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Pier Paolo Pandolfi

Histone deacetylases and transcriptional therapy with their inhibitors

Abstract Acute promyelocytic leukemia (APL) is characterized by the expansion of malignant myeloid cells blocked at the promyelocytic stage of hemopoietic development and is invariably associated with reciprocal chromosomal translocations involving the retinoic acid receptor α (RARα) gene on chromosome 17. RARα variably fuses to PML, PLZF, NPM, NuMA, and Stat5B genes (X genes/proteins). These translocations are balanced and reciprocal, thus leading to the generation of X-RARα and RARα-X fusion genes of which the products coexist in the APL blast. The invariable involvement in these translocations of RARα, a prototypical transcription factor, makes APL a compelling example of aberrant transcriptional mechanisms in the etiopathogenesis of cancer. This paper focuses on the recent progress in defining the molecular mechanisms underlying APL pathogenesis and addresses how this new understanding has allowed the proposal and development of novel therapeutic strategies with compounds such as histone deacetylase inhibitors and inorganic arsenicals such as As_2O_3 which are currently being tested in murine leukemia models as well as in human APL patients. In particular, the crucial role played by the aberrant transcriptional activities of X-RARα and RARα-X fusion proteins in APL pathogenesis is discussed by reviewing the relevant therapeutic implications resulting from this analysis.

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P.P. Pandolfi
Department of Human Genetics and Molecular Biology Program,
Memorial Sloan-Kettering Cancer Center,
Sloan-Kettering Division, Graduate School of Medical Sciences,
Cornell University, 1275 York Avenue,
New York, NY 10021, USA
E-mail:p-pandolfi@ski.mskcc.org
Tel.: + 1-212-7176168
Fax: + 1-212-7173102

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Introduction

Acute promyelocytic leukemia (APL) is characterized by the expansion of malignant myeloid cells blocked at the promyelocytic stage of hemopoietic development and is invariably associated with reciprocal chromosomal translocations involving the retinoic acid receptor α (RARα) gene on chromosome 17 [3]. RARα variably fuses to the PML, PLZF, NPM, NuMA, and Stat5B genes (hereafter referred to as X genes/proteins). These translocations are balanced and reciprocal, thus leading to the generation of X-RARα and RARα-X fusion genes of which the products coexist in the APL blast. The X proteins have no structural similarities. At first glance it appears that their contribution to the two chimeric products is dramatically different at the structural level and that the only common feature among the fusion proteins is the presence of a truncated RARα moiety.

Despite this diversity, the various X-RARα fusion molecules share the capacity to heterodimerize with X genes/proteins, since the regions that can normally mediate the homodimerization of X proteins are retained in the portion of X that fuses to RARα. Similarly, the RARα portion of X-RARα is able to mediate heterodimerization with RXR, and can bind the ligand (retinoic acid, RA) and DNA through the RARα ligand and DNA-binding domains, respectively. Thus the X-RARα fusion product invariably has the ability to interfere with both the X and RAR/RXR pathways.

From a clinical standpoint, APL blasts are exquisitely sensitive to the differentiating action of RA. RA can overcome the block of maturation at the promyelocytic stage and induce malignant cells to mature terminally into granulocytes. This therapeutic approach is conceptually new in that it does not involve chemical or physical agents that eradicate the tumor by "killing" the

neoplastic cells, but rather reprograms these cells to differentiate normally. For this reason, APL has become the paradigm for "cancer differentiation therapy". However, although effective, treatment with RA in APL patients induces disease remission transiently, and relapse, often accompanied by RA resistance, is inevitable. Furthermore, APL associated with translocation between the RARα and the PLZF genes [t(11;17)/PLZF-RARα] shows a distinctly worse prognosis with poor response to chemotherapy and little or no response to treatment with RA, defining a new APL syndrome. New therapeutic strategies are therefore required to potentiate the effects of RA, and to overcome constitutive and/or acquired resistance to RA.

This paper focuses on the recent progress made in defining the molecular mechanisms underlying the pathogenesis of APL in vivo in the mouse. It also addresses how this new understanding has allowed the proposal and development of novel therapeutic strategies with compounds such as histone deacetylase inhibitors (HDACIs) and inorganic arsenicals such as As_2O_3, which are currently being tested in murine leukemia models as well as in human APL patients.

