Molecular Cloning of Six Novel Krüppel-like Zinc Finger Genes from Hematopoietic Cells and Identification of a Novel Transregulatory Domain KRNB*

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To clone zinc finger genes expressed in hematopoietic system, we designed primers based on conserved Cys2/His2 zinc finger sequences to amplify corresponding domains from mRNA of normal bone marrow and leukemia cell line NB4. DNA fragments of novel zinc finger genes were chosen and used as probe pool to screen cDNA libraries or subject to rapid amplification of cDNA ends in order to obtain full-length cDNA. Six cDNAs including whole open reading frame of zinc finger proteins, named as ZNF191, ZNF253 (BMZF-1), ZNF255 (BMZF-2), ZNF256 (BMZF-3), ZNF257 (BMZF-4), and ZNF254 (BMZF-5) were obtained. All six belong to the Krüppel-like zinc finger gene family, and typical transcriptional regulatory motifs exist in the N-terminal moiety, such as the SCAN box in ZNF191, and the KRAB domains in ZNF253, ZNF254, ZNF256, and ZNF257. A previously undefined sequence nominated as Krüppel-related novel box, which may represent a new transregulatory motif, was revealed at the N terminus of ZNF255. The transregulatory function of non-zinc finger regions of ZNF191, ZNF253, and ZNF255 were addressed in yeast and mammalian cells. The results indicated that ZNF255 might be a conditional transactivator, whereas ZNF253 and ZNF191 displayed a suppressive effect on the transcription in yeast and/or mammalian systems.

Transcriptional regulation is controlled through interaction between DNA and protein complex, the latter containing transcription factors with highly conserved protein motifs. The most well known motifs are the helix-turn-helix, helix-loop-helix, and zinc finger. During cell differentiation and development, each of these domains is involved in the binding of transcription factors to their cognate DNA recognition site, resulting in the specific activation or repression of gene expression (1).

Zinc finger gene family belongs to one of the largest human gene families and plays an important role in the regulation of transcription. This large family may be divided into many subfamilies such as Cys2/His2 type, glucocorticoid receptor, ring finger, GATA-1 type, GAL4 type, and LIM family (2–4). In Cys2/His2 type zinc finger genes, there are highly conserved consensus sequence TGEKPYX (X representing any amino acid) between adjacent zinc finger motifs. The zinc finger proteins containing this specific structure are named after Krüppel-like zinc finger proteins because the structure was first found in the Drosophila Krüppel protein (1).

Hematopoiesis is a complex physiological process, which requires fine regulation of gene expression during embryogenesis, fetal life and after birth. Disturbance of this regulation, therefore, may be the cause of hematopoietic disorders. It is interesting to note that some zinc finger proteins may regulate hematopoietic differentiation toward erythroid (GATA-1 and EKLF), megakaryocytic (GATA-1 and -2), lymphoid (GATA-3 and Ikaros), granulocytic (MZF-1), as well as monocytic (EGR-1) lineages (5–7). Moreover, many zinc finger genes have been shown to be involved in the pathogenesis of hematological malignancies. For example, Cys2/His2 type zinc fingers such as HRX (also named as ALL-1 or MLL) (8), PLZF (for promyelocytic leukemia zinc finger gene) (9), and Bel-6 (10); glucocorticoid receptor family members such as retinoic acid receptor α (11); ring fingers such as promyelocytic leukemia (12); and LIM family member such as Ttg-1 and Ttg-2 (13) have been implicated in a variety of chromosomal translocations that play a primordial role in the pathogenesis of hematological malignancies.

This study tries to isolate novel Krüppel-like zinc finger genes expressed in normal bone marrow and promyelocytic leukemia cell line NB4 as part of a long term effort to explore the molecular regulation of hematopoiesis. Using oligonucleotide primers based on conserved sequences of Krüppel-like gene family to drive homologous PCR amplification, a large number of DNA fragments corresponding to the zinc finger regions of these genes were obtained. After subcloning and sequencing, novel genes were defined based on data base search and subject to full-length cDNA cloning and functional analysis.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF038964, AF038965, AF067164, AF067165, AF070651, and AF054180.

