

COMMENTARY ON THE APL SPOTLIGHT

Spotlight on acute promyelocytic leukemia: controversies and challenges

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Fifteen years ago, reports of the efficacy of retinoic acid in the treatment of acute promyelocytic leukemia propelled the study of APL to the forefront of the leukemia field.¹ Since then, discoveries in APL continuously revolutionized and have had a profound impact on the study of cancer biology and experimental therapeutics. Many 'firsts' resulted from these studies including the first successful differentiation therapy, the first molecular targeted therapy against a causative oncoprotein, the first application of principles of eukaryotic transcription to cancer biology, etc. In spite of this wealth of research, many questions remain. Indeed, early models of APL pathophysiology and clinical management are constantly being revised in light of new and increasingly complex data.

A number of issues are still unresolved including, but not limited to such questions as:

- How do APL fusion proteins lead to leukemia?
- What is the role of retinoid receptors in myeloid differentiation and leukemogenesis?
- What biological properties are conferred by the fusion partners of RAR α ?
- What is the function of the normal counterparts of the fusion proteins?
- What other 'hits' are required for leukemia to occur?
- Why do leukemia cells become resistant to retinoids?
- What is the most appropriate manner to monitor response to therapy and how do we use this information?

This Leukemia Spotlight was conceived in discussions between Dr Nicole Muller-Bérat Killmann, Dr Michel Lanotte and myself, to re-assess what we know and to address questions such as those mentioned above. We thought it would be constructive to foster debate and discussion of these topics and hope that this benefits investigators in the field, as well as the readership in general. Consistent with these goals, the contributing authors scrutinized existing scientific and clinical data to provide thoughtful analysis and conclusions that attempt to explain these puzzles and suggest future avenues of research. This first installment of the APL spotlight consists of a diverse set of papers spanning both basic science and clinical research issues with broad appeal for the leukemia field as a whole.

The t(15:17) rearrangement that defines the vast majority of patients with APL was first cloned in 1990.^{2–5} The fact that targeted gene locus on chromosome 17 encodes the retinoic acid receptor alpha was extremely exciting and offered a

potential explanation for the remarkable clinical response of these patients to all-*trans* retinoic acid. Although the drug target was demonstrated posterior to the clinical use of the ATRA, it can be said that this represented the first example of successful molecular targeted therapy directed at a cancer causing oncoprotein. The dramatic differentiation of APL blasts in the presence of pharmacological concentrations of ATRA suggested a critical role for RAR α in differentiation. Furthermore, the identity of a number RAR α target genes such as CEBP/ ϵ and others is consistent with an important regulatory role in myeloid differentiation.⁶ Thus, it is curious that animals lacking RAR α demonstrate normal granulocytic differentiation and that vitamin A-deficient animals have only minor hematopoietic disturbances.⁶ Dr Collins addresses this puzzle in his analysis of the role of RAR α in hematopoietic differentiation, as well its potential roles in APL.

In classical APL, the RAR α locus is fused to a gene locus donated by chromosome 15 that encodes a protein named PML (promyelocytic leukemia). The PML/RAR α fusion protein was first characterized in 1991,^{7–9} and the PML portion was shown to be necessary for its biological and transcriptional properties.^{10,11} Studies of the PML protein led to the re-discovery of a nuclear structure known diversely as PML bodies, nuclear bodies or ND10, the functions of which are still hotly debated and not definitively understood. Furthermore, PML was shown to interact with a bewildering array of protein partners and participate in multiple different cellular processes including differentiation, proliferation, apoptosis and antiviral defense. Its mechanism of action has been ascribed to transcriptional regulation, mRNA transport and stability and others (reviewed in Ref. 12). Although PML-deficient mice are viable – the ubiquitous expression of PML, its apparent participation in multiple cellular processes and its potential candidacy as a tumor suppressor protein – indicate that this protein may play a more general role in cancer biology. We are particularly interested in addressing the numerous controversies in this area. Drs Strudwick and Borden provide a meticulous examination of the evidence pertaining to protein interactions and biological functions of PML and suggest standards to guide future research in this area.¹³

In the past few years a novel cellular response leading to a growth arrested, quiescent state was found to occur in response to oncogenic stimuli; at least in epithelial cell models.¹⁴ Interestingly, several laboratories found that PML participated in cellular senescence pathways related to Ras and p53 oncoprotein function.^{15,16} In his contribution to this issue, Dr Ferbeyre raises intriguing possibilities in the context of leukemia and APL in particular: ie that some of the cellular responses seen in APL and other leukemia cells with oncogenic lesions and/or treated with therapeutic agents, may be related to triggering of senescence mechanisms in hematopoietic cells.¹⁷

