Expression of L-myc and N-myc Proto-oncogenes in Human Leukemias and Leukemia Cell Lines

By Harri Hirvonen, Veijo Hukkanen, Toivo T. Salmi, Tomi P. Mäkelä, Tarja-Taarttu Pelliniemi, Sakari Knuutila, and Riitta Alitalo

The myc proto-oncogenes encode nuclear phosphoproteins, which are believed to participate in the control of cell proliferation and differentiation. Deregulated expression of c-myc has been implicated in several human hematopoietic malignancies. We have studied the expression and mRNA processing of human L-myc, N-myc, and c-myc genes in a panel of human leukemias, leukemia cell lines, and normal hematopoietic cells. L-myc mRNA was expressed in three acute myeloid leukemias (AML) studied and in several myeloid leukemia cell lines. Only low expression levels were observed in adult bone marrow and in fetal spleen and thymus. The K562 and Dami leukemia cell lines showed a unique pattern of L-myc mRNA processing, with approximately 40% of L-myc mRNA lacking exon III and intron I. N-myc was expressed in five of six AML cases studied, in one of nine acute lymphocytic leukemia (ALL) cases, and in several leukemia cell lines, while c-myc mRNA was detected in all leukemias and leukemia cell lines studied. Coexpression of all three myc genes was observed in Dami and MOLT-4 cell lines and in two AMLs, and either L-myc or N-myc was coexpressed with c-myc in several other cases. These results show that in addition to c-myc, the L-myc and N-myc genes are expressed in some human leukemias and leukemia cell lines, and suggest a lack of mutually exclusive cross-regulation of the myc genes in human leukemia cells.

© 1991 by The American Society of Hematology.

Materials and Methods

Clinical specimens and fetal tissue samples. The fetal tissue samples were obtained from therapeutic abortuses at approximately 18 weeks of gestation. Leukemic cells were isolated from...
the peripheral blood or bone marrow of leukemia patients by Ficoll Hypaque (Phar-macia, Uppsala, Sweden) gradient centrifugation before introduction of chemotherapy. Normal bone marrow poly-
morphonuclear and mononuclear cells were isolated by Histopaque
(Sigma, St Louis, MO) double-gradient centrifugation. Clinical data of the patients are given (see Table 1).

Cell culture. K562,26 U937,27 HL-60,27 MOLT-4,27 HEL,26 Jur-
kat,41 KG-1,25 ML-2,24 JOK-1,4 and RC-2A4) cells were grown in RPMI 1640 medium with 10% fetal calf serum (FCS), antibiotics, and L-glutamine. Dami cells40 were grown in Iscoves modified Dulbecco’s Modified Eagle’s medium (DMEM) supplemented with 10% horse serum. Induction of differentiation of K562 and
HL-60 cells was commenced at a cell density of 3 × 10^6/mL with 1.6 to 3.2 nmol/L TPA (12-O-tetradecanoyl-phorbol-13-acetate, dissolved in DMSO). The SCLC cell lines U-16906 and GCL28 (a kind gift from Dr Charles Bays) were cultured in RPMI 1640 medium supplemented with 10% FCS and antibiotics.

Isolation of RNA and Northern analyses. RNAs from the fetal tissue specimens were extracted by the guanidine thiocyanate protocol41 and from the clinical specimens no. 1 to 10 by the lithium chloride method.46 From the cell lines and clinical specimens no. 11 to 17, poly(A)+ RNA was isolated by binding it directly to oligo(dT) cellulose as described.46 RNAs were isolated from freshly isolated cells except from the sample of patient no. 1, from which intact RNA was obtained after culturing the cells for 29 days in RPMI 1640 medium with 10% FCS and antibiotics. Fifteen-microgram aliquots of total cellular RNA were size-fractionated in 1% agarose gels, blotted onto Genescreen Plus (DuPont, NEN, Boston, MA). Hybridizations and washes were performed under stringent conditions as sug-
gested by the supplier. Isolation and analysis of genomic DNA. To analyze myc gene copy number, genomic DNA of patients no. 1 to 10 was isolated by incubating the cells in 500 μg/mL proteinase K with 0.5% sodium dodecyl sulfate (SDS) for 4 to 5 hours, followed by extractions with phenol-chloroform and ethanol precipitation. RNA was removed by RNase A. Ten micrograms of genomic DNA was digested for 4 hours with the restriction enzymes EcoRI or SacI, run in 0.8% agarose gels, blotted onto GeneScreen Plus membrane and hybridized as suggested by the supplier.