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1 The abbreviations used are: PCR, polymerase chain reaction; RT, reverse transcription; BD, binding domain; aa, amino acid(s); bp, base pairs; ORF, open reading frame; EST, expressed sequence tag; CHO, Chinese hamster ovary; RACE, rapid amplification of cDNA ends; contig, group of overlapping clones.
**EXPERIMENTAL PROCEDURES**

**Cell Culture, Bone Marrow, and Cord Blood**

CD34+ Cell Samples—Acute promyelocytic leukemia cell line NB4 was cultured in RPMI 1640 medium supplemented with 10% calf serum in a humidified atmosphere of 95% air, 5% CO2 at 37 °C. Bone marrow samples were obtained from individuals subject to marrow examination while hematological disorders were excluded afterward. Mononuclear cells were isolated by density gradient centrifugation using a lymphocyte separation medium. CD34+ cells were isolated from umbilical cord blood, and a cDNA library was constructed for EST sequencing using previously reported methods (14).

**RNA Preparation and RT-PCR**—The total RNA of NB4 cell and bone marrow were prepared by using TRIzol reagent (Life Technologies, Inc.) and pretreated with DNase I (RNase-free) to eliminate DNA contamination. The transcription reaction was performed with 1 μg total RNA from NB4 or bone marrow, 5 μmol of random primers, 1× RT buffer, 10 mU dithiothreitol, 0.2 μM dNTP, 200 units of Moloney murine leukemia virus (Promega) at 37 °C for 50 min. PCR was performed on a 9600 thermal reactor (Perkin-Elmer) with the primers corresponding to a highly conserved consensus sequence of Krüppel-like zinc finger gene (sense primer, 5'-AGAGAATTCATCATCTCTTCGA-3'; antisense primer, 5'-CATATACTGGTTGCTTTCCTCCCA-3'). Thirty-five cycles were carried out by two steps, in which the first five cycles were composed of denaturation at 94 °C for 5 min, annealing at 53 °C for 1 min, and extension at 72 °C for 1.5 min and the remaining cycles, denaturation at 93 °C for 1 min, annealing at 58 °C for 1 min, and extension at 72 °C for 1.5 min. RT-PCR products were separated by agarose gel, and the bands were subcloned into pGEM-T vector (Promega).

**DNA Sequencing and Bioinformatics Analysis**—DNA sequencing was carried out on 377 DNA automatic sequencer by using a Dye Primer cycle sequencing kit (Perkin-Elmer). After a first analysis with Strider 1.2 software, both DNA and predicted amino acid sequences were searched against GenBank and the Expressed Sequence Tag (EST) data base (release 109.0) for homology comparison by using BLAST software in the GenBank Computer Gene package. DNA fragments corresponding to putative novel Krüppel-like zinc finger genes were chosen for further study.

**Full-length cDNA Cloning**—The following three methods were combined to facilitate the full-length cDNA cloning. 1) cDNA libraries were screened according to the standard protocol. Pooled DNA fragments of novel zinc finger genes were used as probe to screen cDNA libraries from NB4 cell, bone marrow, CD34+ umbilical cord blood cells and brain. Probes were labeled with [32P]random-labeling method by using semi-quantitative RT-PCR (13). The total RNAs from eight different tissues (heart, brain, lung, kidney, stomach, liver, pancreas, placenta, and bone marrow) and six cell lines (acute promyelocytic leukemia cell line NB4, acute myeloblastic leukemia cell line HL60, monocyctic cell line U937, chronic myelocytic leukemia-erythroleukemia blast crisis cell line K562, T-lineage acute lymphoblastic leukemia cell line CEM, and B-lineage acute lymphoblastic leukemia cell line RB1B) were used as template. RT reactions were performed as mentioned above. Gene-specific primers of ZNF191, ZNF253, ZNF254, ZNF255, ZNF256, and ZNF257 were used in PCR with co-amplification of glyceraldehyde-3-phosphate dehydrogenase internal control. The products of PCR were visualized on agarose gel under ultraviolet. Gene expression levels were estimated according to the ratio of density integration between the bands of specific gene and internal controls by scanner (ScanMaker E6, Microtek) and software (Smart View 3.0, Furi Inc.).

