Published OnlineFirst January 21, 2011; DOI: 10.1158/0008-5472.CAN-09-4540

Novel Role for STAT3 in Transcriptional Regulation of NK Immune Cell Targeting Receptor MICA on Cancer Cells

Romain Bedel[1,2,3], Antoine Thiery-Vuillemin[1,2,4], Camille Grandclement[1,2,3], Jeremy Balland[1,2,3], Jean-Paul Remy-Martin[1,2,3], Bernadette Kantelip[5], Jean-René Pallandre[1,2,3], Xavier Pivot[1,2,4], Christophe Ferrand[1,2,3], Pierre Tiberghien[1,2,3], and Christophe Borg[1,2,3,4]

Abstract

The role of natural killer group 2, member D receptor (NKG2D)–expressing natural killer (NK) cells in tumor immunosurveillance is now well established. Nevertheless, tumor progression occurs despite tumor immunosurveillance, leading to cancer persistence in immunocompetent hosts. STAT3 plays a pivotal role both in oncogenic functions and in immunosuppression. In this study, we investigated the role of STAT3 in suppressing NK cell–mediated immunosurveillance. Using a colorectal cancer cell line (HT29) that can poorly activate NK, we neutralized STAT3 with pharmacologic inhibitors or siRNA and found that this led to an increase in NK degranulation and IFN-γ production in a TGF-β1–independent manner. Exposure to NKG2D-neutralizing antibodies partially restored STAT3 activity, suggesting that it prevented NKG2D-mediated NK cell activation. On this basis, we investigated the expression of NKG2D ligands after STAT3 activation in HT29, mesenchymal stem cells, and activated lymphocytes. The NK cell recognition receptor MHC class I chain–related protein A (MICA) was upregulated following STAT3 neutralization, and a direct interaction between STAT3 and the MICA promoter was identified. Because cross-talk between DNA damage repair and NKG2D ligand expression has been shown, we assessed the influence of STAT3 on MICA expression under conditions of genotoxic stress. We found that STAT3 negatively regulated MICA expression after irradiation or heat shock, including in lymphocytes activated by CD3/CD28 ligation. Together, our findings reveal a novel role for STAT3 in NK cell immunosurveillance by modulating the MICA expression in cancer cells. *Cancer Res; 71(5); 1615–26. ©2011 AACR.*

Introduction

The immune system can detect and suppress emerging tumors (1). In addition to their role in pathogen immunity, natural killer (NK) cells have been implicated in tumor surveillance in both mice and human models (1–6). Among NK-activating receptors, natural killer group 2, member D receptor (NKG2D) is a C-type lectin-like transmembrane glycoprotein recognizing self-molecules (referred as NKG2D ligands; NKG2DLs) that emerged as a pivotal signaling pathway supporting cancer immunosurveillance. Indeed, transfected tumor cell lines expressing NKG2DLs are rejected *in vivo* in an NKG2D-dependent manner (7, 8).

Recently, generation of NKG2D-deficient mice confirmed the critical role of these stimulatory NK receptors in immunosurveillance of spontaneous prostate cancer and lymphoma models (9). Contrary to prostate cancer arising in NKG2D-deficient mice, tumor cells isolated from fast-growing carcinoma in control mice (and not in smaller, late-arising tumors) lacked NKG2DLs, suggesting an NKG2D-dependent immunoediting (9) and supporting the hypothesis that oncogenic pathways associated with cancer progression might negatively regulate NKG2DLs.

MHC class I chain–related A and B (MICA and MICB) or UL16 binding proteins (ULBP; refs. 7, 10, 11) are NKG2DLs, weakly expressed on normal cells and upregulated in cancers (12–16). Nonetheless, molecular mechanisms leading to NKG2DL regulation are poorly defined.

The enhanced incidence of colorectal cancer (CRC) in patients affected by inflammatory bowel disease (IBD) had established chronic inflammation as a cornerstone mechanism in tumor suppressor checkpoint subversion (17, 18). Particularly, interleukin 23 (IL-23) was shown to increase tumor incidence in mice (19) and to decrease cancer immunosurveillance through STAT3 (20), which is a transcription factor activated in IBD (21–23) and directly involved both in intestinal inflammation and in cancer progression (24, 25).

In this study, we aimed to investigate the role of STAT3 activation in the regulation of NKG2DL expression and

Authors' Affiliations: [1]INSERM (Institut National de la Santé et de la Recherche Médicale) UMR 645, [2]University of Franche-Comté, IFR133; [3]EFS Bourgogne Franche-Comté; and Departments of [4]Medical Oncology and [5]Pathology, CHU Besançon, Besançon, France

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

R. Bedel and A. Thiery-Vuillemin contributed equally to this work.

Corresponding Author: Christophe Borg, INSERM UMR 645, F-25020, Besançon, France. Phone: 33-3-8161-56-15; Fax: 33-3-8161-56-17; E-mail: christophe.borg@efs.sante.fr

doi: 10.1158/0008-5472.CAN-09-4540

Published OnlineFirst January 21, 2011; DOI: 10.1158/0008-5472.CAN-09-4540

recognition of tumor cells by NK cells. We showed that STAT3 ablation in tumor cells modulates NKG2D-mediated NK cell activation. STAT3 directly interacts with MICA promoter to repress MICA transcription. These results shed light on the negative regulation exerted by STAT3 on MICA expression in different cell types submitted to DNA damage or cellular stress.

Materials and Methods

Reagents

The following antibodies were used: anti-human MICA (BZ-26; Diaclone); anti-human CD107a (LAMP-1; H4A3), isotype PE (MOPC-21; BD Biosciences); anti–MICA-PE (2C10), MICB (9847-1), ULBP1 (Z-9), ULBP2 (F16), ULBP3 (2F9), TGF-β1 (C-16; Santa Cruz Biotechnology), anti-human STAT3 (79D7) and phospho-STAT3 (Tyr705, 3E2; Cell Signaling), neutralizing anti-MICA (clone 159227; RnDsystems). STA-21, a selective inhibitor of STAT3, was purchased from BIOMOL International. Oncostatin M (PeproTech) was used in some experiments. The TGF-β1 receptor inhibitor SB-431542 (Tocris Biosciences) was used in some experiments. Dynabeads Human T-Expander CD3⁄CD28 (Invitrogen) were used for stimulation of peripheral blood lymphocytes (PBL).

Cell lines and primary cells cultures

HT29 (ATCC, HTB-38), SW620 (ATCC, CCL-227), Colo320 (ATCC CCL-220), K562 (ATCC CCL-243), MDA-MB231 (ATCC HTB26), U87 (ATCC HTB-14), and 293T (DSMZ ACC-635) cells were verified by morphology, tested for *Mycoplasma*, and conserved in master cell bank on reception. Cells were never used above passage 10. The stroma cell line SV56 was established as previously described (26). Cells were maintained in either RPMI 1640 (K562 and SV56) or Dulbecco's modified Eagle's medium (DMEM; HT29, SW620, and Colo320; Lonza) supplemented with 10% fetal calf serum (Invitrogen). NK cells were purified from healthy donor peripheral blood mononuclear cells by a negative magnetic selection (Stem Cell). The purity of CD56/CD3 NK cells was assessed by flow cytometry and ranged from 90% to 98%. NK cells were maintained in RPMI 1640 medium (Lonza) supplemented with 10% human serum (Invitrogen).

RNA silencing and plasmids constructs

Specific STAT3 siRNA (sense, 5′-AAAGAACTTCAGACCCGTCAACAAA-3′; antisense, 5′-AAAATTTGTTGACGGGTCTGAAGTT-3′) and scramble siRNA (sense, 5′-AAAGGAGGGGCATGCCACGTTGG-3′; antisense, 5′-AAAACCAACGTGGCATGCCCCTC8-3′) sequences were produced, annealed, and cloned into the *Bbs*I site of the 3′-LTR of pFIV-H1/U6 vector according to manufacturer's instructions (System Biosciences). Lentiviral supernatant production and subsequent infection of cell lines were realized according to manufacturer's instructions. Human STAT3C in pBABE vector was provided by Dr. J. Bromberg (27). pGL3-MICA-pro vector was previously described and kindly given by Dr. Jack D. Bui (28).

Site-directed mutagenesis

STAT3 binding site–directed mutagenesis was done according to manufacturer's protocol (QuikChange II XL Site-Directed Mutagenesis Kit; Stratagene). Four base pairs within the STAT3 binding site were predicted to disrupt STAT3 binding, when mutated, without introducing or removing other binding sites. These changes were as follows: (T/C) (T/G) (C/A) (C/T) turning the normal TTCC**TTCC**A**GG**AC STAT3 consensus binding sequence into TTCC**CGAT**A**GG**AC. Two primers were designed to generate the mutated STAT3 binding site in the MICA promoter region of the pGL3-MICA vector. The sequences were the following: Muta-MICA-sense, 5′-cgcgttgtctgtcctgtaaggaacaagccagtg-3′; Muta-MICA-antisense, 5′-cactggcttgttccttacaggacagacaacgcg-3′.

Real-time quantitative PCR

Total RNA was extracted using RNeasy Mini Kit (Qiagen) and reverse transcribed using random hexamers and Moloney murine leukemia virus reverse transcriptase (Life Technologies. Duplicate samples were subjected to real-time quantitative PCR (RT-qPCR). mRNAs were quantified using primers listed as follows: MICA (Hs00792193_m1; Applied Biosystems). ABL mRNA from each sample was quantified as an endogenous control. Relative mRNA expression was calculated using the $\Delta\Delta C_t$ method, and untreated cells were used as the calibrator.

