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BCR/ABL Oncogene Directly Controls MHC Class I Chain-Related Molecule A Expression in Chronic Myelogenous Leukemia

Nicolas Boissel,2*† Delphine Rea,†‡ Vannary Tieng,*, Nicolas Dulphy,*, Manuel Brun,*, Jean-Michel Cayuela,§ Philippe Rousselot,†§ Ryad Tamouza,*, Philippe Le Bouteiller,‖ François-Xavier Mahon,*, Alexander Steinle,** Dominique Charron,*, Hervé Dombret,‡ and Antoine Toubert*

MHC class I chain-related molecules (MIC) participate in immune surveillance of cancer through engagement of the NKG2D-activating receptor on NK and T cells. Decreased NKG2D expression and function upon chronic exposure to NKG2D ligands and/or soluble forms of MIC (sMIC) may participate in immune escape. In chronic myeloid leukemia, a malignancy caused by the BCR/ABL fusion oncogene, we showed cell surface expression of MICA on leukemic, but not healthy, donor hemopoietic CD34+ cells. At diagnosis, chronic myeloid leukemia patients had abnormally high serum levels of sMICA and weak NKG2D expression on NK and CD8+ T cells, which were restored by imatinib mesylate (IM) therapy. In the BCR/ABL+ cell line K562, IM decreased both surface MICA/B expression and NKG2D-mediated lysis by NK cells. Silencing BCR/ABL gene expression directly evidenced its role in the control of MICA expression. IM did not affect MICA mRNA levels, but decreased MICA protein production and release. Sucrose density gradient fractionation of K562 cytoplasmic extracts treated with IM showed a shift in the distribution of MICA mRNA from the polysomal toward the monosomal fractions, consistent with decreased translation. Among the major pathways activated by BCR/ABL that regulate translation, PI3K and mammalian target of rapamycin were shown to control MICA expression. These data provide evidence for direct control of MICA expression by an oncogene in human malignancy and indicate that posttranscriptional mechanisms may participate in the regulation of MICA expression.


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ataxia-telangiectasia-mutated (ATM) and ATM- and Rad-3-related protein kinases (15). In chronic and acute leukemia, leukemic cells from patients have been shown to express various amounts of surface MIC and ULBP molecules, with a great variability among the different types of leukemias. High levels of sMIC molecules were observed in most patients, but there was no correlation with surface expression or white blood cell count (16). Chronic myeloid leukemia (CML) is a myeloproliferative disease cytogenetically characterized by the t(9;22) translocation. The resulting fusion gene, BCR/ABL, encodes an oncogenic tyrosine kinase with spontaneous activity, which is responsible for enhanced cell proliferation, resistance to apoptosis, and genetic instability (17). In BCR/ABL transgenic mice, the expression of NKG2DL Rae-1 was increased on bone marrow-derived DC and was down-regulated after treatment with imatinib mesylate (IM; STI571; Gleevec; Novartis), a specific BCR/ABL inhibitor (18).

To elucidate the mechanisms of NKG2DL expression in CML, as a privileged example of a human malignancy triggered by a well-characterized oncogene, we evaluated MICA expression on leukemic CD34+ progenitors, and sMIC release and NKG2D expression on NK and CD8+ T cells in a cohort of patients at diagnosis and after treatment with IM. Using the BCR/ABL-positive K562 cell line, we showed the direct role of BCR/ABL in NKG2DL expression. Notably, MICA expression was controlled at the level of mRNA translation involving PI3K and the mammalian target of rapamycin (mTOR) pathways, both activated downstream of BCR/ABL. Such control of protein synthesis has been previously described in CML, but is original in the case of cell surface ligands recognized by activating receptors of the immune system.