Probes. The myc probe fragments are depicted in Fig 1. The c-myc probe was a 316-bp Clal/Alul fragment of human c-myc exon III. This fragment was subcloned into Clal/BamIII sites of Blue-
script KS+ (clone pC3bs316) to allow antisense cRNA generation by T3 polymerase after XhoI linearization. The N-myc probe pN2bs349 was constructed by inserting the 349-bp XhoIl/PstI fragment (exon II) of the clone pNb-6 into Bluescript KS+, to allow antisense cRNA generation by T7 polymerase after HindIII linearization. This cRNA is protected as a 353-nucleotide (nt) fragment, as four flanking nucleotides of the vector MCS match the N-myc sequence. The 2.9-kbp EcoRI insert of the genomic N-myc clone pNb-6 was used in Southern hybridizations only. The L-myc probe pL2GEM450,46 covers 36 bp of intron I and 421 bp of exon II in a pGEM4 vector. Antisense cRNA from HindIII-linearized template was generated by SP6 polymerase. The L-myc clone pU313.blue was constructed by subcloning the 644 bp EcoXl/BamHI fragment of L-myc cDNA clone pU31346 into the corre-
sponding sites of Bluescript KS+. This fragment covers entire exon II (505 bp) flanked asymmetrically by 42 bp and 97 bp of intron I and exon III, respectively. cRNA generated by T3 polymerase from SacI-linearized pU313.blue is thus differentially protected by the different L-myc mRNAs. To generate an RB template clone pRb233bs, the 235-bp BglII/PalI fragment of RB cDNA clone RB3.8M9(32) was inserted into the BamHI/PalI sites of Bluescript KS+, allowing antisense cRNA to be generated from the T3 promoter after linearization with SacI. The glyceraldehyde-3-
phosphate dehydrogenase cDNA clone pHGAPDH21 was used as a reference probe in hybridizations with poly(A)+ RNA. For filter hybridizations, the specific fragments were isolated and purified from agarose gels by isochromophoresis,24 and labeled using [32P]-
dCTP (Amersham, Buckinghamshire, UK, no. PB.10205) accord-
ing to the random priming method23 to specific activities of 1 to 2 × 10^6 cpm/μg. The GAPDH clone was labeled without isolating the specific cDNA fragment. After labeling, the probes were extracted with phenol-chloroform and purified through Sephadex G50 spin columns.

RNAse protection assays. For RNAse protection assays, the cRNA probes were generated from linearized templates (see above) using commercial transcription kits (Transprobe SP and T, Pharmacia) with [32P]-UTP (1,000 Ci/mmol, Amersham, no. PB.20383) as label nucleotide. The full-length antisense transcripts were eluted from 4.5% polyacrylamide gels and hybridized overnight to 15 μg of total cellular RNAs or 1.0 μg of poly(A)+ RNAs in 80% formamide, 400 mmol/L NaCl, 40 mmol/L PIPES (piperazine-N,N’-bis[2-ethanesulfonic acid]), pH 6.4, 1 mmol/L EDTA at 56°C. The L-myc (L2GEM450) and RB probes were mixed and applied as a probe cocktail to the hybridization reactions, the RB probe serving as an internal control. These probes were synthesized and purified separately to avoid possible contamination of the RB cRNA by prematurely terminated L-myc cRNA transcripts. The N-myc and c-myc cRNA probes (353- and 316-nt signals, respec-
tively) were not mixed with the RB probe (322 nt), to avoid blurring of the signals by a residual RB probe observed in some experi-
ments. The hybridizations were terminated by digestion with RNAse A plus RNAse T, followed by proteinase K digestion, purified by phenol-chloroform extractions, ethanol precipitated, and electrophoresed in 4% polyacrylamide/8 mol urea gels with molecular weight ladders composed of end-labeled, MspI-generated fragments of pBR322.

