Research Article

Interleukin-29 Binds to Melanoma Cells Inducing Jak-STAT Signal Transduction and Apoptosis

Kristan D. Günterberg1, Valerie P. Grignol1, Ene T. Raig2, Jason M. Zimmerer2, Anthony N. Chan1, Farriss M. Blaskovits1, Gregory S. Young3, Gerard J. Nuovo4, Bethany L. Mundy2, Gregory B. Lesinski5, and William E. Carson, III1,6

Abstract

Interleukin-29 (IL-29) is a member of the type III IFN family that has been shown to have antiviral activity and to inhibit cell growth. Melanoma cell lines were tested for expression of the IL-29 receptor (IL-29R) and their response to IL-29. Expression of IL-28R1 and IL-10R2, components of IL-29R, was evaluated using reverse transcription-PCR. A combination of immunoblot analysis and flow cytometry was used to evaluate IL-29-induced signal transduction. U133 Plus 2.0 Arrays and real-time PCR were used to evaluate gene expression. Apoptosis was measured using Annexin V/propidium iodide staining. In situ PCR for IL-29R was done on paraffin-embedded melanoma tumors. Both IL-28R1 and IL-10R2 were expressed on the A375, 1106 MEL, Hs294T, 18105 MEL, MEL 39, SK MEL 5, and F01 cell lines. Incubation of melanoma cell lines with IL-29 (10–1,000 ng/mL) led to phosphorylation of signal transducer and activator of transcription 1 (STAT1) and STAT2. Microarray analysis and quantitative reverse transcription-PCR showed a marked increase in transcripts of IFN-regulated genes after treatment with IL-29. In the F01 cell line, bortezomib-induced and temozolomide-induced apoptosis was synergistically enhanced following the addition of IL-29. In situ PCR revealed that IL-10R2 and IL-28R1 were present in six of eight primary human melanoma tumors but not in benign nevi specimens. In conclusion, IL-29 receptors are expressed on the surface of human melanoma cell lines and patient samples, and treatment of these cell lines with IL-29 leads to signaling via the Jak-STAT pathway, the transcription of a unique set of genes, and apoptosis. Mol Cancer Ther; 9(2); OF1–11. ©2010 AACR.

Introduction

IFN-α is commonly used in the treatment of malignant melanoma, both as an adjuvant to surgical resection and in the setting of advanced disease (1). This cytokine enhances the activity of immune effector cells while mediating antiproliferative and proapoptotic effects against tumor cells. The receptor for IFN-α is widely expressed on normal tissues and has been identified on tumor cell lines (2). The binding of IFN-α to its receptor results in the phosphorylation of Janus kinase 1 (Jak1) and tyrosine kinase 2 (Tyk2) on specific tyrosine residues. These phospho-tyrosine residues provide docking sites for cytoplasmic transcription factors belonging to the signal transducer and activator of transcription (STAT) family of proteins. These STAT proteins (STAT1α, STAT2) are phosphorylated by the Janus kinases and subsequently form high-affinity DNA binding complexes that rapidly translocate to the cell nucleus to drive the expression of IFN-responsive genes (3, 4).

Interleukin-29 (IL-29), also known as IFN-λ, is a recently discovered cytokine of the type III IFN family (5, 6). It is thought to have biological properties similar to the type I IFNs. Unlike IFN-α, the receptor for IL-29 (IL-29R) is expressed on a limited number of normal cells, including dendritic cells, T cells, and intestinal epithelial cells. Leukemia cells and colon, prostate, pancreatic, lung, hepatoma, glioblastoma, and breast cancer cells have also been shown to express this receptor (6–12). In these reports IL-29 elicited signal transduction via activation of the Jak-STAT pathway, p-AKT, and the mitogen-activated protein kinase/extracellular signal-regulated kinases (MAPK/ERK; refs. 5, 8, 9, 12, 13).

