Regulation of leukemic cell differentiation and retinoid-induced gene expression by statins

Antonella Sassano,1 Marco Lo Iacono,2 Giovanni Antico,1 Alison Jordan,1 Shahab Uddin,3 Raffaele A. Calogero,2 and Leonidas C. Platanias1

1Robert H. Lurie Comprehensive Cancer Center and Division of Hematology/Oncology, Northwestern University Medical School and Jesse Brown VA Medical Center, Chicago, Illinois; 2Department of Clinical and Biological Sciences, University of Turin, Turin, Italy; and 3Department of Human Cancer Genomic Research, Research Center King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia

Abstract

There is emerging evidence that, beyond their cholesterol-lowering properties, statins exhibit important antileukemic effects in vitro and in vivo, but the precise mechanisms by which they generate such responses remain to be determined. We have previously shown that statins promote differentiation of acute promyelocytic leukemia cells and enhance generation of all-trans retinoic acid (ATRA)–dependent antileukemic responses. We now provide evidence that statin-dependent leukemic cell differentiation requires engagement and activation of the c-Jun NH2-terminal kinase pathway. In addition, in experiments, to define the molecular targets and mediators of statin-induced differentiation, we found a remarkable effect of statins on ATRA-dependent gene transcription, evidenced by the selective induction of over 400 genes by the combination of atorvastatin and ATRA. Altogether, our studies identify novel statin molecular targets linked to differentiation, establish that statins modulate ATRA-dependent transcription, and suggest that combined use of statins with retinoids may provide a novel approach to enhance antileukemic responses in acute promyelocytic leukemia and possibly other leukemias. [Mol Cancer Ther 2009;8(3):615–25]

Introduction

Statins are agents with potent cholesterol-reducing properties, resulting from their abilities to block HMG-CoA reductase activity and inhibit mevalonate synthesis (1–3). As their administration results in rapid lowering of plasma LDL cholesterol levels, statins are used extensively in the treatment of hypercholesterolemia and coronary artery disease in humans (4–9). There is extensive evidence that the introduction of statins in the management and prevention of atherosclerosis had a dramatic effect in the current practice of clinical medicine and has changed the natural history of coronary artery disease in humans (4–9). Notably, extensive work over the years has established that these agents also exhibit important antiinflammatory, proapoptotic, and antineoplastic properties (10–14), raising the possibility that they may ultimately find a place in the prevention and/or treatment of patients with inflammatory or malignant disorders. However, thus far, most of the work to define the antitumor potential of statins has been focused on clinical epidemiologic studies (reviewed in ref. 14), whereas the therapeutic potential of these agents in combination with other chemotherapeutic and antineoplastic agents remains largely unexplored.

An area where the ability of statins to promote antitumor responses was recently directly examined is the treatment of acute myelogenous leukemia (AML) in adults (15). There has been extensive previous work documenting that statins exhibit antileukemic properties in vitro (16–19). Such findings are consistent with previously described observations, establishing that synthesis and import contribute to protective cholesterol increments in AML cells (20). They are also consistent with the notion that a subset of AMLs relies on increased LDL accumulation during treatment with particular drugs, indicating that combined use of statins with chemotherapy in certain cases of acute leukemia could be proved beneficial. Remarkably, it was also recently shown that Ikaros, a transcription factor that directs lymphoid lineage commitment and whose abnormal expression is implicated in the development of leukemias (21), modulates cholesterol uptake (22), further underscoring the relevance of cholesterol regulation in leukemogenesis.