Luciferase assay

HT29 and 293T cells were transfected using Lipofectamine LTX (Invitrogen). In all conditions, *Renilla* luciferase (pRL-TK) and firefly luciferase (triggered by MICA or mutated-MICA promoters in PGL3-MICA vectors) were cotransfected. Firefly luciferase light values were divided by *Renilla* luciferase light values.

ELISA

IFN-γ was detected using commercial ELISA kits (Diaclone). The sensitivity of the human IFN-γ kit was 4.7 pg/mL. MICA was detected using ELISA kits (Diaclone). The sensitivity of the human MICA ELISA kit was 123 pg/mL. All concentrations are expressed as mean ± SEM of triplicates.

NK degranulation assay

NK cells were activated for 24 hours with IL-2 (1,000 UI/mL) and then cocultured in the presence of target cells for 4 hours at 10:1 *E:T* ratio. Degranulation of NK cells was analyzed by flow cytometric analysis of CD107a expression as previously described (29).

Chromatin immunoprecipitation assay

HT29 or 293T cells (5×10^6) were cross-linked with 1% formaldehyde in the presence of protease inhibitors (Complete Mini EDTA Free; Roche) for 15 minutes at room temperature and then treated with 1 mol/L glycine for 5 minutes at room temperature. Cells were harvested and after 2 washing steps with ice-cold PBS, lysed in 500 μL of lysis buffer [50 mmol/L Tris-HCl, pH 7.4, 150 mmol/L NaCl, 1% (v/v) NP40, 0.5% (m/v) Na deoxycholate, 2 mmol/L EDTA, 2 mmol/L NaF, 1 mmol/L vanadate, proteases inhibitor mixture]. A total of 200- to 1,000-bp DNA fragments were generated with 5 times

Published OnlineFirst January 21, 2011; DOI: 10.1158/0008-5472.CAN-09-4540

10-second sonication, using a Vibra Cell sonicator (Sonics & Materials). An aliquot of 100 μL was conserved (total input). Chromatin was immunoprecipitated overnight at 4°C with anti-human STAT3 (clone 79D7) or control rabbit immunoglobulin G (IgG). After a 2 hours incubation with Dynabeads Protein G (Invitrogen), beads were washed twice with wash buffer 1 (0.1% SDS, 1% Triton X-100, 2 mmol/L EDTA, 20 mmol/L Tris, 150 mmol/L NaCl) and then submitted to another washing step with wash buffer 2 (0.1% SDS, 1% Triton X-100, 2 mmol/L EDTA, 20 mmol/L Tris, 500 mmol/L NaCl) and finally 2 washing steps with TE buffer. Beads (and the total input DNA) were subsequently incubated at 65°C overnight to reverse cross-linking. Incubation with Proteinase K (Invitrogen) for 30 minutes at 55°C was done, and DNA samples were purified using QIAamp DNA Mini Kit (Qiagen), collected in 200 μL TE buffer and then assessed by PCR.

Statistical analysis

Results are expressed as the mean ± SEM. Group comparisons were done using Student's *t* test. Differences were considered significant at $P < 0.05$.

Results

Role of STAT3 in colon cancer cell line susceptibility to NK cells

The implication of STAT3 in chronic intestinal inflammation and cancer oncogenesis prompted us to investigate the ability of different colon cancer cell lines to activate NK cells (30–33). For this purpose, NK cells were purified from PBL of normal volunteers and incubated with colon cancer cell lines or with the NK-sensitive K562 cell line for 24 hours. These preliminary experiments indicated that HT29 is a weak activator of NK cell functions *in vitro* compared with Colo320, SW620, and K562 (Fig. 1A and B). The higher expression of STAT3 in HT29 than in K562, Colo320, or SW620 (Fig. 1C) prompted us to investigate the precise role of STAT3 in the recognition of HT29 by NK cells.

To confirm the influence of STAT3 in CRC models, we generated STAT3-deficient cell lines by lentivirus-mediated gene transfer to deliver a specifically designed siRNA for STAT3 into HT29 and to produce a stable cell line ($HT29^{siRNA\text{-}STAT3}$). Western blot analysis confirmed a reduced expression of STAT3 in the knockdown cell line $HT29^{siRNA\text{-}STAT3}$ (Fig. 2A). Moreover, the level of phospo-STAT3 also decreased in $HT29^{siRNA\text{-}STAT3}$ (Fig. 2B).

The next set of experiments was dedicated to assess whether the presence of STAT3 influences HT29 recognition by NK cells. Freshly purified NK cells were cocultured for 24 hours with HT29, $HT29^{siRNA\text{-}CTRL}$, or $HT29^{siRNA\text{-}STAT3}$ and harvested supernatants were then assessed for IFN-γ production (Fig. 2C). $HT29^{siRNA\text{-}STAT3}$ triggered a significantly increased secretion of IFN-γ by NK cells compared with $HT29^{siRNA\text{-}CTRL}$ or HT29 (1,276 ± 82 pg/mL vs. 510 ± 2 pg/mL and 462 ± 38 pg/mL). To further study STAT3 implication on NK functions, we assessed NK degranulation according to STAT3 expression in HT29. Direct CD107a staining was done to reveal NK degranulation against HT29, $HT29^{siRNA\text{-}CTRL}$, or $HT29^{siRNA\text{-}STAT3}$ (Fig. 2D). We observed an increase in CD107a expression in cocultures done with $HT29^{siRNA\text{-}STAT3}$ compared with $HT29^{siRNA\text{-}CTRL}$, suggesting the active degranulation of NK in the condition in which STAT3 is repressed (42% vs. 16% and 14% for HT29 and $HT29^{siRNA\text{-}CTRL}$, respectively). Overall, we can hypothesize that STAT3 is implicated in HT29-altered sensitivity to NK functions.

STAT3 inhibition of HT29 recognition by NK cells involves a TGF-β1–independent mechanism

NK functions result from the integration of activating and inhibitory signaling related to different ligands recognized on target cells. TGF-β1, a cytokine produced by HT29 (34), has been previously characterized as a major inhibitory pathway limiting tumor cell recognition by NK cells (35). Moreover, we and others have shown that STAT3 can directly trigger TGF-β1 transcription (36, 37). Therefore, the expression of TGF-β1 in $HT29^{siRNA\text{-}CTRL}$ and $HT29^{siRNA\text{-}STAT3}$ was analyzed by flow cytometry (Fig. 3A). We noticed a decreased expression of TGF-β1 in $HT29^{siRNA\text{-}STAT3}$ compared with $HT29^{siRNA\text{-}CTRL}$. The quantification of TGF-β1 by RT-qPCR supported previous results at the protein level (Fig. 3B). Indeed, TGF-β1 mRNA was repressed in $HT29^{siRNA\text{-}STAT3}$, suggesting a direct link between STAT3 activity and TGF-β1 expression. As a consequence, we hypothesized that STAT3 activation in HT29 results in a constitutive inhibitory signal for NK cells. Therefore, the presence of a STAT3-dependent TGF-β1 expression in HT29 led us to investigate whether TGF-β1 signaling pathway accounted for STAT3-mediated inhibition of HT29 recognition by NK cells. For this purpose, freshly purified NK cells were cocultured with $HT29^{siRNA\text{-}CTRL}$ and $HT29^{siRNA\text{-}STAT3}$ for 24 hours in the presence or absence of a specific pharmacologic inhibitor of TGF-β1 receptor, SB-431542 (38). SB-431542 treatment during 24 hours abrogated Smad2/3 phosphorylation, prevented TGF-β1 inhibition of IL-2–activated peripheral blood cell (data not shown), and did not influence NK cell–activating receptor expression (Supplementary Fig. 1). As shown previously, we observed a significant increase in IFN-γ production when we compared $HT29^{siRNA\text{-}STAT3}$ with $HT29^{siRNA\text{-}CTRL}$. TGF-β1 inhibition resulted in a weak upregulation of NK activation. In contrast, we could observe that STAT3 neutralization mediated by siRNA improved NK recognition of HT29 even when TGF-β1 signaling was blocked (Fig. 3C). These results suggest that TGF-β1 and STAT3 act independently to repress NK recognition of HT29. As NK activation is known to be the result of an integration of positive and negative signaling pathways following target cell recognition, it is plausible that in the absence of TGF-β1–mediated inhibitory signaling, STAT3-specific inhibition promotes the expression of NK-activating ligands on HT29.

