Identification and Functional Analysis of Ligands for Natural Killer Cell Activating Receptors in Colon Carcinoma

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Natural killer (NK) cells play important roles in the immune defense against tumor cells. The function of NK cells is determined by a balance between activating and inhibitory signals. DNAX accessory molecule-1 (DNAM-1) and NK group 2 member D (NKG2D) are major NK cell activating receptors, which transduce activating signals after binding their ligands CD155, CD112 and major histocompatibility complex class I-related chains A and B (MICA/B). However, the expression and functions of these ligands in colon carcinoma are still elusive. Here, we show the higher expression of CD155, CD112 and MICA/B in colon carcinoma tissues, although no correlations between the ligands expression and patient clinicopathological parameters were found. The subsequent cytotoxicity assay indicated that NK cells effectively kill colon carcinoma cells. Functional blocking of these ligands and/or receptors with antibodies led to significant inhibition of NK cell cytotoxicity. Importantly, expression of DNAM-1 and NKG2D was reduced in NK cells of colon cancer patients, and this reduction could directly suppress the activation of NK cells. Moreover, colon cancer patients have higher serum concentrations of sCD155 and sMICA/B (soluble ligands, secreted or shed from cells) than those in healthy donors (sCD155, 127.82 ± 44.12 vs. 63.67 ± 22.30 ng/ml; sMICA, 331.51 ± 65.23 vs. 246.74 ± 20.76 pg/ml; and sMICB, 349.42 ± 81.69 vs. 52.61 ± 17.56 pg/ml). The up-regulation of these soluble ligands may down-regulate DNAM-1 and NKG2D on NK cells, ultimately leading to the inhibition of NK cytotoxicity. Colon cancer might be a promising target for NK cell-based adoptive immunotherapy.

Keywords: activating receptors; colon cancer; ligands; natural killer cells; tumor immunotherapy

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CD155 and its family member CD112 (PVR-related family 2, also called nectin-2) are ligands for DNAM-1 and they are highly expressed in human carcinomas, melanomas, and neuroblastosomas (Bottino et al. 2003). CD155 and CD112 have also been confirmed to promote and strengthen the anti-tumor effects of NK cells (Chang and Ferrone 2006; Malmberg et al. 2008). The activating receptor NKGD2 is a type II transmembrane-anchored glycoprotein. Known ligands of the NKGD2 receptor are the major histocompatibility complex class I-related chains (MIC) A and B (MICA/B) and the cytomegalovirus UL16-binding proteins 1-4 (ULBP1-4) (Stern-Ginossar and Mandelboim 2009). Previous studies have proven that MICA/B and ULBPs are expressed on a number of human epithelial tumor and leukemia cell lines, and that they have significant roles in rendering these cells susceptible to NK cell-mediated lysis (Pende et al. 2002; Saito et al. 2011). In addition, MICA and ULBP2/3 are expressed by some colorectal cancer cell lines, and the engagement of NKGD2 could influence the pattern of anti-tumor reactivity by T lymphocytes (Maccalli et al. 2003). However, the expression patterns and clinical significance of CD155, CD112 and MICA/B in human colon cancer are still elusive. Moreover, few studies have focused on the interactions between activating receptors and their ligands in the NK cell-mediated killing of colon carcinoma (Masson et al. 2001; Tahara-Hanaoka et al. 2006), but the roles of CD155, CD112, and MICA/B in this killing process are still undetermined.

In this study, we first investigated the expression of CD155, CD112 and MICA/B in colon carcinoma tissues derived from patients with primary colon cancer, and analyzed the susceptibility of colon carcinoma cell lines to NK cell-mediated killing. Furthermore, by functional blocking of the relevant receptors and ligands, we analyzed the roles of the receptor-ligand interactions in NK cell-mediated lysis of tumor cells.

**Materials and Methods**

**Reagents and cells**

The following conjugated or nonconjugated monoclonal antibodies (mAb) were used: Purified mouse anti-human MICA/B mAb (clone number 6D4, Biologend), anti-CD112 mAb (clone R2.525, abcam), anti-CD155 mAb (colon PV404.19, Beckman Coulter), anti-CD56 + , and the

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**Immunohistochemistry**

Before use, the slides were incubated with normal goat serum for 10 min. CD155, CD112, and MICA/B mAb were used as the primary antibody at 5 μg/ml in antibody diluent containing background-reducing components (Dako, Trappes, France). Following washing and incubation for 1 h at room temperature, goat anti-mouse IgG (Sigma) was then added as the secondary antibody. Control for specific staining, an irrelevant monoclonal mouse IgG antibody was used.