RESULTS

L-myc is expressed in some myeloid leukemias. RNAse protection analyses using the probe pL2GEM450 resulted in strong L-myc signals in three of six acute myeloid leukemia (AML) samples studied (Fig 2A and Table 1). Much weaker protected bands were observed in RNAs from two lymphatic leukemias of class L2 (no. 7 and 9). These findings contrast with the lack of expression of L-myc mRNA in the other leukemic samples tested. The 457- and 421-nt bands are protected by L-myc mRNAs, which either contain or lack intron I, respectively. As an internal control, a human retinoblastoma cRNA probe was used in the same
hybridizations with the L-myc probe. The RB cRNA probe is protected as a 233-nt fragment, thus it does not affect the interpretation of the L-myc signals (Fig 2A and B). The leukemia samples and normal adult and fetal hematopoietic tissues appeared to contain similar levels of RB transcripts (Fig 2A). This indicated that the absence of an L-myc signal was not due to lack of RNA in the hybridization reactions. We observed no evidence of transcriptional inactivation of the RB gene in our material. In Northern hybridization analyses with the same RB fragment, a normal-sized 4.7-kb RB mRNA signal was observed (data not shown).

Expression of L-myc in leukemia cell lines. For analysis of L-myc mRNA in leukemia cell lines, 1.0 μg of poly(A')-selected RNA was used in solution hybridization. This amount yielded an RB signal equal to that obtained from 15 μg of total RNA from human fetal tissues. Strong L-myc signals were observed in the cell lines K562, Dami, MOLT-4, and U937 (Fig 2B). In contrast, the KG-1, HL-60, ML-2, and RC-2A cells were negative for L-myc mRNA (Fig 2B; data not shown for ML-2 and RC-2A). Jurkat and HEL cells yielded only a very weak signal (Fig 2B, Table 1). Among the leukemia cell lines studied, K562 contained the highest levels of L-myc mRNA, yet these levels are approximately 100-fold lower than in U1690 cell line, which has a 40-fold amplification of the L-myc gene: 10 ng of U1690 poly(A') RNA yielded a stronger signal than 1,000 ng of K562 poly(A') RNA, as analyzed by RNase protection with the L2GEM450 probe (data not shown).

Unique L-myc mRNA processing pattern in K562 and Dami cells. The cRNA probe derived from the template pL2GEM450 discriminates between intron I-lacking and -retaining L-myc mRNAs, i.e., it distinguishes the 2.0-kb and 3.6-kb from the 2.2-kb and 3.8-kb forms. However, it cannot discriminate between the 2.0-kb versus 3.6-kb and 2.2-kb versus 3.8-kb L-myc mRNAs. To identify each of these mRNAs as a distinct, single protected fragment in RNase protection assays, we designed the cRNA template clone pU313.blue (Fig 3A). As depicted in Fig 3A, pU313.blue-derived cRNA allows simultaneous analyses of all four known differentially processed L-myc mRNAs. U1690 cells express all these L-myc mRNAs, which results in the 644-, 602-, 547-, and 505-nt protected fragments seen in the left panel of Fig 3B. GLC28 cells yield only one protected band of 602 nt, similar to the 3.6-kb L-myc mRNA (Fig 3B, left). This is due to a rearrangement and fusion of a novel gene, rif, to the 5' region of L-myc. The resulting chimeric rif/L-myc mRNA uses the L-myc exon II splice acceptor and splices out L-myc intron II.56

