

CORRESPONDENCE

Relationship Between Degradation of PML-RAR α and Differentiation

To The Editor:

In a recent issue of *Blood*, Fanelli et al¹ reported that constitutive degradation of PML-RAR α through the proteasome pathway mediates retinoic acid (RA)-resistance in a derivative cell line of NB4 (NB4.007/6). The context of this study contradicts our hypothesis that the degradation of PML-RAR α is important as a trigger of RA-dependent differentiation,^{2,3} although the conclusion is reasonable that PML-RAR α expression is crucial to RA-sensitivity. To resolve the complexity of the relationship between the degradation of PML-RAR α and differentiation, we hope to elucidate the following.

Firstly, it must be determined whether the sequences of *PML-RAR α* and *RAR α* genes are normal or mutated in NB4.007/6. In some RA-resistant APL cell lines, mutations of the *PML-RAR α* gene have been reported in the AF-2 domain, which is important to ligand-binding.³⁻⁵ Because a missense mutation and a premature termination potentially cause instability of the protein, the constitutive degradation of PML-RAR α might be due to the mutation.

Secondly, the investigators did not present the immunostaining data of endogenous PML or PML-RAR α in NB4.007/6. Because PML-RAR α was quickly degraded, the immunostaining pattern might resemble the wild pattern. However, the investigators stained exogenous PML-RAR α carrying green fluorescent protein (GFP), which showed a microgranular pattern. This data showed the short life of PML-RAR α but not the subcellular localization.

Finally, our recent data suggested that constitutive degradation of PML-RAR α is not necessarily associated with RA-resistance. We established a new subline of NB4 (NB4/As) that showed resistance to As₂O₃: continuous growth without apoptosis and differentiation in the medium containing 1 μ mol/L As₂O₃. In immunoblot analysis, a PML-RAR α band of this cell line disappeared in the presence with

As₂O₃ and reappeared after the removal of As₂O₃ (data not shown). As₂O₃ is known to modify PML as well as to accelerate the degradation of PML-RAR α .⁶⁻⁸ Although the molecular mechanism of the As-resistance is under investigation, the addition of RA differentiated NB4/As and increased the nitroblue tetrazolium (NBT) reduction activity even in the coculture with As₂O₃ (Fig 1).

We considered that the pathophysiology of NB4.007/6 might have changed from the parental cell line after culture with RA. Additional molecular changes increase the complexity of the RA-resistance in NB4.007/6. Investigation of the RA-resistance cell lines has shown that PML-RAR α is a direct target molecule for RA. However, the relationship between the degradation of PML-RAR α and differentiation remains to be resolved.

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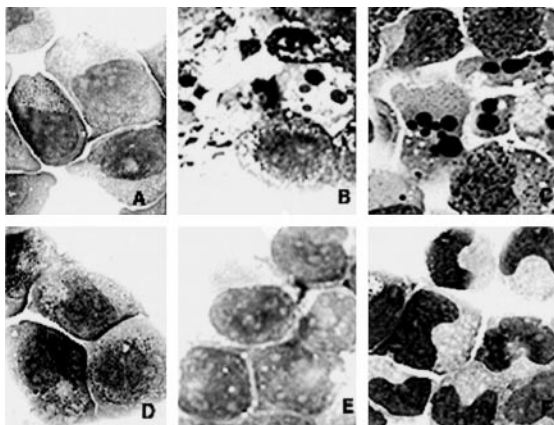


Fig 1. Morphology of NB4 (A, B, and C) and NB4/As (D, E, and F) cell lines on RA or the combination of RA and As₂O₃ treatment. (A and D) Before treatment. (B and E) After 1 day of treatment with 1 μ mol/L As₂O₃. (C) After 1 day of treatment with 1 μ mol/L all-*trans* RA and 1 μ mol/L As₂O₃. (F) After 4 days of treatment with 1 μ mol/L RA and 1 μ mol/L As₂O₃.

Response

One of the conclusions of our report¹ is that PML/RAR α itself mediates RA-dependent differentiation and that its degradation by RA is not crucial for the differentiation process to occur. Naoe and Kitamura disagree.