To date, the biological effects of IL-29 on human melanoma cells have not been investigated. Because this cytokine is postulated to have antitumor properties in other cancer cell lines (12, 14), the direct effects of IL-29 on melanoma cells were explored. In the present report, we characterize IL-29-induced signal transduction, gene expression, and apoptosis in melanoma cells. We also show that IL-29 can act in concert with other antitumor agents to enhance apoptosis of melanoma cells.
Materials and Methods

Cell Lines and Reagents
The A375, Hs294T, and SK MEL 5 human melanoma cell lines were purchased from the American Type Culture Collection. The 1106 MEL, 18105 MEL, MEL 39, F01, and 1174 MEL cell lines were a gift from Dr. Soldano Ferrone (Roswell Park Cancer Institute). IL-29 was obtained from ZymoGenetics. Cell lines were grown as previously described (15). Human natural killer (NK) cells were isolated directly from fresh peripheral blood leukopacks (American Red Cross) by a 25-min incubation with RosetteSep cocktail (Stem Cell Technologies) followed by Ficoll-paque (Sigma) density gradient centrifugation as previously described (16). NK cells were cultured in RPMI 1640 supplemented with 10% heat-inactivated pooled human AB serum (Sigma), 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B (17).

Flow Cytometry of Tumor Cell Lines
Intracellular flow cytometry for phosphorylated STAT1 (P-STAT1) was done as previously described using alexafluor488-labeled antibodies (18). Briefly, total levels of Tyr701-P-STAT1 were measured by flow cytometry on a FACS Caliber (BD Bioscience). A minimum of 10,000 gated events were analyzed for each sample. Data were expressed as specific fluorescence (Fsp = Ft - Fb), where Ft represents the median value of total staining, and Fb represents the median value of background staining with an isotype control antibody (19, 20).

Immunoblot Analysis
Lysates were prepared from melanoma cell lines stimulated with PBS or IL-29 and assayed for the expression of Jak-STAT and MAPK proteins by immunoblot as previously described (15, 19), with antibodies to p-AKT, p-ERK, pSAPK, poly(ADP-ribose) polymerase, and P-STAT1/2/3/5 (Cell Signaling Technology) or β-actin (Sigma).

Cytotoxicity Assays
Purified human NK cells were plated in 96-well V-bottom plates in 10% human AB medium supplemented with 10 to 1,000 ng/mL of IL-29 and incubated overnight at 37°C. 51Cr-labeled cells were added to wells at various effector:target ratios, and following a 4-h incubation at 37°C, supernatants were harvested for quantification of chromium release. Percentage of cell lysis was determined as previously described (21).

cRNA Preparation and Array Hybridization
Probe sets from U133 Plus 2.0 Arrays (Affymetrix), which query approximately 47,000 transcripts, were used in these analyses. The cRNA was synthesized as suggested by Affymetrix. Lysis of cells in TRIzol (Invitrogen), total RNA was isolated by RNeasy purification (Qiagen), cDNA was generated from 2 μg of total RNA using the Superscript Choice System according to the manufacturer’s instructions (Invitrogen). Biotinylated cRNA was generated using the Bio Array High Yield RNA Transcript Labeling System (Enzo Life Sciences Inc.). The cRNA was purified using the RNeasy RNA purification kit. cRNA was fragmented according to the Affymetrix protocol, and the biotinylated cRNA was hybridized to microarrays. Raw data were collected with a GeneChip Scanner 3000 (Affymetrix; ref. 22).

Polymerase Chain Reaction
PCR analysis was conducted to detect transcripts for IL-28R1 and IL-10R2 (the IL-29 receptor components). Briefly, total RNA was isolated using the RNeasy RNA Isolation Kit (Qiagen) and 2 μg of total cellular RNA was used as a template for reverse transcription-PCR (RT-PCR) with random hexamers (Invitrogen). The following primers were used for the PCR reaction: IL-10R2 F 5′ GGCTG-AATT TGCAATGAGCA 3′ and R 5′ GAAGACGCAG-GCCAT GAGG 3′; IL-28R1 F 5′ ACCATTTGGGCTATCA-GACT 3′ and R 5′ CGGCTCCACCTCAA-AAGGTAAT 3′. The amplification scheme used was as follows: 94°C for 5 min, then 35 cycles of 94°C for 45 s, 60°C for 45 s, and 72°C for 45 s, followed by 72°C for 7 min and then 4°C.

Real-time PCR
Real-time PCR was used to assess gene expression in melanoma cells that had been stimulated with either...
PBS or IL-29 (10–1,000 ng/mL) for 12 h. cDNA was prepared as described above and then used as a template for real-time PCR using predesigned primer/probe sets and TaqMan Universal PCR Master Mix (Applied Biosystems) according to the manufacturer’s instructions. Real-time data were analyzed using the Sequence Detector software (version 2.2, Applied Biosystems).

