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Preferential expression of the transcription coactivator *HTIF1α* gene in acute myeloid leukemia and MDS-related AML

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HTIF1α, a transcription coactivator which is able to mediate RARα activity and functionally interact with PML, is encoded by a gene on chromosome 7q32–34, which is a critical region in acute myeloid leukemias (AML). With the assumption that this gene may be related to AML, we investigated the *HTIF1α* DNA structure and RNA expression in leukemic cells from 36 M1–M5 AML patients (28 '*de novo*' and eight 'secondary' to myelodysplastic syndrome (MDS)). Abnormal *HTIF1α* DNA fragments were never found, whereas loss of *HTIF1α* DNA was observed in the patients with chromosome 7q32 deletion and translocation, and in one case without detectable chromosome 7 abnormality. *HTIF1α* RNA was found in acute myelocytic leukemic blasts, and was almost undetectable in normal mononuclear cells. The expression varied among the patients: higher in M1 to M3 subtypes, with the highest values in M1; low levels were constantly observed in M4 and M5 AML. In addition, *HTIF1α* was significantly overexpressed in MDS-related AML (MDR-AML), but not in MDS. We also found that *HTIF1α* expression was high in myeloid cell lines. In myeloblastic HL60 and promyelocytic NB4 cells, induced to differentiate along the monocytic–macrophage pathway by TPA or vitamin D3, *HTIF1α* expression decreased, whereas it was maintained at high levels on induction to granulocytic differentiation by RA or DMSO. In K562 cells, *HTIF1α* RNA levels did not change after hemin-induced erythroid differentiation. These results suggest that *HTIF1α* could play a role in myeloid differentiation, being distinctly regulated in hematopoietic lineages.
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Introduction

Regulation of gene expression is a complex multi-step process that requires the concerted action of many factors. Among these, the transcriptional intermediary factors (TIFs), also designated as co-activators and co-repressors, interact with sequence-specific transcription factors leading to modulation of the chromatin structure and/or activity of the transcriptional machinery.[1,2] The human TIF1α (HTIF1α, human transcription intermediary factor 1α) is a nuclear protein kinase,[3] a TIF family member, that has been identified via its interaction with liganded nuclear receptors, including the retinoid (RXRs and RARs) receptors.[4–6] HTIF1α protein contains an N-terminal RBCC (RING-B-box-coiled-coil) protein–protein interaction motif,[4,7] which is present in many proteins with diverse functions,[8,9] including PML, the protein fused to RARα in the APL subtype of myeloid leukemia.[10–13] It has recently been demonstrated that in the myeloid differentiation PML acts as a ligand-dependent coactivator of RARα/RXRα, by interacting with TIF1α and CBP, in a RA-dependent transcription complex.[14] In addition, HTIF1α like PML, is characterized by tumor and growth suppression activities,[13–14] and is a partner in fusion genes: with a truncated B-Raf gene in murine hepatocellular carcinoma,[4,15] and with a truncated *RET* gene in thyroid papillary carcinoma.[16]

Recently, the *HTIF1α* gene has been mapped to chromosome 7q32–34,[17] which is a critical region of gene loss associated with myeloid disorders.[18–21] In particular, whole or partial losses of chromosome 7 are strongly associated with a dismal outcome of acute myeloid leukemia (AML).[22,23] This is a heterogeneous disease, which is caused by a variety of pathogenetic mechanisms and characterized by variability in the degree of the commitment and differentiation of the myeloid lineage.[23] AML may derive from a preleukemic condition, the myelodysplastic syndrome (MDS).[24] MDS related AML (MDR-AML) and true '*de novo*' AML (TDN-AML) share morphological and clinical characteristics, but they differ in other aspects, such as incidence throughout life, hematopoietic lineage involvement, cytogenetic features, response to therapy and incidence of relapse which is higher in MDR-AML.[24]

The most frequent chromosomal abnormalities identified in MDS are isolated losses of 5q, 20q and Y chromosome, and a variety of chromosome 7 abnormalities (monosomy 7 and rearrangements of 7q22, 7q32 and 7q36),[25,26] which are associated with a poor prognosis.[27] Specific oncogene or a tumor suppressor gene located on 7q could play a role in MDS[25,26] and, in general, in myeloid diseases. Therefore, because of the location on chromosome 7q, and the above mentioned characteristics shared with PML, *HTIF1α* could be one of the genes involved in the pathogenesis of AML. In this study the *HTIF1α* DNA structure and RNA expression have been investigated in both MDR-AML and TDN-AML patients and in leukemia cell lines.