Beyond the proapoptotic properties of statins, there is evidence that these drugs induce differentiation of cells of acute promyelocytic leukemia (APL) origin (23, 24). In addition, recent work from our laboratory has shown that atorvastatin and fluvastatin enhance all-trans retinoic acid (ATRA)–induced differentiation and reverse ATRA resistance of APL-derived cell lines or primary cells (24). These data have raised the possibility that statins can be used as differentiation enhancers, in combination with ATRA. However, the mechanisms by which they promote such differentiation has been largely unknown. In the present study, we provide direct evidence that engagement of the
c-Jun NH₂-terminal kinase (JNK) pathway is essential for statin-dependent leukemic differentiation of cells of APL origin, underscoring the importance of JNK in the induction of statin responses. We also did experiments aimed to compare the effects of statin treatment on the ATRA-dependent induction of expression of differentiation genes. In gene microarray studies, we found a dramatic atorvastatin-dependent enhancement of ATRA-inducible gene transcription in APL cells and were able to identify a group of JNK-linked genes selectively induced by the ATRA and atorvastatin combination. Altogether, these data provide insights on the molecular basis of the enhancing effects of statins on ATRA-induced antileukemic responses and support the hypothesis that combinations of statins with ATRA may lead to novel approaches for the treatment of APL and other leukemias.

Statins and Leukemic Cell Differentiation

Materials and Methods

Cells Lines and Reagents

The NB4 human APL cell line was grown in RPMI 1640 supplemented with 10% fetal bovine serum and antibiotics. The ATRA-resistant NB4.300/6 variant cell line (24, 25) was provided by Dr. Saverio Minucci (European Institute of Oncology) and was also grown in RPMI 1640 supplemented with 10% fetal bovine serum and antibiotics. Atorvastatin was purchased from 21CEC Pharmaceuticals Ltd. ATRA was purchased from Sigma. SP600125 was obtained from Calbiochem. The cell-permeable JNK peptide inhibitor 1 was purchased from Axxora.

In vitro Kinase Assays

NB4 cells were preincubated for 60 min in the absence or presence of the JNK inhibitor SP600125 (20 μmol/L) and then treated with or without fluvastatin (10 μmol/L) or DMSO as solvent control for the indicated times. The cells were subsequently lysed in phosphorylation lysis buffer (26, 27), and lysates were immunoprecipitated with an antibody against JNK1, purchased from Santa Cruz Biotechnology, Inc., using protein G–sepharose. The immunoprecipitated complexes were subsequently washed thrice with phosphorylation lysis buffer containing 0.1% Triton X-100 and twice with kinase buffer (25 mmol/L HEPES, 25 mmol/L MgCl₂, 25 mmol/L β-glycerophosphate, 2 mmol/L DTT, 0.1 mmol/L Na₃VO₄, and 20 μmol/L ATP) and resuspended in 30 μL of kinase buffer containing 3 μg of c-Jun fusion protein purchased from Cell Signaling Technology, Inc., used as an exogenous substrate. [γ-³²P]ATP (10 μCi) was added to the mixture, and after 30 min of incubation at room temperature, the reaction was terminated by the addition of SDS sample buffer. Proteins were analyzed by SDS-PAGE, and the phosphorylated form of c-Jun was detected by autoradiography.

Flow Cytometric Analysis

Granulocytic differentiation of APL cells was assessed by flow cytometric analysis, as previously described (28). Briefly, NB4 cells were pretreated for 1 h with the indicated inhibitors and then treated with atorvastatin or fluvastatin for the indicated times, and cell differentiation was determined by flow cytometric analysis after staining with the anti-CD11b monoclonal antibody. The anti-CD11b monoclonal antibody and a matched isotype control were purchased from BD Biosciences.

Microarray Analysis

Quality and quantity of total RNA from samples was determined using Agilent 2100 Bioanalyzer and a Nanodrop spectrophotometer. cRNA was synthesized using Illumina RNA amplification kit (Ambion) starting from 500 ng of total RNA and following the procedure suggested by the manufacturer. Sentrix Human-6 Expression BeadChip hybridization, washing, and staining were also done, as suggested by the manufacturer. Arrays were scanned on Illumina BeadStation 500.

Microarray Data Analysis

BeadChip array data quality control was done using Illumina BeadStudio software version 1.3.1.5. Probe average intensity signal was calculated with BeadStudio without background correction. Raw data were analyzed with Bioconductor (29). Average probe intensities were log₂ transformed and normalized by lowess method (30). To remove low quality signals, two filters were applied sequentially to the 47,293 reference sequences (RefSeq) available in the Sentrix Human-6 Expression BeadChip: gene-specific detection value should have been ≥0.6 in at least 50% of the samples (25,845 of 47,293 RefSeq passed this filter) and gene-specific interquantile range should have been ≥0.2 (11,287 of 25,845 RefSeq passed this filter).