**Proliferation Assays and Evaluation of Apoptosis**

Cell proliferation was measured using the MTT assay according to manufacturer’s recommendations as previously described (23). Flow cytometric analysis of cells stained with Annexin V/propidium iodide staining was used to measure the percentage of apoptotic cells following various treatments (24).

**In situ RT-PCR**

Using the primers previously listed, seven benign nevi and eight melanoma lesions were tested for IL-10R2 and IL-28R1 mRNA expression using in situ RT-PCR (25, 26). Briefly, optimal protease digestion time was determined using nonspecific incorporation of the reporter nucleotide

![Figure 2. Melanoma cell lines signal via the Jak-STAT pathway. A, the human melanoma cell lines 1106 MEL, A375, F01, and 1174 MEL cells were treated with IFN-α at 10⁴ units/mL or IL-29 at 10, 100, or 1,000 ng/mL and evaluated by immunoblot analysis for P-STAT1/2/3/5. Membranes were probed with β-actin antibody as a loading control. Human melanoma cell lines 1106 MEL, A375, F01, and 1174 MEL were stimulated with increasing doses of IL-29 (0–1,000 ng/mL) for 30 min at 37°C and cells were evaluated for P-STAT1 by intracellular flow cytometry. Fluorescence data are presented as Fsp intensity of P-STAT1 staining (Fsp = Ft - Fb). B, representative histograms for a single experiment and (C) graph for all cell lines evaluated. Columns, mean from a single experiment; bars, SD. D, protein levels of pAKT, pERK, and pSAPK were measured by immunoblot analysis after no treatment (0), or treatment with phorbol 12-myristate 13-acetate (50 ng/mL), or IL-29 (10, 100, 1,000 ng/mL).](http://mct.aacrjournals.org/content/9/2/OF3/F03/F03.large.jpg)
<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>IL-29 dose (ng/mL)</th>
<th>Time (h)</th>
<th>Fold Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>IFI27</td>
<td>Immune response</td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>RSAD2</td>
<td>Viral response</td>
<td></td>
<td></td>
<td>21.1</td>
</tr>
<tr>
<td>DMGDH</td>
<td>Electron transport, glycine catabolic process</td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>IFI44L</td>
<td>Unknown</td>
<td></td>
<td></td>
<td>7.5</td>
</tr>
<tr>
<td>IFIT1</td>
<td>Immune response</td>
<td></td>
<td></td>
<td>22.6</td>
</tr>
<tr>
<td>OAS2</td>
<td>Immune response, nucleic acid metabolism</td>
<td></td>
<td></td>
<td>5.3</td>
</tr>
<tr>
<td>MX1</td>
<td>Regulation of apoptosis, immune response</td>
<td></td>
<td></td>
<td>7.5</td>
</tr>
<tr>
<td>EPST11</td>
<td>Unknown</td>
<td></td>
<td></td>
<td>2.8</td>
</tr>
<tr>
<td>OAS1</td>
<td>Antiviral response, nucleosidase</td>
<td></td>
<td></td>
<td>5.7</td>
</tr>
<tr>
<td>DDX58</td>
<td>Helicase, deoxyribonuclease, ubiquitin pathway</td>
<td></td>
<td></td>
<td>5.3</td>
</tr>
<tr>
<td>ISG15</td>
<td>Immune response</td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>IFI6</td>
<td>Regulator of apoptosis, immune response</td>
<td></td>
<td></td>
<td>3.5</td>
</tr>
<tr>
<td>IFIT3</td>
<td>Immune response</td>
<td></td>
<td></td>
<td>4.3</td>
</tr>
<tr>
<td>IFITM1</td>
<td>Immune response, negative regulation of proliferation</td>
<td></td>
<td></td>
<td>2.3</td>
</tr>
<tr>
<td>PRIC285</td>
<td>Regulation of transcription</td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>SULT1C2</td>
<td>Amine metabolic process</td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>OASL</td>
<td>Immune response, nucleic acid metabolism</td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>XAF1</td>
<td>Zinc ion binding</td>
<td></td>
<td></td>
<td>4.6</td>
</tr>
<tr>
<td>TMPRSS3</td>
<td>Proteolysis, sensory reception</td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>LOC129607</td>
<td>dTDP/dTTP biosynthetic process</td>
<td></td>
<td></td>
<td>2.3</td>
</tr>
<tr>
<td>IFIH1</td>
<td>Helicase, deoxyribonuclease</td>
<td></td>
<td></td>
<td>2.8</td>
</tr>
<tr>
<td>HFE2</td>
<td>GPI anchor binding</td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>RTP4</td>
<td>Sensory reception</td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>SAMD9L</td>
<td>Unknown</td>
<td></td>
<td></td>
<td>5.3</td>
</tr>
<tr>
<td>PARP9</td>
<td>Protein ADP ribosylation, cell migration</td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>CCNC</td>
<td>Cell cycle regulation</td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>IFIT2</td>
<td>Immune response</td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>TLX2</td>
<td>Regulation of transcription, negative regulation of dendrite morphogenesis</td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>FLJ20035</td>
<td>Nucleic acid binding, helicase activity</td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>IRF9</td>
<td>Regulation of transcription, immune response, viral response</td>
<td></td>
<td></td>
<td>5.7</td>
</tr>
<tr>
<td>STAT1</td>
<td>Transcription factor, JAK-STAT pathway</td>
<td></td>
<td></td>
<td>2.6</td>
</tr>
<tr>
<td>ACOT11</td>
<td>Intracellular signaling cascade, response to temperature stimulus</td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>HERC5</td>
<td>Regulation of cyclin dependent protein kinase activity, ubiquitin pathway</td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>IRF7</td>
<td>Transcription factor, antiviral response, apoptosis</td>
<td></td>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td>TRIM22</td>
<td>Regulation of transcription, immune response, response to virus</td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>USP18</td>
<td>Ubiquitin-dependent protein catabolism</td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>FAM125A</td>
<td>Unknown</td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>HERC6</td>
<td>Ubiquitin-protein ligase</td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>HTRA4</td>
<td>Proteolysis</td>
<td></td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>