**Functional Analysis of Putative Transregulatory Domain of Construction Expression Plasmid**—To define the transregulatory properties of ZNF191, ZNF253, and ZNF255, non-zinc finger regions of these genes were inserted into yeast plasmid pGB79 and mammalian cell plasmid pCM (CLONTECH). Both pGB79 and pCM contain DNA-binding domain (GAL4-BD) (1–147 aa) of GAL4, which was driven by ADH1 and SV40 promoters, respectively. Three pairs of primers containing restriction enzyme sites and gene-specific sequences corresponding to non-zinc finger regions of ZNF191, ZNF253, and ZNF255 were designed to amplify the target fragments of these genes. The resultant PCR fragments were inserted into pGEM-T9 or pGEM-SalI, SmalPstI, and EcoRI/Pst restriction enzyme sites to generate fusion genes encoding GAL4-ZNF191, GAL4-ZNF253, and GAL4-ZNF255 chimera, respectively. The amplified regions and the junctions in these constructs were verified by DNA sequencing.

**Mammalian Cell Transfection**—In the mammalian assay system, the recombinant pM with different GAL4 fusions, the negative control pM and the positive control pM3-VP16 encoding herpesvirus protein were cotransfected by LipofectAMINE (Life Technologies, Inc.) into NIH3T3, COS-7 and primary hamster cells with a terminal concentration of 80% and 20% of transfection efficiency. After transfection, the cells were cultured in DMEM medium supplemented with 10% calf serum in a humidified atmosphere of 95% air, 5% CO2 at 37 °C. Bone marrow samples were obtained from individuals subject to marrow examination while hematological disorders were excluded afterward. Mononuclear cells were isolated by density gradient centrifugation using a lymphocyte separation medium. Pooled DNA fragments of non-zinc finger regions of these genes were used as probe pool to screen cDNA libraries of NB4 cells or bone marrow, respectively. Mononuclear cells were isolated by denaturing and adaptation primers from CLONTECH and homemade gene-specific primers. The products of PCR were then separated on polyacrylamide gel electrophoresis and visualized by autoradiography.

**Northern Blot Analysis and Semi-quantitative RT-PCR**—Northern blotting were carried out according to protocol (CLONTECH Inc.) The blots were probed with ZNF191, ZNF253, ZNF254, ZNF255, ZNF256, and ZNF257 cDNA that had been labeled using random primer method. In addition, expression patterns of these genes also were carried out by using semi-quantitative RT-PCR (13). The total RNAs from eight different zinc finger genes were used as probe to screen cDNA libraries of NB4 cells or bone marrow, respectively, for obtaining full-length cDNA.

**Cloning and Characterization of ZNF191, ZNF253, ZNF254,**

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### RESULTS

**DNA Fragments of Zinc Finger Genes Obtained from NB4 Cell Line and Normal Bone Marrow**—Totally, 179 DNA fragments of Cys2/His2 type zinc finger genes were obtained from NB4 cell line and normal bone marrow by RT-PCR using primers corresponding to the consensus sequences of the Krüppel-like zinc finger genes. Sequence analysis showed that there were 17 DNA fragments that encoding zinc finger genes after searching against GenBank. Three DNA fragments of novel genes expressed in NB4 and four fragments of novel genes expressed in bone marrow were used as probe pool to screen cDNA libraries of NB4 cells or bone marrow, respectively, for obtaining full-length cDNA.
ZNF255, ZNF256, and ZNF257 Full-length cDNA—Five pure positive clones were isolated after screening cDNA library of NB4 cells using mixed probes. These fragments contained overlapping sequences and could thus be assembled into a contig. Bioinformatic analysis of this cDNA suggested that it contained an ORF (136–1243 nucleotides) encoding 368 amino acid residues (Fig. 1A). This gene was initially named as RSG-A (for retinoic acid suppressed gene-A) because its mRNA could be amplified by homologous RT-PCR only in retinoic acid-untreated but not in retinoic acid-treated NB4 cells. However, a cDNA for the same gene, with longer 3′-untranslated region and thus 2.5 kilobase pairs in length was also cloned by a different approach, and obtained the international nomenclature ZNF191.

