Induction of class II major histocompatibility complex expression in human multiple myeloma cells by retinoid

Takaomi Sanda, Shinsuke Iida, Satoshi Kayukawa, Ryuzo Ueda

Class II major histocompatibility complex (MHC II) is normally silenced in plasma/multiple myeloma (MM) cells at the transcriptional level through downregulation of class II transactivator (CIITA), allowing MM cells to escape from immunological responses. Here we demonstrate that a retinoic acid receptor-α/β-selective retinoid Am80 (tamibarotene) could induce the expression of functional MHC II molecules in human MM cell lines. Am80 upregulated expression of the interferon regulatory factor-1 gene, followed by enhancement of CIITA expression. This is the first report demonstrating that retinoid can induce the expression of MHC II in terminally-differentiated plasma/MM cells.

Key words: multiple myeloma, retinoid, MHC II, CIITA

Haematologica 2007; 92:115-120
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Manuscript received May 18, 2006. Accepted August 3, 2006.

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Design and Methods

MHC II-negative human MM cell lines, U266, ILKM-2, ILKM-3, ILKM-8, NOP1, XG7, FR4, SK-MM1, SACHI, NCUMM-2 and NCUMM-3, a B-cell lymphoma cell line, Daudi, and a CD4-positive T-cell leukemia cell line, Jurkat, were used in this study.10,13,14 Retinoids were prepared as described previously.15 A cDNA microarray analysis was performed using IntelliGene HS Human expression chips containing 16,600 probes (Takara).15 Briefly, U266 cells were or were not treated 1 μM Am80 for 24 h, conditions in which Am80 shows approximately 5-10% growth inhibition on U266 cells as previously reported by us.10 After incubation, total RNA was purified and subjected to a microarray analysis. The results are presented as the ratio of gene expression in the Am80-treated sample to that in the untreated control (T/C ratio). The genes with a T/C ratio of ≥2.00 or ≤0.50 were selected. To validate the results of gene expression analysis, we performed quantitative reverse transcriptase polymerase chain reaction (RT-PCR) using Taqman Gene Expression Assays and the AB7300.

Table 1. Upregulated/downregulated genes after treating U266 cells with Am80.

<table>
<thead>
<tr>
<th>Accession No.</th>
<th>Gene Symbol</th>
<th>Gene name</th>
<th>Treated/Control Ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>NM_078467.1</td>
<td>CDKN1A</td>
<td>cyclin-dependent kinase inhibitor 1A (p21, Cip1)</td>
<td>2.86</td>
</tr>
<tr>
<td>NM_005428.2</td>
<td>VAV1</td>
<td>vav 1 oncogene</td>
<td>2.12</td>
</tr>
<tr>
<td>NM_014330.2</td>
<td>PPR1R15A</td>
<td>protein phosphatase 1, regulatory (inhibitor) subunit 15A</td>
<td>2.12</td>
</tr>
<tr>
<td>NM_002777.2</td>
<td>PRRT3</td>
<td>protease 3</td>
<td>2.02</td>
</tr>
<tr>
<td>NM_000308.1</td>
<td>PPG8</td>
<td>protective protein for β-galactosidase</td>
<td>2.01</td>
</tr>
<tr>
<td>NM_002961.2</td>
<td>S100A4</td>
<td>S100 calcium binding protein A4</td>
<td>0.47</td>
</tr>
<tr>
<td>NM_006113.3</td>
<td>VAV3</td>
<td>vav 3 oncogene</td>
<td>0.47</td>
</tr>
</tbody>
</table>

**The results are indicated as the ratio of expression by the Am80-treated sample to expression by the untreated control.**
To induce MHC II expression by retinoid, the cells were incubated with staining buffer containing the fluorescein isothiocyanate-conjugated mouse monoclonal antibody which can cross-react with HLA-DP, -DQ and -DR, or isotype-matched control antibody (BD Biosciences), followed by flow cytometry as described elsewhere. For interaction analysis between T-cell receptor (TCR) and MHC II, the reporter plasmid, 4κB-Luc, containing four nuclear factor-κB (NF-κB) binding sites, and the internal control plasmid, pRL-TK, expressing Renilla luciferase, were transfected into Jurkat cells using Nucleofector (Amaxa Biosystems). Twenty-four hours after transfection, Jurkat cells were co-cultured with U266 or Daudi cells, which had or had not been pre-treated with 1 μM Am80 for 5 days. The cells were left unstimulated or stimulated with 300 ng/mL of staphylococcal toxin SEE (Toxin Technology). After 24 h incubation, luciferase activity was measured by a luminometer as described previously. In this system, when TCR signaling occurs, luciferase activity in Jurkat cells is induced through NF-κB activation.

