 specifically recognizes the strand-exchanged homodimeric interface of E-cadherin, indicating that KLRG1 only binds to the monomeric form of E-cadherin, with an
exposed N-terminal region, to regulate immune function, such as NK cell cytotoxicity.

In normal epithelial tissues, two E-cadherin molecules interact with each other to form strand-exchanged homodimeric complexes constituting adherent junctions, as described above, and thus they are not easily accessible to NK cells or T cells (Fig. 4, left). However, E-cadherin may be exposed on the surfaces of disrupted or infected epithelial cells. In turn, NK cells and T cells can directly detect E-cadherin by utilizing KLRG1, but this recognition occurs in a more sophisticated manner. Here, we show that KLRG1 recognizes the N-terminal strand-exchanged homodimer interface of E-cadherin and thus can only bind to the monomeric form to inhibit KLRG1-mediated cytotoxicity. This monomeric form is thought to be expressed on abnormal epithelial tissues. Therefore, NK cells and T cells can mediate suitable inhibitory responses via KLRG1 binding to only monomeric E-cadherin. The KLRG1-E-cadherin interaction is presumably safe and efficient for inhibiting the excess functional responses of NK cells as well as those of the SLECs (Fig. 4, right). This function is similar to that of inhibitory costimulatory molecules, such as CTLA4 (cytotoxic T-lymphocyte antigen 4).

The αvβ7 integrin (CD103) receptor also recognizes E-cadherin (22). The αvβ7 integrin is found on intraepithelial lymphocytes, where it mediates their adhesion to epithelial cells and enhances their function (23). The αvβ7 integrin recognizes the conserved residue Glu31 and its surrounding area at the top of E-cadherin domain 1 (supplemental Fig. S3) (23), adjacent to the KLRG1 binding site. Thus, KLRG1 may physically interfere with αvβ7 integrin binding. Although the expression of KLRG1 in activated intraepithelial lymphocytes has not been determined, if KLRG1 is expressed simultaneously with αvβ7 integrin, it may inhibit excess immune responses both by trans-

FIGURE 3. Effects of E-cadherin mutagenesis on KLRG1 tetramer binding, reporter cell assays, and KLRG1-expressing NK cell killing assays. A, untransduced BWS147 cells or BWS147 cells transduced with wild-type or mutant E-cadherin (D1A, W2A, V3A, I4A, and P5A) were stained with KLRG1 tetramer (shaded histograms) or PE-streptavidin (thin lines). B, KLRG1 reporter cells were stimulated with the indicated target cells for 16 h and assayed for β-galactosidase activity. The bars represent the relative activities. C, KLRG1-expressing NK03 cell killing assays against BWS147 cells expressing wild-type or mutant E-cadherins. The assays were performed in the presence or absence of the anti-KLRG1 3D4 F(ab’2). Representative data for wild-type, D1A, and V3A E-cadherins are shown. D, bar (mean ± S.D.) indicates the differences of KLRG1-expressing NK03 cell cytotoxicity against target cells expressing the indicated E-cadherins in the presence and absence of the anti-KLRG1 3D4 F(ab’2). The difference of target cells expressing wild-type E-cadherin was set as 100%.
The mutation or lack of E-cadherin in some cancer cells causes the loss of cell adhesion and the subsequent acquisition of cellular motility, facilitating tumor metastasis. Such abnor-
mal cells may be susceptible to NK cells and T cells, because of the lack of KLRG1-mediated inhibitory signals. On the other hand, some tumors may exploit the KLRG1-E-cadherin interaction to their advantage. Such tumor cells might initially down-regulate E-cadherin to acquire motility and metastatic potential but then re-express E-cadherin to establish adher-
ent metastatic foci and avoid immune attack by NK cells and T cells (24–27). Therefore, the develop-
ment of inhibitors of KLRG1-E-cadherin recognition is important for eliminating these cancer cells. Our results demonstrate that KLRG1 recognizes the N-terminal amino acids of monomeric E-cad-
herin domain 1, which are well con-
served in the type I classical cad-
herins (Fig. 2D). In fact, KLRG1 can also bind to the other type I classical cadherins, neuronal and retinal (N- and R-cadherins) (5), strongly sug-
uggesting that the N-terminal amino acids are a major determinant for the KLRG1 binding. Therefore, the N-terminal region may be a useful template for designing inhibitors of the KLRG1-E-cadherin interaction.

CONCLUSIONS

This study demonstrates that KLRG1 recognizes the N-terminal region of E-cadherin, known to be critical for homophilic adhesion. Therefore, we propose that KLRG1 cannot bind to the homodimeric form of E-cadherin but rather binds to the monomeric form. The monomeric form of E-cadherin is exposed on the adherens junction in special condi-
tions, such as damaged epithelial tissues. Under these circumstances, it may serve as a target for KLRG1 recognition to mediate inhibitory signals, raising the activation threshold of NK cells and T cells and preventing excessive immune response.

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