Materials and methods

Patients and cell lines

Samples of bone marrow (BM, 36) and peripheral blood (PB, 13) were obtained from 36 AML patients, 28 of whom presented with *de novo* AML, and eight who had evolved from an antecedent MDS. This series included 16 males (ages 30–79, mean 64) and 20 females (ages 32–88, mean 61). BM and PB were analyzed in control cases (four healthy males and four females) and in 10 patients with MDS (four males and six females). Diagnosis was based on morphological, clinical and immunological criteria. Conventional cytogenetic analysis was performed in 34 AML cases showing the following clonal abnormalities: chromosome 7 abnormalities in nine

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cases (five with MDR-AML); t(15;17) in five; +13 in three; t(8;21) in two; +11, 5q–, 9q–, t(6;11), i(Xq) in one patient each. There were normal karyotypes in six patients and no mitoses in the remaining four.

Cell lines (myeloblastic HL-60, promyelocytic NB4, erythroleukemic K562, B-lymphoid Raji and EBV-immortalized lymphoblastoid) were studied. The differentiating agents used were 16 nM 12-O-tetradecanoylphorbol 13-acetate (TPA) and 100 nM 1,25-dihydroxyvitamin D3 (D3) (monocyte/macrophage pathway), 1.25% dimethylsulphoxide and 1 μM all-*trans* retinoic acid (RA) (granulocytic pathway), and 30 μM hemin (erythroid pathway), as previously described.[28,29] Cell differentiation into neutrophils and monocytes was assessed by morphology. Macrophage-like differentiation induced by TPA was assessed by adhesion of the cells to the tissue culture plastic and by the morphology of the adherent cells.

DNA and RNA extraction and analysis

Experiments were carried out on cultured cells and patient blast cells obtained by Ficoll separation. Nucleic acid extraction, Southern and Northern blotting were performed as previously described.[30–32] Filters were hybridized with the probes shown in Figure 1a: *HPRR-1* (a 750 bp RT-PCR-derived probe) and *HPRR-2* (a 1.5 kb *Eco*RI-*Eco*RI cDNA fragment).[7] The amount of RNA was assessed by hybridization with a *GAPDH* gene probe,[32] and level of gene expression was determined by densitometric analysis. *TIF1α* expression values were defined by the *TIF1α/GAPDH* ratio.

Statistical analysis

Data are expressed as the mean ± s.e.m. Statistical analysis was performed using the unpaired and paired Student's *t*-test, as applicable.