In general, we think that there is much direct and indirect evidence to support these conclusions: (1) PML/RAR α is an RA-dependent transcriptional activator^{2,3}; (2) PML/RAR α mediates RA-induced differentiation in both APL and non-APL cells; in particular, PML/RAR α restores RA-sensitivity in RA-resistant cell lines carrying mutations of RAR α ^{4,5}; (3) commitment to differentiation in the presence of RA occurs within the first 12 hours, when PML/RAR α is still detectable⁶; (4) inhibition of PML/RAR α degradation by caspase inhibitors (another mediator of RA-induced degradation of the fusion protein) increases RA-induced differentiation in APL cells⁷; and (5) PML/RAR α re-expression restores RA-sensitivity in RA-resistant APL cells with constitutive degradation of the fusion protein.¹

With respect to some specific criticisms of Naoe and Kitamura: (1) we did not sequence RAR α and PML/RAR α transcripts in NB4.007/6 cells. Therefore, as suggested, we cannot exclude that mutations of the PML/RAR α coding sequence might affect fusion protein stability. However, we think that other mechanisms are more likely, because exogenous PML/RAR α (GFP-PML/RAR α) or PML/RAR α from parental NB4 cells were equally degraded in NB4.007/6 cells in infection and *in vitro* degradation assay, respectively. (2) Immunostaining of NB4.007/6 cells showed a micropunctuated pattern, typical of PML/RAR α expression. Because we did not detect PML/RAR α by Western blotting, this might reflect a better efficiency of our PG-M3 anti-PML monoclonal antibody on undenatured, versus denatured, antigens.

Finally, Naoe and Kitamura mentioned the isolation of an arsenic-resistant/RA-sensitive NB4 subline. Because the signaling pathway triggered by arsenic is yet undefined, we do not think that this model system can provide information as to the role of PML/RAR α in RA-signaling.

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Successful Healing of Hydroxyurea-Related Leg Ulcers With Topical Granulocyte-Macrophage Colony-Stimulating Factor

To the Editor:

Hydroxyurea (HU) is a cytoreductive agent commonly used in the treatment of chronic myeloproliferative disorders. HU is usually well tolerated; however, long-term HU therapy has been associated with cutaneous side effects, such as alopecia, diffuse hyperpigmentation, erythema, skin atrophy, and nail changes.¹ Painful skin ulcers have been also reported and their treatment modalities mainly consisted of HU discontinuation,²⁻⁴ which was generally followed by the complete or almost complete healing.⁵ We report here a successful treatment of HU-related leg ulcers with topical granulocyte-macrophage colony-stimulating factor (GM-CSF) in 4 patients affected by chronic myeloid leukemia (CML), whose clinical features are shown in Table 1. Diagnosis of CML was confirmed by both cytogenetic (Ph⁺ chromosome) and molecular (bcr-abl) findings. All of the patients, after a preliminary cytoreduction of peripheral leukemic burden with HU, were assigned to receive conventional therapy with α -interferon (α -IFN), and 3 of them were also enrolled in a clinical trial of the Italian Cooperative

Table 1. Patients' Clinical and Laboratory Characteristics at Skin Ulcers' Onset

Patient No.	Age/Sex	Time From Diagnosis (mo)	HU		WBC (10 ⁹ /L)	PLT (10 ⁹ /L)	LDH (U/L)	Spleno-megaly	Hepato-megaly
			Duration (mo)	Therapy					
1	68/M	54	27	73.5	728	1,155	No	Yes	
2	62/M	75	36	29.8	264	184	No	No	
3	65/F	54	37	26.5	1,086	968	Yes	Yes	
4	44/F	50	19	40.0	312	1,286	Yes	Yes	

Abbreviations: WBC, white blood cell count; PLT, platelet count; LDH, lactate dehydrogenase.

Study Group on CML.⁶ However, because of adverse side effects caused by α -IFN,⁷ we first had to reduce and then to stop it in all patients. They were then reverted to chemotherapy with HU, which was administered at a mean daily dosage of 2 g to maintain complete hematological response. After a mean time of 29.7 months (range, 19 to