Rearrangements and Aberrant Expression of the Retinoic Acid Receptor α Gene in Acute Promyelocytic Leukemias

By Letizia Longo,* Pier Paolo Pandolfi,* Andrea Biondi,‡ Alessandro Rambaldi,§ Amedea Mencarelli,* Francesco Lo Coco,‖ Daniela Diverio,‖ Luigi Pegoraro,¶ Giancarlo Avanzi,¶ Antonio Tabilio,* Daniela Zangrilli,** Myriam Alcalay,* Emilio Donti,* Fausto Grignani,* and Pier Giuseppe Pelicci*

*From the *Istituto di Clinica Medica I, University of Perugia, Policlinico Monteluce, 06100 Perugia; the ‡Clinica Pediatrica, University of Milan, Ospedale S. Gerardo, 20052 Monza; the §Divisione di Ematologia, Ospedali Riuniti Bergamo e Istituto Ricerche Farmacologiche "M. Negri", 24100 Bergamo; the ‖Dipartimento di Biopatologia, Divisione di Ematologia, I University of Rome, 00161 Roma; the ¶Istituto di Medicina Interna, University of Turin, Ospedale Maggiore di S. Giovanni, 10126 Torino; and the **Dipartimento di Medicina Interna, Cattedra di Ematologia, II University of Rome, 00100 Roma, Italy*

Summary

Although acute promyelocytic leukemias (APLs) are consistently associated with a reciprocal chromosome 15;17 translocation, the gene(s) directly affected by the breakpoints have never been isolated. The chromosome 17 breakpoint maps to near the retinoic acid receptor α (RARα) locus. Investigation of 20 APLs and a large series of other neoplastic patients and normal controls revealed RARα gene rearrangements and aberrant transcripts only in the APL cases. These findings suggest that the RARα gene is involved in the APL chromosome 17 breakpoint, is implicated in leukemogenesis, and could be used as a marker for identifying leukemic promyelocytes.

Acute promyelocytic leukemia (APL;[1] M3 of the FAB classification) is a distinct, well-characterized clinical and morphological subtype of acute myeloid leukemia (AML) (1, 2). It is cytogenetically distinguished by a reciprocal chromosome 15;17 translocation, which is present in 70–90% of cases and never seen in other AMLs or other types of malignancy (3, 4). Although the high frequency and specificity of the t(15;17), and the fact that it is often the only karyotypic aberration present (4), is strong evidence that it plays a crucial role in the pathogenesis of APL, the gene(s) directly involved in the chromosome 15 and 17 breakpoints have never been identified. We have previously shown that the APL chromosome 17 breakpoint lies between the c-erbB-2 and the retinoic acid receptor α (RARα) loci (5). We report here the findings of RARα gene rearrangement and aberrant expression in APLs.

Materials and Methods

Molecular Studies. The K/S, IT, and P/R RARα cDNA probes were obtained by subcloning portions of the p63 plasmid (6) in the pGEM-3 vector. The K3 probe is a KpnI/BamHI 1.8-kb genomic fragment derived from the RARα-Fixα1 phage, isolated from a λ-FixII human genomic library (Stratagene, La Jolla, CA) (unpublished data). It includes the intron located between the RARα exons 281–430 and 431–574 (exon nucleotide positions refer to the RARα cDNA sequence of reference 6), Southern and Northern blot analyses were performed according to established procedures (7).

Cytogenetic Analysis. Chromosome preparations were obtained from unstimulated bone marrow (BM) cultures as previously described (5).

Results

The RARα K/S cDNA Probe Hybridizes to Extra-fragments in 13 of 20 APL DNAs. High molecular weight DNA was extracted from the blasts of 20 leukemia patients who met all the morphological criteria for the FAB M3 classification (2). 18 displayed the typical t(15;17), and two had a normal karyotype (Table 1). DNAs were digested with the EcoRI, HindIII, or BamHI restriction enzymes and hybridized to DNA probes representative of different portions of the RARα cDNA in Southern blot experiments (probes are diagramatically shown in Fig. 1). The K/S probe representative of the 5′ end of the RARα p63 cDNA revealed additional hy-

[1] *Abbreviations used in this paper:* ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; BM, bone marrow; RARα, retinoic acid receptor α.

