Expression of Cellular Oncogenes in Primary Cells from Human Leukemias

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Alterations of cellular oncogene expression have been reported in numerous neoplasias, particularly of the hematopoietic system. However, a cause-effect relationship between cellular oncogene abnormalities and oncogenesis is still unclear. In order to explore the involvement of cellular oncogenes in human cancer cells, we studied the levels of the expression of three cellular protooncogenes in the primary leukemic cells from 53 leukemic patients with different types and stages.

A. Materials and Methods

Thirty-five patients with acute myeloblastic leukemia (AML), 14 with acute lymphoblastic leukemia (ALL), 2 with chronic myelocytic leukemia (CML), 2 with chronic lymphocytic leukemia (CLL), and 8 normal individuals as controls were studied. Diagnosis was based on the morphological evaluation of bone marrow smears according to the French-American-British (FAB) criteria. Leukemic cells, obtained from peripheral blood after diagnosis and before initiation of chemotherapy, were enriched up to 90% by centrifugation on Ficoll-Hypaque density gradient [1]. HL-60, a human promyelocytic leukemia cell line, was used as reference.

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I. Probes

The following cellular or viral oncogene probes were used: a 1.6-kb DNA sequence (pMC41) of human c-*myc*; 1.0 kb *Pvu*I-*Pvu*I fragment (pA T 8.8) of N-*ras*, and 5.8 kb of *Hind*III-*Hind*III fragment (pFBJ-2) of v-*fos*. These probes were all obtained as gifts from Dr. Land H.

II. mRNA Analysis

The mRNA of the corresponding protooncogene in all fresh leukemic cells from patients or control cells was detected by Quick-blot assay [2]. Briefly: cells → deproteinized by proteinase K → add detergents (Brij-35, Doc) and NaI → filter through BA-85 membrane → wash membrane consecutively in H_2O, EtOH/H_2O, dilute acetic anhydride → hybridization with corresponding radioisotope-labeled probes → autoradiography → detect intensity of spots by densitometer (Shimadzu CS930).

B. Results

I. Storage of Samples

In order to repeat the experiment, we stored the cell samples in $1 \times$VRC (vanadyl ribonucleaside complex) solution in liquid nitrogen for 1–2 months. There was no difference in mRNA content between 15 cases of fresh and stored samples (Fig. 1).

II. Expression Levels of Protooncogenes in Normal Leukocytes

The expression levels of c-*myc*, N-*ras*, and c-*fos* in peripheral leukocytes from

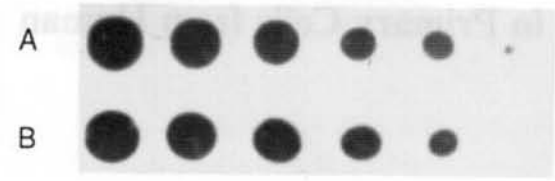

Fig. 1. Comparison of the mRNA content in fresh and stored cells: *A*, fresh leukemic cells from AML-M3; *B*, sample from the same patient but stored in liquid nitrogen for 45 days. Cell number from left to right spot: 2.0×10^6, 1.0×10^6, 5.0×10^5, 2.5×10^5, 1.25×10^5, 7.5×10^4

Table 1. Oncogene expression in HL-60 and WBCs from eight normal individuals

Cell source	c-*myc*	N-*ras*	c-*fos*
WBCs	$< +1$ [a]	+	−
HL-60	+5	$+1 \sim +2$	−

[a] Using "+5" to indicate the expression level of c-*myc* in HL-60, and one-fifth of the level as "+1," and so on.

eight different normal individuals, and the HL-60 cell line were detected. The level of the c-*myc* transcripts in HL-60 was denoted as "+5" [3], and the one-fifth of the level as "+1" and so on. Figure 2 and Table 1 show that the *myc* gene was slightly expressed and N-*ras* was marginally expressed, whereas the expression of c-*fos* was undetectable in the normal leukocytes.

III. Expression Levels of Protooncogenes in Leukemic Cells

In ten AML-M3, four AML-M2, four AML-M4, and ten ALL patients, before chemotherapy, the c-*myc* was obviously expressed in almost all leukemic cells irrespective of the cell types, while N-*ras* and c-*fos* were unconstantly expressed. However, the c-*fos* was expressed in all four cases of AML-M4 (Tables 2, 3). The c-*myc* transcripts were detected but the N-*ras* and c-*fos* were not in four chronic

Table 2. Protooncogene expression levels in 18 AML[a] patients

	No. studied	No. positive	Degree of expression				
			+1	+2	+3	+4	+5
AML-M3							
c-*myc*	10	7	4	2		1	
N-*ras*	9	4	1		2	1	
c-*fos*	8	2	1	1			
AML-M2&M4							
c-*myc*	8	6	4	1	1		
N-*ras*	8	3	2	1			
c-*fos*	8	4	3	1			

[a] Before chemotherapy.

Table 3. Protooncogene expression levels in eight ALL patients

c-*onc*	No. studied	No. positive	Degree of expression				
			+1	+2	+3	+4	+5
c-*myc*	8	6	2	3	1		
N-*ras*	8	3	3				
c-*fos*	6	1	1				

[a] Before chemotherapy.