STAT3 knockdown restores NKG2DL expression

Raulet and colleagues showed an increased tumor incidence in $NKG2D^{-/-}$ mice (9). As a consequence, avoidance of this specific NKG2D/NKG2DL pathway is a hallmark of many malignancies against NK immunity (28, 39, 40). Consequently, we choose to determine whether STAT3 was implicated in NKG2D-based tumor cell recognition. To clarify this point,

Published OnlineFirst January 21, 2011; DOI: 10.1158/0008-5472.CAN-09-4540

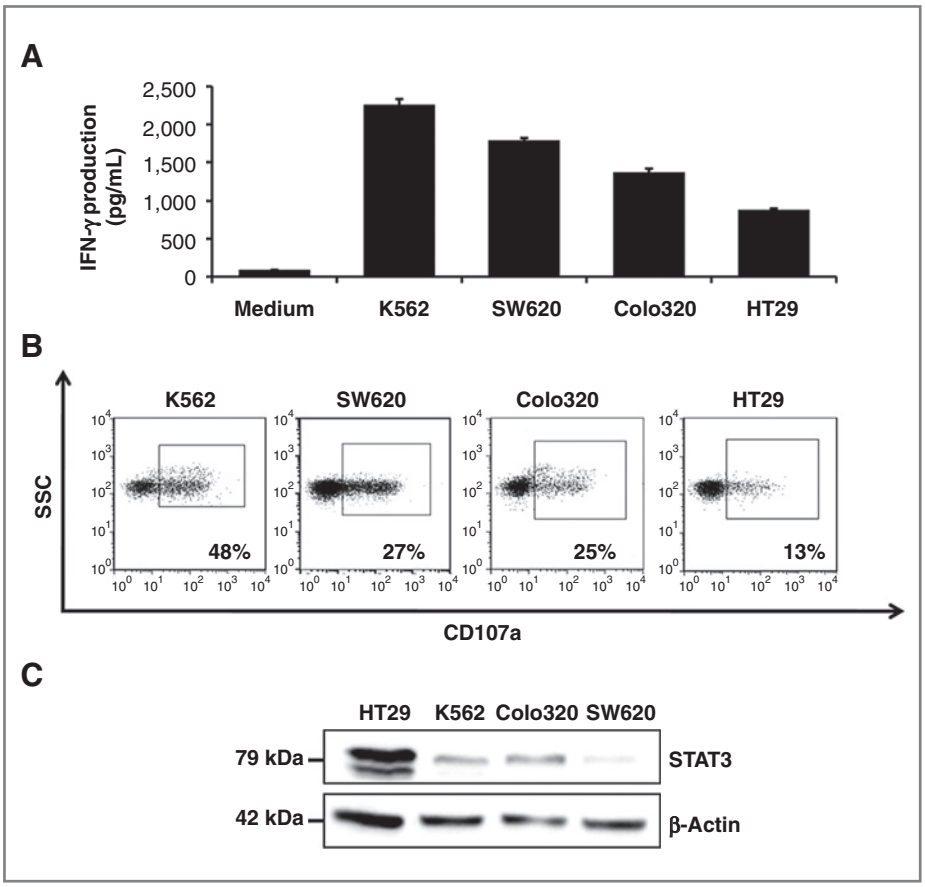

Figure 1. Investigation of colon cancer cell line susceptibility to NK cells. A, IFN-γ concentration assessed by ELISA of freshly purified NK cells cocultured with tumor cell lines for 24 hours at *E*:*T* ratio (10:1; representative experiment of $n = 4$). B, flow cytometric analysis of CD107a expression on IL-2–activated NK cells cocultured for 4 hours with candidate tumor cells at *E*:*T* ratio (10:1; representative blot of $n = 3$). C, Western blot analysis for STAT3 on whole cell extracts. β-Actin was used as a control of protein loading (20 μg per lane; representative experiment of $n = 3$). Bars, SD.

previous experiments were reproduced in the presence of neutralizing anti-NKG2D or IgG control antibodies. NK cells were pretreated for 30 minutes at 37°C with blocking antibodies and then cocultured with HT29$^{siRNA\text{-}CTRL}$ or HT29$^{siRNA\text{-}STAT3}$ for 24 hours and harvested supernatants were assessed for IFN-γ production. As shown in Figure 4A (left), IFN-γ production by NK cells in the coculture with HT29$^{siRNA\text{-}STAT3}$ was significantly decreased in the presence of NKG2D neutralization while not affected by control antibodies. Of note, NKG2D blockade reduced NK degranulation against HT29 (Fig. 4A, right). The magnitude of this inhibition by anti-NKG2D was significantly higher in the presence of specific STAT3 siRNA (Fig. 4A, right). These results strongly suggested a role for NKG2DLs in the NK activation function conferred by STAT3 inhibition in HT29. Subsequently, expression of NKG2DLs was analyzed by flow cytometry in HT29$^{siRNA\text{-}CTRL}$ and HT29$^{siRNA\text{-}STAT3}$ (Fig. 4B). Although MICA expression was influenced by STAT3 modulation, we failed to identify any variation in either MICB or ULBPs in HT29$^{siRNA\text{-}CTRL}$ and HT29$^{siRNA\text{-}STAT3}$. To confirm this hypothesis, we carried out Western blotting analysis on total protein extracts from HT29, HT29$^{siRNA\text{-}CTRL}$, and HT29$^{siRNA\text{-}STAT3}$ to control the presence of MICA. These experiments revealed a marked increase in MICA protein in HT29$^{siRNA\text{-}STAT3}$ (Fig. 4C).

Several authors mentioned that the shedding of MICA and the release of soluble protein (sMICA) are thought to promote tumor evasion (41, 42). Consequently, we assessed the supernatants coming from HT29$^{siRNA\text{-}CTRL}$ and HT29$^{siRNA\text{-}STAT3}$ cultures by ELISA and did not observe a significant difference in sMICA quantified in both conditions (Supplementary Fig. 2). Thereafter, we decided to assess whether the correlation between the abrogation of STAT3 signaling and enhancement in MICA expression was detectable at the transcriptional level. RT-qPCR analyses were done on total mRNA extracts from HT29 cells treated with the STAT3 pharmacologic inhibitor STA21 at different time points (Fig. 4D). We detected a 9-fold increase in MICA mRNA expression in the presence of STA21. Similar results were obtained using the JAK2-specific inhibitor AG490 that predominantly repress STAT3 activity. These results show an inverse correlation between STAT3 activity and MICA expression.

Direct influence of STAT3 on MICA transcription

To extend previous results, we selected 2 tumor cell lines U87 and MDA-MB231 constitutively expressing an active form of STAT3 (Fig. 5A). MICA expression increased in U87 and MDA-MB231 cells treated with STA21 for 48 hours (Fig. 5B). The functional significance of STAT3 in tumor cell recognition by NK cells was studied in the coculture of NK with HT29, U87, and MDA-MB231 cells previously