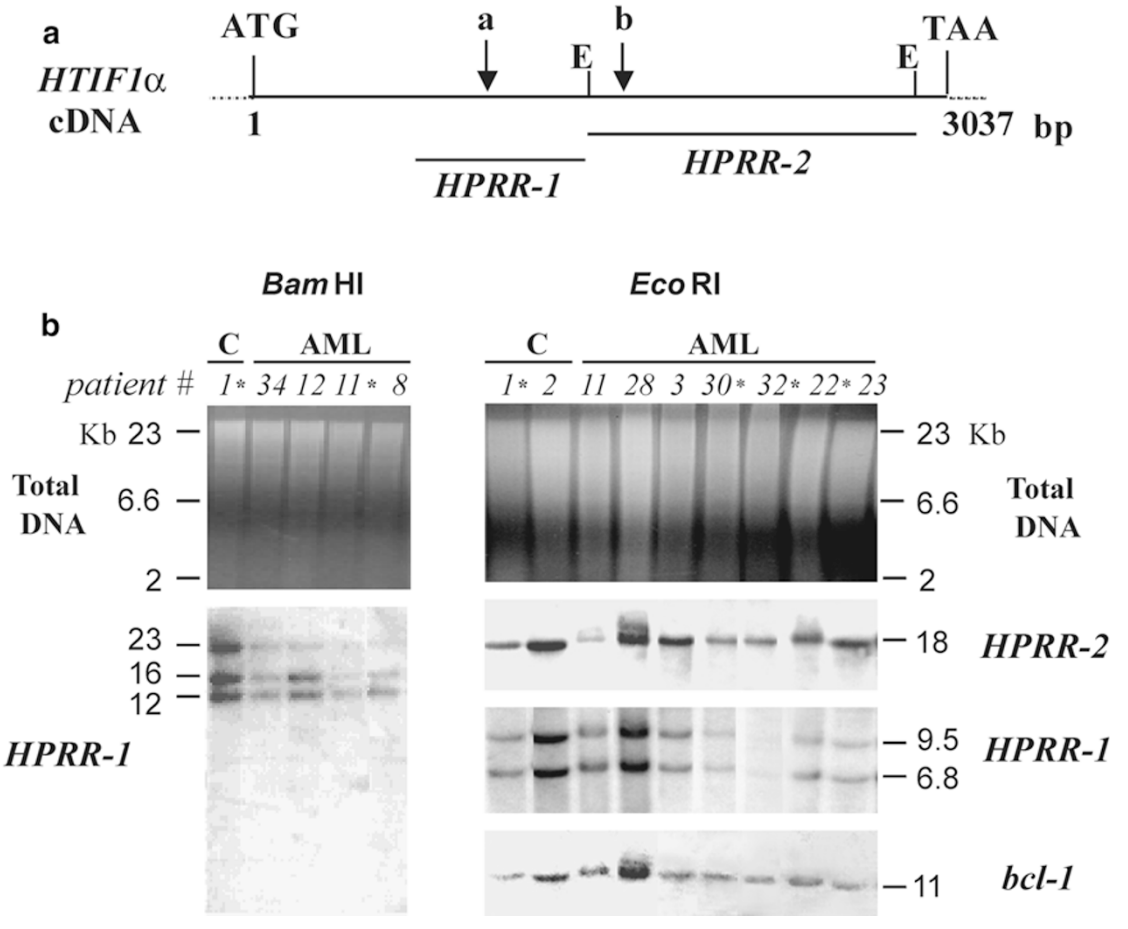

Figure 1 Southern blot analysis of *HTIF1α* DNA in AML. (a) Schematic representation of *HTIF1α* cDNA. E: *Eco*RI restriction sites. The a and b arrows indicate the previously reported breakpoint sites.[15,16] The *HPRR-1* probe was produced by RT-PCR using primers *HPRR*-F2: 5′-AAGACCACACTGTCAGACAG-3′ and *HPRR*-R2: 5′-ATTCTGTTCCACGACAGGAT-3′ on reverse-transcribed HL60 cell total RNA. *HPRR-2* is a cDNA probe.[7] (b) DNA from PB (asterisk) and BM samples of leukemic (AML) and non-leukemic (C) cases were restricted with *Bam*HI or *Eco*RI enzymes. Patient numbers correspond to cases indicated in Figure 2a and Table 1, with the exception of No. 34, an AML M1 patient showing a –7 chromosome abnormality. After gel fractionation and blotting of the DNA, filters were hybridized with *HPRR* probes and rehybridized with the *bcl-1* pB probe for the major cluster region, as previously described.[32] The amount of *HPRR* DNA was related to that of total DNA and *bcl-1* DNA. Reduction in *HPRR*-specific bands was found in cases 34, 11, 8 and 32. In the latter case a marked reduction in *HTIF*α DNA was detected with the *HPRR-1*, and not with the *HPRR-2* probe. Similar restriction patterns were observed in PB and BM samples when available, as shown for case 11.