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/90/12/1571/05 $2.00
Volume 172 December 1990 1571–1575

Table 1. *Synthesis of Cytogenetic, and Southern and Northern Blot Results in APL Cases*

APLs	Cytogenetic	Southern			Northern
		E	H	B	
1MPa	t15;17	G	G	G	ND
2MGu	t15;17	G	G	G	4.0; 3.8; 3.5; 2.8
3MGa	t15;17	R(13.7)	R(5.5)	G	ND
4MCh	46,XX	G	G	G	ND
5MFe	t15;17	R(16.5)	R(23)	ND	ND
6MMa	t15;17	G	R(17)	ND	4.4; 3.8; 2.8
7MPi	t15;17	G	G	G	4.0; 3.5; 2.8
8MRo	t15;17	G	G	G	4.0; 3.8; 3.5; 2.8
9MSp	t15;17	G	G	R(16.5)	ND
10MMo	t15;17	G	R(9)	R(9.5)	4.4; 3.8; 2.8
11MGu	t15;17	G	R(18)	G	4.0; 3.5
12MVa	t15;17	G	G	G	ND
13MTr	t15;17	R(11.5)	R(6.6)	R(7)	ND
14MSa	t15;17	G	R(16)	G	4.4; 3.8; 2.8
15MPa	t15;17	R(16.4)	R(6)	G	4.0; 3.8; 3.5; 2.8
16RDe	t15;17	G	G	G	ND
17TGp	t15;17	R(3.6)	R(7.5)	G	ND
18RLa	t15;17	R(6)	G	R(9.4)	ND
19T48	t15;17	R(13.3)	R(8.5)	G	ND
20PRe	46,XY	R(9.7)	R(6.5)	R(6.7)	ND

G, germline hybridization fragments; R, rearranged fragments (kilobase length is given between parentheses). Numbers in the Northern column indicate the length of RARα transcripts (the typical 3.8- and 2.8-kb transcripts are underlined).

bridizing fragments that differed from the placental germline pattern with one (four cases), two (seven cases), or all three (two cases) of the restriction enzymes used (Fig. 2). The additional fragments were of a different size in each case (Table 1). No deviation from the germline configuration was seen in any case with either the RARα IT or P/R cDNA probes, which contain the intermediate or the 3′ portions of the RARα p63 cDNA, respectively (not shown).

The K/S-hybridizing Extra-fragments Are Not the Consequence of Genetic Variation. 19 DNAs from normal PBL and 45 non-APL samples were digested with the EcoRI, HindIII, or BamHI restriction enzymes and hybridized to the K/S DNA probe. All cases showed the germline K/S hybridization pattern with all three restriction enzymes (not shown). In addition, DNA was extracted from the BM cells of two APL patients (3MGa and 14MSa) who had entered morpholog-

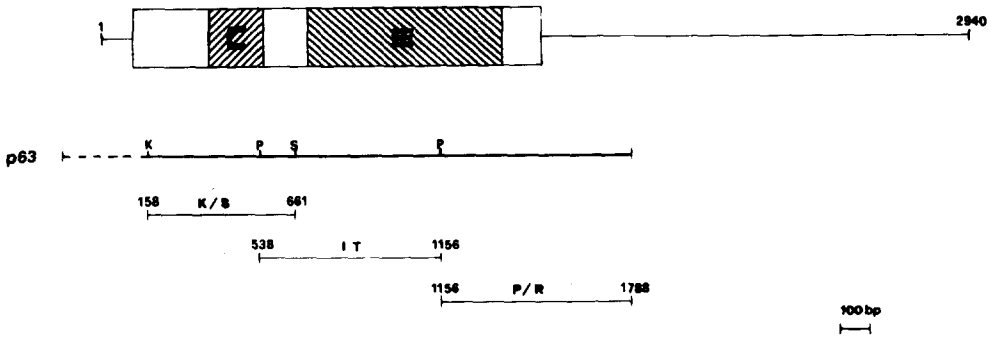

Figure 1. RARα DNA probes. A near full-length RARα cDNA is shown in the top line (1–2940) (10). The block is a schematic representation of the coding region. The DNA (*C*) and retinoic acid (*E*) binding domains are shown. A limited restriction map of the p63 plasmid (6) used to generate the RARα probes is shown in the middle line. The broken line indicates that the sequence of the region has not been reported. Numbers on the bars below indicate the sequence limits of each probe with respect to the RARα cDNA.