Protection analyses of L-myc positive leukemia cells and normal fetal tissues using the U313.blue probe showed a different L-myc mRNA processing pattern in the Dami and
Table 1. Expression of c-myc, N-myc, L-myc, and RB Genes in Human Leukemias

<table>
<thead>
<tr>
<th>Pt No.</th>
<th>Sex/Age</th>
<th>FAB Class</th>
<th>PB Leukocyte Blasts (%)</th>
<th>Immunophenotype</th>
<th>Karyotype</th>
<th>c-myc</th>
<th>N-myc</th>
<th>L-myc</th>
<th>RB</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M/38</td>
<td>L2</td>
<td>8</td>
<td>72</td>
<td>CD7+, 2+, 19-</td>
<td>ND</td>
<td>+</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>2</td>
<td>M/81</td>
<td>NHL</td>
<td>35</td>
<td>10</td>
<td>CD19+, 10+, 19-</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>M/30</td>
<td>M2</td>
<td>20</td>
<td>34</td>
<td>CD7+, 19-, 15+</td>
<td>46,XY (6;11)*</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>4</td>
<td>F/25</td>
<td>L1</td>
<td>23</td>
<td>87</td>
<td>CD10+, 19+, 7-</td>
<td>ND</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>M/54</td>
<td>M4</td>
<td>60</td>
<td>80</td>
<td>CD10+, 19+, 7-</td>
<td>ND</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>6</td>
<td>F/45</td>
<td>M4</td>
<td>162</td>
<td>72</td>
<td>CD7-, 19+, 15+</td>
<td>ND</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>F/13</td>
<td>L2</td>
<td>98</td>
<td>85</td>
<td>CD10+, 19+, 20+</td>
<td>46,XX</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>F/3</td>
<td>L2</td>
<td>14</td>
<td>100</td>
<td>CD7-, 10-, 19-</td>
<td>46,XX, 22a-</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>9</td>
<td>F/2</td>
<td>L2</td>
<td>29</td>
<td>90</td>
<td>CD10+, 19+, 20+, 7-</td>
<td>ND</td>
<td>++</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>M/6</td>
<td>L1</td>
<td>220</td>
<td>90</td>
<td>CD7+, 10-</td>
<td>ND</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>F/86</td>
<td>M2</td>
<td>97</td>
<td>100</td>
<td>CD33+, 2+, 7-, 10-, 11-, 13-, 14-, 19-, 20-</td>
<td>46,XX</td>
<td>+</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>F/19</td>
<td>L2</td>
<td>200</td>
<td>99</td>
<td>CD7+, 10+, 14+ (45%), 8+ (56%), 2+, 13-, 14-, 19-, 20-, 33-, 36-</td>
<td>No mitotic cells obtained</td>
<td>+++</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>F/48</td>
<td>CLL</td>
<td>400</td>
<td>—</td>
<td>CD19+, 20+, 10-</td>
<td>No mitotic cells obtained</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>F/30</td>
<td>L1</td>
<td>199</td>
<td>97</td>
<td>Smlg x light chain +</td>
<td>46,XX, t(4;11)(q21;q23)</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>M/37</td>
<td>M2</td>
<td>6</td>
<td>99</td>
<td>CD33+, 2+, 7-, 10-, 11-, 13-, 14-, 20-, 33-, 36-</td>
<td>ND</td>
<td>+</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>M/35</td>
<td>M3</td>
<td>90</td>
<td>95</td>
<td>CD13+, 14+, 33+, 2+ (50%), 7-, 11-, 20-, 26-</td>
<td>46,XY, t(15;17)</td>
<td>+++</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>M/3</td>
<td>L2</td>
<td>8</td>
<td>70</td>
<td>CD10+, 19+, 2-, 7-, 13-, 14-, 20-, 33-</td>
<td>46,XY, 46, XY 3q-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Expression levels are expressed semiquantitatively as −, +, ++, or ++++. Note that the different myc mRNA levels cannot be compared with each other. In patient no. 2, c-myc expression was not evaluable due to lack of RNA for further analyses. Lack of some data for patients no. 8 (N-myc) and 9 (N-myc) is also due to paucity of RNA. Specimens no. 7 and 15 were obtained at relapse.

