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

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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. 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.

The HUMAN myc family of proto-oncogenes consists of three well-characterized members, the c-myc, N-myc, and L-myc genes, which code for nuclear DNA-binding phosphoproteins. Accumulating evidence suggests that they have significant roles in the control of cell proliferation and differentiation. the N-myc and L-myc genes have genomic three-exon structures similar to c-myc. They were identified as amplified, myc homologous DNA sequences in cell lines from neuroblastoma (N-myc) and human small cell lung carcinoma (SCLC) (L-myc). Recently, novel putative members to the myc family, the rat B-myc and s-myc genes, have also been reported. The myc proteins are highly related, with several features characteristic of transcription factors (putative DNA-binding domain, helix-loop-helix, and leucine zipper motifs). These carboxy-terminal domains are also indispensable for the immortalizing and transforming activities of the myc proteins, and have been found to bind DNA in a sequence-specific manner alone or more effectively in association with Max.

Overexpression of the myc genes can immortalize a wide range of cell types and reduce their growth factor requirements for continuous proliferation. However, only c-myc has thus far been implicated in human hematopoietic malignancies. In Burkitt lymphomas and some acute leukemias, c-myc is oncogenically activated due to chromosomal translocations that place c-myc coding regions under immunoglobulin or T-cell receptor enhancers. Translocations can also abolish the c-myc 3' mRNA destabilization sequences, resulting in prolonged mRNA half-life and mRNA accumulation. Sporadic amplifications of c-myc in human leukemias have been described, although this appears to be an infrequent mechanism of c-myc activation: rothberg et al observed no c-myc amplifications among 106 human leukemias and lymphomas studied.

The myc genes are differentially regulated. the c-myc gene is active in almost all proliferating cells, but also in some postmitotic, differentiating cells, eg, in the cortical plate of fetal brain. N-myc expression is more restricted (developing brain, retina, and kidney), while L-myc is expressed in developing brain, kidney, spleen, thymus, pancreas, lung, and skin. Transgenic mice with N-myc expression driven by the immunoglobulin heavy-chain (IgH) enhancer develop lymphoid malignancies at a high frequency, showing the ability of deregulated N-myc expression to transform lymphoid cells. Deregulated L-myc expression from an IgH enhancer in transgenic mice can abrogate T-cell development and cause T-cell lymphomas. High-level L-myc expression can also effectively substitute for c-myc in arresting mouse erythroleukemia cell differentiation. These lines of experimental evidence show that L-myc and N-myc can interfere with hematopoietic cell development and lead to malignant cell growth. However, no studies have thus far addressed the expression of L-myc and N-myc genes in naturally occurring human leukemias. Therefore, we studied L-myc, N-myc, and c-myc expression and L-myc mRNA processing in a panel of human leukemias, leukemia cell lines, and normal hematopoietic tissues by RNase mapping and Northern analyses.

MATERIALS AND METHODS

Clinical specimens and fetal tissue samples. The fetal tissue samples were obtained from therapeutic abortions at approximately 18 weeks of gestation. Leukemic cells were isolated from...
the peripheral blood or bone marrow of leukemia patients by Ficoll Hypaque (Pharmacia, Uppsala, Sweden) gradient centrifugation before introduction of chemotherapy. Normal bone marrow polymorphonuclear and monocellular 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, U937, HL-60, MOLT-4, HEL, Jurkat, KG-1, ML-2, JOK-1, and RC-2A cells were grown in RPMI 1640 medium with 10% fetal calf serum (FCS), antibiotics, and L-glutamine. Dami cells were grown in Iscoves modified Dulbecco’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 to 5 × 10^6/mL with 1.6 to 3.2 nmol/L TPA. Dami cells 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 to 5 × 10^6/mL with 1.6 to 3.2 nmol/L TPA. Dami cells were grown in Iscoves modified Dulbecco’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 to 5 × 10^6/mL with 1.6 to 3.2 nmol/L TPA.