Statistical analysis for the time-course experiments was done using maSigPro Bioconductor library (31). MaSigPro follows a two-step regression strategy to find genes with significant temporal expression changes and significant differences between experimental groups. The method defines a general regression model for the data wherein the experimental groups are identified by dummy variables (i.e., ATRA, atorvastatin, ATRA + atorvastatin). The procedure first adjusts this global model by the least-squared technique to identify differentially expressed genes and selects significant genes applying false discovery rate control procedures (≤0.05). Secondly, backward stepwise regression is applied as a variable selection strategy to study differences between experimental groups and to find statistically significant different profiles (P ≤ 0.05). The final list of significant differentially expressed genes was defined using the $R^2$ values of this second regression model. RefSeq probes characterized by an $R^2$ of ≥0.6 of the regression model were selected for further analysis. RefSeq probes identified by regression analysis were filtered to have at least one of the experimental point characterized by an $\log_2$(fold change) ≥ 1. Clustering analysis was done using TMEV v3.1 suite. Functional analysis was done using Ingenuity knowledge database.

---

4 http://www.tigr.org
5 http://www.ingenuity.com
Cells were treated with ATRA (0.5 μmol/L) or atorvastatin (2 μmol/L) or both agents for 8, 24, and 48 h, and RNA was isolated using the RNeasy kit from Qiagen. Total cellular RNA (1 μg) was reverse transcribed into cDNA using the Omniscript reverse transcription kit, and oligo(dT) primer was purchased from Invitrogen. Real-time reverse transcription–PCR (RT-PCR) for CCL3, CCL4, IL1B, BTG-2, and NCF2 genes was carried out by an ABI7900 sequence detection system from Applied Biosystems (32), using commercially available FAM-labeled probes and primers from Applied Biosystems. Relative quantification of mRNA levels was plotted as fold increase compared with untreated samples. Glyceraldehyde-3-phosphate dehydrogenase was used for normalization (33).

**Quantitative Reverse Transcription– PCR (Taqman)**

Cells were treated with ATRA (0.5 μmol/L) or atorvastatin (2 μmol/L) or both agents for 8, 24, and 48 h, and RNA was isolated using the RNeasy kit from Qiagen. Total cellular RNA (1 μg) was reverse transcribed into cDNA using the Omniscript reverse transcription kit, and oligo(dT) primer was purchased from Invitrogen. Real-time reverse transcription–PCR (RT-PCR) for CCL3, CCL4, IL1B, BTG-2, and NCF2 genes was carried out by an ABI7900 sequence detection system from Applied Biosystems (32), using commercially available FAM-labeled probes and primers from Applied Biosystems. Relative quantification of mRNA levels was plotted as fold increase compared with untreated samples. Glyceraldehyde-3-phosphate dehydrogenase was used for normalization (33). ΔC_t values (target gene C_t minus glyceraldehyde-3-phosphate dehydrogenase C_t) for each triplicate sample were averaged, and ΔΔC_t was calculated as previously described. mRNA amplification was determined by the formula 2^ΔΔC_t(32).

**IPA 4.0 Analysis**

IPA 4.0 searches for the presence of relations existing between the differentially expressed genes and the genes annotated in the Ingenuity knowledge database were analyzed using the data mining tool IPA 4.0. For each treatment, each time point was subjected to IPA 4.0 analysis, and subsequently, the results were subjected to a comparative analysis to identify functional classes associated with differentially expressed genes in response to different treatments. The data shown in Figs. 3B to D and 6 were generated with an updated IPA 7.0 version.
Results