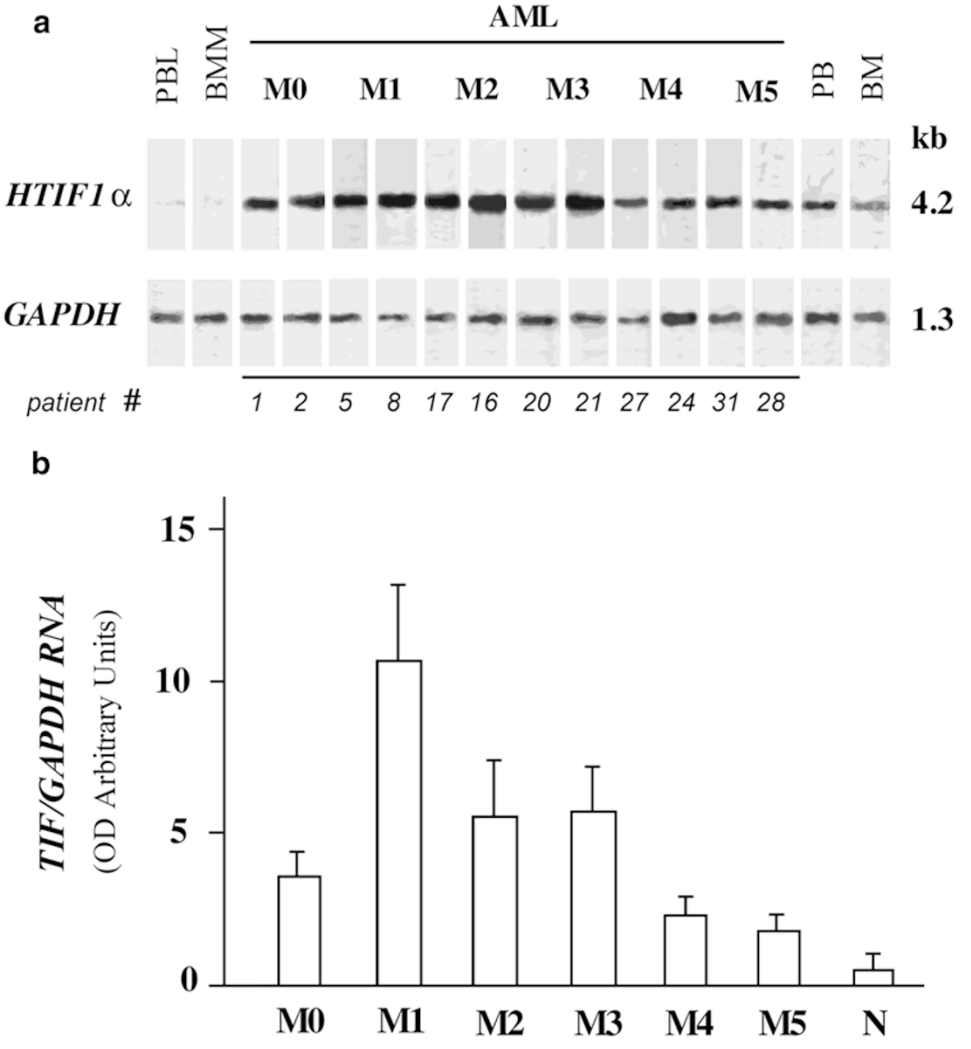

Figure 2 *HTIF1α* gene expression in AML patients. (a) Northern blot of *HTIF1α* RNA in BM samples from AML patients. Total RNA from two cases for each FAB subtype are shown. Patient numbers correspond to those indicated in Figure 1b and Table 1. PBL, normal peripheral blood lymphocytes; BMM, normal bone marrow mononuclear cells; BM, whole normal bone marrow; PB, whole normal peripheral blood. After hybridization with the ^{32}P-labelled *HPRR-2* probe, filters were rehybridized with the ^{32}P-labelled *GAPDH* probe (see Methods). (b) Mean ± s.e.m. of *HTIF1α* expression level in each AML FAB subtype. Only BM samples were included in this analysis.

Results

Loss of HTIF1α DNA in AML with abnormal chromosome 7q

The *HTIF1α* gene was analyzed in 23 AML cases by Southern blotting, after restriction with *Bam*HI and *Eco*RI, and hybridization with the *HPRR-1* and *HPRR-2* c-DNA probes (Figure 1a). The probes cover 75% of the *HTIF1α* coding sequences including the two breakpoints associated with previously reported *TIFα* gene rearrangements.[4,15,16] No rearrangements were detected within the 50 kb region of the *HTIF1α* analyzed. However, weaker bands were found in patients with monosomy 7 or deletion of 7q32 consistent with loss of one allele as expected, but also in cases with translocation involving 7q32 band (cases 34, 11 and 8, respectively, see Figure 1b). No abnormality was observed in the case with a translocation involving 7q22 (patient 12). In case 32, an AML M5 patient with no detectable abnormality of chromosome 7, a marked reduction in *TIFα* DNA was detected with the *HPRR-1* probe, and not with the *HPRR-2*, suggesting a partial loss of the gene.

Variable HTIF1α RNA expression in AML patients

Considering the possibility that small mutations, which are undetectable by Southern blotting, could lead to abnormal transcripts, *HTIF1α* expression was analyzed by Northern blotting in 33 AML cases and in a series of hemopoietic cell lines. Only the expected transcript of approximately 4.2 kb in size was detected with both probes in all the AML cases (Figure 2a). Surprisingly, the *HTIF1α*-specific band was almost undetectable in mononuclear cells obtained by separation over a Ficoll gradient of normal peripheral blood (PB) and bone marrow (BM) samples. However, it was present in normal whole (PB) and (BM) samples containing a majority of neutrophilic cells.