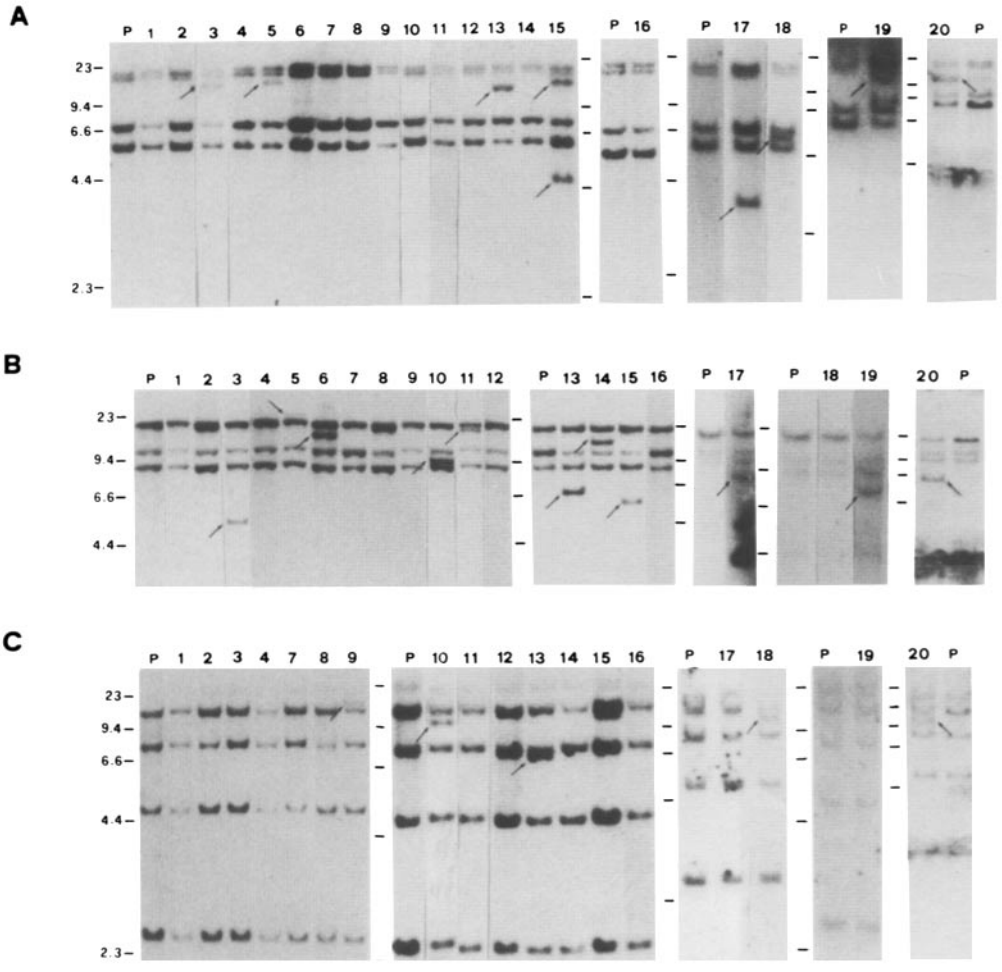

Figure 2. Southern blot analysis of the RARα gene in APLs. DNAs from 20 APLs were digested with EcoRI (*A*), HindIII (*B*), or BamHI (*C*) restriction enzymes and hybridized to the K/S probe. The cases are identified above the lanes by the same numbers as used in Table 1. *P*, placenta DNA. Arrows indicate RARα-rearranged bands. Molecular weight markers are indicated at the left of each panel.