Our previous studies (24) had shown that treatment of cells with statins results in activation of the JNK kinase pathway in leukemic cells and that such activation is required for statin-induced apoptosis. To determine whether engagement of the JNK kinase plays a role in the induction of differentiation of APL cells, experiments were done wherein JNK activity was blocked by either pharmacologic or molecular means, and induction of differentiation was subsequently determined. Initially, the effects of a JNK pharmacologic inhibitor, SP600125, were determined. When NB4 cells were pretreated with SP600125, leukemic cell differentiation, in response to either atorvastatin or fluvastatin, was blocked (Fig. 1A) whereas, as expected, SP600125 blocked statin induction of JNK kinase activity detected by immune complex kinase assays (Fig. 1B). To definitively establish whether JNK activity is essential for statin-dependent leukemic cell differentiation, the effects of targeting JNK, using a peptide inhibitor (GRKKRRQRRRP-PKRPRTTLNLFPQVPRSDQ-NH2) known to block JNK activation and downstream engagement of c-jun (34–36), were determined. As in the case of pharmacologic inhibition, pretreatment of cells with the peptide inhibitor abrogated the induction of differentiation of leukemic cells by atorvastatin (Fig. 1C), definitively establishing that such statin-regulated responses are JNK dependent.

To determine whether the effects of statin treatment on ATRA-induced leukemic cell differentiation reflect induction of specific genes, the patterns of gene expression induced by ATRA, atorvastatin, or the atorvastatin + ATRA combination were subsequently examined using DNA microarrays. We analyzed three prototypic situations (ATRA, atorvastatin, and ATRA + atorvastatin) using Illumina Sentrix Human-6 Expression BeadChips over a three-point time course (8, 24, and 48 hours), and time-course points were replicated in three independent experiments done at different days. A total of 36 arrays were hybridized. After average probe intensity calculation, log₂ transformation, and normalization (29), probes characterized by a |log₂(fold change)| variation of at least one of the points of the time-course treatments. Seven hundred five RefSeqs were found directly associated to treatment with ATRA alone, whereas 250 RefSeqs were found directly associated to treatment with atorvastatin alone. The combination of ATRA + atorvastatin could be associated with the highest number of RefSeq at 782. The overlaps existing between various time points for each treatment, as well as the overlaps between all treatments for RefSeq characterized by the presence of an absolute log₂(fold change) variation of at least 1 are summarized as Venn diagrams (Fig. 3A). As expected, the Venn diagrams clearly established that the transcriptional effects induced by atorvastatin alone are limited (Fig. 3A, top right). In addition, they showed a strong transcriptional synergic effect by the ATRA + atorvastatin combination (Fig. 3A, bottom left).

The three treatment groups were then analyzed using the data mining tool IPA 4.0.5 Using IPA 4.0, we searched for relationships between differentially expressed genes identified by us in these microarray studies and genes annotated in the Ingenuity knowledge base, the largest manually gene annotation database based on functional information available in published studies. A comparative analysis was done to identify the variation of expression of functional classes associated to differentially expressed genes with time. There were 15 top-ranked enriched functional classes (Supplementary Table S1)6, and seven of them were common to the three different treatments.

6 Supplementary material for this article is available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).
Among the genes belonging to the 15 enriched classes, 332 Illumina IDs were associated to 254 Entrez Gene IDs. Loading those genes in IPA 4.0, 13 networks (Supplementary Table S2) were obtained, and the three top-ranked networks were all made of differentially expressed genes. It should be emphasized that networks were defined as groups of two or more genes linked by a functional association based on peer-reviewed published data whereas hub genes were those sharing a high number of relations with other components of networks.

In a subsequent analysis, using an updated IPA 7.0 version, the effects of the different combination treatments at 48 hours on changes for the three top-ranked networks were assessed. The 48-hour time point was selected for these studies, as it represents the time wherein maximum transcriptional effects were seen. Clearly, marked synergistic effects were induced by the combination of ATRA with atorvastatin (Fig. 3B–D). In cluster 1 (Fig. 3B), treatment of cells with atorvastatin alone regulated expression of very few genes in the network. This is consistent with the fact that this network is centered on IL1B. Up-regulation of IL1B expression can be efficiently seen only within the combined ATRA + atorvastatin treatment (Fig. 3B). In cluster 2 (Fig. 3C), a different example of a centered gene (MYC) is shown, which is down-regulated by ATRA alone, but whose expression is not further modified by the addition of atorvastatin.