Twelve positive clones were isolated through screening cDNA library of bone marrow. Eight clones with insert size longer than 500 bp were chosen for further study. Sequencing and data base search of these eight clones revealed that four corresponded to known genes and the four others were cDNA fragments of novel zinc finger genes, nominated as ZNF253, ZNF255, ZNF256, and ZNF257. Although in silico cloning and RACE were used to extend the 5′ end of ZNF253 cDNA, no stop codon could be found in frame upstream of an 825-bp ORF encoding 275 amino acid residues (Fig. 1B). The in vitro transcription and translation of ZNF253 gene revealed a protein product of 30 kDa in good agreement with the predicted amino acid sequence derived from its cDNA (data not shown).

ZNF255 gene full-length cDNA was obtained through the following steps. First, cDNA library screening allowed two overlapping clones to be isolated, which, however, only contained the 3′ part of an ORF. The in silico cloning method was then utilized, which identified two ESTs (GB-EST5, N99348; and GB-EST7, AA011208) and extended largely the 5′ end from the existing sequence. An assembled sequence of 3006 bp was thus acquired, which may contain an entire ORF. Finally, a cDNA containing full-length ORF of ZNF255 was amplified from total RNA of bone marrow by RT-PCR using primers based on the assembled sequence. This clone was sequenced again to get the final sequence. As a result, an 1869-bp ORF of ZNF255 gene may encode a protein of 623 amino acids (Fig. 1D).

A clone from cDNA library of bone marrow was a partial 3′ end of ZNF257. Primers were synthesized for getting full-length cDNA of ZNF257 by RACE because neither cDNA library screening nor data base search found clones overlapping with the initial sequence. The sequences from product of RACE PCR could form a contig with that of the original clone. The collective 2181-bp nucleotide sequence thus obtained included a 1605-bp ORF encoding 535 amino acid residues (Fig. 1E).

ZNF254 (also named HD-ZNF1) was identified through EST sequencing of a cDNA library from human CD34+ cells. This cDNA was 1619 bp long, and a 1059-bp ORF was found that encoded 353 amino acid residues (Fig. 1F).

Among the six genes, all have a consensus polyadenylation signal AATAAA in the 3′-end. However, only four (ZNF191, ZNF255, ZNF256, and ZNF257) have “in-frame” stop codon upstream of the first ATG, while two genes (ZNF253 and ZNF254) have ORFs, which could continue to the 5′ end. Although this is so, the two genes have several ESTs matched, which, nevertheless, do not extend to the 5′ end. The amino acid sequences of the two genes were compared with known genes by search against swissprot data base. Both genes share KRAB domain in the N terminus. However, the sequences upstream of the first ATG in KRAB are not homologous with known ones. Thus, we believe that the two genes may contain whole ORF, although further evidence is needed.

All Six Genes Belong to Krüppel-like Zinc Finger Gene Family—Bioinformatics analyses revealed that ZNF191, ZNF253, ZNF254, ZNF255, ZNF256, and ZNF257 were novel genes belonging to the Krüppel-like zinc finger gene family. The deduced amino acid sequences of these genes contain 3–18 tandemly repeated zinc finger motifs related to Drosophila Krüppel gene family at the C terminus and possible transcriptional regulatory elements such as KRAB and SCAN box at their N termini (Fig. 1). The amino acid “knuckle” between zinc finger motifs, typified by the amino acid sequence TGE(R/K)P(F/Y), was also highly conserved in all six deduced amino acid sequences. From these features it was reasonable to predict that all six genes could encode DNA-binding proteins with transcriptional regulatory properties.

