CONCISE REPORT

c-myc and c-fos Expression During Interferon-α Therapy for Hairy Cell Leukemia

By Pierre Lehn, François Sigaux, David Grausz, Pascale Loiseau, Sylvie Castaigne, Laurent Degos, Georges Flandrin, and François Dautry

Low-dose interferon-α (IFN-α) therapy is consistently effective in the treatment of hairy cell leukemia (HCL). In two cases of resistance to IFN-α administration, we diagnosed variant HCL, a form of HCL with intermediate features between typical HCL and B cell prolymphocytic leukemia. We tried to distinguish variant and typical hairy cells (HCs) by Northern blot analysis of the oncogenes expressed in vivo. We report that variant HCs contain c-myc transcripts in contrast to typical HCs, whereas c-fos transcripts are detected in both cell types. We also report that the mRNA levels of c-myc are not modified in variant HCs by IFN-α treatment, whereas the level of c-fos mRNA is modulated in both types of HCs. Our findings suggest that the failure to modulate c-myc expression in vivo might indicate the limits of low-dose IFN-α therapy.

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Table 1. Initial Hematologic Data and Composition of Cell Samples

<table>
<thead>
<tr>
<th>Patients</th>
<th>Diagnosis</th>
<th>Splenectomy</th>
<th>Origin of Cells</th>
<th>Initial Blood Counts</th>
<th>Cell Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>WBC (10^3/dL)</td>
<td>Monocytes (%)</td>
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<tr>
<td>1</td>
<td>Typical HCL</td>
<td>–</td>
<td>blood</td>
<td>18.3</td>
<td>72</td>
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<tr>
<td>2</td>
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<td>–</td>
<td>blood</td>
<td>17.3</td>
<td>58</td>
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<tr>
<td>3</td>
<td>Typical HCL</td>
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<td>blood</td>
<td>59.5</td>
<td>85</td>
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<td>4t</td>
<td>Typical HCL</td>
<td>+</td>
<td>blood</td>
<td>12.5</td>
<td>54</td>
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<tr>
<td>5</td>
<td>Typical HCL</td>
<td>+</td>
<td>blood</td>
<td>13.1</td>
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<tr>
<td>6</td>
<td>Typical HCL</td>
<td>+</td>
<td>spleen</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>Typical HCL</td>
<td>+</td>
<td>spleen</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>Variant HCL</td>
<td>+</td>
<td>blood</td>
<td>43.0</td>
<td>78</td>
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<tr>
<td>9</td>
<td>Variant HCL</td>
<td>–</td>
<td>blood</td>
<td>217.0</td>
<td>97</td>
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</tbody>
</table>

*IFN-α therapy: 1. 3 x 10^8 units daily of recombinant IFN-α2a from Hoffmann-La Roche Inc, Basel, Switzerland; 2. 2 x 10^8 U/m² three times each week of recombinant INF-α2 from Schering Corp, Kenilworth N.J.

†In case 4, the mononuclear cells were depleted of T lymphocytes by sheep erythrocyte rosetting.

HC samples. The blots were further standardized by hybridizing with a mouse β-actin probe.

RESULTS AND DISCUSSION

We could detect c-myc transcripts only in the variant HCs. These transcripts are of the normal size. Only in sample 9 is their level as high as that seen in the control cell lines K562, Ramos, and Molt4, but it remains lower than in the HL60 cell line (Fig 1B). Contrary to c-myc, normal-sized (2.2 kb) c-fos transcripts are present in HCs from all patients tested, although at variable levels (Fig 1A). The level of c-fos mRNA is typical HCs seems to depend on the presence or absence of the spleen (Fig 1A and Table 1). In addition (data not shown), we could not detect c-myb transcripts in both types of HCs, although they were present in two of the control cell lines, HL60 and Molt4. Harvey-ras mRNA accumulates at similar levels in the HCs and in the control cell lines; this is consistent with previous studies of Ha-ras gene expression in acute and chronic leukemias.17 Finally, we could not detect c-sis messengers. This is of particular interest because of the relationship between c-sis protein and platelet-derived growth factor (PDGF) and the well-known myelofibrosis in HCL.

Our detection of c-myc transcripts exclusively in variant HCs suggests that variant HCL is a more proliferative disease than typical HCL. Because we could also detect
c-myc transcripts in two cases of B cell prolymphocytic leukemias (data not shown), our results strengthen the classification of variant HCL as an intermediate disorder between typical HCL and B cell prolymphocytic leukemia.7 The heterogeneity of these rare intermediate diseases, already reflected in the difference in the c-myc mRNA levels in our two cases, could be further analyzed by the study of additional cases.

Our findings of c-fos expression in both typical and variant HCs could be related to their mature phenotype since a stable expression of c-fos has been reported only in some differentiated cells.12 In the hematopoietic system in which a stable expression of c-fos is known in macrophages,12,13 our results extend the domain of investigation to the B lymphocyte lineage. Recent observations indicate that c-fos transcripts can be detected during activation of normal human peripheral blood lymphocytes.18,19 In addition we observed noticeable c-fos mRNA levels in prolymphocytic leukemias (data not shown).

To assess further the possible link between c-myc expression in variant HCs and their resistance to IFN-α therapy, we studied the c-myc mRNA levels during treatment. We observed that even after 6 weeks of IFN-α administration, c-myc transcripts persisted at constant levels in variant HCs (Fig 2C), although they remained undetectable in typical HCs at several time points following the first administration of IFN-α (data not shown). In Fig 2B, we also show that the level of c-fos mRNA in variant HCs was modified despite the ineffectiveness of IFN-α therapy. In four typical HCL cases in which IFN-α was effective, a sequential study revealed two patterns of c-fos expression after the first administration of IFN-α (Fig 2A). In two nonsplenectomized patients, we noticed a rapid disappearance of the c-fos transcripts, whereas IFN-α induced a transient accumulation of c-fos mRNA in two splenectomized patients. These variations of c-fos mRNA levels are not due to a generalized change in the overall level of mRNA as indicated by the nearly constant levels of β-actin mRNA (Figs 2A and 2D).

Thus, the expression of c-fos, but not c-myc, in variant HCs is modulated by IFN-α administration. The c-myc gene is of particular interest because not only is its expression closely associated with cellular proliferation but it also has been shown in vitro that in the case of growth inhibition of cell lines by IFN, a decrease in c-myc mRNA accumulation correlates with the antiproliferative activity.20 Therefore, the failure to modulate c-myc expression might be a good indicator of the limits of low-dose IFN-α therapy, even if c-myc expression is not solely responsible for the resistant phenotype. The meaning of the variation of c-fos expression in response to IFN-α remains unclear.
In conclusion, the study of c-myc expression will be helpful in the identification of variant HCLs. Our observations suggest that the particular sensitivity of typical HCs to low-dose IFN-α therapy is related to their very low level of proliferation because a specific event of the G0-G1 phase might be the target of IFN action. Thus, although one cannot preclude other mechanisms, we suggest that the cytostatic effect of IFN-α on typical HCs leads to the progressive disappearance of the leukemic cell population by impairing its renewal. Recent data showing that HCs synthesize B cell growth factor (BCGF) and that IFN-α inhibits BCGF-induced proliferation of HCs are consistent with our hypothesis.

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