Two investigators and a pathologist graded the CD155, CD112, and MICA/B expression in a blinded fashion. The degree of staining was categorized by the extent and intensity of the staining. The immunoreactive score was determined by the sum of extension and intensity as reported previously (Koomagi and Volm 1999; Rahman et al. 2001).

**Ligand expression and NK degranulation assays**

A ligand expression assay was conducted by flow cytometry. Single cell suspensions from four colon carcinoma cell lines and K562 were analyzed for CD155, CD112, and MICA/B expressions by immunostaining with mAbs, followed by analysis by flow cytometry (Beckman Coulter EPICS-XL). Cells were incubated with 1 μg of human IgG per 10⁵ cells for 30 min on ice to block the FC receptors. Thereafter, cells were stained with non-conjugated primary mAbs (PV404.19, R2.525, and 6D4, 1 μg per 1 × 10⁵) for 30 min on ice followed by staining with FITC-labeled goat anti-mouse IgG. Irrelevant isotype-matched mouse IgG was used as the negative control. Data were acquired on the flow cytometer and were analyzed using software.

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SW116, SW480, SW620, and Colo205 colon carcinoma cell lines were grown in RPMI-1640 containing L-glutamine and sodium bicarbonate (Sigma, Saint Quentin Fallavier, France) supplemented with 10% heat-inactivated fetal calf serum (FCS, Gibco), penicillin (100 U/ml) and streptomycin (0.1 mg/ml). Cells were maintained at 37°C in 5% carbon dioxide and passage twice a week. Both K562 and P815 were cultivated in complete RPMI-1640 medium. For the isolation of NK cells,uffy coats from healthy donors and colon cancer patients were separated by density gradient centrifugation (Ficoll Hypaque). NK cells were then enriched by the magnetic bead negative selection (NK selection kit, Miltenyi Biotech) of the nonadherent fraction. NK cells used in assays were > 95% CD3/CD56⁺, and the CD3⁺ cell contamination was < 1%. NK cells were either used directly in experiments or activated with IL-2 (100 IU/ml) for 5 to 7 days before use. The collection of all blood and tumor samples was approved by the Ethics Committee of Fourth Military Medical University, and informed consent was obtained.

**Tumor samples**

Tissue samples were obtained from 42 patients (25 male and 17 female with a mean age of 62 years and a range of 45-76) that underwent surgical resections for primary colon carcinoma diagnosed at the Department of Pathology, Xi‘jing Hospital (affiliated with Fourth Military Medical University, China). All 42 patients were diagnosed with adenocarcinoma; tumor tissues and adjacent normal mucosa (about 10 cm from the tumor) from each patient were excised and collected. Samples were embedded in paraffin and cut into 5 μm-thick sections.
Analysis of NK cell degranulation (CD107a is considered as a marker of degranulation) was also conducted. NK cells from healthy donors were coincubated with the target cells (K562, SW116, SW480, SW620, and Colo205) at a ratio of 1:1 in a final volume of 200 μl in round-bottomed 96-well plates at 37°C and 5% CO₂ for 6 h. PE-Cy5-conjugated anti-CD107a mAb or the corresponding IgG1 isotype control was added at the initiation of the assay. After coincubation for 1 h, Golgistop (monensin: BD) was added at a 1:100 dilution. NK cell surface markers were stained with anti-CD3 and anti-CD56 mAbs for 15 min on ice. The cells were then washed and resuspended in cellfix and analyzed by flow cytometry.

**DNAM-1 and NKG2D expressions on NK cells**

Flow cytometry was used to analyze DNAM-1 and NKG2D expressions on NK cells from the peripheral blood of healthy donors and colon cancer patients. Freshly isolated NK cells (obtained from 20 healthy donors and 17 colon cancer patients after informed consent) were stained with PE-labeled DNAM-1 and NKG2D antibodies; their expression levels were determined on the NK cells. An isotype control was used in the assay.