(Continued on the following page)
digoxigenin dUTP. Protease digestion was followed by overnight incubation in RNase-free DNase (10 U per sample; Boehringer Mannheim) and one-step RT PCR using the rTth system and digoxigenin dUTP. Following PCR, nitroblue tetrazolium and bromochloroindolyl phosphate were used to stain the tissue with nuclear fast red as the counterstain. Controls for the in situ PCR reaction included use of tissues negative for IL-28R1 and IL-10R2 (e.g., benign nevi and normal skin), omission of the primers (negative controls), and omission of the DNase step which yields an intense nuclear-based signal in all cell types. This is due to amplification of genomic DNA that has been exposed by the protease digestion step and serves as a positive control (25, 26). Images were taken with an Olympus DP-10 camera (Olympus) using a Nikon Labphotomat-2 microscope (Nikon) and adjusted with Adobe Photoshop CS4 (Adobe).

### Statistics

Linear mixed effects models were used to compare the levels of phosphorylated STAT1 (as determined by flow cytometry) for the various doses of IL-29 for each of the cell lines. Linear mixed effects models were used to model the dose-response relationship for the IL-29 and bortezomib/temozolomide combination experiments. As each experiment was run in triplicate, a random effect was included for each of the replications allowing for correlation in the response. For the combination experiments, doses were considered as categorical variables and the error was assumed linear in log(E/(1-E)) where E represents the fraction of cells surviving. Synergy was assessed via interaction contrasts at specific dose combinations. In addition, interaction indices were calculated based on Loewe additivity (27) and the median effect equation of Chou and Talalay (28). An index value of 1 indicates additivity of the two agents, whereas a value <1 indicates synergism. Fisher’s exact test was used to determine significance of IL-29R expression in melanomas as compared with benign nevi. Results were considered significant if \( P < 0.05 \).