In the AML patients, *HTIF1α* expression was specifically related to the FAB subtype (Figure 2b and Table 1): the mean

Table 1 Comparison of *HTIF1α* RNA expression in TDN-AML and MDR-AML

	AML							MDS (1–10)	Nor (2–8)
	M0 (1–4)	M1 (5–10)	M2 (11–18)	M3 (19–23)	M4 (24–27)	M5 (28–33)			
TDN-AML (25)	1.7 2.2 4.3	2.5 10.4 11.3	1.7[d] 2.2[t] 2.5 2.6[d] 6.0 6.5	2.2 3.8 6.6 6.8 10.6	1.1 2.2 3.3 2.8	0.7 1.3 2.6 2.6		0.0 0.3 0.5 0.5 0.7 1.0 1.4 1.6 1.8 2	0.0 0.0 0.0 0.5 0.5 1.1 1.5
MDR-AML (8)	6.0[m,d,t]	6.1[t] 16.5[m] 23	5.5 18[t]			1.2 3[d]			
Total (Mean ± s.e.m.)	3.5 ± 0.8	11.6 ± 2.6	5.6 ± 1.8	6.0 ± 1.4	2.3 ± 0.5	1.9 ± 0.3	(*P* = 0.004)	0.98 ± 0.22	0.51 ± 0.2
							Total		
TDN-AML (Mean ± s.e.m.)	2.7 ± 0.6	8 ± 2.2	3.5 ± 0.7	6.0 ± 1.4	2.3 ± 0.5	1.8 ± 0.4	4 ± 0.6		
MDR-AML (Mean ± s.e.m.)	6.0	15.2 ± 3.9	11.7 ± 4.3			2.1 ± 0.6	9.9 ± 2.6		
TDN-AML *vs* MDR-AML							0.4 (*P* = 0.002)		

Values represent the amount of *HTIF1α* RNA in mononuclear cells from patients (AML, MDS) and control subjects (Nor) that are indicated by numbers in italics (see Methods for technical details). M1–M5 indicate FAB AML subtypes. Patient cases 3, 6–12, 15, 17, 18, 20, 22–24, 28, 30, 32, have been analyzed by Southern blotting. [m], –7; [d], del(7q), [t], translocations involving 7q. MDS patients include one RAEBt (1) and two CMML (2, 3).

value of expression was higher in M1 (11.6 ± 2.6), five times lower in M5 subtype (1.9 ± 0.3) ($P = 0.003$), and 20 times lower in normal samples. In BM and PB samples from 11 patients, *HTIF1α* displayed a comparable expression. No association with patient age or sex was observed. The data indicate a decrease in *HTIF1α* expression with monocytic differentiation, in agreement with the undetectability of *HTIF1α* expression in normal mononuclear cells, including monocytes and lymphocytes.

Different HTIF1α RNA expression in myeloid and lymphoid cell lines

The detection of *HTIF1α* RNA in myeloid cells led us to investigate whether it was related to a specific cell lineage, and we examined the gene expression in myeloid and lymphoid cell lines. A marked *HTIF1α* expression was observed in myeloid HL-60, NB4 and K562 cells, a clearly lower level in B lymphoid Raji cells and an almost undetectable level in two B-lymphoblastoid cell lines (LCL 1 and 2) (Figure 3). This result confirms a prevalence of *HTIF1α* expression in myeloid *vs* B-lymphoid cells.

HTIF1α RNA is markedly down-regulated in TPA-induced macrophage differentiation

The possible relation between *HTIF1α* expression and cell differentiation was investigated in cells treated with differentiating agents (Figure 4a and b).

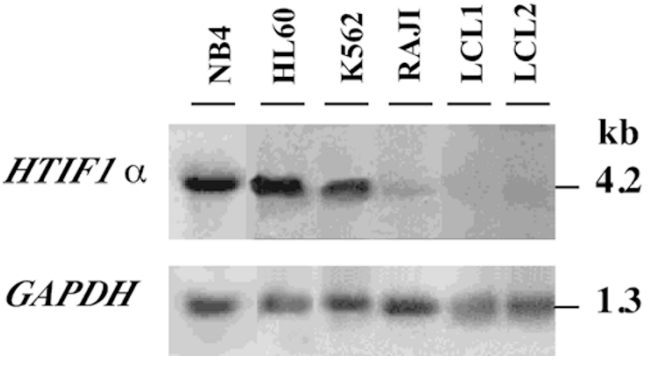

Figure 3 *HTIF1α* gene expression in hematopoietic cell lines. Northern blot of *HTIF1α* RNA in various hematopoietic cell lines. Acute promyelocytic leukemia, NB4; acute myeloblastic leukemia, HL60; chronic myeloid erythro-leukemia, K562; B lymphoid cells: Raji; B-lymphoblastoid cell line: LCL1 and LCL2. See Methods and legend of the previous figure for technical details.

Treatment of HL60 cells for 48 h with 16 nM TPA, that commits the cells to macrophage-like lineage, markedly reduced *HTIF1α* mRNA levels (Figure 4a). Compared to untreated cells, *HTIF1α* RNA values found in adherent and non-adherent cells were 10 and three times lower, respectively (*a* and *n a* of Figure 4a); similar, but less pronounced results were obtained in NB4 cells (Figure 4b).