Materials and Methods

Primary CML samples and healthy controls

Patients > 18 years old with chronic phase (CP) CML were eligible for this study. Previously treated patients (except those with hydroxyurea) and patients with an associated malignant solid tumor, chronic infection, or immune disorder were excluded. CML diagnosis was confirmed by cytogenetics and molecular biology. Sera were collected at diagnosis in 49 patients (26 men and 23 women; median age, 57 years; range, 25–87 years). In 19 of these 49 patients, PBMCs were collected at diagnosis and after 6 mo of daily treatment with 400 mg of IM. Bone marrow from nine patients was tested at diagnosis for MICA expression. This work was approved by the Institutional review board of St. Louis Hospital (Paris, France). Blood and marrow samples from healthy donors (HD) and cord blood were obtained at the St. Louis Hospital Transfusion Center. RNA was prepared from total peripheral leukocytes after erythrocyte lysis with 1 M NaCl, 1 mM K2HPO4, and 0.1 mM EDTA buffer.

Cell lines and culture conditions

The p210 BCR/ABL-positive K562 cell line was provided by American Type Culture Collection and was maintained in RPMI 1640 medium with 10% FCS and 0.5% Triton X-100. The p210 BCR/ABL-negative K562 cell line was provided by American Type Culture Collection and was maintained in RPMI 1640 medium with 10% FCS and were cultured for up to 4 days, then harvested by 0.5% Triton X-100.

Antibodies

The anti-MICA mAb AMO1 was obtained from Immatics. The anti-MICB mAb BMO1 was described previously (16). Anti-ULBP-1, -ULBP-2, and -ULBP-3 mAbs were purchased from R&D Systems. A rabbit polyclonal anti-MICA Ab developed in our laboratory was used for intracellular staining (20). The goat anti-mouse PE conjugate was obtained from Jackson ImmunoResearch Laboratories. The goat anti-rabbit Alexa 594 secondary Ab was purchased from Molecular Probes. Anti-NKG2D (1D11), -CD54, -CD34, -CD34 and -CD3-PerCP, -CD8-FITC, - and -CD56-allophycocyanin mAbs and the IgG1 and IgG2b isotype controls were obtained from BD Pharmingen. Anti-CD16 and -NKG2D-PE (ON72) mAbs were purchased from Beckman Coulter. The anti-BCR Ab was obtained from Santa Cruz Biotechnology. The biotinylated goat anti-mouse conjugate was purchased from Amersham Biosciences.

Flow cytometry

K562 cells, bone marrow, and cord blood cells were incubated with the anti-NKG2D mAb or the corresponding isotype control, then washed and incubated with the secondary goat anti-mouse PE conjugate. Stained cells were analyzed on a FACSscan cytometer using CellQuest software (BD Biosciences). The increase in mean fluorescence intensity (ΔMFI) was calculated as: (MFI(specific mAb) − MFI(isotype control))/MFI(isotype control).

Soluble MICA ELISA

Soluble MICA in sera from patients, HD, and K562 cell culture supernatants was quantified using the MICA sandwich ELISA (Immatics) according to the manufacturer’s protocol. Supernatants from K562 cells were concentrated 10-fold using a Vivaspin centrifugal concentrator with a 10-kDa molecular mass cutoff (Vivasor). Briefly, plates were coated with the AMO1 capture mAb against MICA at a concentration of 2 μg/ml in PBS, then blocked with 15% BSA overnight at 4°C and washed. Standard recombinant sMICA, serum samples, and culture supernatants (diluted 1/2) were added to the plates, which were then incubated at room temperature for 2 h. After washing, the detection mAb IgG1 was added, and the samples were incubated at room temperature for 2 h, then for 1 h at room temperature with HRP-conjugated anti-mouse IgG2a (Southern Biotechnology Associates). The chromogenic substrate for HRP, tetramethylbenzidine, was added, and the reaction was terminated with 1 M phosphoric acid. Absorbance was measured at 450 nm. A standard curve of the logarithmic relationship between concentration and absorbance was used to calculate the sMICA concentration in samples.