### Results

#### Human Melanoma Cell Lines Express IL-29 Receptor Transcripts

The expression of IL-28R1 and IL-10R2 was evaluated by RT-PCR in a panel of eight human melanoma cell lines. Each of the eight cell lines expressed the IL-10R2
mRNA; however, expression of the IL-28R1 subunit was variable between cell lines and was absent in the 1174 MEL cell line (Fig. 1A). These results were confirmed via semiquantitative real-time PCR. This analysis confirmed that both receptors were expressed in all the cell lines except 1174 MEL, which showed no expression of IL-28R1, and SK MEL 5, which had very low expression of IL-28R1 (Fig. 1B). In addition, real-time PCR was used to evaluate the presence of IL-10R1 and IL-20R1, receptors coexpressed with IL-10R2. Both receptors were found to be present in all eight melanoma cell lines (data not shown).

**IL-29 Induces Jak-STAT Signal Transduction in Melanoma Cells**

Melanoma cell lines were stimulated with IL-29 and the activation of downstream signal transduction pathways was evaluated. Following stimulation of melanoma cell lines for 20 minutes with IL-29 (10–1000 ng/mL), phosphorylation of STAT1 (at Tyr 701) and STAT2 (Tyr 690) was induced in all the cell lines tested that expressed both IL-29R components (Fig. 2A). IL-29–induced phosphorylation of STAT1 was confirmed using intracellular flow cytometry (Fig. 2B and C). STAT1 and STAT2 phosphorylation in response to IL-29 was variable across the individual melanoma cell lines. For example, the 1106 MEL cell line exhibited strong induction of P-STAT1 and P-STAT2 following IL-29 treatment, whereas the A375 cell line required high doses of IL-29 to elicit maximal phosphorylation of STAT1 and STAT2. There was a statistically significant increase in P-STAT1 signaling in the 1106 MEL (P = 0.0003), A375 (P = 0.0014), and F01 cell lines (P = 0.0379) following treatment with 1,000 ng/mL IL-29 as compared with media treatment. There was no significant increase in Jak-STAT signaling in the 1174 MEL cell line in response to any dose of IL-29, which is consistent with its lack of the IL-28R1.

Basal phosphorylation of the STAT3 and STAT5 transcription factors is common in melanoma cell lines and is thought to contribute to the oncogenic phenotype (29–33). As expected, there was basal phosphorylation of STAT3 in all of the cell lines except for 1106 MEL (20). However, in contrast to stimulation with IFN-α, stimulation of cells with IL-29 did not lead to a further increase in P-STAT3 except in the 1106 MEL cell line. Phosphorylation of STAT5 in response to IL-29 treatment was also observed in the 1106 MEL and 1174 MEL cell lines. Although 1174 MEL lacks the IL-28R1 component it does express the IL-10R2 subunit. We hypothesize that the interaction of the IL-10R2 component and other cytokine receptor components such as IL-10R1 or IL-20R1 may have led to the increased phosphorylation of STAT5. The ability of IL-29 to modulate the activation of AKT, ERK, and stress-activated protein kinase/Jun-amino-terminal kinase (SAPK/JNK) was also investigated in this panel of melanoma cell lines. There was no activation of these pathways irrespective of the dose of IL-29 employed (Fig. 2D).

**Microarray Analysis of IL-29–Induced Gene Expression**

Microarray analysis was conducted to determine the transcriptional profile of melanoma cells following IL-29 stimulation. The 1106 MEL cell line was stimulated for 5 or 18 hours with IL-29 (10 and 1,000 ng/mL) or PBS (control). The predominant genes expressed in response to IL-29 treatment were IFN-stimulated genes (ISG; e.g., Mx1, OAS, IFI27; Table 1). This is consistent with prior studies conducted in IL-29–stimulated somatic cells (34). The number of genes induced increased both with increasing dose of IL-29 and with increasing duration of treatment. At the 18-hour time point there was upregulation of 60 genes as compared with the 41 genes that were upregulated at the 5-hour time point. For example, in response to a 5-hour treatment with IL-29 at doses of 10 and 1,000 ng/mL expression of radical s-adenosyl methionine domain containing protein 2 (RSAD2) increased by 21.1- and 48.5-fold, respectively, as compared with 19.7- and 84.4-fold following an 18-hour treatment. In response to a 5-hour treatment with 10 and 1,000 ng/mL IL-29, expression of 2′-5′-oligoadenylate synthetase 2 (OAS2) increased by 5.3- and 11.3-fold, respectively, as

![Figure 3. IL-29 induces transcription and protein expression of IFN-stimulated genes. Transcript levels of various genes were measured using real-time PCR analysis after the treatment of 1106 MEL, A375, and F01 human melanoma cell lines with IL-29 at 10 to 1,000 ng/mL for 12 h (A) or 1,000 ng/mL for 12 h (B). Columns, mean from three separate experiments; bars, SD.](image-url)
compared with 27.9- and 64-fold at 18 hours. In addition, IL-29 induced the expression of multiple ISGs that regulate transcription and apoptosis (e.g., MX1, IFI6, IFITM1, Pric 285, TLX2, IRF9, and IRF7).