A Possible Novel Transregulatory Domain KRNB Analysis of Non-ZF Regions—The deduced 368-amino acid sequence of ZNF191 has four continuous typical Krüppel-like zinc finger motifs in C terminus and contains rich acidic amino acids (15 Asp, 36 Glu) in non-zinc finger region. The non-zinc finger region shared high homology with those in some other Cys2/His2 type zinc finger genes such as human ZNF174, ZNF165, and Q15776 and mouse ZNF38. An 81-amino acid stretch at the N termini of these genes was highly conserved and has been...
Cloning of Six Novel Krüppel-like Zinc Finger Genes

A

| Amino acid alignment of non-zinc finger region from some novel zinc finger proteins. Conserved amino acid residues are in underline. A, amino acid alignment of SCAN box from ZNF191 and its homologous proteins. B, amino acid alignment of KRAB domain from ZNF253 and its homologous proteins. C, amino acid alignment of non-zinc finger region (named KRNB) of ZNF255 and related proteins.

B

FIG. 2. Amino acid alignment of non-zinc finger region from some novel zinc finger proteins. Conserved amino acid residues are in underline. A, amino acid alignment of SCAN box from ZNF191 and its homologous proteins. B, amino acid alignment of KRAB domain from ZNF253 and its homologous proteins. C, amino acid alignment of non-zinc finger region (named KRNB) of ZNF255 and related proteins.

ZNF255

| Amino acid alignment of non-zinc finger region from some novel zinc finger proteins. Conserved amino acid residues are in underline. A, amino acid alignment of SCAN box from ZNF191 and its homologous proteins. B, amino acid alignment of KRAB domain from ZNF253 and its homologous proteins. C, amino acid alignment of non-zinc finger region (named KRNB) of ZNF255 and related proteins.

ZNF191, ZNF253, ZNF255, ZNF256, and ZNF257—Northern blots were performed to explore tissue expression pattern of these genes. However, except for ZNF191 (Fig. 3A), the analysis was not always successful due to relatively low expression level of other genes. Therefore, semi-quantitative RT-PCR was used to determine the expression patterns of these genes in various tissues and leukemia cell lines. All genes were expressed in bone marrow in good agreement with their origin. ZNF191 gene was expressed in almost all tissues and cell lines except for heart, and in fact its expression level was stable in NB4 cells during the course of ATRA treatment. ZNF253, ZNF255, ZNF256, and ZNF257 genes were selectively expressed in different tissues (Fig. 3B). Within the hematopoietic system, ZNF191 and ZNF255 were expressed in all lineages, whereas ZNF253 expression was restricted to monocytic (U937) and immature erythroid (K562) lines. ZNF256 and ZNF257 tended to be expressed in myelomonocytic lineages (HL-60 and U937), although a low expression level could be detected in T lymphocytes (MOLT-4) and early erythroid cells (K562). ZNF253 expression was observed in all lineages except for K562 cells.