NK cell-mediated cytotoxicity assay

NK cells from HD were isolated using NK Cell Isolation Kit II (Miltenyi Biotec) according to the manufacturer’s protocol. NK cells were incubated for 24 h in 100 U/ml IL-2 before cytotoxicity assay. NK cell-mediated cytotoxicity was measured using a standard chromium release assay. K562 target cells were labeled with 50 μCi of [51Cr]sodium chromate (Amer sham Biosciences) for 1 h and then washed three times. For blocking experiments, anti-NKG2D Ab or IgG1 isotype control was added at 10 μg/ml to NK cells 30 min before incubation with the targets. NK cells and target cells were the coincubated at various E:T cell ratios for 4 h at 37°C in RPMI 1640 medium and 10% FCS. Spontaneous release was determined with target cells alone. Maximum release was obtained by target cells lysis with 0.5% Triton X-100. The percentage of specific lysis was calculated as follows: 100 × (experimental release − spontaneous release) / (maxim um release − spontaneous release).

Small RNA assays

K562 cells were transfected using the Nucleofector device and Cell Line Nucleofector Kit V (Amaxa) according to the K562-ATCC manufacturer’s protocol (American Type Culture Collection). The siRNA targeting BCR/ABL and the control (nonsilencing) siRNA were obtained from Qiagen-Solgte (21). Briefly, 1 × 106 cells were washed three times in PBS and resuspended in 100 μl of prewarmed Nucleofector Solution V with 0.5–1 μM siRNA. Cells were immediately resuspended in 2 ml of prewarmed RPMI 1640 medium and 10% FCS and were cultured for up to 4 days, then MICA expression was evaluated. Transfection efficiency was assessed after 4 h by measuring control-FITC siRNA incorporation by flow cytometry. Western blot

K562 cells were lysed in lysis buffer containing 50 mM Tris (pH 8.0), 150 mM NaCl, 1% Nonidet P-40, 5 mM EDTA, and protease inhibitor mixture (Roche). Cell lysates were incubated on ice for 20 min, with vortexing every 5 min, and then were centrifuged at 12,000 × g for 5 min. Protein concentrations were determined by Bradford assay. Protein lysates were dissolved in 2 × Laemmli sample buffer by boiling for 5 min. Whole-cell lysates were resolved on an SDS-5% polyacrylamide gel, and proteins were
transferred to a Hybod ECL nitrocellulose membrane (Amerham Biosciences). The membrane was blocked overnight at 4°C with 5% nonfat dry milk in PBS, then probed for 2 h at 25°C with anti-BCR Ab in PBS and 0.05% Tween 20 buffer (PBS-T). After three washes with PBS-T, membranes were incubated for 1 h at 25°C with biotinylated anti-mouse Ig. After three more washes, membranes were incubated for 45 min with streptavidin-HRP (Amerham Biosciences). The membrane was washed three times in PBS-T buffer, and bound Abs were detected by chemiluminescence (ECL Western Blotting Analysis System; Amerham Biosciences).

**Polysonome fractionation**

After a 10-min incubation in 100 µg/ml cycloheximide, K562 cells were lysed in polysome buffer containing 300 mM KCl, 5 mM MgCl₂, 10 mM HEPES, 0.5% Triton X-100, 500 µg/ml heparin, 5 mM DTT, and 100 IU/ml RNasin at 4°C. After removal of nuclei and mitochondria by centrifugation, supernatants were loaded on a 15–50% sucrose gradient. Gradients were centrifuged for 120 min at 40,000 rpm in a Beckman SW55Ti rotor at 4°C. Centrifuge gradients were fractionated into 10 fractions of equal volume. UV absorbance at 256 nm was measured in each fraction. RNA was isolated from each fraction with the TRIzol reagent according to the manufacturer’s protocol (Invitrogen Life Technologies).