**IL-29–Induced IFN Stimulated Gene Expression**

Real-time PCR was done on three melanoma cell lines to confirm the expression of genes that were most strongly induced by IL-29 on microarray analysis. There was a marked increase in the expression of IFI27, RSAD2, OAS1/2, DDX58, ISG15, IFI6, IFIT3, IFITM1, and Mx1 in response to 10 to 1,000 ng/ml IL-29 for the 1106 MEL, A375, and F01 cell lines (Fig. 3A and B). Based on previous studies showing that overexpression of SOCS-1 protein in neuroendocrine and hepatoma cells abrogates IL-29–induced Jak-STAT signaling (12, 13), the expression of SOCS genes was tested. SOCS-1 was upregulated 2.0-fold in the F01 cell line in response to IL-29 and SOCS-4 was downregulated by 0.5-fold. SOCS-6 was induced by 1.0- to 1.6-fold in all cell lines (Fig. 3B).

**IL-29 Does Not Enhance NK Cell Cytotoxicity against Melanoma Target Cells**

Because immune effector cells are known to express IL-28R1 and IL-10R2 and respond to IL-29, we postulated that this cytokine could potentially prime NK cells to mediate enhanced lysis of tumor cells. To test this hypothesis, NK cells were treated overnight with IL-29 and tested for their ability to lyse a panel of three melanoma tumor cell lines in a standard 4-hour 51Cr release assay. IL-29 did not enhance NK cytotoxic activity in this setting, despite the fact that NK cells were found to express both IL-10R2 and IL-28R1 and to induce Jak-STAT signal transduction (data not shown). Similar results were found with IL-29–treated peripheral blood mononuclear cells against the F01 cell line. In addition, melanoma cells pretreated with 1,000 ng/ml.
of IL-29 exhibited no change in their susceptibility to NK cell–mediated cytotoxicity (data not shown).

**IL-29–Induced Apoptosis of Melanoma Cells Is Enhanced in the Presence of Bortezomib (a Proteasome Inhibitor) or Temozolomide**

There was no change in the proliferation of melanoma cell lines following a 24- to 72-hour treatment with IL-29 (10–1,000 ng/mL) as assessed by either the MTT or [H] thymidine incorporation methods (data not shown). The ability of IL-29 to induce apoptosis was next assessed in the F01 melanoma cell line. Flow cytometric analysis by Annexin V/propidium iodide staining revealed a dose-dependent increase in apoptosis in response to 48-hour treatment with IL-29 (Fig. 4A). Based on previous work showing that proteasome inhibition could enhance the proapoptotic effects of IFN-α in melanoma cells (24), the apoptosis of F01 cells was measured following treatment with IL-29 in combination with bortezomib. As expected, IL-29–induced apoptosis was enhanced following exposure to bortezomib (Fig. 4B). Chou and Talalay interaction indices were calculated for the combination of IL-29 and bortezomib (index values = 0.36–1.03). At the 20 nmol/L dose of bortezomib this combination induced synergistic apoptosis of F01 cells which was statistically significant ($P < 0.018$ for all dose levels). For example, IL-29 at...
10 ng/mL induced 8.8% apoptosis and bortezomib at 20 nmol/L induced 50% apoptosis, whereas the combination caused apoptosis in 83% of the cells. Apoptosis was enhanced in response to these treatment combinations as confirmed by immunoblot analysis for the presence of cleaved poly(ADP-ribose) polymerase (Fig. 4C). A similar synergistic apoptotic effect was observed following treatment of F01 cells with temozolomide plus IL-29 (Fig. 4D). Synergistic apoptosis occurred with IL-29 at concentrations of 100 and 1,000 ng/mL at all doses of temozolomide (all interaction contrast P values <0.003, interaction indices = 0.003–0.18). For example, single-agent IL-29 at 1,000 ng/mL caused 15.2% apoptosis and single-agent temozolomide at 150 μmol/L caused 15.7% apoptosis. The combination resulted in 52.2% apoptosis, which was greater than the combined effects of both agents. Marginally significant synergy took place in response to IL-29 at 10 ng/mL and temozolomide at 50, 100, and 150 μmol/L (all interaction contrast P values <0.056, interaction indices = 0.03–0.82).