Functional Analysis of KRNB in Comparison with SCAN and KRAB Transcriptional Regulatory Domain—To further address the function of the six genes isolated in the present work, ZNF191, ZNF253, and ZNF255 were chosen to study their transcriptional regulatory activities, since these genes contain SCAN, KRAB, and KRNB, respectively. In order to assess whether ZNF191 can affect the transcriptional expression of other genes, the non-zinc finger region (1–251 aa) of ZNF191 (Fig. 3B) was amplified by PCR and subcloned into the expression vectors pGBl (Fig. 4A). When a recombinant pGBl containing GAL4-ZNF191 chimera and control plasmids pGBlt, pGBlt-HA, and pC1 were then used to transform yeast strain Y187. The qualitative and quantitative analyses of β-galactosidase did not show obvious transregulatory activity of ZNF191 in Y187 (Fig. 4A). However, when a recombinant pGBl containing GAL4-ZNF191 chimera was cotransfected with a luciferase reporter plasmid into mammalian cells CHO and NIH3T3, it showed significant transrepression activity (p < 0.05–0.01) (Fig. 4B and C). Analysis using yeast one-hybrid system and mammalian cell transfection for defining the functions of KRAB domain from ZNF253 generated quite coherent results. After Y187 was
transformed with pGBT9-ZNF253 containing GAL4 BD-ZNF253 (1–174 aa) chimera, both qualitative and quantitative assays of β-galactosidase displayed a suppressive effect of ZNF253 non-zinc finger region on the transcription of reporter gene lacZ, making the galactosidase activity lower than that from pGBT9 with minimal basal stimulation (p < 0.01). A similar transcriptional repressor effect was also observed in mammalian cells in that recombinant pM containing GAL4-ZNF253 fusion gene inhibited significantly the expression of reporter plasmid pGAL45tkLUC in CHO, NIH3T3, and COS-7 cell lines (p < 0.05–0.01) (Fig. 4, A–D).

To approach the property in transcriptional regulation of ZNF255 containing KRNB domain, the same experimental procedures were performed. Non-zinc finger region (1–81 aa) including the KRNB domain was subcloned into the pGBT9 and pM to form in-frame fusions, which were used to transform Y187 and transfect mammalian cell lines, respectively. It is interesting to note that the fusion protein GAL4-ZNF255 can stimulate the expression of reporter gene lacZ in yeast (p < 0.001) (Fig. 4A). Moreover, a slight but statistically significant transactivation activity could be observed in NIH3T3 when recombinant pM containing GAL4-ZNF255 chimera was co-transfected into the cell line with reporter gene (p < 0.05–0.01) (Fig. 4, C and E). However, no significant transcriptional activity was found in both CHO and COS-7 cell lines (p > 0.05) (Fig. 4, B and D).

**DISCUSSION**

Cys_{2}/His_{2} type zinc finger gene family is one of the largest gene families, and each member has repeated zinc finger motifs containing finger-like structure by two cysteines and two histidines covalently binding to one zinc ion (18). It is estimated...
that in this huge family, about one third of the members are Krüppel-like genes as characterized by the presence of highly conserved connecting sequences "TGEKPYX" between adjacent zinc finger motifs. Substantial evidence indicates that Krüppel proteins are important players in many physiological processes as transcriptional regulators. Krüppel-like zinc finger genes are key transcriptional repressors in development of Drosophila. In mammalian animal and human, these genes are found involved in embryo development and hematopoiesis. For example, mouse Krox20 gene and both mouse and human PLZF genes have been associated with development of rhombomere

**Fig. 4. Functional analysis of putative transregulatory domain of ZNF191, ZNF253, and ZNF255 by yeast one-hybrid system and mammalian cell transfection.** Each value represents the mean of three replicate assays. The error bars indicate standard deviation from the mean. Where the error bars are not visible, the standard deviation was very small. A, quantitative analysis of β-galactosidase in yeast reporter strain Y187 transformed with hybrid expression plasmids containing different GAL4 fusion protein such as GAL4 BD-ZNF191 (1–251 aa), GAL4 BD-ZNF253 (1–174 aa), and GAL4 BD-ZNF255 (1–81 aa). Y187 were also transformed with the negative control pGBT9, weak positive control pGBT9-HA (hemagglutinin) and strong positive control pCL1 encoding the full-length wild-type GAL4 simultaneously. B and C, analysis of luciferase in CHO and NIH3T3 cells cotransfected by constructive plasmids derived from pM containing GAL4 BD with reporter plasmid pGAL4-tkLUC, respectively. The recombinant pM including GAL4 BD-ZNF191 (1–251 aa), GAL4 BD-ZNF253 (1–174 aa), and GAL4 BD-ZNF255 (1–81 aa), the negative control pM and the positive control pM3-VP16 encoding herpesvirus protein were cotransfected into CHO and NIH3T3 cells with different molar ratios of plasmids to be tested and reporter plasmid. Open columns and filled columns represent ratio of 1:1 and ratio of 1:3, respectively. D and E, the dose-response curves of ZNF191, ZNF253, and ZNF255 in COS-7 and NIH3T3 cells, respectively. The same recombinant pM were transfected into COS-7 and NIH3T3 cells with molar ratio of plasmids to be tested and reporter plasmid as 1:1.