**Real-time PCR quantification**

MICA mRNA and reference mRNAs for 18S rRNA, TATA box binding protein (TBP), and β-actin were assayed using a fluorescence-based real-time PCR. RT was performed using the SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen Life Technologies). Real-time PCR was performed with an ABI PRISM 7000 Sequence Detection System with SYBR Green reagents (Applied Biosystems) using 40 cycles of a two-step PCR (15 s at 95°C and 60 s at 60°C) after initial denaturation (95°C for 10 min). Threshold cycles (Cₜ) were determined as the mean of duplicate determinations for patient samples and triplicate determinations for cell line samples. For K562 cell assays, differences in relative abundances of mRNA were calculated as 2⁻ΔΔCₜ, where ΔCₜ is Cₜ(MICA)-Cₜ(REFERENCE gene), and ΔΔCₜ is ΔCₜ(IM) - ΔCₜ(DMSO). For quantification in patient and HD samples, relative MICA expression was normalized for the median value obtained in HD. The primer sequences for PCR were: 18S rRNA forward, 5'-GCT GGA ATT ACC GCC GCT-3'; TBP forward, 5'-CAC GAA CCA CGG CAC TGA TT-3'; TBP reverse, 5'-TTT TCT TGC TGC CAG TCT GGA C-3'; β-actin forward, 5'-TAC CTC ATG AAG ATC CTC A-3'; β-actin reverse, 5'-TTC GTG GAT GCC ACA GGA C-3'; MICA forward, 5'-CCT CGG AGG CCT CCC TGC G-3'; and MICA reverse, 5'-CCT TGG CCA TGA ACG TCA GG-3'.

**Statistical analysis**

Comparisons between groups were performed using Fisher’s exact test for binary variables and Mann-Whitney U test for continuous variables.

**Results**

**MICA expression by leukemic CD34⁺ progenitors and modulation of NKG2D expression by T and NK cells in CML patients**

CML is a myeloproliferative disease with a proportional expansion of polynuclear cells and granulocytic progenitors without a relative excess of immature blasts. To evaluate MICA expression associated with this disease, we measured surface MICA on hematopoietic CD34⁺ progenitors and sMICA concentrations in sera from CML patients. Six of nine CML patients showed surface MICA expression on bone marrow CD34⁺ cells at diagnosis (Fig. 1, A and B). MICA was not detected at the CD34⁺ cell surface in two HD bone marrow and six cord blood samples.

Sera from 49 patients with CP CML were tested for the presence of sMICA before and after IM therapy. Of these 49 patients, 44 were in early CP (within 15 days to 9 mo after diagnosis), and five were in late CP (diagnosis between 15 and 43 mo previously). After 6 mo of IM therapy (400 mg daily), all patients showed complete hematological remission. Before IM therapy, patients displayed significantly higher levels of sMICA (median, 289 pg/ml; range, 0–1233 pg/ml) than 13 HD (median, 162 pg/ml; range, 9–312 pg/ml; p = 0.01; Fig. 1C). Patients with a higher WBC than the median value (>33 10⁹/l) had significantly higher sMICA levels (median, 378 vs 250 pg/ml; p = 0.01). Defining the upper limit of the normal sMICA range as 360 pg/ml (mean ± 2 SD), 19 of 49 (39%) patients displayed high sMICA levels (Fig. 1C). Fourteen of these 19 patients could be evaluated after 6 mo of IM therapy and showed a substantial decrease in sMICA levels.

Soluble MICA modulates NKG2D expression at the CD8⁺ T cell and NK cell surface in cancer patients (10). The proportion of peripheral CD3⁺CD8⁺ T lymphocytes and CD16⁺CD56⁺ CD3⁻ NK cells that were NKG2D⁺ was higher in patients with CML at diagnosis (n = 11) than in HD (n = 7; Fig. 2). Moreover,

**FIGURE 1.** MICA expression and release in CML patients. MICA expression was studied on CD34⁺ cells in bone marrow from nine CML patients at diagnosis (Diag), two HD bone marrow samples, and six cord blood (CB) samples. A, Staining in two positive CML patients, one HD sample, and one cord blood is shown. B, MICA sMICA are shown for all samples. C, Serum levels of sMICA were measured by ELISA in 13 HD and 49 CP-CML patients at diagnosis. The 19 CML patients (35%) with elevated sMICA (greater than the mean of HD ± 2 SD) at diagnosis and the 14 evaluable (of 19) patients after 6 mo of IM therapy. Results are the mean of duplicate determinations.
after 6 mo of IM therapy, NKG2D expression returned to normal levels at the T cell surface and improved on NK cells.