ical remission after conventional chemotherapy. These DNAs, and DNAs extracted from blasts of the same patients before remission, were digested with the HindIII restriction enzyme, hybridized to the K/S probe, and compared. The results showed that the K/S extra-fragments seen in the APL blasts had disappeared in one case (3MGa) and become fainter in the other (14MSa) (Fig. 3). Taken together, these data indicate that the K/S extra-fragments are not the consequence of RFLPs, but of somatic mutations that had occurred in the APL blasts. In the 14MSa case, where the K/S extra-fragment was less intense after chemotherapy, promyelocytes

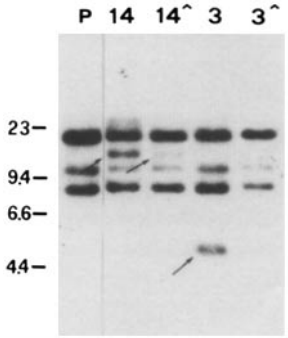

Figure 3. Southern blot analysis of the RARα gene in two APL cases at diagnosis and during remission. DNAs from placenta (*P*), BM APL blasts of two patients (14MSa, *14*; 3MGa, *3*), and BM cells from the same two patients after chemotherapy (*14*, *3*) were digested with the HindIII restriction enzyme and hybridized to the K/S probe. Arrows indicate RARα-rearranged bands.

may have persisted in the BM despite the diagnosis of morphological remission. It is well known that, due to the difficulty of establishing whether promyelocytes are normal or leukemic, it is far from easy to accurately assess remission in APL (8).

The K/S-hybridizing Extra-fragments Are the Consequence of RARα Gene Rearrangements. The RARα gene is a member of the steroid and thyroid hormone receptor family (9). The members of this family share certain structural features: they contain the highly conserved domain that mediates receptor binding to specific sequences of DNA in target genes and the less conserved domain required for ligand binding. As a consequence, the K/S probe, which includes the DNA binding domain, hybridizes to other than RARα fragments in Southern blot experiments (10). To exclude that the extra-bands seen in the APL blasts were due to crosshybridizations of the K/S probe, three APL DNAs were hybridized to the K3 probe, which includes intron sequences of the RARα locus (see Materials and Methods for a description of the K3 probe). The K3 probe hybridized to a single EcoRI fragment in placenta DNA and to extra-fragments in all three APL EcoRI-digested DNAs (Fig. 4). Note that the K3 EcoRI-hybridizing extra-fragments were of the same size as the K/S

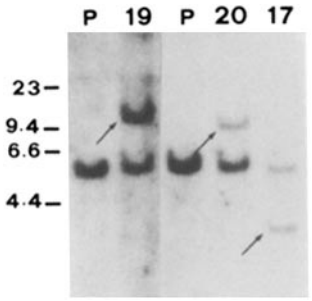

Figure 4. Hybridization of the K3 genomic RARα probe to three APL cases. Three APL (19T48, *19*; 20PRe, *20*; 17TGp, *17*) and placental (*P*) DNAs were digested with the EcoRI restriction enzyme and hybridized to the K3 genomic RARα probe.

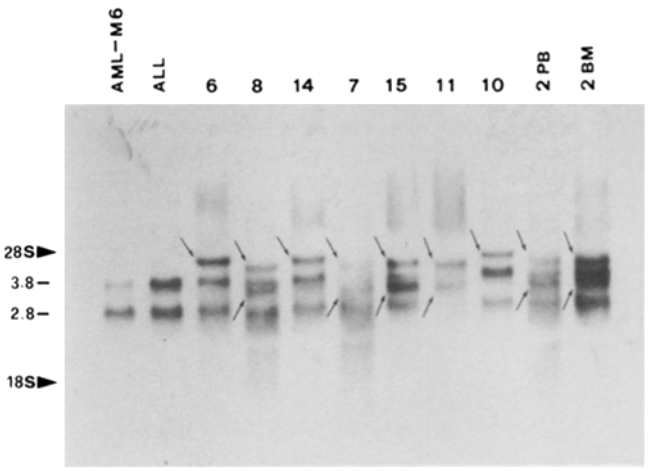

Figure 5. RARα gene expression patterns in APLs. RNA samples from BM blasts of eight APLs, one AML-M6, and one ALL case were analyzed by Northern blotting using the IT probe. Both PBL and BM blasts were analyzed for patient 2MGu (2PB, *2BM*). The patient numbers are the same as those used in Table 1. Ribosomal RNAs (*28S* and *18S*) are given as size markers. The estimated length (3.8 and 2.8 kb) of the RARα typical double transcript is also given. Arrows indicate aberrant RARα transcripts.