**Flow cytometry-based NK cell killing assays**

Colon carcinoma cell lines and the NK-resistant P815 cell line were used as targets. NK cells derived from the blood of healthy donors (n = 20) and colon cancer patients (n = 17) were used as effectors. The target cells were labeled with carboxyfluorescein diacetate, succinimidyl ester, CFSE (Cat # V-12883, Molecular probes, USA) for 15 min at 37°C and 5% CO₂ using a final concentration of 7.5 μmol/l. After labeling, the same volume of FCS was used to stop the CFSE and cells were washed 2 times. Freshly isolated resting NK cells (nonactivated peripheral blood NK cells) or IL-2-activated NK cells (to maximize cytotoxicity) were coincubated with target cells at a ratio of 5:1 in a final volume of 200 μl. The tubes were mixed and centrifugated at 120 ×g for 2 min, and samples were then incubated at 37°C and 5% CO₂ for 6 h. At the end of the incubation time, the samples were placed in an ice water bath and 50 μl of 50 μg/ml propidium iodide (PI, Sigma, Sweden) was added for DNA labeling of dead cells. Samples were then incubated for 5 min and analyzed by flow cytometry within 60 min. The percentage of cytolysis was calculated as follows: 100×[(dead targets in the sample(%)) – spontaneously dead targets (%))/(100 – spontaneously dead targets (%))]. (Marcusson-Stahl and Cederbrant 2003). For the receptor and ligand blocking experiments, NK cells from healthy donors (IL-2 activated) and target cells were preincubated with functional blocking antibody (DX11, 1D11, SKII.4, R2.525, or 6D4) or the isotype control antibody for 30 min at room temperature before coculture.

**Assessment of sCD155 and sMICA/B in human serum**

A standard sandwich enzyme-linked immunosorbent assay (ELISA) was conducted to detect the levels of soluble CD155 and soluble MICA/B (ligands secreted into serum and have similar structures with membrane-anchored types) in sera of healthy donors (n = 30) and colon cancer patients (n = 26). The procedures were performed according to the manufacturer’s instructions and the final concentration was determined by optical density according to the standard curves.

**Statistical analysis**

Mann-Whitney U test was used to evaluate correlations between clinicopathological parameters and ligand expression, as shown in Table 1 (without patient gender and lymph node invasion). The significant difference in patient gender and lymph node invasion of ligands negative and positive were determined via Chi-square and Fisher’s exact tests using SPSS software (version 13). Statistical significance was defined as P < 0.05.

**Results**

**The expression of ligands**

We analyzed expression of the activating NK cell receptor ligands CD155, CD112, and MICA/B on 42 human colon carcinoma tissues and four colon carcinoma cell lines. As shown in Fig. 1, to determine whether CD155, CD112, and MICA/B are expressed in colon cancer tissues, immunostaining experiments were performed on 5μm-thick sections with monoclonal antibodies (PV404.19, R2.525, and 6D4). The expression of each ligand was detected in tumor samples and strong brown signals were present on the membranes. In each case, the sections used for the negative control, which were stained with an irrelevant antibody, were negative. In adjacent normal mucosa, CD155 rather than CD112 and MICA/B showed weak staining on the epithelial cells. The positive rate of CD155, CD112, and MICA/B on tumor tissues was 81, 52.4, and 47.6%, respectively. Simultaneous expression of CD155, CD112, and MICA/B was found in only 9 cases (21.4%). Conversely, the expression of the three ligands was not detected in 4 cases (9.5%). Up to 19 (45.2%) cases were double positive for CD155 and CD112, and 18 (42.9%) expressed CD155 and MICA/B simultaneously. In the comparative analysis of clinicopathological parameters of colon cancer and the ligands expression, all staining was independent of patient gender and Duke’s stage of the

![Fig. 1. IHC analysis of CD155, CD112, and MICA/B expressions in colon carcinoma tissues.](image-url)
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Table 1. Ligand expression and relationship with various clinicopathological parameters.

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<tr>
<th>Gender a</th>
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<th>CD112</th>
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<th>MICA/B</th>
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<th>CD112</th>
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*Chi-square and Fisher’s exact tests. ©P value, CD155 or CD112 or MICA/B expression versus patient’s gender. ©P value, CD155 or CD112 or MICA/B expression versus lymphoid invasion.

©Mann-Whitney U test. ©P value, CD155 or CD112 or MICA/B expression versus tumor differentiation. ©P value, CD155 or CD112 or MICA/B expression versus Duke’s stages.

Fig. 2. Expression of CD155, CD112, and MICA/B in colon carcinoma cell lines.