**MICA/B surface expression on K562 cells is down-regulated by IM**

Because of the heterogeneity of the leukemic myeloid population in CML, a cell line model was needed to investigate in detail the role of BCR-ABL in NKG2DL expression. We thus studied MI-
CAML tox

**IM reduces susceptibility of K562 cells to NKG2D-mediated NK cell cytotoxicity**

To analyze the effects of IM-induced NKG2DL down-regulation on NKG2D-mediated cytotoxicity, we exposed IM-treated K562 cells to the NK cell line NKL in a chromium release assay. NKL expresses high levels of NKG2D, but minimal levels of the activat

**BCR/ABL oncogene controls MICA surface expression.**

IM inhibits the BCR/ABL kinase and also c-ABL and class III receptor tyrosine kinases, including c-Kit and the receptor to plate-

**Posttranscriptional control of MICA expression by BCR/ABL**

We next tested whether BCR/ABL inhibition results in the down-regulation of MICA gene transcription. We determined MICA mRNA levels by quantitative RT-PCR using three control mRNAs: 18S rRNA, TBP, and β-actin. Surprisingly, we did not observe significant changes in the MICA expression during the 24 h after exposure to 1 μM IM compared with controls (Fig. 5A). This suggested a posttranscriptional control of MICA gene expression. A well-described mechanism of surface down-regulation of MICA is the shedding that results from the action of metalloproteinases (11). To test this possibility, we measured spontaneous release of sMICA in K562 cell culture. Soluble MICA release was 1.5 times lower after 24 h and 1.8 times lower after 48 h of 1 μM IM exposure than that in samples treated with control siRNA (Fig. 5B). Recovery of MICA surface expression was observed 96 h after transfection. This modulation was dose dependent and confirmed the involvement of the BCR/ABL oncogene in MICA cell surface expression.
MICA and actin mRNA were determined in 10 consecutive equal fractions by RT-PCR. There was a significant shift in the polysomal distribution toward monosomal fractions for MICA, but not actin, mRNA after IM exposure (Fig. 5, C and D). These results indicate translational control of MICA expression by BCR/ABL.

Role of the PI3K/mTOR pathway in regulating MICA expression

BCR/ABL triggers various pathways, including PI3K/mTOR and MAPK kinase (MEK)/ERK, which have both been described to promote translation. We first explored the PI3K/mTOR pathway that controls mRNA translation through the activation of both p70 S6 kinase (p70S6K) and 4E-binding protein 1 (4E-BP1) translational regulators. Previous studies describe BCR/ABL downstream regulation of PI3K activity and phosphorylation of p70S6K and 4E-BP1 in CML cells and particularly K562 cells (25, 26). To determine whether this pathway is involved in MICA up-regulation, we exposed cells to the PI3K inhibitor LY294002 (1–30 μM IM) and to rapamycin (1–100 pg/ml), which specifically targets mTOR.

MICA and actin mRNA were determined in 10 consecutive equal fractions by RT-PCR. There was a significant shift in the polysomal distribution toward monosomal fractions for MICA, but not actin, mRNA after IM exposure (Fig. 5, C and D). These results indicate translational control of MICA expression by BCR/ABL.

FIGURE 4. BCR/ABL controls MICA surface expression. K562 cells were transfected with 0.5–1 mM siRNA targeting BCR/ABL or control siRNA. A, Twenty-four and 48 h after transfection, cytoplasmic proteins were analyzed by Western blotting using an anti-BCR Ab. Bands corresponding to p210 BCR/ABL and native BCR (160 kDa) are indicated. B, The relative MICA MFI (rMFI) with reference to the DMSO control is shown as the result of three independent experiments.
We observed a dose-dependent decrease in MICA expression (Fig. 6) with concentrations of these inhibitors that are nontoxic but inhibit PI3K and mTOR in K562 cells (26). The MAPK MEK/ERK pathway has also been suggested to be involved in MICA up-regulation on activated T lymphocytes (27), and we therefore also targeted ERK1/2 kinases spontaneously activated downstream of BCR/ABL (28). The inhibition of ERK1/2 by PD98059 (1–50 μM) did not affect MICA expression. None of these inhibitors affected CD54/ICAM-1 expression on the cell surface. These findings suggest that the PI3K/mTOR pathway may be the one primarily involved in BCR/ABL downstream control of MICA expression.