**Primary Melanomas Express the IL-29 Receptor**

Paraffin-embedded tissue samples of benign nevi and primary melanoma lesions were evaluated for expression of the IL-29R components by in situ PCR (Fig. 5). Seven benign nevi were examined and all were negative for both components of the IL-29R. Six of eight primary melanoma lesions were positive for both receptor components and two primaries were negative for both components of IL-29R (P = 0.007 versus benign nevi). The signal localized primarily to the cytoplasm of the neoplastic cells.

**Discussion**

It is shown in the present study that the receptor components needed for IL-29 signal transduction are present on several human melanoma cell lines. In cells with intact IL-29R signaling machinery (IL-28R1 and IL-10R2), IL-29 treatment led to phosphorylation of STAT1 and STAT2 and an increase in the expression of genes involved with the antiviral response, immune response, and regulation of transcription. IL-29–induced apoptosis in a melanoma cell line was synergistically enhanced following the addition of temozolomide or bortezomib. Additionally, the receptor for IL-29 was found to be present on human melanoma primaries but not on benign nevi.

The receptor components for IL-29 are present on dendritic cells, T cells, intestinal epithelial cells, and several human cancer cell lines (6–12). Brand et al. evaluated signal transduction of intestinal epithelial cells stimulated with IL-29. They found that IL-29 activated the ERK-1/2, SAPK/c-jun, AKT, and Jak-STAT pathways (6, 8). Other authors have shown Jak-STAT pathway signaling in neuroendocrine tumors (12), human keratinocytes (35), and hepatoma cells following treatment with IL-29 (9, 36). In a murine model, Sommereyns et al. found that IFN-λ (mouse ortholog of IL-29) was strongly induced in the liver in response to viral infections. They also showed that mice with systemic viral infections expressed IFN-λ and this resulted in a marked increase in IFN-stimulated genes in the stomach, intestines, and lungs (37). The present article is the first to report the presence of IL-29R in human melanoma cells and delineate the signal transduction pathways that are initiated in response to this cytokine. The induction of P-STAT1, P-STAT2, P-STAT3, and P-STAT5 in response to IL-29 suggests a complex yet robust effect. The lack of MAP-kinase activation in IL-29–treated melanoma cells was unexpected and is being confirmed in additional cell lines.

Prior studies have evaluated the response of lymphoma and hepatocellular carcinoma cells to IL-29 stimulation via microarray analysis and have shown an upregulation of multiple ISGs (9, 38). Using Affymetrix S130 high-density microarray chip analysis, Zhou et al. showed lower induction of ISGs in IFN-λ-stimulated Raji cells (10 ng/mL) compared with IFN-α-stimulated cells (38). In contrast, ISG induction by IL-29 was stronger than that of IFN-α in human hepatitis C virus-transfected hepatoma cells (36). Our studies showed an increase in antiviral proteins such as OAS and Mx1 along with many other immune and antiproliferative proteins. A prior study by our group evaluating the effects of high-dose IFN-α treatment on the expression levels of genes in the peripheral blood mononuclear cells of patients with malignant melanoma showed a pattern of gene induction that was similar to that observed in the present study (39). These results lend support to the idea that IL-29 and IFN-α induce a similar set of genes and therefore could have similar antitumor effects. Several studies have shown that type III IFNs and IFN-α have overlapping antiviral activity (9, 14, 40, 41). Overall, the antiviral effects of IL-29 are slower in onset, weaker, and last longer than those of IFN-α (7, 35, 36). IL-29 acts in an additive manner when combined with IFN-α in blocking the replication of vesicular stomatitis and hepatitis C virus (42). The precise role of IL-29 in host antitumor responses and immune surveillance has yet to be defined in the context of malignant melanoma, but the available data suggest that its effects are similar to those of IFN-α. Studies by other groups have shown that IL-29 inhibits proliferation in glioblastoma cells (7) and both inhibits proliferation and induces apoptosis in a human neuroendocrine cell line (12). Whether IL-29 has unique antitumor effects or can exert additive effects with IFN-α in the setting of malignant melanoma is currently under investigation.