Treatment of HL60 and NB4 cells for 5 days with 100 nM D3, that is reported to commit HL60 cells to monocytic differentiation,[28,33,34] induced only minimal changes in morphology and cell growth (cell counts were 75 and 85% of

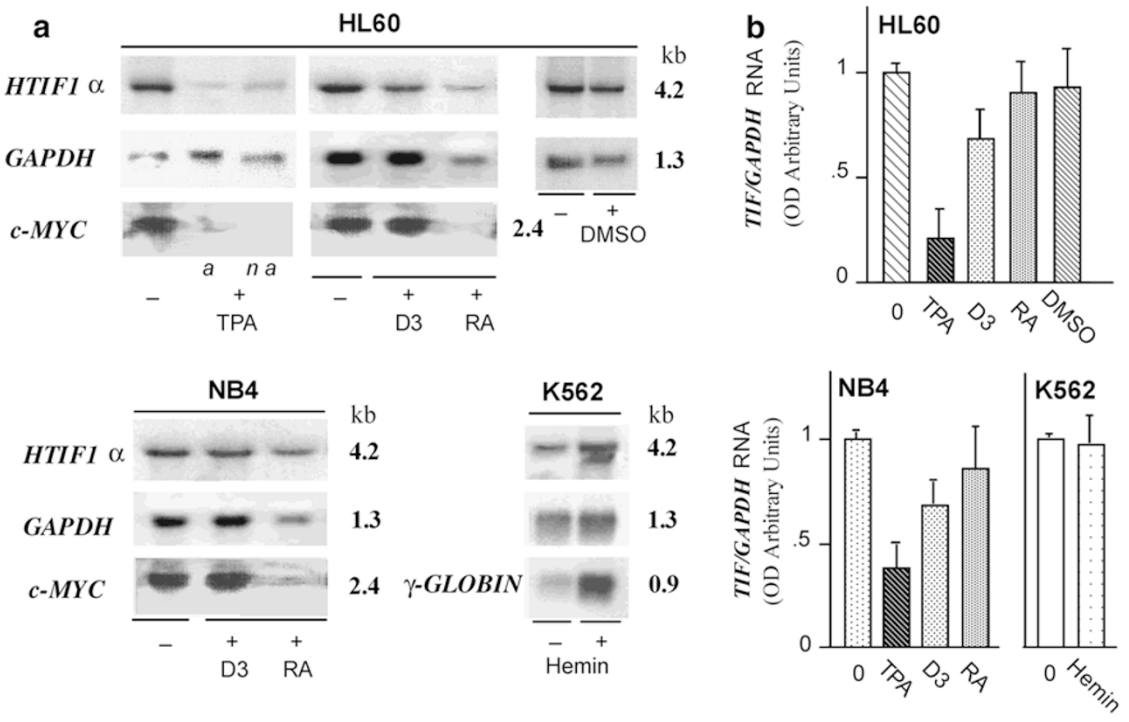

Figure 4 Decrease in *HTIF1α* expression upon monocytoid–macrophage differentiation of HL60 and NB4 cells. (a) Autoradiograms of a Northern blot of total RNA from HL60, NB4 and K562 cells, untreated (–) and treated (+) with differentiating agents (TPA, D3, RA, DMSO, Hemin, as described in Materials and methods). Filters were hybridized with the *HTIF1α HPRR-2* probe and subsequently re-hybridized with *GAPDH* probe, *c-myc* or *γ-globin* probes, as previously described.[32,29] *a* and *n a* indicate adherent and non-adherent cells. (b) Mean values (± s.e.m.) of *HTIF1α*/*GAPDH* RNA ratios in treated and untreated cells, from at least three different experiments.

control values). c-myc RNA levels, that were down-regulated by TPA, remained unchanged after treatment with D3, as shown in Figure 4a. Nevertheless, in both cell types, a reduction in *HTIF1α* RNA levels was observed in treated cells.

Treatment of HL60 and NB4 cells for 4 days with 1 μM RA, that commits cells to granulocyte-like differentiation,[28,35,36] produced significant morphological changes (about 50–70% of the cells aggregated to form clusters), a reduction in cell proliferation (55 and 45% of control) and disappearence of c-myc RNA levels. However, a significant reduction in *HTIF1α* RNA levels, relative to *GAPDH* RNA levels, was not observed when compared with untreated control cells (Figure 4b). Treatment of HL60 cells for 6 days with DMSO inducing granulocyte differentiation, did not produce a significant reduction in *HTIF1α* RNA levels (Figure 4a and b). In K562 cells induced to erythroid differentiation by treatment with hemin for 5 days, as previously reported,[29] *HTIF1α* RNA levels remained unchanged while a marked increase in γ-globin mRNA levels was observed (Figure 4a).