In cluster 3 (Fig. 3D), a different pattern is shown, involving the EGR1 hub gene. In that case, the expression of EGR1 is decreased by either ATRA or atorvastatin alone but is further down-regulated by the combination of ATRA + atorvastatin (Fig. 3D).

Altogether, our data established that atorvastatin treatment modulates ATRA-induced gene expression, raising the possibility that modulation of transcription may be a key mechanism by which statins enhance generation of antileukemic responses. This prompted us to classify the genes selectively induced by the ATRA + atorvastatin combination.
Figure 3. Induction of gene expression by ATRA and/or atorvastatin in NB4 cells. A, Venn diagrams of differentially expressed RefSeqs characterized by an absolute log2 (fold change) of at least 1. Overlaps of the genes expressed at different time points for ATRA alone (top left), atorvastatin alone (top right), and ATRA + atorvastatin (bottom left). Bottom right, differentially expressed RefSeqs characterized by an absolute log2 (fold change) of at least 1 in at least one of the different time points of treatment. B-D, relation found within differentially expressed genes in at least one of the treatments and present in the enriched functional classes. Cluster 1, centered on IL1B gene at time point 48 h (B); cluster 2, centered on MYC gene at time point 48 h (C); cluster 3, centered on EGR1 gene at time point 48 h (D).
based on their functional relevance. Among the genes induced by the ATRA + atorvastatin combination, there were several genes involved in cell differentiation (Supplementary Table S3) and apoptosis (Supplementary Table S4). In those tables, only genes whose expression was selectively modified/regulated by the combination of ATRA and atorvastatin at 48 hours are shown. Genes, regulated synergistically by statins and ATRA and involved in

Figure 4. Synergistic effect of ATRA and atorvastatin on gene expression. NB4 cells were treated for 8, 24, and 48 h with ATRA (0.5 μmol/L), atorvastatin (2 μmol/L), or the combination of ATRA and atorvastatin, as indicated. Left, patterns of gene expression for CCL3 (A), CCL4 (B), IL1B (C), BTG2 (D), and NCF2 (E) were investigated using Illumina Sentrix Human-6 Expression BeadChips. Three independent microarray experiments were done, and two-step regression strategy was applied as statistical analysis. Right, expression of mRNA for CCL3 (A), CCL4 (B), IL1B (C), BTG2 (D), and NCF2 (E) were evaluated by quantitative real-time RT-PCR (Taqman). Glyceraldehyde-3-phosphate dehydrogenase was used for normalization. Data are expressed as fold increase over untreated samples and represent means ± SE of three independent experiments.
apoptosis (Supplementary Fig. S1) or differentiation (Supplementary Fig. S2), were identified, suggesting the existence of cellular cascades and/or networks that regulate apoptosis or differentiation induced by the combination.

To further confirm the expression of key differentiation genes regulated by the ATRA + atorvastatin combination, the induction of expression of CCL3, CCL4, IL1B, BTG2, and NCF2 were assessed by real-time RT-PCR and compared with the patterns of expression seen in the microarray studies. There was strong induction of the expression of these genes after 48 hours of treatment with the ATRA + atorvastatin combination (Fig. 4A–E, left), whereas similar results were obtained when real-time RT-PCR studies were done (Fig. 4A–E, right).

It is well established that resistance to ATRA develops in several cases of APL despite initial sensitivity to the agent (36). In previous work, we had shown that statins reverse ATRA resistance and enhance leukemic differentiation of ATRA-resistant NB4 variants (24).