Only a limited amount of in vivo work has been done to evaluate the effects of IL-29 in melanoma. In a transient transfection model, Sato and colleagues showed that overexpression of the murine IFN-λ receptor ligand in B16F10 cells caused increased expression of MHC class I. Additionally, they found that the transfected cell line had lower levels of proliferation and exhibited significantly enhanced activation of caspase 3 and caspase 7 at 36 hours. The induction of p21 and dephosphorylated Rb was also enhanced (as compared with mock transfected...
cells; ref. 41). Administration of IFN-λ-expressing B16F10 cells to mice via tail vein injection led to decreased pulmonary metastases at 14 days and reduced mortality as compared with control mice. This effect was dependent on NK cells, but not on CD4+ and CD8+ T-cells (41). In a separate study, Sato et al. showed that systemic overexpression of IFN-λ by hydrodynamic injection of IFN-λ cDNA resulted in increased numbers of NK and NKT cells in the livers of mice and resulted in antitumor activity against a colon cancer cell line (41). The applicability of these findings to the clinical situation is unclear as there are no reports of IL-29 being produced by human melanoma cells, although it might be present in the tumor microenvironment under certain conditions. Our analysis of primary melanomas indicates that these lesions routinely express the receptor components for IL-29 and would likely respond to IL-29 treatment with the induction of ISG transcription.

Like IFN-α, IL-29 activates several components of the immune system. IL-29 stimulates monocytes and macrophages to release cytokines (e.g., IL-6, IL-8, and IL-10) resulting in a shift from a type II T helper cell response to a type I T helper cell response (11). Similarly, exposure of lipopolysaccharide-treated monocytes to IL-29 enhances the release of IL-12 (43, 44). IFN-λ treatment resulted in increased expression of the MHC class I proteins in human keratinocyte and murine melanoma cell lines (14, 41), an effect that could enhance their recognition by T cells. Of note, IL-29 treatment of NK cells did not enhance their cytotoxicity against melanoma cells nor did IL-29 treatment of melanoma target cells render them more susceptible to lysis by NK cells (data not shown).

We hypothesized that IL-29 could render melanoma cells more susceptible to proapoptotic therapies such as chemotherapy or radiation therapy. Temozolomide has documented activity against metastatic melanoma and bortezomib has been tested in this setting as well (24, 45). IL-29 enhanced the apoptotic effects of both drugs, which suggests that combination therapies might be clinically effective. Of note, not all melanoma cell lines responded equally well to these combinations. Additional studies are underway to determine the cause of this variation.

We have shown that the receptor for IL-29 is expressed on melanoma cell lines and that activation with this cytokine leads to Jak-STAT signal transduction, expression of multiple genes, and an increase in apoptosis. The addition of either bortezomib or temozolomide resulted in a synergistic enhancement of apoptosis. Primary melanomas showed increased expression of the genes for IL-29R as compared with benign nevi. The present data suggest that the IL-29 can exert direct effects on melanoma cells.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank the Ohio State University Comprehensive Cancer Center Analytic Cytometry, Centers for Biostatistics, Microarray, and the Nucleic Acid Shared Resources for their assistance.

Grant Support

NIH Grants P01 CA95426 (M. Caligiuri), P30 CA16058 (M. Caligiuri), CA84402, K24 CA93670 (W.E. Carson, III), K22 AI134551 (G.B. Lesinski), The Valvano Foundation for Cancer Research (G.B. Lesinski), T32 CA09038. 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 5/28/09; revised 12/8/09; accepted 12/21/09; published OnlineFirst 1/26/10.

References

Molecular Cancer Therapeutics

Interleukin-29 Binds to Melanoma Cells Inducing Jak-STAT Signal Transduction and Apoptosis


Mol Cancer Ther  Published OnlineFirst January 26, 2010.

Updated version  Access the most recent version of this article at:
doi:10.1158/1535-7163.MCT-09-0461

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.