High HTIF1α RNA expression in MDR-AML

In order to evaluate whether the different level of expression in AML subtypes could correlate with the clinical history of the patients, we compared *HTIF1α* expression in TDN-AML *vs* MDR-AML cases (Table 1). A total mean value of 4.0 ± 0.6 was found in TDN-AML, while a markedly higher mean value of 9.9 ± 2.6 was found in MDR-AML, with a statistically significant difference between the subgroups ($P = 0.002$). Thus, overexpression of *HTIF1α* appears to correlate mostly with the MDR-AML. In the 10 cases of MDS analyzed, levels of *HTIF1α* expression were comparable to those of control cases (Nor, Table 1).

Since the *HTIF1α* gene has been localized on chromosome 7q32–34, we considered the possible association between *HTIF1α* expression and chromosome 7 abnormalities, analyzing eight of the nine patients presenting the involvement of chromosome 7 (superscript letters in Table 1), including five MDR-AML. The abnormalities were prevalent in MDR-AML cases, but also present in TDN-AML cases with low levels of *HTIF1α* RNA expression (Table 1). Thus, high levels of *HTIF1α* expression seem to be a feature of the MDR-AML population, regardless of the presence of 7q abnormality.

Discussion

TIF1α is a chromatin-associated factor with a conserved structural motif, the RBCC domain,[4,8] present in fusion oncoproteins like PML.[9] Because of this structural similarity and the ability of TIF1α to interact with RARα and PML,[14] that have been demonstrated to be critical proteins in growth inhibition and myeloid differentiation,[37–38] we investigated the possible involvement of *HTIF1α* in AML.

Southern analysis in 23 AML cases revealed no rearrangements of *HTIF1α*, at least in the regions previously found rearranged in a papillary thyroid carcinoma[16] and hepatocellular carcinoma.[15] Nevertheless, the group of investigated cases was not large enough to exclude the occurrence of *TIF1α* rearrangements in AML, considering that well known rearrangements, such as those involving *DEK-CAN*, occur at a frequency lower than 4%.[39] The reduction in the level of *HTIF1α* DNA found in some cases appeared to correlate with

chromosome 7q abnormalities, including loss of 7q, deletions and translocation at 7q32 and not at 7q22, in accordance with the mapping of *HTIF1α* gene to the 7q 32–34 region.[17] The partial loss of the *HTIF1α* DNA 5′ region observed in a MDR-AML case with no apparent chromosome 7 abnormalities, would indicate the occurrence of a submicroscopic chromosome 7 lesion involving the gene. This is in accordance with results previously reported in AML and MDS patients, demonstrating loss of 7q by PCR-based techniques[19,26] and hidden translocations of 7q by spectral karyotyping,[25] otherwise not revealed by conventional cytogenetics. The case presented here remains to be analyzed in more detail. In spite of this sample, disruption of *HTIFα* seems to be a rare event in AML patients.

Although DNA analysis did not implicate *HTIF1α* gene rearrangement as a mechanism associated with the development of AML, the relative expression of *HTIF1α* RNA in leukemic cells of AML patients and in myeloid cell lines, suggests an important role of this gene in the myeloid lineage.

Previous studies have shown that *TIF1α* is expressed in various human tissues, including whole peripheral blood.[17] In the mouse,[40] during embryogenesis it is ubiquitously expressed until mid-gestation, then, it remains highly expressed in developing nervous system and within the proliferating regions of the kidneys and teeth, whereas, in the adult, it is predominantly expressed in both the male and female gonads. No data are available on *TIF*1α expression in hematopoietic cells. Our data show *HTIF1α* to be specifically expressed in granulocytic cells, both in the PB and in the BM (Figure 2a). In our patients *HTIF1α* was found to be predominantly expressed in those leukemias reflecting an early and intermediate stage of myeloid differentiation (M1–M3 of the FAB classification), in M2-derived myeloblastic HL60, in M3-promyelocytic NB4 and chronic K562 myeloid cell lines. On the contrary, low levels of expression were found in Raji B-lymphoid and EBV-transformed B-lymphoblastoid cell lines (see Figure 3).