Flow cytometry analysis for the expression of CD155, CD112, and MICA/B in four colon carcinoma cell lines. A, SW116, SW480, SW620, and Colo205 cells were stained with non-conjugated primary mAbs, followed by staining with a FITC-labeled goat anti-mouse IgG (solid line). An irrelevant isotype matched mouse IgG was used as the negative control (filled) and cells were then analyzed using a flow cytometer. K562 is a positive control for all stainings. B, the expression of the three ligands by four carcinoma cell lines. C, CD155 was expressed at higher levels than CD112 and MICA/B. Column, mean of the ligands expression by four colon carcinoma cell lines obtained from 10 experiments respectively; bar, SD. MFI: Mean Fluorescence Intensity.

tumor, differentiation, and lymph node invasion. No statistically significant correlations were found between the ligands expression and clinicopathological parameters (Table 1). To investigate further the expression of these ligands in the cell lines, four human carcinoma cell lines derived from different Duke’s stages were used and the erythroleukemia cell line K562 served as the positive control for all stainings. As illustrated in Fig. 2, CD155, CD112, and MICA/B were expressed in all cell lines. However, CD155 showed higher expression levels compared to those of CD112 and MICA/B.

NK cell-mediated killing of colon carcinoma cell lines

To assess the ability of NK cells in killing colon carci-
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In colon cancer cells, a flow cytometric NK-cytotoxicity assay was used, and the specific killing rates were calculated (Marcusson-Stahl and Cederbrant 2003). As shown in Fig. 3A, each experimental system has three control groups (a-c) and one (d) experimental group. Interestingly, we found that the specific killing rates mediated by NK cells from colon cancer patients were much lower than those mediated by isolated NK cells from healthy donors. Resting NK cells have cytotoxic activity against a limited number of human tumor cell lines. All four colon cancer cells were lysed by freshly isolated resting NK cells. Activation of NK cells with IL-2 increased the efficacy of lysis of colon cancer cells by NK cells (Fig. 3B).

We conducted blocking experiments to investigate further the roles of receptor-ligand interaction in this killing process. After blocking the DNAM-1 and NKG2D receptors simultaneously, the specific killing was dramatically decreased under the same experimental conditions. In contrast, the killing rates of the Colo205 and SW480, which highly express CD155 and CD112 but lacks MICA/B expression, were mainly dependent on DNAM-1 rather than NKG2D. Furthermore, SW116, which has strong expression of CD155 and MICA/B but lower expression of CD112, was dependent on both receptors pathways. However, the control group in the blocking experiment showed no significant change in killing rate (Fig. 4A). These findings were partially similar to those of a previous research in myeloma (El-Sherbiny et al. 2007). To know more about the interaction between receptors and ligands, neutralizing anti-CD155, anti-CD112, and anti-MICA/B were used in the blocking assay (Fig. 4B). The results confirmed that CD155, CD112, and MICA/B are required for the NK cell-mediated tumor killing process. After blocking CD155, the specific killing dramatically decreased in all cell lines. However, in SW480 and Colo205, which have higher CD112 expression than CD155, the specific killing after blocking CD112 was not decreased to the levels obtained after blocking CD155. After blocking all ligands, the NK-mediated killing was significantly inhibited.

**NK cell expression of DNAM-1 and NKG2D**

It has been reported that DNAM-1 expression density is reduced in the NK cells of myeloma patients with active disease compared with patients in remission (El-Sherbiny et al. 2007). Moreover, NK cells also showed NKG2D down-regulation in patients with gastric cancer (Saito et al. 2011). Intriguingly, we found a similar phenomenon when attempting to explain why the cytotoxicity of NK cells from colon cancer patients decreased (Fig. 5). Compared with those derived from healthy donors, expression levels of both DNAM-1 and NKG2D were reduced in NK cells derived from colon cancer patients.

**Serum levels of sCD155 and sMICA/B in patients and healthy donors**

Suppression of NK cell function in cancer patients has been associated with reduced expression of activating NK cell receptors, but the mechanisms for receptor modulations...
are not fully understood (Costello et al. 2002; Lee et al. 2004; Krockenberger et al. 2008). Chronic receptor-ligand interactions may cause loss of DNAM-1 expression by NK cells in the tumor environment, thereby contributing to poor NK cell-mediated elimination of ovarian carcinoma cells (Carlsten et al. 2009). Moreover, researchers speculate that ligands released from tumor cells and soluble ligands in serum would competitively inhibit the normal NK cell-activating signal and induce the internalization and degradation of activating receptors (Groh et al. 2002; Oppenheim et al. 2005; Salih et al. 2006). Therefore, we compared the serum levels of sCD155 and sMICA/B between colon cancer patients and healthy donors (Fig. 6). The mean value of sCD155 was significantly higher among patients than among healthy donors (127.82 ± 44.12 ng/ml vs. 63.67 ± 22.30 ng/ml). The concentration of sMICA and sMICB was also found to be up-regulated in cancer patients compared with healthy donors (sMICA: 331.51 ± 65.23 pg/ml in patients vs. 246.74 ± 20.76 pg/ml in healthy donors; sMICB: 349.42 ± 81.69 pg/ml in patients vs. 52.61 ± 17.56 pg/ml in healthy donors).