In addition to PML, four other gene loci are fused to RAR α

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in rare cases of APL. Although the number of cases is very small, it appears that the novel fusion partner may confer different clinical and biological characteristics. The most characterized example of this phenomenon is the case of t(11;17)(q23;q21) APL. In this case the fusion partner of RAR α is the PLZF protein, a transcriptional repressor that in its wild-type form acts as a powerful growth suppressor of myeloid cells. Patients with PLZF/RAR α APL generally present with the M3v type of APL and are refractory to retinoids. This is believed to be caused by the ability of the PLZF moiety to directly recruit corepressors and histone deacetylases to RAR α target genes. The presence of HDACs deacetylate local chromatin and confer a repressed state on gene loci. This mechanism bypasses the release of corepressors and HDACs that occurs in the case of PML/RAR α in the presence of high-dose ATRA. The discovery that repression by PLZF/RAR α could be defeated by combining HDAC inhibitor drugs with ATRA was the first potential medical application of this class of drugs.^{18,19} This led to the coining of the now familiar term of 'transcription therapy'. Therefore, much can be learned by studying the different properties of the APL fusion proteins. Dr. Redner compares and contrasts the functions of these alternate variants with the classical APL to ask important questions related to APL pathogenesis.²⁰

Chromosomal translocations in AML most often involve transcription factors. Correspondingly, the vast majority of molecular studies in acute myeloid leukemias focus on the aberrant transcriptional properties of fusion proteins. The fact that almost none of these translocations are sufficient to cause leukemias strongly implicates the importance of second hits for full transformation to occur. Such events are still poorly known. However, a growing body of work suggests that signal transduction pathway abnormalities may be quite common in leukemias and represent a bona fide therapeutic target. Of particular interest is the case of activating mutations of the flt3 receptor protein, which were first described in this journal in 1996.²¹ These are the most common genetic mutations in AML and occur in one third of patients with APL (reviewed in Ref. 22). Transgenic mice expressing PML/RAR α in their bone marrow frequently develop a myeloproliferative disease, while only a minority of mice develop APL-like disease and this only occurs after long latency.^{23,24} Conversely, mice transplanted with bone marrow cells transduced to express activating flt3 mutants also develop a myeloproliferative disease, but do not develop leukemias.²⁵ However, in marked contrast to these two situations, transplantation of t(15;17) transgenic bone marrow cells transduced to express activated flt3 causes all recipient mice to develop rapid onset APL.²⁶ This represents the most compelling documentation to date of multi-hit pathogenesis in acute leukemia biology. In the current APL spotlight, Noguera *et al*²⁷ describe the impact of activating mutations of flt3 in their cohort of patients from the AIDA APL study. In addition to this, there are several other signaling transduction pathways that intersect physically and functionally with PML/RAR α and may thus play a role in APL pathogenesis. In this spotlight section, Dr Lutz and Dr Cayre provide an exciting analysis of these intersection points and challenge the field to provide more in depth studies of these events in the future.²⁸ Such studies will be of particular importance to the field given promising preclinical results using flt3 tyrosine kinase inhibitors, MAP kinase inhibitors and others.^{29,30}

In the clinical leukemia arena, there has been no greater success in the past 15 years than the remarkable story of retinoid therapy in APL. However, one of the greatest puzzles

in APL is the question of why ATRA does not induce lasting remissions and even more so, why do patients cease to respond to retinoids in subsequent courses of therapy? In this issue, Dr Robert Gallagher presents a thoughtful and comprehensive examination of existing data pertaining to mechanisms of retinoid resistance in APL and their clinical implications.³¹

Finally, although the prognosis of most APL patients is good once they have been treated with chemotherapy and retinoids, approximately 30–50% of them will eventually suffer a relapse (reviewed in Ref. 32³²). Clinical and morphological relapse can often be predicted by molecular identification of bone marrow cells harboring the t(15;17) translocation. Furthermore, a subgroup of patients never achieve full molecular remission of the disease. The question of what the presence of such minimal residual disease means for each patient is complicated by differences in the procedures used to detect these lesions in different laboratories. Thus, the proper clinical response to MRD may ultimately depend on the methodologies that are used to detect them. In this issue, Drs Grimwade and Lo Coco compare and contrast these different detection techniques and discuss their advantages and disadvantages.³³ They also analyze the importance of detecting MRD in setting of APL and suggest future standards of care for the implementation of such monitoring.

This spotlight attempts to contribute useful perspectives on biomedical advances in the APL field. A second part will follow shortly and focus on other important issues related to both the molecular and clinical aspects of this disease. Readers interested in contributing original research or discussions of particular topics are invited to contact the editorial office of *Leukemia* for more information. All spotlight papers are peer-reviewed prior to acceptance for publication.

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