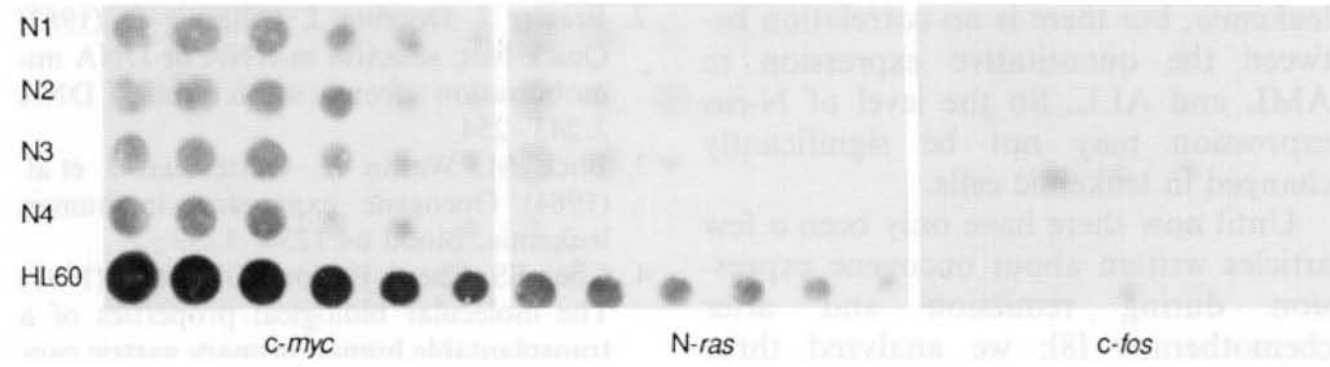

Fig. 2. Oncogene expression in normal leukocytes. N1–N4: four different normal individuals. HL-60 used as a reference. Cell number the same as in Fig. 1

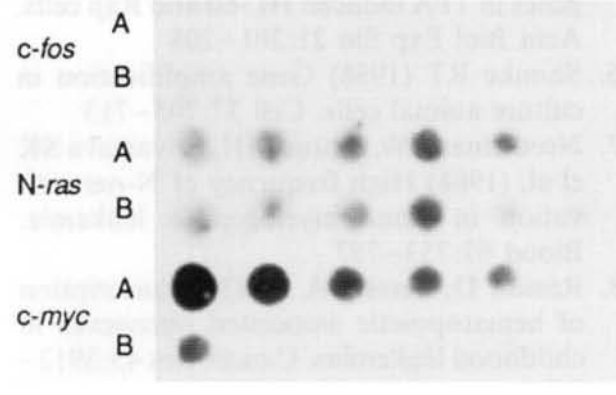

Fig. 3. Protooncogene expression in remitted AML-M2 patient: *A*, before chemotherapy; *B*, remission phase

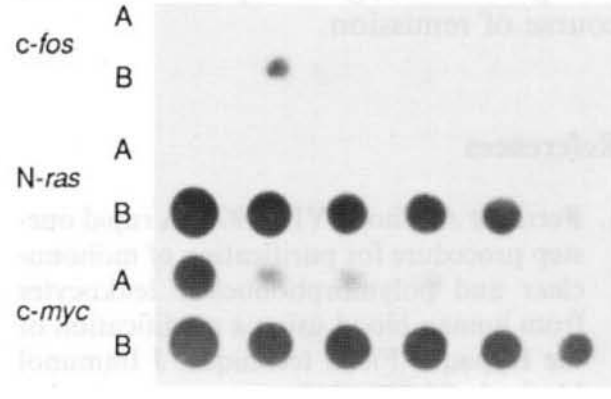

Fig. 4. Protooncogene expression in a CLL patient in blast crisis: *A*, before blast crisis; *B*, in blast crisis

leukemic cases. The samples were obtained from the three AML patients 2–3 months after initial therapy, while the patients were in remission and on maintenance chemotherapy. The results showed that the levels of c-*myc* mRNA decreased obviously in one of three remission samples (Fig. 3), while the levels of c-*myc* mRNA remained essentially unchanged in the others. Figure 4 demonstrates that c-*myc* and N-*ras* mRNA increased dramatically in CLL during blast crisis.

C. Discussion

In this experiment, we selected three cellular protooncogenes – c-*myc* and c-*fos* gene coding for nuclear proteins, the former relating to early cell differentiation and the latter linking to monocytic differentiation and N-*ras* for putative intermediate transducers of mitogenic signals – for detecting their expression levels in primary cells from human leukemia. Most previous studies on the expression of cellular oncogenes in leukemias have been carried out on neoplastic lines, which may not faithfully represent the primary cancer cells [4–6]. So in this study we observed the expression of protooncogenes in primary cells from human leukemias. Our results showed that only c-*myc* gene expressed slightly in normal leukocytes while expressed obviously in almost all leukemic cells irrespective of the cell types. The c-*myc* expression level is higher in acute leukemia than in chronic leukemia. But the levels of N-*ras* and c-*fos* transcripts were variable (Tables 2, 3). An interesting finding is that the c-*fos* gene was expressed in all four AML-M4. The recent data indicate that the N-*ras* gene may be activated by mutation in AML [7]. Our results showed the N-*ras* was strictly expressed in acute

leukemia, but there is no correlation between the quantitative expression in AML and ALL. So the level of N-*ras* expression may not be significantly changed in leukemic cells.

Until now there have only been a few articles written about oncogene expression during remission and after chemotherapy [8]; we analyzed three cases of AML and one of CLL, but no definite suggestion can be put forward. We think that it is important to study the changes of oncogene expression in the course of remission.

References

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