Colon carcinoma cells trigger NK cell degranulation

In a previous study, Carlsten et al. (2007) proved that freshly isolated ovarian carcinoma cells could trigger NK cell degranulation. This method was utilized to investigate whether NK cells mediate the lysis of colon carcinoma cells by releasing perforin and granzyme B. To determine if NK cells could mediate the killing of the target cells by releasing perforin and granzyme B, the surface expression of CD107a on NK cells following contact with colon carcinoma cell lines were analyzed because surface expression of CD107a closely correlates with degranulation and release of perforin and granzyme B by NK cells (Betts et al. 2003; Alter et al. 2004). As shown in Fig. 7, 0.82% of the NK cells expressed CD107a under the condition of no stimulating target cells. However, the CD107a expression in NK cells was dramatically increased when induced with MHC-devoid K562 target cells (20.3%). Colon cancer cell lines also triggered significant NK cell degranulation, albeit at lower levels compared with K562 cells. This outcome indicated that the colon carcinoma cell lines could effectively stimulate the degranulation of NK cells. In the process of NK cell-mediated killing of colon carcinoma, the releasing pathway may serve as an essential and/or critical mode.

Discussion

As our understanding of the molecular mechanisms governing NK cell activity increases, their potential in cancer immunotherapy is growing increasingly prominent. NK cells are activated by cytokines and by interactions with specific molecules expressed on target cells. Upon activation, NK cells produce cytokines and chemokines and can exert strong cytolytic effects. Hence, knowledge of the activating receptor ligands expression on solid tumor cells is essential for introducing a more effective NK cell-based immunotherapy protocols into clinical practice. Numerous studies have demonstrated NK cell killing of many different types of murine and human tumor cell lines in vitro (Smyth et al. 2002; Wu and Lanier 2003). NK cell activating receptors DNAM-1 and NKG2D participate in NK cell-mediated killing of some tumors, such as myelomas, thyroid carcinomas, and ovarian carcinomas, among others (Costello et al. 2002; Castriconi et al. 2004; Pende et al. 2005; Carlsten et al. 2007; El-Sherbiny et al. 2007). However, much less is known about the expression patterns of activating NK receptor ligands and these receptor-ligand interactions in colon cancer.

In this study, the results confirmed the presence of
CD155, CD112, and MICA/B in colon carcinoma. To the best of our knowledge, this study is the first to broadly investigating the expression of CD155, CD112, and MICA/B in a cohort of colon cancer tissues and a panel of tumor cell lines. Interestingly, the CD155 expression was stronger than that of CD112 and MICA/B in the cell lines and tumor tissues. CD155 might be more frequently up-regulated in colon cancer than CD112 and MICA/B and the mechanisms behind this phenomenon remain undetermined. Further statistical analysis indicated up-regulated expressions of DNAM-1 and NKG2D ligands in colon cancer tissues compared with adjacent normal mucosal tissues. However, no
correlations between the expression of these ligands and patient clinicopathological parameters were found. Thus, the overexpression of these ligands may merely correlate with tumor transformation.

Previous studies have demonstrated that the overexpression of the CD155 gene in human tumors, including colorectal carcinomas, neuroblastomas, and myeloid leukemias (Masson et al. 2001; Castriconi et al. 2004; Pende et al. 2005). The expression of DNAM-1 ligands may be inducible by "stress," such as tumorigenesis and infection. Recently, researchers found that CD155 and CD112 expressed on RMA (a murine T-cell lymphoma cell line) tumors potentially trigger innate immunity mediated by NK cells and CD8α+ DCs, ultimately resulting in tumor rejection (Tahara-Hanaoka et al. 2006). CD155 also appear to be a key ligand recognized by DNAM-1 in NK cell-mediated suppression of melanoma metastases, and this suppression coincided with perforin activity (Chan et al. 2010). In summary, emerging evidence suggest that DNAM-1 ligands play an important role in NK cell- and T cell-mediated immunity to a variety of tumors. NKG2D ligands are expressed on diseased or stressed cells, and numerous stress pathways lead to the up-regulation of these ligands. Up-regulation of NKG2D ligands during tumorigenesis may render the emerging tumor cells sensitive to NKG2D-dependent elimination. Recently, NKG2D has been demonstrated to play a critical role in tumor immunosurveillance in vivo (Guerra et al. 2008). Interestingly, the expression of high levels of MICA in colon cancer is associated with good prognosis, suggesting that this molecule may make tumors more susceptible to immune attack (Watson et al. 2006).