Results and Discussion

In order to examine the effects of Am80 on MM cells, we first performed gene expression profile analysis using a cDNA microarray containing 16,600 human genes. Table 1 shows the representative results of gene expression profiles after treatment of U266 cells with Am80. Am80 upregulated p21 expression and downregulated IL-6 expression, supporting the previous results. In addition,
Figure 2. Induction of expression of IRF-1 and CIITA by Am80. A. The effect of Am80 on expression of the IRF-1, CIITA, PRDI-DF1 and STAT1 genes. U266, XG7 and Daudi cells were treated with Am80 (0-10 µM) for 24 h. Total RNA was isolated and subjected to quantitative RT-PCR using primers for IRF-1, STAT1, PRDI-DF1 or CIITA. Expression levels were normalized to the expression level of β-actin and indicated as fold-change compared to the expression of the untreated control. Experiments were done in triplicate; column, mean; bars, standard deviation. B. Temporal profile of gene expression after treatment with Am80. U266 cells were treated with 1 µM of Am80 for the indicated times (0-24 h).
Am80 upregulated gene expressions of MHC II molecules including HLA-DRA, HLA-DMB, CD74, HLA-DQB1, HLA-MA, HLA-DPA1 and HLA-DRB5, which prompted us to examine the effect of Am80 on the cell surface expression of MHC II. We used 11 MHC II-negative MM cell lines and a control MHC II-positive B-cell line, Daudi. As shown in Figure 1A, U266 cells did not express MHC II molecules on their surface. Am80 treatment induced the expression of these molecules in a time-dependent manner. On the other hand, in Daudi cells, constitutive expression of MHC II was detected but not modified by Am80 treatment. Among the MM cell lines examined, distinct induction of MHC II was observed in U266 and ILKM-2 cells (Figure 1B), both of which are IL-6-dependent cell lines. We then compared the effect of Am80 with that of other retinoids including ATRA and 9-cis retinoic acid (9-cis RA), which can bind to RAR-γ and RXR in addition to RAR-α/β. As shown in Figure 1C, ATRA and 9-cis RA also induced MHC II expression. There was no significant difference among these retinoids, suggesting that RARα/β is important for the induction of MHC II.

Next, we examined whether the MHC II molecules induced by Am80 can functionally interact with TCR. We transfected the luciferase reporter plasmid into a CD4-positive T-cell line, Jurkat, and co-cultured them with U266 or Daudi cells, which were or were not pre-treated with Am80. In order to bridge MHC II and TCR, the staphylococcal toxin SEE, a superantigen, was added. As shown in Figure 1D, in the presence of Daudi cells, luciferase activity was increased and driven by addition of SEE, whereas U266 cells could not stimulate luciferase activity without pre-treatment with Am80. Importantly, luciferase activity of U266 cells pre-treated with Am80 was increased by the addition of SEE. These results demonstrate that Am80 can induce the expression of functional MHC II molecules in U266 cells.

We then examined the effect of Am80 on CIITA gene expression to understand the mode of action of Am80 on MHC II induction. We used U266, Daudi and a MM cell line XG7, in which MHC II expression could not be induced by Am80 (Figure 1B). As shown in Figure 2A, PRDI-DF1 was highly expressed in U266 and XG7 cells, and CIITA expression was suppressed. Although Am80 treatment did not modify the expression of PRDI-DF1, Am80 induced a 4-fold increase in CIITA expression in U266 cells, but not in XG7 cells. In contrast, PRDI-DF1 was completely silenced in Daudi cells, and CIITA was highly expressed. Am80 treatment did not upregulate CIITA expression in Daudi cells. ATRA and 9-cis RA could induce expression of IRF-1 and CIITA in U266 cells (data not shown).

We then examined the effect of Am80 on expression of the interferon-inducible genes such as IRF-1 and STAT1. In U266, but not XG7 or Daudi cells, Am80 induced a 1.6-fold increase in IRF-1 expression, whereas Am80 had no effect on STAT1 expression in either cell line (Figure 2A). The temporal profile of gene expression by U266 cells showed upregulation of IRF-1 at 1 h after the treatment with Am80 and upregulation of CIITA from 4 h after treatment (Figure 2B). IRF-1 has been reported to be induced by retinoid at the transcriptional level through the binding of RAR to the IRF-1 promoter.2,18,19 Although it remains unclear why Am80 could not induce the expression of IRF-1 in XG7 cells, these findings suggest that Am80 directly enhances the expression of IRF-1, which is followed by secondary induction of CIITA expression without changing the PRDI-DF1.

In conclusion, Am80 has an immunomodulatory effect in addition to anti-proliferative and anti-angiogenic activities. Although Am80 did not induce MHC II expression in all MM cell lines, it might enhance the immunogenicity of MM cells in vivo through the induction of MHC II expression. Since Am80 appears to be a safe and practical agent, which has many therapeutic advantages over other retinoids, it could be used as a chemopreventive agent from the earliest stage of MM.

Author Contributions
SI: directed this study and performed several experiments; SK: performed microarray analysis; RU: directed this study.

Conflict of Interest
The authors reported no potential conflicts of interest.

References
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