As our data established that groups of genes associated with differentiation and/or apoptosis are selectively induced by the ATRA + atorvastatin combination, we sought to determine whether the expression of key differentiation genes by the combination also occurs in NB4.300/6 cells. NB4.300/6 cells were treated for either 24 hours (Fig. 5, left) or 48 hours (Fig. 5, right) with either ATRA, atorvastatin, or the combination of ATRA and atorvastatin, and the induction of expression of the CCL3,

![Graphs showing expression of CCL3, IL1B, BTG2, and NCF2](image-url)

Figure 5. Expression of genes associated with differentiation in ATRA-resistant APL cells. NB4.300 cells were treated for 24 h (left) or 48 h (right) with ATRA (0.5 μmol/L), atorvastatin (2 μmol/L), or the combination of ATRA and atorvastatin, as indicated. Expression of mRNAs for CCL3 (A), IL1B (B), BTG2 (C), and NCF2 (D) was evaluated by quantitative real-time RT-PCR (Taqman) using glyceraldehyde-3-phosphate dehydrogenase for normalization. Data are expressed as fold increase over untreated samples. Columns, means of two independent experiments; bars, SE.
**Figure 6.** Key connection links of the JNK kinase pathway to statin-induced genes associated with differentiation. IPA 7.0 analysis was done, as described in Materials and Methods. Functional relations were derived by connecting JNK to various genes selectively regulated after 48 h of treatment with the atorvastatin and ATRA combination.

**Discussion**

APL is a subtype of AML characterized by the presence of the t (15;17) chromosomal translocation, resulting in the abnormal fusion of the retinoic acid receptor α gene with the PML gene and the abnormal PML–retinoic acid receptor α fusion protein (38–40). It is well established that ATRA is a potent inducer of differentiation of APL cells in vitro and in vivo (reviewed in refs. 41–43), and after its introduction in the management of the disease in the 1980s, it has become a key component of standard combination regimens for the treatment of this leukemia. However, it is now also well known that leukemic cell resistance to ATRA develops with time, and this has led to the introduction and development of another agent with potent anti-APL properties, arsenic trioxide, for the management of ATRA-resistant APL (reviewed in ref. 44). The development of retinoic acid resistance in a subset of patients with APL underscores the need for the development of novel therapeutic approaches to overcome such resistance. Moreover, beyond their potential usefulness in APL, development of similar novel approaches may prove to be of value in other AML subtypes that are normally resistant to the effects of ATRA.

We have recently shown that atorvastatin and fluvastatin enhance the induction of differentiation and apoptosis of APL cells by ATRA (24). In addition, we have established that these statins reverse the development of ATRA resistance in both NB4 variant APL cell lines and in primary resistant APL cells (24). Such results have raised the possibility that combined use of ATRA with late generation statins could provide a novel approach to overcome leukemic cell resistance. Importantly, our previous studies have also shown that statins enhance the...
suppressive effects of ATRA on bone marrow–derived or peripheral blood–derived primary leukemic progenitors from patients with non-APL forms of AML, raising the possibility that statins may sensitize other resistant AML subtypes to the antileukemic effects of ATRA in vivo (24). Although the clinical testing of such a hypothesis is pending, understanding the mechanisms by which statins promote induction of ATRA-dependent antileukemic effects is highly relevant and potentially important. Identifying such mechanisms could result in the identification of novel cellular elements that could be targeted in a more selective and specific manner for the treatment of acute leukemias.

The potent effects of new generation statins as inducers of APL cell differentiation and their ability to reverse ATRA resistance prompted us to perform studies to define the cellular mechanisms involved in the generation of such responses. In initial studies, we examined the effects of pharmacologic or molecular inhibition of the JNK kinase pathway in the induction of differentiation of the NB4 APL cell line that express the t(15;17) translocation. These studies showed that JNK activity is essential for the atorvastatin-dependent or fluvastatin-dependent leukemic cell differentiation, as evidenced by the reversal of such differentiation by either the pharmacologic JNK inhibitor SP600125 or by a peptide JNK inhibitor. Thus, activation of JNK by statins is essential for the generation of both proapoptotic responses (24) and leukemic cell differentiation in APL. It should be noted that JNK is the only major pathway activated by statins in APL cells, and as we have shown in previous work, the p38–mitogen-activated protein kinase pathway is not modulated by statins whereas the extracellular signal-regulated kinase–mitogen-activated protein kinase is inhibited (24).