Isolation of RNA and Northern analyses. RNAs from the fetal tissue specimens were extracted by the guanidine thiocyanate-protocol19 and from the clinical specimens no. 1 to 10 by the lithium chloride method.18 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.20 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/formaldehyde gels and blotted onto nylon membrane (GeneScreen Plus20, DuPont, NEN, Boston, MA). Hybridizations and washes were performed under stringent conditions as suggested 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 (1:1) and chloroform and ethanol precipitation. RNA was removed by RNAse A. Ten micrograms of genomic DNA were 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 ClaI/AluI fragment of human c-myc exon III. This fragment was subcloned into ClaI/BamHI sites of Bluescript 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 XhoI/PstI fragment of N-myc exon II of the clone pNb-6 into Bluescript KS+, to allow antisense cRNA generation by T7 polymerase after KpnI 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 2.0-kbp EcoRI insert of the genomic N-myc clone pNB-6 was used in Southern hybridizations only. The L-myc probe pL2GEM450,25 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 EcoRI/BamHI fragment of L-myc cDNA clone pU313 despite19 into the corresponding 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/PstI fragment of RB cDNA clone Rb3.8M(93) was inserted into the BamHI/PstI 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 pHIGAPDH17 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 isochophoresis,24 and labeled using [32P]-dCTP (Amersham, Buckinghamshire, UK, no. PB.10205) according to the random priming method29 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 (Transcribe 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, respectively) were not mixed with the RB probe (322 nt), to avoid blurring of the signals by a residual RB probe observed in some experiments. 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 leukemias (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
Fig 2. L-myc expression in human leukemias and in leukemia cell lines. (A) RNase protection analysis of human leukemias and normal hematopoietic tissues with L-myc and RB antisense cRNA probes. The two protected fragments of 457 and 421 nt represent L-myc mRNAs, which either contain or lack intron I, respectively, as a result of alternative mRNA processing. Strong signals are observed in three AML patients (no. 3, 5, and 6, types M2, M4, and M4, respectively). Weak L-myc signals are seen in normal bone marrow polymorphonuclear and mononuclear cells, as well as in fetal spleen and thymus. The RB cRNA probe was included in the same hybridizations as internal standard to verify the amount of RNA used. The 322-nt band results from residual undigested RB probe, and is seen also with the probe mix hybridized to carrier RNA (lane 3). The strong 233-nt RB signal is seen in all samples studied, but not with carrier RNA. (BM-polym/BM-monon., normal adult bone marrow polymorphonuclear/mononuclear cells.) The liver, spleen, and thymus samples are from a normal human second trimester fetus (see Materials and Methods). (B) RNase protection analysis of L-myc mRNA expression in human leukemia cell lines. Strong L-myc signals are observed in Dami, MOLT-4, U937, and K562 cells. The L-myc signal appears as a doublet, indicating the presence of both intron I splicing variants. The faint 322-nt band (indicated by *) is residual incompletely digested RB probe.

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). As controls, normal adult bone marrow (polymorphonuclear and mononuclear cells) and human fetal spleen, thymus, and liver RNAs were analyzed. Weak L-myc signals were obtained from fetal spleen and thymus (Fig 2A), while liver RNAs from five different fetuses contained only trace amounts of L-myc mRNA (two specimens shown in Fig 2B).

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, ie, 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, rlf, to the 5' region of L-myc. The resulting chimeric rlf/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

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<th>Pt No.</th>
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<th>Blasts (%)</th>
<th>Immunophenotype</th>
<th>Karyotype</th>
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<td>CD10+, 14+, 33+, 2+</td>
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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 (NIL-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 contains a very high amount of L-myc mRNA. These results are summarized in Fig 3C.

N-myc and c-myc expression in the leukemia cells. To analyze coexpression of the myc genes, we performed similar analyses for N-myc and c-myc mRNAs. For N-myc analyses, the cRNA template pN2b349 was used. In RNase protection, five of six AML and one of nine acute 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...
Fig 3. Unique processing pattern of L-myc mRNAs in K562 and Damí cells. (A) Structure of the human L-myc gene, the four alternatively processed L-myc mRNAs, and the cRNA template clone pU313.blue. The probe covers 42 bp of intron I, entire exon II (505 bp), exon II/exon III splice junction, and 97 bp of exon III. The four known L-myc mRNAs of 2.0, 2.2, 3.6, and 3.8 kb protect 505-, 547-, 602-, and 644-nt cRNA fragments, respectively. E, Exon II coding region; 0, exon III coding region. (B) RNase protection analysis of L-myc mRNA structure. The 736-nt band represents residual undigested probe. The 644- and 602-nt bands correspond to the 3.8-kb and 3.6-kb mRNAs, respectively. Virtually no 2.2-kb mRNA is seen in any cells analyzed except in U1690. The lung adenocarcinoma cell line GLC28 results in a single protected band at 602 nt. This is due to a translocation of a novel gene (dlf gene, Mäkelä et al., 1991) 5' of the L-myc gene and a resulting fusion mRNA, which uses exclusively the L-myc exon II splice acceptor and splices out L-myc intron II. Note that a 505-nt fragment is protected only in Damí and K562 cells. It corresponds to the shortest (2.0 kb) L-myc mRNA that lacks intron I and exon III sequences. Densitometric scanning of the autoradiograms showed that approximately 40% of L-myc mRNA in these cells is of the 2.0-kb form. (C) Summary of the alternatively processed L-myc mRNAs in human leukemia cells, fetal brain, and the SCLC cell line U1690. The relative amounts of the different L-myc mRNA forms are expressed semiquantitatively as −, +, ++, or ++++, with respect to each other.

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.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.29 These mRNAs differ from each other due to alternative splicing of intron I and alternative use of the intron II polyadenylation site.
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.84 Indeed, such short L-myc proteins have been demonstrated in SCLC cell lines with amplified and overexpressed L-myc genes.85,86 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.

RNAse 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>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>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 RNAse 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. 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 myelomono cytotic malignancies at high frequency, but after long latency periods.

In normal cells, c-myc expression is under negative feedback regulation at the level of transcriptional initiation. This negative autoregulation is frequently lost in malignant cells. 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.

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L-myc AND N-myc IN HUMAN LEUKEMIAS


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

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