SUPPLEMENTARY INFORMATION

Title: p38α MAPK interacts with and inhibits RARα: suppression of the kinase enhances the therapeutic activity of retinoids in acute myeloid leukemia cells

Maurizio Gianni¹, PhD, Marco Peviani², PhD, Nathalie Bruck³, PhD, Alessandro Rambaldi⁴, MD, Gianmaria Borleri⁴, BS, Mineko Terao¹, PhD, Mami Kurosaki¹, PhD, Gabriela Paroni¹, PhD, Cecile Rochette-Egly³ PhD, and Enrico Garattini¹, MD.

¹ Laboratory of Molecular Biology, Istituto di Ricerche Farmacologiche “Mario Negri”, via La Masa, 19. 20156 Milano, Italy. ² Laboratory of Neurobiology, Istituto di Ricerche Farmacologiche “Mario Negri”, via La Masa, 19. 20156 Milano, Italy. ³ IGBMC (Institut de Génétique et de Biologie Moléculaire et Cellulaire), INSERM, U964; CNRS, UMR7104; Université de Strasbourg, 1 rue Laurent Fries, BP 10142, 67404 Illkirch Cedex, France. ⁴ Division di Ematologia, Ospedali Riuniti di Bergamo, Largo Barozzi 1.
**Suppl. Fig. 1** PD169316 enhances ATRA-dependent myeloid differentiation of HL-60 cells and reduces degradation of RARα

*HL-60* cells were treated with ATRA (1 µM) and PD169316 (10 µM) alone or in combination for 1 or 3 days. (A) NBT-reducing activity was measured in cell extracts (3 days). Each value is the mean±SD of 3 replicate cultures. (B) Expression of CD11b, CD11c and CD38 was determined...
by FACS (Fluorescence Activated Cell Sorter) analysis (3 days). (C) Expression of RARα, STAT1α, cEBPβ and β-actin was evaluated by Western blot analysis (1 day).
Suppl. Fig. 2 *Inhibition of p38α increases the anti-proliferative and differentiating activity of ATRA in freshly isolated blasts from AML patients*

(A) Primary cultures of leukemic blasts from an AML patient (Pt 2) were treated with ATRA (0.01 µM) and/or PD169316 (10.0 µM) for 3 days. The expression of the indicated myeloid surface markers was determined by FACS (Fluorescence Activated Cell Sorter) analysis. CD11b and CD11c results are expressed in % positive cells, while CD38 data are expressed in mean associated fluorescence (MAF). (B) Primary cultures of leukemic blasts from another AML patient (Pt 3) were treated as in (A). The expression of CD11b, CD11c and CD38 was determined by FACS analysis. (C) Primary cultures of leukemic blasts from a last AML patient (Pt 4) were treated as in (A) for 1 day. The expression of CD11c and CD38 was determined by FACS analysis using also the mean associated fluorescence values (MAF). The expression of STAT1α, cEBPβ, p38α and β-actin was determined by Western Blot analysis.
Suppl. Fig. 3  p38α inhibits the ligand-dependent transcriptional activity of over-expressed RARα and PML-RARα and induces degradation of the two retinoid receptors in COS-7 cells. Subcellular localization of RARα, PML-RARα and p38α in NB4 cells

(A) COS-7 cells were transfected with increasing concentrations of p38α, RARα (0.1 μg, left) and PML-RARα (0.1 μg, right) expression plasmids or the corresponding void vector in the presence of
the retinoid-responsive reporter construct, \textit{DR5-tk-Luc}, based on the firefly-luciferase gene. Renilla luciferase was always co-transfected as the normalizing reporter. Twenty four hours after transfection, cells were treated for a further 16 hours with ATRA (1.0 µM). Firefly and renilla luciferase activities were measured in cell extracts and the results are expressed as fold induction relative to the corresponding vehicle treated control after normalization for transfection efficiency, using renilla luciferase activity. Each value is the Mean±SD of three replicate cultures. The results are representative of two independent experiments. (B) COS-7 cells were transfected as in (A) with RARα (left) or PML-RARα (right). Twenty four hours after transfection, cells were treated for a further 16 hours with ATRA (1.0 µM), PD169316 (10.0 µM) or the combination of the two compounds. Firefly and renilla luciferase activity were measured and the results expressed as in (A). The results are representative of 3 independent experiments. (C) COS-7 cells were transfected and treated as in (A) with RARα or PML-RARα and the retinoid-responsive reporter construct, \textit{DR5-tk-Luc}, in the absence and presence of a plasmid encoding a dominant-negative form of p38α (\textit{dnP38}). Firefly and renilla luciferase activity were measured and the results expressed as in (A). Each value is the mean±SD of 3 replicate cultures. (D) NB4 cells were treated with ATRA (1.0 µM) for 24 hours. The cytoplasmic and nuclear fractions were isolated, as described \cite{1}. The levels of RARα, p38α and tubulin were determined by Western blot analysis.
Suppl. Fig. 4  