The selective engagement of JNK by statins and its important role in leukemic cell differentiation prompted us to perform further work aimed to identify statin-dependent induction of genes that participate in leukemic cell differentiation or apoptosis and may be linked to JNK-regulated pathways. For that purpose, we did extensive microarray studies using Illumina BeadChips to define the gene expression profiles seen in NB4 cells in response to statin treatment alone or the combination of statin treatment and ATRA. A number of genes that seem to be regulated by ATRA and atorvastatin and have relevance to apoptosis and differentiation were identified by using such approach. Although atorvastatin alone induced expression of a minimal number of genes, it exhibited a remarkable effect in promoting ATRA-inducible gene expression. A large number of genes associated with apoptosis or differentiation were found to be selectively induced by the combination of atorvastatin and ATRA, a finding consistent with the potent-enhancing effects of statins on ATRA-induced differentiation and proapoptotic responses in leukemic cells (24). Among the genes selectively expressed by the combination were a number of chemokines, including macrophage inflammatory protein-1α (CCL3) and macrophage inflammatory protein-1β (CCL4), which are well known to play important regulatory effects on hematopoietic progenitor growth and differentiation (45, 46). Similarly, IL1B, a key regulator of hematopoiesis (47), was a prominently induced gene by the ATRA + atorvastatin combination. Our microarray data analysis also showed that the expression of a number of other genes was drastically augmented in response to the treatment with ATRA + atorvastatin. Among those genes are BTG2 and NCF2. BTG2 is involved in growth inhibition and cell cycle regulation (48), and its overexpression increases retinoic acid receptor α transcriptional activity and cell differentiation in response to ATRA in myeloid leukemia cells and CD34+ hematopoietic progenitors (49). NCF2 expression has also been linked to myelopoiesis and differentiation (50), underscoring the functional relevance of gene induction by the ATRA and statin combination.

Importantly, in experiments wherein the expression of gene targets was examined using real-time RT-PCR, we were able to directly show up-regulation of CCL3, IL1B, BTG2, and NCF2 in the variant cell line NB4.300/6, which is completely refractory to the effects of ATRA (25), but undergoes differentiation and apoptosis in response to treatment by combinations of ATRA and statins (24). Thus, statins enhance ATRA-dependent expression of key genes involved in the differentiation and apoptosis in both ATRA-sensitive and ATRA-resistant APL cells. Although the precise contributions of distinct elements in the generation of the antileukemic effects of ATRA remain to be defined in future studies, our data establish a unique mechanism by which statins reverse ATRA antileukemic responses via regulation of the JNK pathway. Further work in that direction is warranted and may lead to the definition of new target proteins for the development of novel approaches for the treatment of ATRA-resistant APL and other AML subtypes.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Acknowledgments
We thank Dr. Saverio Minucci (European Institute of Oncology) for the NB4.300/6 cell line and Dr. Daniela Cantarella (Oncogenomics Center, Institute for Cancer Research and Treatment) for microarray data acquisition.

References


Molecular Cancer Therapeutics

Regulation of leukemic cell differentiation and retinoid-induced gene expression by statins

Antonella Sassano, Marco Lo Iacono, Giovanni Antico, et al.

Mol Cancer Ther 2009;8:615-625. Published OnlineFirst February 24, 2009.

Updated version
Access the most recent version of this article at:
doi:10.1158/1535-7163.MCT-08-1196

Supplementary Material
Access the most recent supplemental material at:
http://mct.aacrjournals.org/content/suppl/2009/02/19/1535-7163.MCT-08-1196.DC1

Cited articles
This article cites 49 articles, 28 of which you can access for free at:
http://mct.aacrjournals.org/content/8/3/615.full.html#ref-list-1

Citing articles
This article has been cited by 1 HighWire-hosted articles. Access the articles at:
/content/8/3/615.full.html#related-urls

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.