The low levels of *HTIF1α* RNA expression in leukemias with predominant monocytic differentiation seem to be in accordance with the reduction in *HTIF1α* RNA expression found in HL60 and NB4 cells induced to differentiate down the monocyte-macrophage pathway. The less pronounced reduction in *HTIF1α* RNA after D3 treatment, in comparison with the TPA treatment, might be explained by the previously observed weak effectiveness of D3 on both leukemia cell lines;[35] furthermore, it has been shown that D3 or TPA in NB4 cells treated individually, result in only a partial or incomplete differentiation along the monocyte–macrophage pathway, and that both are required to achieve optimal macrophage function.[33] Accordingly, the down-regulation of the c-myc proto-oncogene which parallels terminal differentiation,[33,36] was not observed in D3-treated cells (Figure 4a). *HTIF1α* RNA expression was not significantly decreased when HL60 and NB4 cell lines differentiated along the granulocytic pathway after treatment with DMSO or RA. However, in NB4 cells treated with RA, down-regulation of the c-myc was observed, associated with the decrease in cellular proliferation and progression of maturation and differentiation.

Therefore, the downregulation of *HTIF1α* expression in macrophage and not in granulocytic maturation, obtained after treatment of different cell lines with agents acting at different molecular levels, supports the hypothesis of a differential regulation of *HTIF1α* gene according to a different cell lineage.

In myeloid differentiation PML acts as a ligand-dependent coactivator of RARα/RXRα, by interacting with TIF1α and CBP, in a RA-dependent transcription complex.[14] The presence of PML, therefore, is crucial for the growth-inhibitory activity of RA, as well as for RA induction of myeloid differentiation.[41] Intriguingly, it has been reported that, in HL60 cells, RA increases the level of PML.[42] It is, therefore, possible that RA could also sustain the *HTIF1α* expression to maintain the cellular homeostasis of the two co-receptors.

The hypothesis of PML and HTIF1α co-expression is also supported by the sustained levels of *HTIF1α* RNA detected in K562 cells induced to erythroid differentiation (Figure 4). In fact, PML which has been found to functionally interact with nuclear proteins in K562 cells,[43,44] appears to be highly expressed through the erythroid pathway, as observed in differentiating hematopoietic progenitor cells.[44]

Thus, *HTIF1α* expression appears to be not only associated with immature proliferating cells, but also with cells deriving from precursors with specific differentiation commitments. Further studies of *HTIF1α* expression in GEMM- and GM-CFU could confirm this hypothesis.

The distinction of '*de novo*' AML from MDS-derived AML is extremely useful because, despite the fact that the two conditions share a number of characteristics, they differ in other aspects such as sensitivity to conventional therapy.[23,24] Morphological, clinical and genetic differences are valuable tools for better differentiation of these two conditions. The observation of a significant correlation between MDS-derived AML and a higher level of *HTIF1α* expression, in comparison to the lower level detected in TDN-AML and MDS, is of interest, suggesting that *HTIF1α* expression could be a typical feature of MDS progressing to AML and that the MDS transition to AML could be mediated by transcriptional mechanisms requiring high levels of *HTIF1α* and/or its interacting proteins. On the other hand, deregulation of *HTIF1α* expression could be associated with the abnormal DNA methylation frequently observed in AML.[45–47] For instance, methylation of p15 gene promoter has frequently been reported in AML at presentation,[48] and in MDS it is highly associated with leukemic transformation.[49] However, it becomes difficult to reconcile the overexpression of *HTIF1α* with DNA hypermethylation which is generally associated with gene expression silencing, unless it occurs on a hypothetical gene encoding a repressor of *HTIF1α* gene expression.

On the other hand, it has been shown that the activation of the *HOX11* proto-oncogene in T-ALL occurs in the absence of translocation, but in association with extensive demethylation of the proximal *HOX11* promoter.[50] Examination of the methylation status of the *HTIF1α* promoter sequence, when available, will be necessary to verify its methylation status in AML cases with high *HTIF1α* expression.

The association of myeloid leukemia with chromosomes 7–/7q–,[18–22] and their involvement in MDS as a well known negative prognostic factor,[22–27] supports the concept that these chromosomal regions contain novel tumor suppression genes. In the AML patients examined here, chromosome 7 abnormalities, although prevalent in the MDR-AML cases, did not show an invariable association with the high expression of *HTIF1α* gene, located at 7q32–34.

In conclusion, the absence of abnormal *HTIF1α* DNA fragments or transcripts suggests that gross genomic recombinations of *HTIF1α* do not occur frequently in AML. Nevertheless, the differential expression in myeloid and B-lymphoid cells, and its overexpression in some AML subtypes, strongly indicate that the *HTIF1α* gene could play an important role in myeloid differentiation. Therefore, at variance with the

housekeeping expression previously reported,[17] we found that *HTIF1α* gene shows a cell- and differentiation-specific expression.

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