Abbreviations: FAB, French-American-British; PB, peripheral blood; ND, not done.

*The t(6;11) translocation of patient no. 3 is congenital.

K562 cells as compared with other cells and tissues analyzed (Fig 3B, right). In K562 and Dami cells, three protected fragments of 644, 602, and 505 nt were detected (Fig 3B). The major protected fragments of 602 and 505 nt correspond to the 3.6- and 2.0-kb mRNAs. The 2.0-kb mRNA appears unique to K562 and Dami cells. Indeed, approximately 40% of L-myc mRNA in K562 and Dami cells is of the 2.0-kb form, which lacks exon III and intron 1, in addition to the 3.8-kb and 3.6-kb L-myc mRNAs found in other cell types. The weaker 644-nt band corresponds to the 3.8-kb mRNA. In contrast, we observed virtually no signal corresponding to the 2.2-kb mRNA except in the U1690 cell RNAs. Figure 3 also demonstrates that in AML case no. 5 (M4), L-myc is expressed at several-fold higher levels than in human fetal brain (Fig 3B), which among normal tissues lymphoid leukemias (ALL) showed strong N-myc signals (Fig 4A and data not shown). The N-myc signal intensities were of the same order of magnitude as in human fetal brain. A weak but definite N-myc signal was observed in fetal spleen, while fetal liver, fetal thymus, and adult bone marrow appeared devoid of N-myc mRNA (Fig 4A). Strong N-myc signals were obtained from MOLT-4, Dami, KG-1, Jurkat, and HEL cells, whereas U937, HL-60, K562, JOK-1, ML-2, and RC-2A cells contained no N-myc mRNA (Fig 4B).

Expression of c-myc was analyzed by Northern hybridization and RNase protection. The c-myc transcripts were detected in all leukemic cells, as well as in normal human hematopoietic tissues studied (data not shown). The highest c-myc mRNA levels did not correlate with lack of N-myc or L-myc expression in the leukemia specimens or in the cell lines (see Table 1). We found no amplifications of the myc genes in any of the patients studied by Southern hybridization (data not shown). The data on L-myc, N-myc, c-myc, and RB mRNA expression are summarized in Tables 1 and 2.

**DISCUSSION**

We have studied the expression of the c-myc, N-myc, and L-myc mRNAs in a panel of human leukemias, leukemia
cell lines, and normal hematopoietic tissues, using RNase protection and Northern hybridization analyses. We show for the first time that in addition to c-myc, N-myc and L-myc are also expressed in some human leukemias and leukemia cell lines. High levels of L-myc mRNA were expressed in three cases of AML and in one ALL and three myeloid leukemia cell lines studied. The L-myc mRNA levels were comparable to those found in developing fetal brain, which contains high amounts of L-myc mRNA.\textsuperscript{28} Adult bone marrow polymorphonuclear and mononuclear cells contained very low amounts of L-myc mRNA, at levels similar to fetal spleen and thymus, while fetal liver appeared devoid of L-myc mRNA.