p38α interacts with RARα and PML-RARα in a direct fashion

COS-7 cells were transfected with a RARα expression plasmid in the absence and presence of a p38α expression plasmid. (A) Pull-down experiments. Extracts of COS-7 cells transfected with RARα or PML-RARα were pulled-down after incubation with a GST-tagged version of p38α or GST alone and glutathione-coupled Sepharose beads. Precipitates were subjected to Western blot analysis with anti-RARα or anti-GST antibodies. The levels of RARα and PML-RARα present in cell extracts before the pull-down procedure were determined in parallel by Western blot analysis (input). (B) Far-Western experiments. COS-7 cells were transfected with plasmid constructs encoding RARα and p38α subsequently treated with vehicle or ATRA (1.0 µM) for 16 hours. Cell extracts were immuno-precipitated with anti-RARα antibodies. Immuno-precipitates were
electrophoresed and blotted on nitrocellulose filters. The filters were challenged with recombinant p38α and developed with an anti-p38α antibody. The position of the bands visualized by the anti-p38α antibody corresponds to the RARα bands, as indicated by subsequent washing and challenge of the filter with an anti-RARα antibody.
**Suppl. Fig. 5** Effect of p38α mutants on ligand-dependent activation of RARα and PML-RARα

COS-7 cells were transfected with plasmid constructs encoding the indicated wild-type (WT) and mutants of p38α along with RARα (left) and PML-RARα (right) expression plasmids in the presence of the retinoid-responsive reporter construct, DR5-tk-Luc, based on the firefly-luciferase gene as well as the renilla luciferase normalizing reporter. Twenty four hours after transfection, cells were treated for a further 16 hours with ATRA (1.0 μM). Firefly and renilla luciferase activities were measured in cell extracts and the results are expressed as fold induction relative to the corresponding vehicle treated control after normalization for transfection efficiency, using renilla luciferase activity. Each value is the mean±SD of 3 replicate cultures.
Suppl. Fig. 6 Effects of p38α on the transcriptional activity and degradation of RARα deletion and site-directed mutants of RARα and PML-RARα

COS-7 cells were co-transfected with p38α and the indicated deletion mutants of RARα in the presence of the DR5-tk-luc reporter. Renilla luciferase was always co-transfected as the normalizing reporter. Twenty four hours after transfection, cells were treated with vehicle (DMSO) or ATRA (0.1 µM) for a further 16 hours. (A) Firefly and renilla luciferase activities.
were measured in cell extracts and the results are expressed as fold induction relative to the corresponding vehicle treated control after normalization for transfection efficiency, using renilla luciferase activity. Each value is the Mean±SD of three replicate cultures. The results are representative of two independent experiments. (B) The levels of wild-type (WT) RARα and indicated mutants, as well as p38α and β-actin were determined by Western blot analysis after 1 day of treatment with ATRA. (C) COS-7 cells were co-transfected with p38α and S369A mutant of RARα as in (A), treated with ATRA for the indicated amount of time and processed also as in (A). Each value is the Mean±SD of three replicate cultures. WT = wild-type hRARα; ΔA = hRARα deleted for the A region; ΔB = hRARα deleted for the B region; ΔAB = hRARα deleted for the A and B regions; ΔF = hRARα deleted for the F region. The characteristics of all these deletion mutant constructs used are available in C. Rochette-Egly et al., 2000 ². S369A = site directed mutant of hPML-RARα with substitution of Ser-369 into Ala ³.
**Suppl. Table 1 Genotypic characteristics of the AML patients**

<table>
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<th>Patient</th>
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<tr>
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<td>46,XY, t(3:5)(q31;q21)</td>
<td>ITD</td>
<td>MLF-1/NPM-1</td>
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</tbody>
</table>

The genotypic characteristics of the obtained from the AML blasts used for the primary culture experiments are illustrated. FAB = French American British classification; ITD = internal tandem repeat; mut = mutated form of NPM-1 (4-nucleotide duplication in exon 12); MLF1/NPM1 = t(3:5) translocation involving the MLF1 and the NPM1 genes.
SUPPLEMENTARY REFERENCES