EcoRI extra-fragments in all three APL cases (Figs. 2 and 4). We conclude that RARα rearrangements are responsible for the K/S-hybridizing extra-fragments.

RARα Gene Is Aberrantly Expressed in all APLs. To determine whether the rearrangements of the RARα locus interfere with its expression pattern, Northern blot experiments were performed on RNAs extracted from APL blasts. When the I/T and K/S probes were hybridized with RNAs from one AML-M6 and one acute lymphoblastic leukemia (ALL) case, the typical RARα double transcript (3.8 and 2.8 kb) was seen (see Fig. 5 for IT hybridizations) (11). However, when the RNAs from eight APL cases were hybridized with the same two probes, the two typical RARα transcripts appeared at an intensity that varied both within and between samples in seven cases. Neither transcript was detected in one case (11MGu). All eight cases exhibited one or two RARα-aberrant transcripts of three different sizes: 4.4 kb in three cases (6MMa, 10 MMo, 14MSa); and 4.0 and 3.5 kb in five (2MGu, 7MPi, 8MRo, 11MGu, 15MPa) (Fig. 5 and Table 1). The eight APL cases included two (2MGu, 7MPi) that carried the germline configuration of the RARα gene (Table 1).

RARα-aberrant Transcripts Are Specific to APLs. To investigate the specificity of the aberrant transcripts, the RARα expression pattern was analyzed in a variety of non-APL RNAs that included both normal (granulocytes, monocytes, lymphocytes, mesothelial cells, amnion cells) and neoplastic cells (21 AMLs, five chronic myelogenous leukemias, six ALL, one chronic lymphoid leukemia, one Burkitt lymphoma, one Hodgkin lymphoma, two lung cancers, two brain tumors, one cervix cancer, and one bladder cancer). Only the typical 3.8- and 2.8-kb transcripts were detected in all cases (not shown).

Discussion

The RARα locus maps in the proximity of the chromosome 17 breakpoint of the APL-specific t(15;17) (5, 12). However, the relationship between the chromosome 17 breakpoint and the RARα locus is not known. 13 of the 20 APL DNA samples analyzed bore rearrangements of the RARα gene, and eight of eight RNA samples showed aberrant RARα transcripts. These findings provide insights into both the molecular architecture of the t(15;17) and the pathogenesis of APLs.

As 12 of the 13 rearranged RARα alleles were identified in APLs with the t(15;17), the rearrangements could be indicative of a breaksite within, or in the proximity of, the RARα locus. The fact that the remaining case had a normal chromosome 17 at cytogenetic analysis does not contradict this proposal, since the RARα rearrangement could be associated with submicroscopic chromosome 17 aberrations, similar to those encountered in chronic myeloid leukemias that bear bcr gene rearrangements in the absence of the typical 9;22 chromosome translocation (13). Although no RARα rearrangements were detected in six APLs with the t(15;17), they could have occurred in regions of the gene not explored by presently available RARα DNA probes. The finding of aberrant RARα transcripts in every APL case examined, including two with no RARα rearrangements, supports the hypothesis that structural abnormalities occur in the RARα gene of all APLs. The physiological function of the RARα gene product supports the hypothesis that such abnormalities are involved in the pathogenesis of APLs. RARα is an intracellular receptor that functions to regulate gene expression in response to the binding of retinoic acid (RA) (6, 10). RA exerts profound effects on embryonic development and cell differentiation in many systems (9), and is also a potent inducer of the differentiation of myeloid leukemia blasts (14), including APL (15).