Since L-myc is expressed as four distinct mRNA species with different protein coding capacities, it is necessary to analyze the expression of each of the four transcript forms separately. Our approach in studying L-myc mRNA expression and structure enabled us to differentiate between the various structures of mature L-myc mRNAs, which were initially characterized in SCLC cell lines.\textsuperscript{29} These mRNAs differ from each other due to alternative splicing of intron I and alternative use of the intron II polyadenylation site.
L-myc AND N-myc IN HUMAN LEUKEMIAS

Fig 4. RNAsse protection analysis of N-myc mRNA in leukemias and leukemia cell lines. (A) Leukemia patients and normal hematopoietic tissues. A very strong N-myc signal is obtained from one AML (no. 3), and somewhat weaker signals from two other leukemic samples. For comparison, an equal amount of fetal brain RNA with high N-myc mRNA content was analyzed simultaneously. This results in a signal equal to that obtained from patient no. 3. A very weak signal is seen in fetal spleen, while the liver and thymus appear negative. (B) Leukemia cell lines. Strong N-myc signals are seen in the cell lines MOLT-4, Dami, KG-1, Jurkat, and HEL, while the other cell lines appear negative for N-myc mRNA. In these experiments, the N-myc probe was not mixed with the RB probe, as the incompletely digested RB probe (322 nt) observed in some experiments (see Fig 2) would have interfered with the N-myc signal.

This results in the 3.8-, 3.6-, 2.2-, and 2.0-kb L-myc mRNAs (see Fig 3A). The long form mRNAs (3.6 and 3.8 kb) code for a full-length L-myc protein. The short form L-myc mRNAs (2.0 and 2.2 kb) lack exon III. Thus, they have the capacity to code for a short form L-myc protein whose carboxyl terminus extends 41 amino acids into intron II.58 Indeed, such short L-myc proteins have been demonstrated in SCLC cell lines with amplified and overexpressed L-myc genes.59,60 The majority of L-myc mRNA in normal human cells or tissues is of the 3.6- and 3.8-kb forms. Interestingly, two leukemia cell lines (K562 and Dami) showed a unique pattern of L-myc mRNA processing: approximately 40% of total L-myc mRNA in K562 and Dami cells was of the 2.0-kb form, which may represent a tumor cell-specific alteration of L-myc mRNA processing in these leukemia cells. Virtually no 2.2-kb L-myc mRNA was detected, except in the U1690 SCLC cells, which have a 40-fold amplified L-myc gene.61 The short L-myc mRNA in K562 and Dami is of interest due to its different protein coding capacity. We are currently attempting to determine whether a truncated L-myc protein is translated from the short L-myc transcripts in these cells. However, current detection methods are not sensitive enough for such analyses; the levels of L-myc mRNA in these cells are very low when compared with SCLC cells with amplified L-myc genes, which have been used in all L-myc protein analyses so far.

RNAsse protection analyses showed high N-myc mRNA levels in three AML patients (no. 3, 6, 11) and one T-cell ALL patient (no. 10), as well as in Dami, KG-1, MOLT-4, Jurkat, and HEL cell lines. These findings contrast with the lack of N-myc expression in normal hematopoietic cells and tissues, where only fetal spleen yielded a weak N-myc signal. Thus, one could speculate that N-myc expression in leukemia cells is not an event secondary to transformation, but may contribute directly to the malignant phenotype. However, the normal hematopoietic specimens studied here represent mixed cell populations. There may exist a minor subpopulation of N-myc expressing cells in normal bone marrow, but its N-myc mRNA may be diluted beyond detection by RNA from N-myc negative cells. Similarly, the low level of L-myc mRNA expression observed, eg, in bone marrow, may also result from as yet unidentified subpopulation(s) of hematopoietic precursor cells with higher L-myc expression.

Table 2. Expression of c-myc, N-myc, L-myc, and RB Genes in Human Leukemia Cell Lines and Normal Hematopoietic Tissues

<table>
<thead>
<tr>
<th>Cell Line/Tissue</th>
<th>c-myc</th>
<th>N-myc</th>
<th>L-myc</th>
<th>RB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukemia cell lines</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dami</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>K562</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>KG-1</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>MOLT-4</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Jurkat</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>U937</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HEL</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>HL-60</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>JOK-1</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>ML-2</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>RC-2A</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Normal tissues/cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone marrow, polymorphonuclear cells</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Bone marrow, mononuclear cells</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Regenerating bone marrow*</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Fetal liver</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Fetal spleen</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Fetal thymus</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Expression levels are expressed semiquantitatively as –, +, ++, or ++++. Note that mRNA levels for the different myc genes cannot be compared with each other. The c-myc mRNA analyses were performed by Northern hybridization; L-myc, RB, and N-myc mRNA analyses by RNAsse protections.