Published OnlineFirst January 21, 2011; DOI: 10.1158/0008-5472.CAN-09-4540

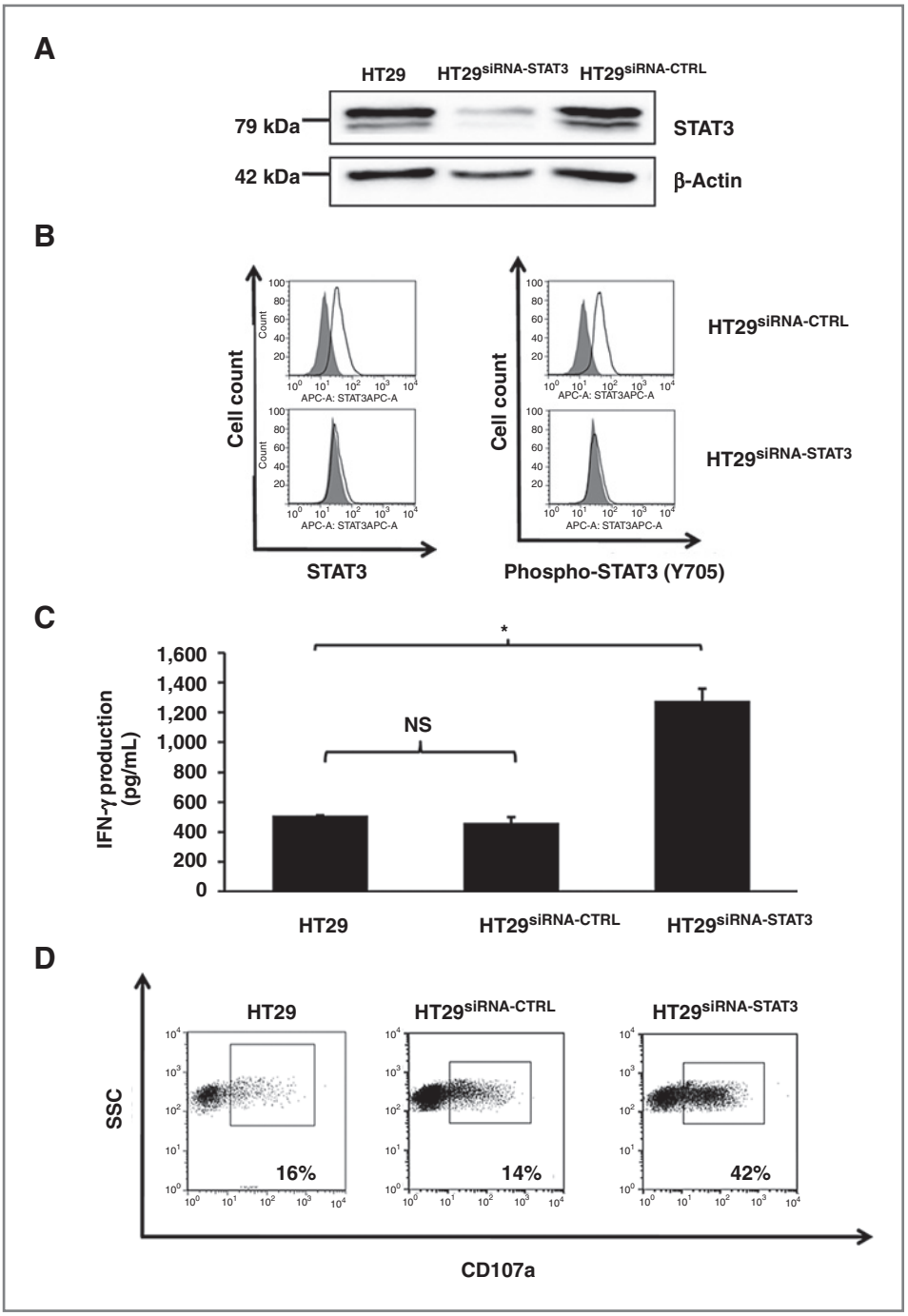

Figure 2. STAT3 knockdown restores NK cell activation by HT29. A, Western blot analysis for STAT3 on whole cell extracts from HT29, HT29$^{siRNA\text{-}CTRL}$, or HT29$^{siRNA\text{-}STAT3}$. β-Actin was used as a control of protein loading (20 μg per lane; representative experiment of $n = 3$). B, flow cytometric analysis of STAT3 and phospho-STAT3 expression by HT29$^{siRNA\text{-}CTRL}$ or HT29$^{siRNA\text{-}STAT3}$. Gray and white histograms represent isotype or STAT3 staining, respectively. C, IFN-γ concentration assessed by ELISA of freshly purified NK cells cocultured with HT29, HT29$^{siRNA\text{-}CTRL}$, or HT29$^{siRNA\text{-}STAT3}$ for 24 hours in DMEM medium at *E*:*T* ratio (10:1). D, flow cytometric analysis of CD107a expression on IL-2–activated NK cells cocultured for 4 hours with HT29, HT29$^{siRNA\text{-}CTRL}$, or HT29$^{siRNA\text{-}STAT3}$ at *E*:*T* ratio (10:1; representative blot of $n = 5$). Bars, SD. *, $P < 0.05$.

treated during 48 hours with 2 different STAT3 pharmacologic inhibitors. These experiments confirmed that treatment of tumor cells with either STA21 or AG490 enhances NK IFN-γ production (Fig. 5C). As previous observations revealed that STAT3 influence seems to be restricted to MICA expression, we added anti-MICA–neutralizing antibodies in coculture experiments. Interestingly, MICA blockade decreased the IFN-γ production observed in NK cocultures with HT29, U87, or MDA-MB231 previously treated with STA21 or AG490 (Fig. 5C). To confirm the direct influence of STAT3 on MICA transcription in tumor cells, a pGL3-MICA vector containing the luciferase gene under the control of the 1-kb MICA promoter was transfected in HT29, U87, and MDA-MB231. Pharmacologic inhibition of STAT3 in all transfected cell lines resulted in an enhanced MICA promoter activity (Fig. 5D).

Published OnlineFirst January 21, 2011; DOI: 10.1158/0008-5472.CAN-09-4540

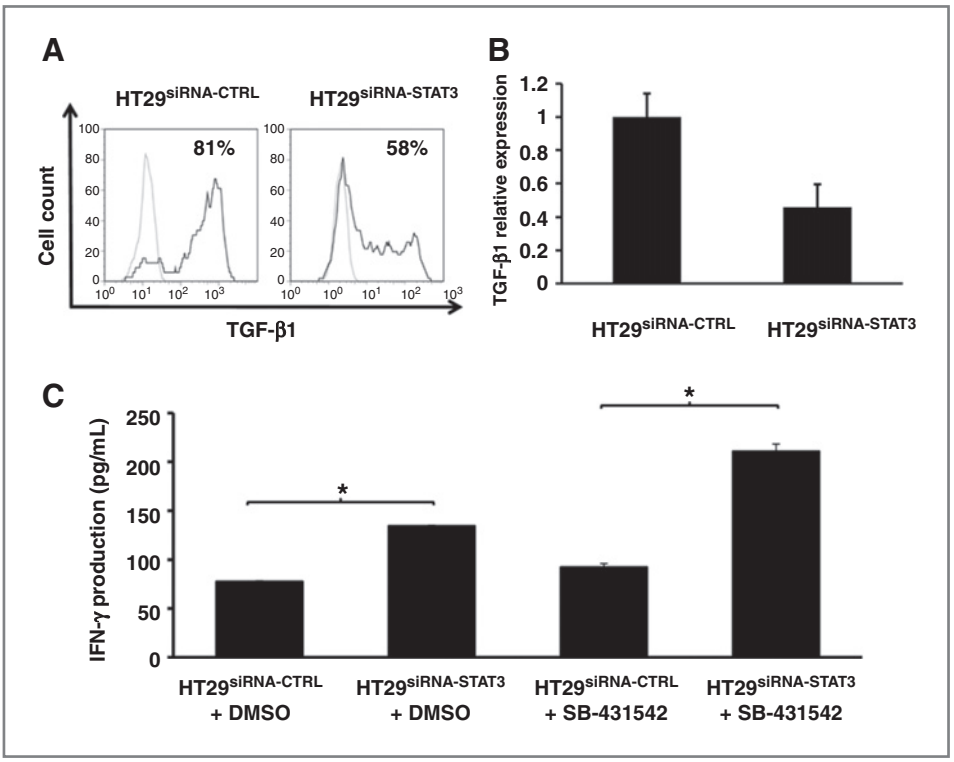

Figure 3. STAT3 inhibition of HT29 recognition by NK cells does not involve TGF-β1. A, flow cytometric analysis of TGF-β1 expression by $HT29^{siRNA\text{-}CTRL}$ or $HT29^{siRNA\text{-}STAT3}$. Here, we show isotype (gray) versus specific staining (black). B, RT-qPCR analysis of total mRNA extracts from $HT29^{siRNA\text{-}CTRL}$ or $HT29^{siRNA\text{-}STAT3}$. Raw data were analyzed with the $\Delta\Delta C_t$ method and results are shown as relative expression to the control $HT29^{siRNA\text{-}CTRL}$ (representative experiment of $n = 2$). C, freshly purified NK cells were cocultured for 24 hours with $HT29^{siRNA\text{-}CTRL}$ or $HT29^{siRNA\text{-}STAT3}$ in the presence or absence of the TGF-β1 receptor inhibitor SB-431542 (10 μmol/L) at *E:T* ratio (10:1). Supernatants were harvested and IFN-γ concentration was assessed by ELISA (representative experiment of $n = 3$). Bars, SD. *, $P < 0.05$.

STAT3 directly interacts with specific binding sites in MICA promoter

To exert its action as a transcriptional factor, STAT3 forms a cytoplasm homodimer, translocates into the nucleus, and interacts with functional transcription factor binding sites (TFBS) in the promoter of regulated genes. We sought to investigate the presence of STAT3 TFBSs in MICA promoter to further elucidate the mechanisms governing previous observations. We used the predictive software MatInspector for TFBSs and found a significant match (5′-TTCTTCCAGGACAGACAA-3′) for a more recently discovered sequence (core nucleotide is underlined: TTCCNGG; ref. 43). Complementary analyses were realized on MICB and ULBPs promoter sequences as well. We obtained specific sequences for MICB (3′-TTCCTTCCGGGACAGACAA-5′) and ULBP2 (3′-CATCTTCCAGGCTCTCCTT-5′) promoters, whereas no specific matches were retrieved from ULBP1 and ULBP3 promoter investigations (Supplementary Data 1 and 2). We initiated a chromatin immunoprecipitation (ChIP) assay to control whether STAT3 could indeed bind the TFBS given by MatInspector. HT29 cells were fixed with paraformaldehyde, sonicated, and total proteins were harvested. Specific immunoprecipitation with STAT3 monoclonal antibodies allowed for recovery and enrichment of STAT3-bound DNA. PCR, designed to amplify the sequence comprising NKG2DL potential TFBSs, was realized on immunoprecipitated DNA. An internal control for STAT3 binding was used with a PCR designed to amplify STAT3 TFBS in the IL-10 promoter (44). We noticed a signal for the MICA and MICB promoters in DNA isolated from STAT3 ChIP. Interestingly, there was no signal observed for the ULBP2 promoter analysis (Fig. 6A).

To further develop our hypothesis, the highly transfectable 293T cells, in which STAT3 activity also influence MICA promoter activity (Fig. 6B), was used to address the precise role of STAT3-TFBS on STAT3 and MICA promoter interactions

Then, we conducted a site-directed mutagenesis to remove STAT3-TFBS in the promoter region of pGL3-MICA vector, displaying MICA promoter. After successful mutagenesis, we obtained a mutated version of pGL3-MICA (pGL3-MICAmut) that did not harbor any STAT3-TFBS. 293T cells were transfected with the normal or mutated luciferase construct. Forty-eight hours posttransfection, cells were stimulated for 2 hours with oncostatin M (OSM; 100 ng/mL) at 37°C to amplify STAT3 phosphorylation. ChIP experiments were done subsequently. PCR, designed to specifically amplify the pGL3 vector sequence, was realized on immunoprecipitated DNA (Fig. 6C). We observed a specific band in the pGL3-MICA condition, suggesting the binding of STAT3 to its target sequence on the vector. When STAT3-TFBS was mutated, there was no specific sequence amplification. These results support the specificity of the binding sequence in MICA promoter. Finally, 293T and HT29 cell lines were transfected with pGL3-MICA or pGL3-MICAmut vector. Specific mutation hampering STAT3 binding to MICA promoter increased luciferase activity in both HT29 and 293T cells (Fig. 6D). Of note, similar results were obtained using U87 and MDA-231 cell lines (data not shown). Collectively, these results suggest that STAT3 regulates MICA expression at the transcriptional level.