Finally, the combined Southern and Northern blot analyses of the RARα gene provides us with a molecular marker for neoplastic promyelocytes. The clinical implications of such a marker are obvious, since it would both allow neoplastic promyelocytes to be unambigously distinguished from normal promyelocytes at diagnosis, and would permit the monitoring of APL cases during induction chemotherapy; neither of which can be done by presently available methods.

We thank Drs. L. Luzzatto, L. Lanfrancone, A. Mantovani, G. Saglio, and P. Mannoni for critical reading of the manuscript, and Dr. P. Chambon for providing the p63 RARα DNA probe used in this research.

This work was supported by the Associazione Italiana Ricerca sul Cancro (AIRC) to P. G. Pelicci, F. Grignani and A. Rambaldi, and by a "Biotechnologie E Biostrumentazione" grant (C.N.R.).

Address correspondence to Pier Giuseppe Pelicci, Istituto Clinica Medica I, Policlinico Monteluce, 06100 Perugia, Italy.

Received for publication 10 July 1990.

References

1. Sultan, C., M. Heilmann-Gouaultme, and M. Tulliez. 1973. Relationship between blast morphology and occurrence of a syndrome of disseminated intravascular coagulation. *Br. J. Haematol.* 24:255.
2. Bennett, J.M., D. Catovsky, M.T. Daniel, G. Flandrin, D.A.G. Galton, H.R. Gralnick, and C. Sultan. 1976. Proposals for the classification of the acute leukemias. *Br. J. Haematol.* 33:451.
3. Rowley, J.D., H.M. Golomb, and C. Dougherty. 1977. 15/17 translocation, a consistent chromosomal change in acute promyelocytic leukemia. *Lancet.* i:549.
4. Mitelman, F. 1988. Catalog of Chromosome Aberrations in Cancer, Third edition, Alan R. Liss, Inc., New York.
5. Longo, L., E. Donti, A. Mencarelli, G. Avanzi, L. Pegararo, G. Alimena, A. Tabilio, G. Venti, F. Grignani, and P.G. Pelicci. 1990. Mapping of chromosome 17 breakpoints in acute myeloid leukemia. *Oncogene.* In press.
6. Petkovich, M., N.J. Brand, A. Krust, and P. Chambon. 1987. A human retinoic acid receptor which belongs to the family of nuclear receptors. *Nature (Lond.).* 330:444.
7. Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
8. Stone, R.M., M. Maguire, M.A. Goldberg, J.H. Antin, D.S. Rosen-thal, and R.J. Mayer. 1988. Complete remission in acute promyelocytic leukemia despite persistence of abnormal bone marrow promyelocytes during induction therapy: experience in 34 points. *Blood.* 71:690.
9. Evans, R.M. 1988. The steroid and thyroid hormone receptor superfamily. *Science (Wash. DC).* 240:889.
10. Giguére, V., E.S. Ong, P. Segui, and R.M. Evans. 1987. Identification of a receptor for the morphogen retinoic acid. *Nature (Lond.).* 330:624.
11. de Thé, H., A. Marchio, P. Tiollais, and A. Dejean. 1989. Differential expression and ligand regulation of the retinoic acid receptor α and β genes. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:429.
12. Mattei, M.G., M. Petkovich, J.F. Mattei, N. Brand, and P. Chambon. 1988. Mapping of the human retinoic acid receptor α to the q21 band of chromosome 17. *Hum. Genet.* 80:186.
13. Bartram, C.R., E. Kleihauer, A. de Klein, G. Grosveld, J.R. Teyssier, N. Heisterkamp, and J. Groffen. 1985. c-abl and bcr are rearranged in a Ph-negative CML patient. *EMBO (Eur. Mol. Biol. Organ.) J.* 4:683.
14. Breitman, T.R., S.E. Selonick, and S.J. Collins. 1980. Induction of differentiation of the human promyelocytic leukemia cell line (HL-60) by retinoic acid. *Proc. Natl. Acad. Sci. USA.* 77:2936.
15. Huang, M.E., Y.C. Ye, S.R. Chen, J.R. Chai, J.X. Lu, L. Zhoa, L.J. Gu, and Z.Y. Wang. 1988. Use of all-trans retinoic acid in the treatment of acute promyelocytic leukemia. *Blood.* 72:567.