Discussion

The expression of NKG2DL is strongly associated with oncogenic transformation in many tissues. The immune consequences of this neoexpression have been well described in various tumor models (29). However, the molecular mechanisms that lead to NKG2DL expression during tumorigenesis are poorly understood. We used CML to investigate this issue because of the genetic homogeneity of this disease and the established knowledge about the oncogenic processes related to the spontaneous activity of the fusion BCR/ABL kinase (17). For the first time we directly incriminate an oncogene in the regulation of NKG2DL expression. The originality of this mechanism is that it is translational regulation involving the PI3K/mTOR pathway.

We studied MICA expression in a large cohort of CML patients. The report by Salih et al. (16) concerned a broad variety of acute and chronic leukemias, including four CML patients. Staining bone marrow cells revealed that MICA was expressed at the CD34+ leukemic cell surface, but was down-regulated during granulocytic maturation. The levels of sMICA correlated with the white blood cell count, reflecting tumor expansion. In this context of sMICA release and chronic exposure to NKG2DL-expressing tumor cells, NKG2D was down-modulated at the T and NK cell surfaces in CML as in other cancer patients (9, 10, 30–32). After 6 mo of IM therapy, sMICA levels decreased substantially in parallel with the return to a BCR/ABL-negative hemopoiesis and normalization of NKG2D expression on T cells.

Evidence that the immune system plays a role in the control of CML was available before the use of IM therapy from the response to immune-mediated therapies such as IFN-α, allogeneic stem cell transplantation, and donor lymphocyte infusion (33, 34). Several tumor Ags have been identified, including the BCR/ABL oncogene
itself, and overexpressed self-proteins, including proteinase 3 and the Wilm’s tumor protein (35–37). In vivo, the emergence of CD8⁺ T cells directed against proteinase 3 correlates with cytogenetic remission in patients treated with IFN-α and allogeneic stem cell transplantation (33, 38). In contrast, the lack of such T cells at diagnosis suggests tumor escape from host immune surveillance by mechanisms such as the production of immunosuppressive factors, a dominant Th2-type immunity or a decreased production of DCs (39, 40). The presence of MICA at the CD34⁺ leukemic cell surface illustrates in CML the complex role of NKG2DL, which is also observed in other cancers (9, 10, 30–32). NKG2D interactions with its ligands provide activating signals for NK and CD8⁺ T cells. In an allogeneic setting, especially in the BCR/ABL transgenic mouse model, it was shown recently that leukemic DCs could activate NK cells. This activation was related to NKG2DL overexpression induced by BCR/ABL on DCs (18). The role of NKG2D in the antileukemia effect mediated by NK cells in CML has also been recently supported (41). However, sMICA (9) and/or chronic stimulation by NKG2DL (12) could impair NKG2D expression and signaling. Our data, especially the NKG2D down-modulation we observed on CD8⁺ T and NK cells, are consistent with an impairment of autologous antitumor T and NK cell responses in CML, as reported in solid tumors.

Several immunological effects of IM have been described; most concern effectors or DCs, but not target cells. The in vitro effects of IM on NK cells, T cells, and DCs and their interactions are controversial (42–44). However, the induction of a CD8⁺ T cell response against proteinase 3 is lower in CML patients treated with IM than in those treated with IFN-α (38). We show in this study that IM directly affects tumor expression of NKG2DL, key molecules in both innate and adaptive immunity (45). By decreasing NKG2DL expression, IM may theoretically contribute to reduce the immunogenicity of BCR/ABL-positive cells and thus compromise the development of a specific immune response. Rather, normalization of NKG2DL expression and decreased sMICA production under IM may counteract NKG2D down-regulation and the subsequent impairment of NK cell and CTL activity. Attempts to increase NKG2D expression on cytotoxic effectors via IL-15 or by counteracting TGF-β could tune the response more effectively (46).