**Fig. 5. Chromosome localization of ZNF191 by fluorescence in situ hybridization.** Arrows indicate the location of the gene.

**Table I**

| Name of genes | Linkage marker | CR10000 | Chromosome |
|---------------|----------------|---------|------------|
| ZNF253        | AFM214YF8      | 1.31    | 19p13.1–13.2 |
| ZNF254        | SHGC-31335     | 30.29   | 19p13.1–13.12 |
| ZNF255        | SHGC-33269     | 39.75   | 19q13.1–13.2 |
| ZNF256        | WI-7361        | 14.52   | 19q13.1–13.13 |
| ZNF257        | SHGC-9450      | 27.78   | 19p13.1–13.13 |

in hindbrain (19, 20). EKLF protein regulates expression of β-globin by binding cis-acting elements to facilitate the switch of hemoglobin from fetal γ-globin to adult β-globin (21–22), whereas MZF1 participates in the progression of differentiation toward granulocytic lineage through high affinity binding to promoters of some specific genes expressed in myelocytic lineage such as CD34, transferrin, and myeloperoxidase (23). On the other hand, PLZF is associated with the pathogenesis of acute promyelocytic leukemia with t(11;17) chromosomal translocation, while Bcl-6 is one of the key genes involved in large cell lymphoma (9–10). The importance of Krüppel-like zinc finger genes in physiological and pathological hematopoietic regulation has prompted us, in the present work, to clone novel members of this family from blood cells of normal and leukemia origins. Since the nucleic acid sequences encoding typical Cys2/His2 motifs and the connecting sequences are well conserved, it was possible to identify new members of this family by homologous RT-PCR amplification/library screening strategy. A large num-
ber of homologous sequences, including novel ones, were indeed obtained from bone marrow and NB4 cell line. This approach, together with cDNA library screening, in silico cloning, and RACE, allowed an efficient working system to be established to clone novel zinc finger genes.

One important clue to the possible functions of these novel zinc finger genes was their expression pattern. It is interesting to note that ZNF253, ZNF254, ZNF256, and ZNF257 are selectively expressed in certain leukemia cell lines representing different lineages, and thus could be related to the differentiation and maturation of hematopoiesis. In contrast, ZNF191 and ZNF255 show ubiquitous expression in leukemia cell line.

Krüppel-like zinc finger family has many subfamilies based on non-zinc finger regions, and these subfamilies play distinct roles in terms of transcriptional regulation of target genes. So far, several domains are found in non-zinc finger regions, such as KRAB, FAX (finger-associated box), POZ (poxvirus and zinc finger), SCAN, FAR (finger-associated repeats), and PR domains (17, 24–27). These domains may affect transcription directly or indirectly. For instance, POZ domain of PLZF may affect transcription by heterodimer formation with SMART, and observed during early time of evolution. These genes are usually involved in cell differentiation and morphogenesis and expressed during embryonic development. According to the bioinformatics analysis, the six genes obtained in this study all belong to type I subfamilies and show relative late appearance in the phylogenetic tree (Fig. 6). Chromosomal localization also supports this conclusion because five of them are aggregated on chromosome 19p13 or 19q13 regions, except for ZNF191, which has been mapped to chromosome 18. The precise functions of these six genes should be further elucidated.

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