X-RARα proteins are necessary, but not sufficient, to cause leukemia, are biologically distinct RARα mutants, and directly mediate differential response to RA

We have generated transgenic mice (TM) in which the expression of the X-RARα or RARα-X fusion genes is under the control of a myeloid-promyelocytic-specific human cathepsin-G (hCG) minigene [1, 2, 5]. Our characterization of PML-RARα, PLZF-RARα, and NPM-RARα TM has revealed that the X-RARα fusion proteins play a critical role in leukemogenesis as well as in determining responses to RA in APL, since PLZF-RARα mice develop RA-resistant leukemia, while PML-RARα develop APL-like leukemias that respond to RA. Furthermore, the comparative analysis of the phenotypes in PML-RARα and PLZF-RARα TM demonstrated that X-RARα molecules do not represent identical RARα mutants since both hemopoiesis and leukemogenesis in these mice are biologically distinct.

RARα-X proteins are not sufficient for, but do play a crucial role in, leukemogenesis

We have shown that PML-RARα TM develop leukemia with some of the features of APL, while PLZF-RARα TM develop myeloid leukemias that completely lack the distinctive differentiation block at the promyelocytic stage that characterizes human APL. We have recently recreated in vivo the molecular complexity of human APL by generating TM that express RARα-PLZF and PLZF-RARα protein in their myeloid-promyelocytic cellular compartment. We have shown that two concomitant genetic events are required to recreate the disease in its uniqueness. While one of the two events is oncogenic, neither event by itself can cause a disease that can be recognized as APL [4]. RARα-PLZF TM do not develop overt leukemia, nor a block in myeloid differentiation, but display marked hyperplasia of the myeloid compartment. Strikingly, however, PLZF-RARα/RARα-PLZF double TM develop leukemia with classic APL features. Furthermore, RARα-PLZF renders the leukemic blasts further unresponsive to the differentiating activity of RA. RARα-PLZF can act as an aberrant transcription factor that can interfere with the transcriptional repressive ability of PLZF. These findings demonstrate for the first time the crucial role of both products of a cancer-associated translocation not only in contributing to the multistep process toward malignant transformation, but also in determining the phenotypic characteristics of the disease in its native form, including differential response to treatment, in a "double-hit model" for promyelocytic leukemogenesis.

X-RARα molecules are transcriptional repressors

We have demonstrated that both PML-RARα and PLZF-RARα fusion proteins can act as transcriptional repressors and are able to interact with nuclear receptor transcriptional corepressors such as SMRT and N-CoR [2, 7]. PML-RARα can act as a dominant negative transcriptional repressor of RARα through a nuclear corepressor association that is less sensitive to RA. PLZF-RARα can form, via its PLZF moiety, corepressor complexes that are insensitive to RA. HDACIs such as trichostatin A (TSA), sodium phenylbutyrate (PB), and suberanilohydroxamic acid (SAHA) in combination with RA can overcome the transcriptional repressive activity of PML-RARα and PLZF-RARα. HDACIs can also overcome the unresponsiveness of PLZF-RARα leukemic cells to RA.

HDACIs might be utilized in the treatment of APL

The crucial role for transcriptional silencing in APL pathogenesis and resistance to RA in APL suggests that HDACIs alone or in combination with RA might be utilized for the treatment of APL. This therapeutic approach, which we have termed "transcription therapy", represents the first example by which specifically targeting aberrant transcription renders it possible to antagonize the activity of oncogenic transcription factors. Despite their general effects on transcription, the toxicity of HDACIs administered in vivo to mice is negligible. Results obtained with combinations of SAHA and RA in our PLZF-RARα/RARα-PLZF models of RA-resistant leukemia, as well as in human APL patients with combinations of RA and HDACIs, demonstrate the efficacy of this novel therapeutic approach [7, 9].

Arsenic trioxide in combination with RA is effective in the treatment of APL

The inorganic arsenic compound As_2O_3 used in traditional Chinese medicine appears to be highly and specifically effective in the treatment of t(15;17) (PML-RARα) APL [8]. The efficacy of As_2O_3 treatment in APL patients with variant translocations has not been demonstrated. It is also unclear whether As_2O_3 can potentiate the therapeutic effects of RA, and if these two drugs can be administered in combination for the treatment of APL. Utilizing our mouse models of APL, we provide evidence suggesting that the association of As_2O_3 and RA may be beneficial in the treatment of t(15;17) APL, but not for the treatment of t(11;17) APL [6]. On the basis of these findings, clinical trials with HDACIs and As_2O_3 plus RA combinations have been initiated in human APL and in other leukemias and cancers.

Conclusion

In conclusion, it is becoming apparent that mouse models of human diseases go far beyond the simple mimicry of the human pathological condition in providing invaluable insights into the definition of the molecular mechanisms underlying any etiopathogenetic process, while allowing the validation in vivo under physiological conditions of novel therapeutic strategies.

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