To investigate further the efficacy of NK cells in recognizing and killing of colon carcinoma cells, an NK cytotoxicity assay was conducted. CD155, CD112, and MICA/B have been found to play important roles in the process of NK cell-mediated killing of colon carcinoma cells, and CD155 seems to be more effective than CD112 and MICA/B in this process. After binding the corresponding receptor DNAM-1 and NKG2D, these ligands significantly enhanced the NK cell-mediated killing activity. Furthermore, as proven by the blocking experiment, the DNAM-1 and NKG2D may have significantly improved tumor cell lysis by NK cells, especially when both of them are expressed. More importantly, we observed that the resting NK cells can efficiently lyse colon cancer cells, and the cytotoxicity of NK cells from colon cancer patients decreased, which may correlates with the reduced expression of DNAM-1 and NKG2D in NK cells. As far as we know, this is the first report wherein DNAM-1 and NKG2D expressions decreased in NK cells of patients with colon cancer. To further detect the existence and variation of soluble ligands in colon cancer patients and healthy donors, ELISA assays were conducted. The higher concentration of sCD155 and sMICA/B in patients may reflect the probability of the speculation that soluble ligands in serum would competitively inhibit the normal NK cell-activating signal and induce the internalization and degradation of activating receptors. Whereas, we could not confirm whether there exists a direct correlation between the down-regulation of activating receptors and the up-regulation of soluble ligands based on the existing data because the present study just observed the up-regulation of these soluble ligands. It needs more intense researches and delicate analysis. Moreover, some previous researches reported the reversibility of cytotoxicity and even the up-regulation of activating receptors after cultivating patient’s NK cells for a time in vitro (Saito et al. 2011). We did not analyze this issue in the present study, and further studies are needed. In the process of NK cell-mediated killing of colon carcinoma,
NK cell degranulation may serve as an essential and/or critical mode, which is consistent with the findings of a study on melanoma (Chan et al. 2010).

Besides DNAM-1 and NKG2D, natural cytotoxicity receptors (NCRs; including three receptors: Nkp46, Nkp44 and Nkp30) is another group of important activating receptors. Although the cellular ligands for Nkp46, Nkp44 and Nkp30 are still elusive, it has been confirmed that these receptors also mediate important role in anticancer activity (Moretta et al. 2001; Arnon et al. 2008; Kim et al. 2009). Moreover, ULBPs (UL-16 binding proteins) are expressed in colon cancer and as NKG2D ligands they have lower significance than MICA/B in judging the patient’s prognosis (McGilvray et al. 2009). It should be noted that we only focused on CD155, CD112 and MICA/B in the present study. A systemic analysis of NK cell activating receptors and all its ligands in colon cancer should be made in the future.

Questions are also raised. Previously, extensive intratumoral infiltration of NK cells has been associated with favorable tumor outcomes (Coca et al. 1997). However, why are the NK cells unable to suppress tumor growth efficiently? Are there changes of NK cell subsets in patients with colon cancer? Now that the DNAM-1 and NKG2D ligands are expressed on colon carcinoma, why do they not promote NK cell-mediated killing in vivo and what factors hamper this process? If we can find ways to eliminate the unknown obstructions and to improve the expression of activating receptors in NK cells of cancer patients, then it will be a great improvement in NK-based tumor immunotherapy. However, present data and speculations could not answer all questions; further studies are needed.

Taken together, activating receptor ligands CD155, CD112, and MICA/B are overexpressed in colon carcinoma. NK cell-mediated killing of colon carcinoma cell lines is dependent on the receptor-ligand interactions, and NK degranulation to release perforin and granzyme B may serve in the killing process. The sCD155 and sMICA/B were increased in serum of colon cancer patient, and this up-regulation may cause the down-regulation of DNAM-1 and NKG2D on NK cells and ultimately lead to the inhibition of NK cytotoxicity. These results may have implications for the design of future protocols of adoptive NK cell immunotherapies for colon carcinoma and possibly other human tumors.

Acknowledgments
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Conflict of Interest
The authors declare no conflict of interest.

References