Published OnlineFirst January 21, 2011; DOI: 10.1158/0008-5472.CAN-09-4540

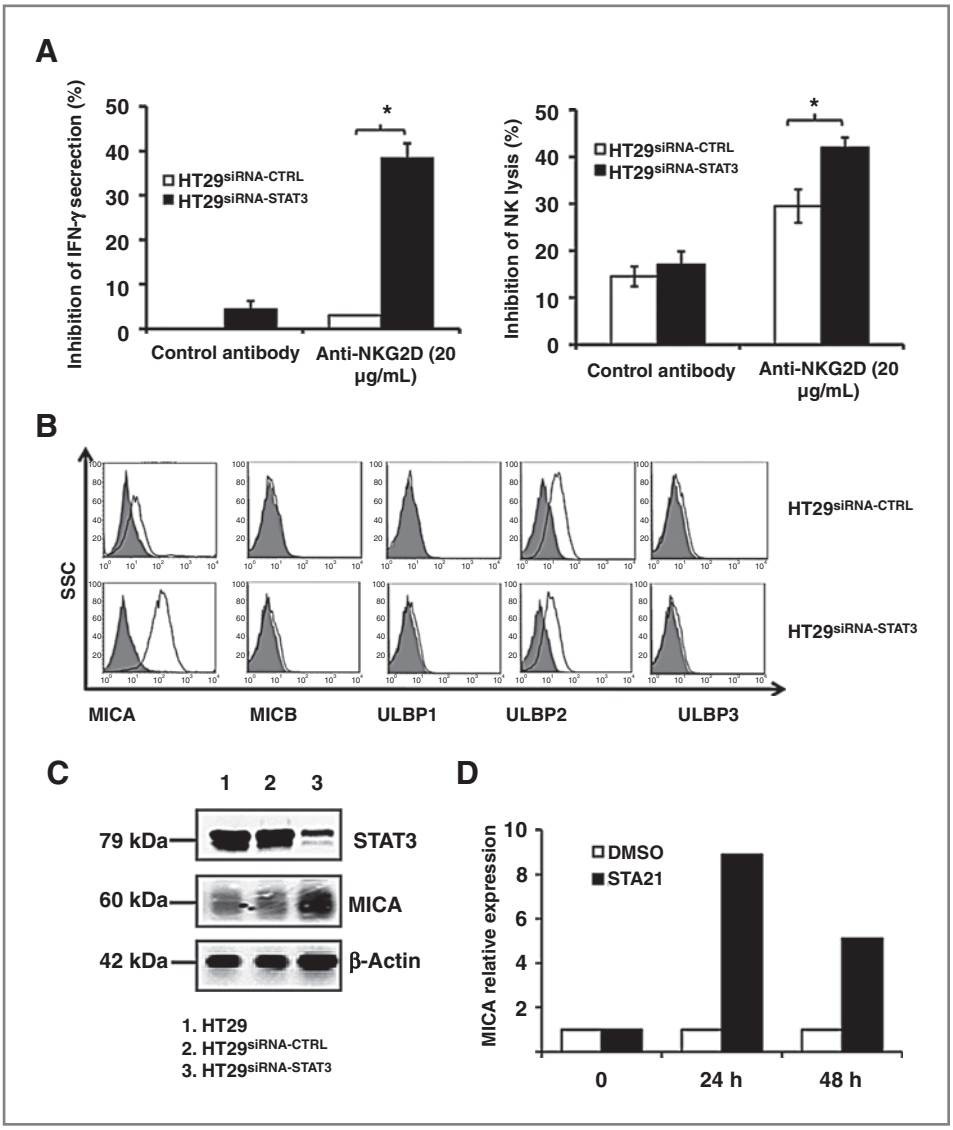

Figure 4. STAT3 knockdown restores NKG2D ligands expression. A, freshly purified NK cells were cocultured for 24 hours with HT29$^{siRNA-CTRL}$ or HT29$^{siRNA-STAT3}$ in the presence or absence of either an IgG or an NKG2D-blocking antibody (20 μg/mL for each condition) at *E*:*T* ratio (10:1). After 24 hours of cocultures, supernatants were harvested and IFN-γ concentration was assessed by ELISA (representative experiment of $n = 2$). B, flow cytometric analysis of CD107a expression on IL-2–activated NK after cocultures with HT29$^{siRNA-CTRL}$ or HT29$^{siRNA-STAT3}$ (*E*:*T* = 10:1) for 4 hours in the presence or absence of an IgG or an NKG2D-blocking antibody (representative experiment of $n = 3$). C, flow cytometric analysis of MICA, MICB, ULBP1, ULBP2, and ULBP3 on HT29$^{siRNA-CTRL}$ or HT29$^{siRNA-STAT3}$. Here, we show isotype (gray) versus specific staining (white). D. Western blot analysis for STAT3 and MICA on whole cell extracts from HT29, HT29$^{siRNA-CTRL}$, or HT29$^{siRNA-STAT3}$. β-Actin was used as a control of protein loading (20 μg per lane; representative experiment of $n = 3$). E, HT29 cells were treated with the STAT3 pharmacologic inhibitor STA21 (30 μmol/L) for 0, 24, and 48 hours. mRNA was extracted and MICA expression was assessed by RT-qPCR. Bars, SD. *, $P < 0.05$.

Influence of STAT3 on MICA regulation by DNA damage pathways

DNA damage response pathway was reported to play a role in upregulation of NKG2DLs and this molecular signaling is a possible candidate bridging cellular transformation and innate immunosurveillance (16). For this purpose, the role of STAT3 in NKG2DL induction by DNA damage response or heat shock was investigated in mesenchymal stem cells and activated lymphocytes known to express MICA (45, 46). The SV56 stroma cell line (26) was transduced with a retroviral vector containing a sequence for a modified and constitutive active form of STAT3 (STAT3C) or the empty vector (pBabe) and exposed to ionizing radiation or heat shock stress. STAT3 constitutive activation led to the downregulation of MICA and prevented the induction of MICA following ionizing radiation or heat shock exposition (Fig. 7A).

Furthermore, ataxia telangiectasia mutated (ATM)-mediated DNA damage response pathway also induces MICA expression on activated T-cell lymphocytes (46). Because we and others have previously reported a STAT3 phosphorylation driven by CD28 costimulation in $CD4^+$ lymphocytes (37, 47), we next investigated the influence of STAT3 on MICA expression following CD3/CD28-mediated activation of T lymphocytes. For this purpose, T-cell lymphocytes were purified, exposed with or without different STAT3 pharmacologic inhibitors, and activated by CD3/CD28 during 48 hours. As shown in Figure 7B, pharmacologic inhibition of STAT3 promoted higher level of MICA expression in T lymphocytes following CD3/CD28 stimulation (Fig. 7B). These results confer to STAT3 a pivotal role in MICA regulation in both cancer and nonmalignant cells.

Published OnlineFirst January 21, 2011; DOI: 10.1158/0008-5472.CAN-09-4540

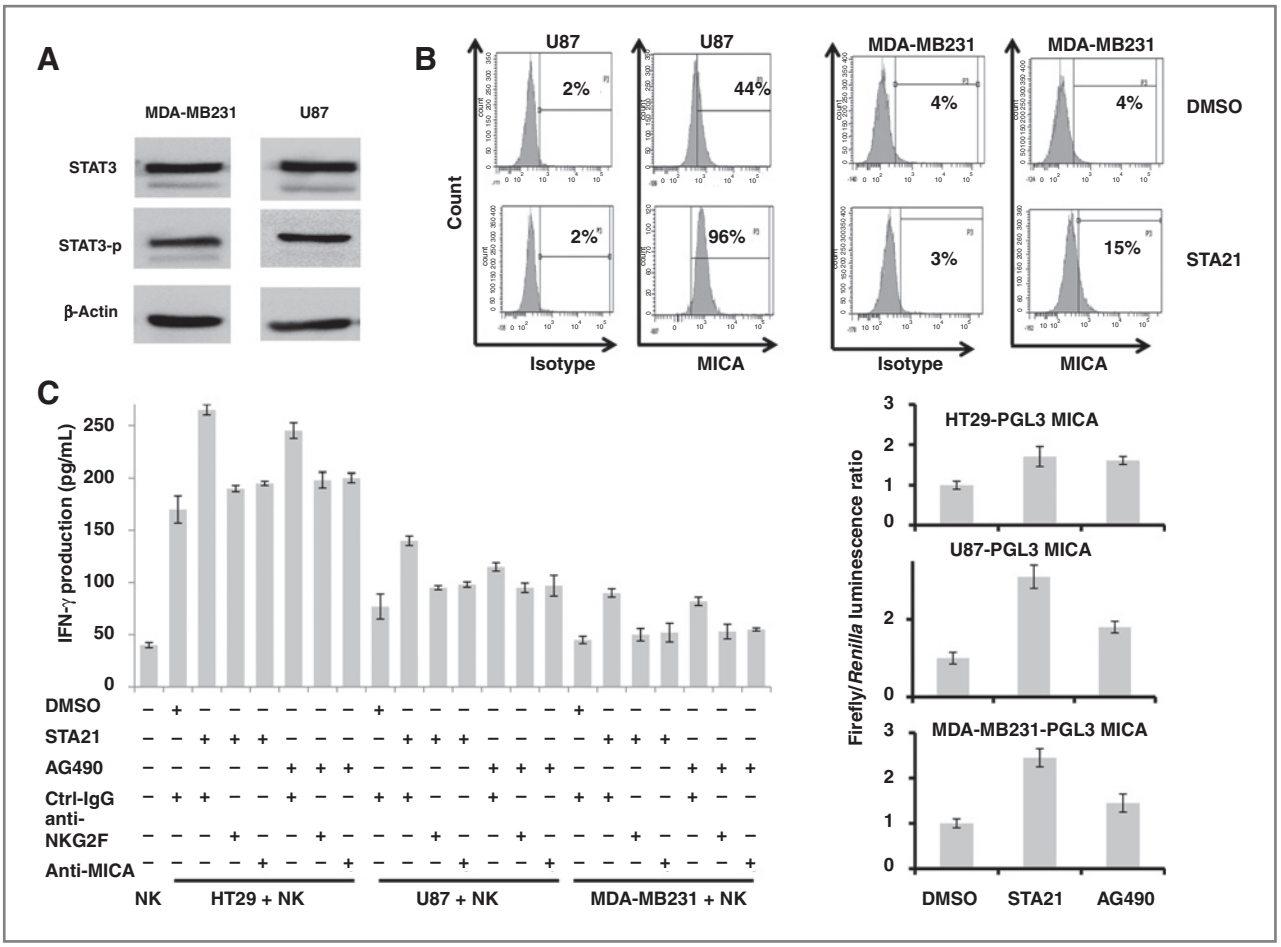

Figure 5. STAT3 pharmacologic inhibition specifically interferes with MICA transcription. A, Western blot analysis for STAT3 and STAT3-p on whole cell extracts from U87 and MDA-MB231 cell lines. β-Actin was used as a control of protein loading (20 μg per lane; representative experiment of $n = 2$). B, flow cytometric analyses of MICA expression on U87 and MDA-MB231 treated with or without DMSO, STA21 (30 μmol/L) during 48 hours. C, IFN-γ concentration assessed by ELISA of freshly purified NK cells cocultured with HT29, U87, and MDA-MB231, in the presence or absence of IgG, anti-MICA, or NKG2D-blocking antibodies (20 μg/mL for each condition) for 24 hours in DMEM medium at *E:T* ratio (10:1). In some conditions, tumor cells were treated with or without DMSO, STA21 (30 μmol/L), or AG490 (50 μmol/L) during 48 hours before experiment ($n = 2$). D, luciferase MICA promoter assay. HT29, U87, and MDA-MB231 were transiently transfected with pGL3-MICA vector. After 24 hours, cells were treated with or without STA21 at 30 μmol/L or AG490 (50 μmol/L). Results are presented as a ratio between the firefly luciferase activity and the *Renilla* luciferase activity for each condition ($n = 2$). Ctrl, control.

Discussion

NKG2D is an activating receptor shared by NK cells and T-cell lymphocytes, now identified as a pivotal mechanism to prevent the emergence of cancer cells arising following DNA damage induction or spontaneous transformation (7, 9, 16). In this study, we investigated the role of STAT3, a transcription factor harboring both oncogenic and immunosuppressive functions (27, 37), in NKG2DL expression regulation.

In this study, we found that specific repression of STAT3 with RNA interference promoted the recognition of HT29 by NK cells. We showed that this mechanism involves the activating receptor NKG2D (Fig. 4). We revealed an inverse correlation between STAT3 activation and expression of MICA. Finally, ChIP analyses and luciferase promoter assays have shown for the first time MICA as a target for STAT3 transcriptional regulation (Fig. 6).

The role of NKG2D in NK immunosurveillance has been well documented. NKG2D is involved in the prevention of cancer initiation (9) and control of tumor progression (8). DNA damage pathway checkpoints ATM and ATR (ataxia telangiectasia and Rad3 related) could upregulate the expression of NKG2DLs in epithelial cells, thus alerting the immune system (16). These results established MICA/NKG2D axis as an early mechanism of tumor suppression. The higher incidence of spontaneous cancers observed in NKG2D-deficient mice confirmed the tumor-suppressive role of NKG2D *in vivo* (9). Moreover, the disappearance of NKG2DLs on tumor cells derived from aggressive tumors in mice expressing wild-type NKG2D, but not from tumors derived from NKG2D-deficient mice, highlighted the presence of NKG2DL immunoediting.

Another mechanism was previously shown to prevent NKG2D-mediated recognition. Tumor-secreted metalloprotease induce proteolytic shedding of MICA molecules and

Published OnlineFirst January 21, 2011; DOI: 10.1158/0008-5472.CAN-09-4540

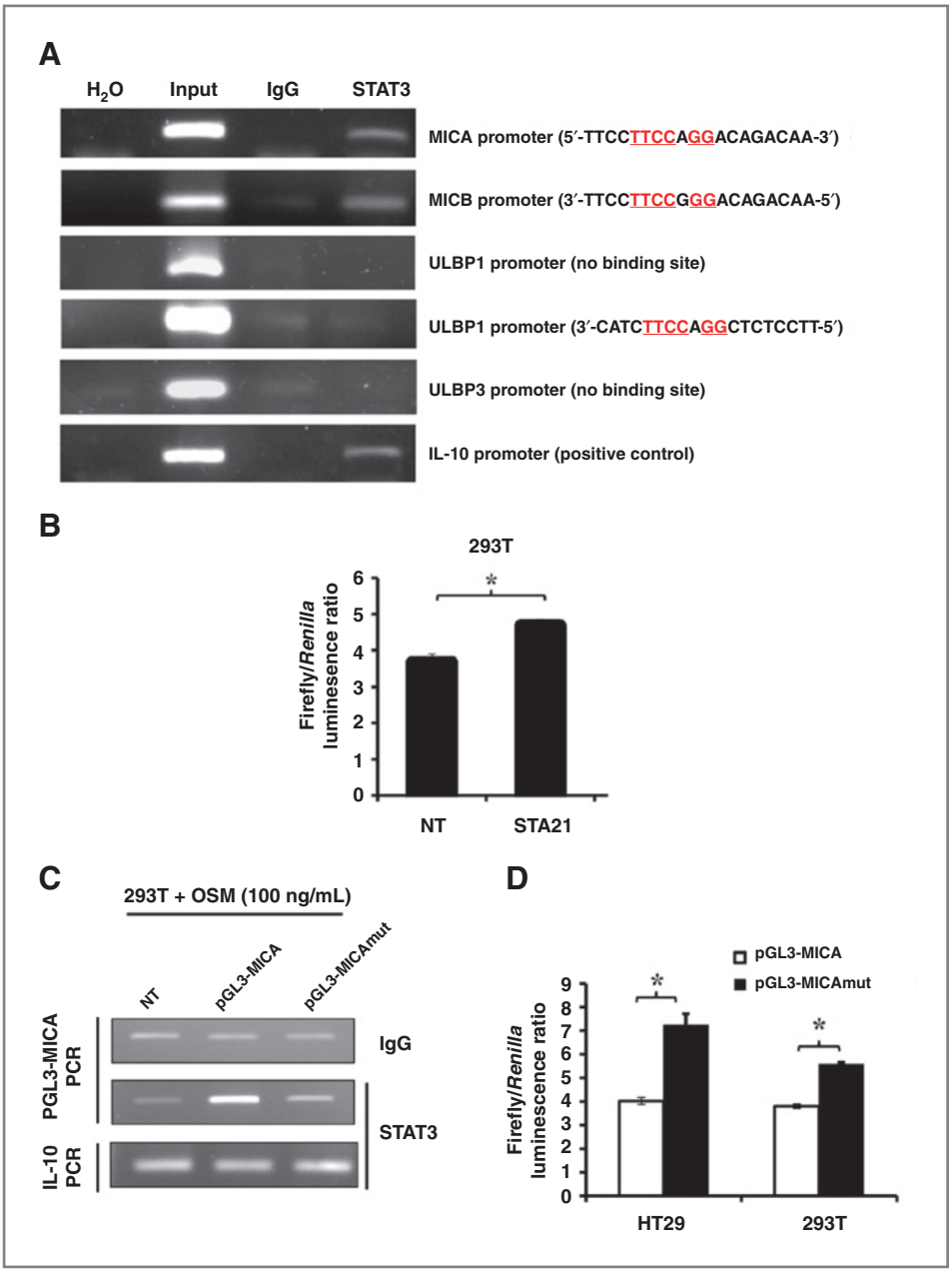

Figure 6. STAT3 directly interacts with specific binding sites in MICA promoter. A, PCR analysis was done on DNA retrieved from specific STAT3 ChIP experiments. Potential site sequence is indicated along with the PCR results (representative of $n = 3$). B, luciferase MICA promoter assay. 293T cells were transiently transfected with pGL3-MICA vector. After 24 hours, cells were treated with or without STA21 at 30 μmol/L. Results are presented as a ratio between the firefly luciferase activity and the *Renilla* luciferase activity (Ren/Luc) for each conditions ($n = 2$). C, 293T cells were transiently transfected with either the pGL3-MICA or the pGL3-MICAmut vector. After 48 hours, cells were treated with OSM (100 ng/mL) for 2 hours. After that incubation, cells were fixed and a ChIP experiment was realized as previously described. PCR analyses were done on DNA retrieved from specific STAT3 ChIP experiments (representative experiment of $n = 2$). D, 293T and HT29 cells were transiently transfected with pGL3-MICA or pGL3-MICAmut. After 48 hours, luciferase activity was quantified using the manufacturer's protocol. Results are presented as a ratio between the firefly luciferase activity and the *Renilla* luciferase activity for each conditions ($n = 2$). NT, not treated.

downregulation of NKG2DL expression (48). However, STAT3 modulation using siRNA or specific pharmacologic inhibitors did not influence the level of soluble MICA detected in HT29 culture supernatants (Supplementary Fig. 2).

Furthermore, both the induction of MICA transcription following exposition to STAT3 pharmacologic inhibitors and direct binding of STAT3 on MICA promoter support a direct influence of STAT3 on the transcriptional regulation of MICA.

Because STAT transcription factors are expected to promote gene transcription, our findings unraveled a possible involvement of STAT3 in the negative regulation of transcription. Experiments using directed mutation of the STAT3 binding sites in MICA promoter indicated that STAT3 exerts its repressor activity on MICA transcription through DNA binding (Fig. 6). STAT3 has been shown to act as a transcriptional repressor of p53 and IL-8 (49, 50). Of note, a direct binding of STAT3 to p53 promoter was also required for STAT3-mediated inhibition of p53 transcription (49).

STAT3 has been described as a potential mediator in chronic inflammatory disorders and oncogenesis. The

Published OnlineFirst January 21, 2011; DOI: 10.1158/0008-5472.CAN-09-4540

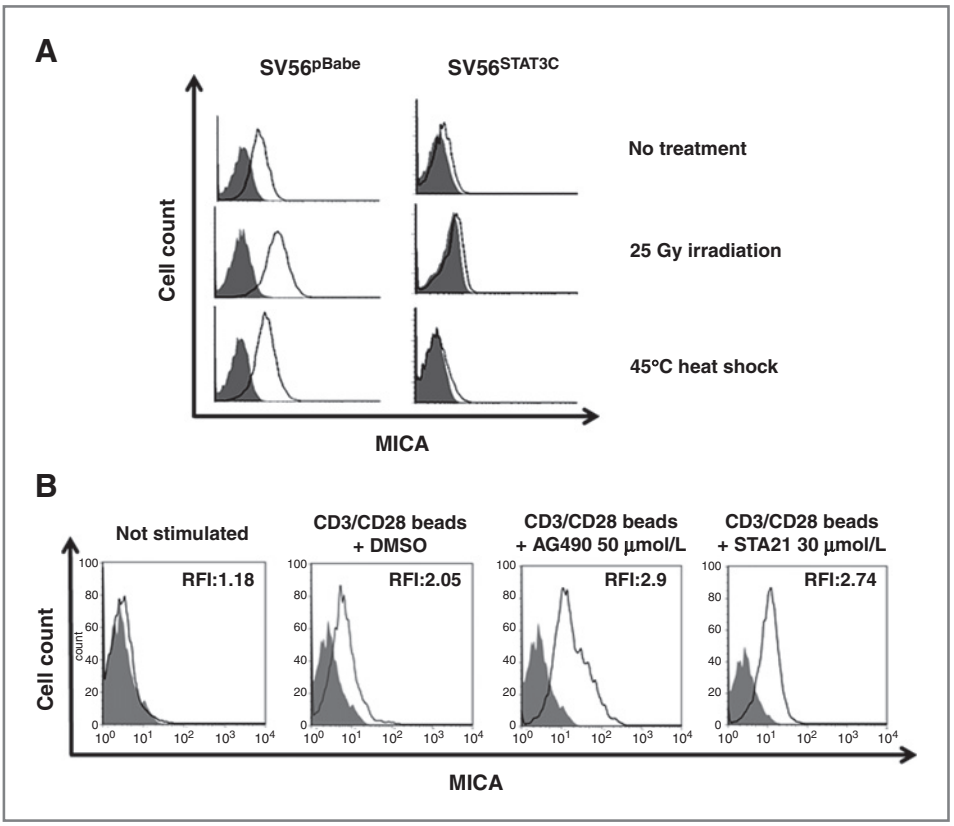

Figure 7. STAT3 influences MICA expression in nonmalignant cells. A, stroma cell lines SV56^{pBabe} and SV56^{STAT3C} were stimulated with either ionizing irradiation (25 Gy) or heat shock (45°C for 1 hours). Sixteen hours posttreatment, MICA expression was assessed by flow cytometric analysis (representative of $n = 3$). B, PBLs isolated from healthy donor were stimulated for 48 hours with CD3/CD28 beads at a bead/T-cell ratio of 1:1 in the presence or absence of STA21 (30 μmol/L) or AG490 (50 μmol/L). MICA expression was assessed by flow cytometric analysis and reported as relative fluorescent intensity (RFI), which was calculated by dividing the mean fluorescent intensity of test antibody by the fluorescent intensity of isotype control-treated cells from the same well ($n = 3$).

relationship between chronic inflammation and tumor progression has been documented through clinical trials showing that long-term use of nonsteroidal anti-inflammatory drugs reduce the relative risk of developing CRC by 40% to 50% (17). Many studies revealed the IL-6/STAT3 signaling pathway to be critical for IBD development (51). Thus, it is plausible that STAT3 could be activated at the time of epithelial cell transformation or DNA damage exposition and binds MICA promoter to prevent its expression and promote escape to NKG2D-mediated immunosurveillance.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Author Contributions

C. Borg designed the research, analyzed experiments, and wrote the manuscript. R. Bedel, C. Grandclement, J. Balland, J.-P. Remy-Martin, B. Kantelip, and A. Thiery-Vuillemin conducted research and analyzed experiments. C. Ferrand, X. Pivot, and P. Tiberghien contributed to the design and writing.

Grant Support

R. Bedel received a fellowship from the conseil regional de Franche Comté. This work has been supported by the "Ligue contre le cancer inter-regionale-Grand Est," associations Laurette Fugain and cent pour sang la vie, and the conseil regional of Franche Comté.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received December 14, 2009; revised December 13, 2010; accepted January 4, 2011; published OnlineFirst January 21, 2011.

References

1. Zitvogel L, Tesniere A, Kroemer G. Cancer despite immunosurveillance: immunoselection and immunosubversion. Nat Rev Immunol 2006;6:715–27.
2. Hayakawa Y, Smyth MJ. Innate immune recognition and suppression of tumors. Adv Cancer Res 2006;95:293–322.
3. Shankaran V, Ikeda H, Bruce AT, White JM, Swanson PE, Old LJ, et al. IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity. Nature 2001;410:1107–11.
4. Imai K, Matsuyama S, Miyake S, Suga K, Nakachi K. Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population. Lancet 2000;356:1795–9.
5. Ljunggren HG, Karre K. In search of the "missing self": MHC molecules and NK cell recognition. Immunol Today 1990;11:237–44.
6. Koh CY, Blazar BR, George T, Welniak LA, Capitini CM, Raziuddin A, et al. Augmentation of antitumor effects by NK cell inhibitory receptor blockade *in vitro* and *in vivo*. Blood 2001;97:3132–7.
7. Diefenbach A, Jensen ER, Jamieson AM, Raulet DH. Rae1 and H60 ligands of the NKG2D receptor stimulate tumour immunity. Nature 2001;413:165–71.

8. Cerwenka A, Baron JL, Lanier LL. Ectopic expression of retinoic acid early inducible-1 gene (RAE-1) permits natural killer cell-mediated rejection of a MHC class I-bearing tumor *in vivo*. Proc Natl Acad Sci U S A 2001;98:11521–6.
9. Guerra N, Tan YX, Joncker NT, Choy A, Gallardo F, Xiong N, et al. NKG2D-deficient mice are defective in tumor surveillance in models of spontaneous malignancy. Immunity 2008;28:571–80.
10. Cosman D, Mullberg J, Sutherland CL, Chin W, Armitage R, Fanslow W, et al. ULBPs, novel MHC class I-related molecules, bind to CMV glycoprotein UL16 and stimulate NK cytotoxicity through the NKG2D receptor. Immunity 2001;14:123–33.
11. Diefenbach A, Jamieson AM, Liu SD, Shastri N, Raulet DH. Ligands for the murine NKG2D receptor: expression by tumor cells and activation of NK cells and macrophages. Nat Immunol 2000;1:119–26.
12. Groh V, Rhinehart R, Secrist H, Bauer S, Grabstein KH, Spies T, et al. Broad tumor-associated expression and recognition by tumor-derived gamma delta T cells of MICA and MICB. Proc Natl Acad Sci U S A 1999;96:6879–84.
13. Pende D, Cantoni C, Rivera P, Vitale M, Castriconi R, Marcenaro S, et al. Role of NKG2D in tumor cell lysis mediated by human NK cells: cooperation with natural cytotoxicity receptors and capability of recognizing tumors of nonepithelial origin. Eur J Immunol 2001;31:1076–86.
14. Pende D, Rivera P, Marcenaro S, Chang CC, Biassoni R, Conte R, et al. Major histocompatibility complex class I-related chain A and UL16-binding protein expression on tumor cell lines of different histotypes: analysis of tumor susceptibility to NKG2D-dependent natural killer cell cytotoxicity. Cancer Res 2002;62:6178–86.
15. Groh V, Bahram S, Bauer S, Herman A, Beauchamp M, Spies T. Cell stress-regulated human major histocompatibility complex class I gene expressed in gastrointestinal epithelium. Proc Natl Acad Sci U S A 1996;93:12445–50.
16. Gasser S, Orsulic S, Brown EJ, Raulet DH. The DNA damage pathway regulates innate immune system ligands of the NKG2D receptor. Nature 2005;436:1186–90.
17. Smalley WE, DuBois RN. Colorectal cancer and nonsteroidal anti-inflammatory drugs. Adv Pharmacol 1997;39:1–20.
18. Bernstein CN, Blanchard JF, Kliewer E, Wajda A. Cancer risk in patients with inflammatory bowel disease: a population-based study. Cancer 2001;91:854–62.
19. Langowski JL, Zhang X, Wu L, Mattson JD, Chen T, Smith K, et al. IL-23 promotes tumour incidence and growth. Nature 2006;442:461–5.
20. Kortylewski M, Xin H, Kujawski M, Lee H, Liu Y, Harris T, et al. Regulation of the IL-23 and IL-12 balance by Stat3 signaling in the tumor microenvironment. Cancer Cell 2009;15:114–23.
21. Szkaradkiewicz A, Marciniak R, Chudzicka-Strugala I, Wasilewska A, Drews M, Majewski P, et al. Proinflammatory cytokines and IL-10 in inflammatory bowel disease and colorectal cancer patients. Arch Immunol Ther Exp (Warsz) 2009;57:291–4.
22. Musso A, Dentelli P, Carlino A, Chiusa L, Repici A, Sturm A, et al. Signal transducers and activators of transcription 3 signaling pathway: an essential mediator of inflammatory bowel disease and other forms of intestinal inflammation. Inflamm Bowel Dis 2005;11:91–8.
23. Pickert G, Neufert C, Leppkes M, Zheng Y, Wittkopf N Warntjen M, et al. STAT3 links IL-22 signaling in intestinal epithelial cells to mucosal wound healing. J Exp Med 2009;206:1465–72.
24. Kortylewski M, Kujawski M, Wang T, Wei S, Zhang S, Pilon-Thomas S, et al. Inhibiting Stat3 signaling in the hematopoietic system elicits multicomponent antitumor immunity. Nat Med 2005;11:1314–21.
25. Kim DJ, Chan KS, Sano S, Digiovanni J. Signal transducer and activator of transcription 3 (Stat3) in epithelial carcinogenesis. Mol Carcinog 2007;46:725–31.
26. Loeuillet C, Bernard G, Rémy-Martin J, Saas P, Hervé P, Douay L, et al. Distinct hematopoietic support by two human stromal cell lines. Exp Hematol 2001;29:736–45.
27. Bromberg JF, Wrzeszczynska MH, Devgan G, Zhao Y, Pestell RG, Albanese C, et al. Stat3 as an oncogene. Cell 1999;98:295–303.
28. Yadav D, Ngolab J, Lim RS, Krishnamurthy S, Bui JD. Cutting edge: down-regulation of MHC class I-related chain A on tumor cells by IFN-gamma–induced microRNA. J Immunol 2009;182:39–43.
29. Alter G, Malenfant JM, Altfeld M. CD107a as a functional marker for the identification of natural killer cell activity. J Immunol Methods 2004;294:15–22.
30. Garcia R, Bowman TL, Niu G, Yu H, Minton S, Muro-Cacho CA, et al. Constitutive activation of Stat3 by the Src and JAK tyrosine kinases participates in growth regulation of human breast carcinoma cells. Oncogene 2001;20:2499–513.
31. Dhir R, Ni Z, Lou W, DeMiguel F, Grandis JR, Gao AC. Stat3 activation in prostatic carcinomas. Prostate 2002;51:241–6.
32. Niu G, Bowman T, Huang M, Shivers S, Reintgen D, Daud A, et al. Roles of activated Src and Stat3 signaling in melanoma tumor cell growth. Oncogene 2002;21:7001–10.
33. Kusaba T, Nakayama T, Yamazumi K, Yakata Y, Yoshizaki A, Nagayasu T, et al. Expression of p-STAT3 in human colorectal adenocarcinoma and adenoma; correlation with clinicopathological factors. J Clin Pathol 2005;58:833–38.
34. Halder SK, Beauchamp RD, Datta PK. A specific inhibitor of TGF-beta receptor kinase, SB-431542, as a potent antitumor agent for human cancers. Neoplasia 2005;7:509–21.
35. Castriconi R, Cantoni C, Della Chiesa M, Vitale M, Marcenaro E, Conte R, et al. Transforming growth factor beta 1 inhibits expression of NKp30 and NKG2D receptors: consequences for the NK-mediated killing of dendritic cells. Proc Natl Acad Sci U S A 2003;100:4120–25.
36. Kinjyo I, Inoue H, Hamano S, Fukuyama S, Yoshimura T, Koga K, et al. Loss of SOCS3 in T helper cells resulted in reduced immune responses and hyperproduction of interleukin 10 and transforming growth factor-beta 1. J Exp Med 2006;203:1021–31.
37. Pallandre JR, Brillard E, Crehange G, Radlovic A, Remy-Martin JP, Saas P, et al. Role of STAT3 in $CD4^{+}CD25^{+}FOXP3^{+}$ regulatory lymphocyte generation: implications in graft-versus-host disease and antitumor immunity. J Immunol 2007;179:7593–604.
38. Hjelmeland MD, Hjelmeland AB, Sathornsumetee S, Reese ED, Herbstreith MH, Laping NJ, et al. SB-431542, a small molecule transforming growth factor-beta-receptor antagonist, inhibits human glioma cell line proliferation and motility. Mol Cancer Ther 2004;3:737–45.
39. Jinushi M, Vanneman M, Munshi NC, Tai YT, Prabhala RH, Ritz J, et al. MHC class I chain-related protein A antibodies and shedding are associated with the progression of multiple myeloma. Proc Natl Acad Sci U S A 2008;105:1285–90.
40. Wiemann K, Mittrucker HW, Feger U, Welte SA, Yokoyama WM, Spies T, et al. Systemic NKG2D down-regulation impairs NK and CD8 T cell responses *in vivo*. J Immunol 2005;175:720–29.
41. Salih HR, Antropius H, Gieseke F, Lutz SZ, Kanz L, Rammensee HG, et al. Functional expression and release of ligands for the activating immunoreceptor NKG2D in leukemia. Blood 2003;102:1389–96.
42. Waldhauer I, Goehlsdorf D, Gieseke F, Weinschenk T, Wittenbrink M, Ludwig A, et al. Tumor-associated MICA is shed by ADAM proteases. Cancer Res 2008;68:6368–76.
43. Chen X, Xu H, Yuan P, Fang F, Huss M, Vega VB, et al. Integration of external signaling pathways with the core transcriptional network in embryonic stem cells. Cell 2008;133:1106–17.
44. Benkhart EM, Siedlar M, Wedel A, Werner T, Ziegler-Heitbrock HW. Role of Stat3 in lipopolysaccharide-induced IL-10 gene expression. J Immunol 2000;165:1612–17.
45. Spaggiari GM, Capobianco A, Becchetti S, Mingari MC, Moretta L. Mesenchymal stem cell-natural killer cell interactions: evidence that activated NK cells are capable of killing MSCs, whereas MSCs can inhibit IL-2-induced NK-cell proliferation. Blood 2006;107:1484–90.
46. Cerboni C, Zingoni A, Cippitelli M, Piccoli M, Frati L, Santoni A. Antigen-activated human T lymphocytes express cell-surface NKG2D ligands via an ATM/ATR-dependent mechanism and become susceptible to autologous NK-cell lysis. Blood 2007;110:606–15.
47. Larmonier N, Janikashvili N, LaCasse CJ, Larmonier CB, Cantrell J, Situ E, et al. Imatinib mesylate inhibits $CD4^{+}CD25^{+}$ regulatory T cell activity and enhances active immunotherapy against BCR-ABL-tumors. J Immunol 2008;181:6955–63.
48. Groh V, Wu J, Yee C, Spies T. Tumour-derived soluble MIC ligands impair expression of NKG2D and T-cell activation. Nature 2002;419:734–8.

Published OnlineFirst January 21, 2011; DOI: 10.1158/0008-5472.CAN-09-4540

49. Niu G, Wright KL, Ma Y, Wright GM, Huang M, Irby R, et al. Role of STAT3 in regulating p53 expression and function. Mol Cell Biol 2005;25:7432–40.
50. de la Iglesia N, Konopka G, Lim KL, Nutt CL, Bromberg JF, Frank DA, et al. Deregulation of a STAT3-Interleukin 8 signaling pathway promotes human glioblastoma cell proliferation and invasiveness. J Neurosci 2008;28:5870–78.
51. Rose-John S, Mitsuyama K, Matsumoto S, Thaiss WM, Scheller J. Interleukin-6 trans-signaling and colonic cancer associated with inflammatory bowel disease. Curr Pharm Des 2009;15:2095–103.

Cancer Research
The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

AACR American Association for Cancer Research

Novel Role for STAT3 in Transcriptional Regulation of NK Immune Cell Targeting Receptor MICA on Cancer Cells

Romain Bedel, Antoine Thiery-Vuillemin, Camille Grandclement, et al.

Cancer Res 2011;71:1615-1626. Published OnlineFirst January 21, 2011.