The precise mechanisms of MICA expression in tumors have been unclear. Recently, it has been suggested that NKG2DL expression could be controlled by the genotoxic stress and the DNA damage that participate in oncogenic transformation (15). In this model, mouse NKGDL Rae-1 and MULT1 transcription were up-regulated by ATM and ATM- and Rad-3-related kinases that regulate the cell cycle checkpoint. We repeatedly did not see any change in MICA mRNA transcription that could explain the modulation of MICA cell surface levels upon IM blockade of BCR/ABL activity. The frequent discrepancy between MICA mRNA levels and cell surface expression previously reported (14) suggested that mechanisms other than transcription modulation could also be involved. In this study we provide evidence that BCR/ABL regulates MICA expression posttranscriptionally through the control of MICA mRNA translation. The abnormal control of mRNA translation in cancer pathogenesis is a growing field of investigation (47). Many oncogenes, including BCR/ABL, regulate malignant progression by affecting the protein synthesis rate (47, 48). Constitutively activated by BCR/ABL, both Ras/ERK and PI3K/mTOR pathways are critical in the control of translation (25, 28, 49). Our data implicate PI3K/mTOR signaling in the regulation of MICA expression. PI3K/mTOR controls 1) ribosomal translation by phosphorylation of ribosomal p70S6K and 2) cap-dependent translation by phosphorylation of 4E-BP1 (50). The phosphorylation of 4E-BP1 prevents the binding and inactivation of eukaryotic translation initiation factor 4E, a critical factor in cap-dependent translation, which is itself phosphorylated through the Ras/ERK pathway. Thus, factors 4E-BP1 and eukaryotic translation initiation factor 4E interact to regulate translation at the end of PI3K/mTOR and Ras/ERK pathways, respectively. However, inhibition of MEK/ERK did not affect MICA expression, suggesting that p70S6K may be more responsible for the control of MICA expression. Such translational regulation downstream of BCR/ABL has been observed for C/EBPα and MDM2 mRNAs (51, 52). This rapid mechanism to modulate the level and activity of proteins is particularly consistent with the concept of MICA as a danger signal molecule (20, 53). This translational regulation of MICA expression may be additive to transcriptional regulation mechanisms such as those induced by DNA damage (15), because...
translational control may only occur downstream from efficient mRNA transcription.

To our knowledge, this is the first report in the field of cancer that the cell surface expression of an immune recognition molecule, in this case NKG2DL MICA, is directly controlled by mTOR and is down-regulated by its specific inhibitor, rapamycin. Rapamycin has been primarily used as an immunosuppressive drug. However, it is also a potent cytostatic inhibitor that arrests cells in the G1 phase of the cell cycle with potential antitumoral effects in several cancers, including acute myeloid leukemias and CML (54–56). The decrease in MICA protein expression by rapamycin is, in contrast to its well-known action on activated lymphocytes, another effect of the drug on the target side of the immune response. This should be taken into account when considering the antitumor or immunosuppressive uses of rapamycin and its analogues.

In conclusion, our data provide new insight into MICA regulation in cancer cells. In CML, MICA expression appears to include posttranscriptional control by the oncogene BCR/ABL. The PI3K/mTOR pathway is recurrently activated among tumors, and its involvement in MICA regulation should be investigated in other cancers as well. In light of these findings, the immune consequences of emerging oncogene-targeted therapies should be explored to develop associated immunotherapies.

The authors have no financial conflict of interest.

References

3. Groh, V., A. Steinle, S. Bauer, and T. Spies. 1997. Role of BCR/ABL tyrosine kinase in the G1 phase of the cell cycle with potential antitumoral effects in several cancers, including acute myeloid leukemias and CML (54–56). The decrease in MICA protein expression by rapamycin is, in contrast to its well-known action on activated lymphocytes, another effect of the drug on the target side of the immune response. This should be taken into account when considering the antitumor or immunosuppressive uses of rapamycin and its analogues.

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cells in chronic myeloid leukemia correlate with high plasmatic VEGF and are not normalized by imatinib mesylate. *Leukemia* 18: 1656–1661.