*The regenerating bone marrow specimen was obtained from an otherwise healthy child during recovery from neutropenia associated with viral infection.
mRNA levels. However, we consider it unlikely that RNA from the normal peripheral blood cells of, eg, patient no. 3 with a low percentage of blasts (34%), could contribute to the intense L-myc signal obtained in this case, as we only observe a barely detectable L-myc signal in RNAse protection analyses of normal peripheral blood (H. Hirvonen, unpublished observations).

N-myc and L-myc mRNAs were expressed only in combination with c-myc mRNA, which was readily detected in all specimens (see Table 1). This observation is of interest, as negative cross-regulation of myc genes has been shown in several experimental systems with abundant myc expression.1,2,3,6,8 It suggests that at lower expression levels, such negative cross-regulation may not be manifested, since we detected coexpression of two to three myc genes in several types of leukemia cells (Table 1). Further investigations are required to find out whether the combinatorial expression of myc genes has a role in the growth regulation and/or malignant transformation of hematopoietic cells.

It should be emphasized that we did not observe any distinct clinical characteristics associated with L-myc or N-myc expression in this small set of patients. Larger clinical materials and follow-up studies are therefore required to establish possible clinical correlates and significance of L-myc and N-myc expression in human leukemias. The observation of L-myc expression in three AML cases, as well as in three myeloid and one ALL cell line, also warrants further studies on the possible role of L-myc in hematopoietic cell growth and differentiation. Experimental evidence suggesting a role for L-myc in transformation of myeloid cells has been obtained from transgenic mice; Eμ-L-myc transgenic mice show perturbation of myeloid cell development and develop myelomonocytic malignancies at high frequency, but after long latency periods.57

In normal cells, c-myc expression is under negative feedback regulation at the level of transcriptional initiation.44 This negative autoregulation is frequently lost in malignant cells.45 However, possible negative autoregulation mechanisms of N-myc and L-myc and their eventual failure in malignant cells remain to be established. These questions must be elucidated in order to distinguish whether N-myc and L-myc expression in leukemic cells results from pro primo enhanced expression, or whether activation of these genes occurs secondary to differentiation arrest. Further mapping of N-myc and L-myc expression to eventually distinct hematopoietic precursor cells and developmental stages will undoubtedly advance our understanding of the roles of myc genes in hematopoietic cell growth and differentiation.

ACKNOWLEDGMENT

Drs Kari Alitalo and Eero Vuorio are gratefully acknowledged for valuable discussions and critical reading of the manuscript, Dr Yen-Kai Fung for the RB cDNA clone Rb38M(93), and Drs Leif Andersson, Tapani Ruutu, and Ulla Saarinen for their help and advice. The authors also thank Tuula Oivanen, Merja Lakkisto, and Hilkka Toivonen for expert technical assistance.

REFERENCES


59. Ikegaki N, Minna J, Kennett RH: The human L-myc gene is expressed as two forms of protein in small cell lung carcinoma cell lines: Detection by monoclonal antibodies specific to two myc homology box sequences. EMBO J 8:1793, 1989


Expression of L-myc and N-myc proto-oncogenes in human leukemias and leukemia cell lines

H Hirvonen, V Hukkanen, TT Salmi, TP Makela, TT Pelliniemi, S Knuutila and R Alitalo

Updated information and services can be found at:
http://www.bloodjournal.org/content/78/11/3012.full.html
Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml