

研究报告

Cloning and Characterization of a New Gene Encoding C2H2 Zinc Finger Protein Which May Play an Important Role in T Lymphocyte Development*

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Abstract Some of proteins associated with differentiation and development of cells and tissues contain zinc finger domain. To clone and characterize proteins that are related to the hemopoietic cell differentiation and development. C2H2 zinc finger domains were amplified by RT-PCR with degenerated primers designed according to the conserved amino acids. Total RNA was extracted from human bone marrow. Some expressed sequence tags (ESTs) containing the zinc finger motif were acquired and one of them was used as probe to screen human bone marrow cDNA library. As a result, a new gene (GenBank accession number: AF246126) was cloned and designated HZF2. The full length of the cDNA is 3 888 bp and the open reading frame encodes a protein with 686 amino acid residues containing 17 typical and 2 atypical C2H2 zinc finger motifs. Northern blot and Human RNA Master Blot analysis suggested that HZF2 might play an important role in T cell development and amplification. The fragment encoding the complete HZF2 peptide was inserted into the eukaryotic expression vector pEGFP-N1 and introduced into 3T3 cells. The fusion protein was located in cell nuclei, which was a consistent with the conjecture that HZF2 may function as a DNA-binding protein to regulate gene transcription.

Key words zinc finger protein, cDNA library screening, expression spectrum, T cell development

Differentiation and development of cell, tissue and organ is a complex process and it is associated with the special gene expression. Transcriptional factors play very important role in regulation of specific gene expression. Transcription factors perform at least two functions: They bind specifically to *cis*-acting elements of genes and they regulate transcription initiation. The structure analysis of the transcription factors revealed the DNA-binding domains are usually modular in nature. Zinc finger motif is one of typical DNA binding motifs and it can be divided into three types, C2H2, C4 and C6. C2H2 type is the most common one. Sometimes cysteine (C) and histidine (H) can be substituted with aspartates (R) and glutamic acids (Q). Several C2H2 type zinc finger proteins that have important functions have been identified and characterized.

In order to identify new zinc finger proteins that may be involved in blood cells differentiation and development, we designed degenerated primers according to conserved amino acids in C2H2 motifs to amplify cDNA fragment encoding the zinc finger motifs using the human bone marrow cDNA as template^[1]. We used rapid amplification of cDNA end (RACE) combining with cDNA library screening to overcome the false positive that may be caused by DNA homology^[2]. As a result, a new gene (GenBank accession number: AF246126), named HZF2, was identified. The full

length of HZF2 is 3 888 bp with an open reading frame encoding a peptide of 686 amino acid residues containing 19 C2H2 zinc finger motifs. We analyzed and deduced its function according to the result of its mRNA transcription in human tissues.

1 Materials and methods

1.1 Materials and reagents

Trizol, M-MLV reverse transcriptase, RNasin, DNase I, LipofectamineTM 2000 and human bone marrow cDNA library are products of Gibco BRL Company. DNA fragment purify kit is from BioDev. T-vector and ³²P labeling kit are from Promega. Primers were synthesized by Shanghai Sangon. The Multiple Tissues Northern (MTNTM) Blots and Human RNA Master Blot were obtained from Clontech Company. [α -³²P]-dCTP was from Beijing Yahui Company.

1.2 Methods

1.2.1 Extract of total RNA from bone marrow: About 2 ml bone marrow was obtained from a normal volunteer total cellular RNA was extracted from bone

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marrow cells using Trizol.

1.2.2 Reverse transcription PCR, subcloning and sequencing: After treatment of the RNA with DNase I mRNA was reverse transcribed into cDNA with M-MLV reverse transcriptase using Oligo (T15) as primer. The cDNA was used as template for PCR amplification of the sequences encoding C2H2 zinc finger domain. The sequences of the degenerated primers were: 5' GG(TC) TT(TC) TC(ATCG) CC(ATCG) GT(AG) TG 3'; 5' TG(TC) CC(ATCG) GA(GA) TG(TC) GG(ATCG) AA 3'. The procedure of PCR is different from normal PCR. The reaction was split into three parts. The steps for the first part were at 94°C for 1 min, at 37°C for 2 min, heating to 72°C in 2 min and extending for 3 min. Totally 5 circles were included in this part. There were 35 circles in part 2 and the steps were at 94°C for 1 min, at 45°C for 2 min, at 72°C for 3 min, and it was followed by an extension time for 10 min as part 3. PCR product was verified by low melting agarose gel electrophoresis and the DNA fragments were cloned into pGEM-T easy vector. DNA sequencing reactions were performed using the BigDye terminator cycle sequencing kit.

1.2.3 Nested-PCR and human bone marrow cDNA library screening: One EST was used for screening human bone marrow cDNA library. Based on the EST sequence and the result of homology comparison by NCBI blast, nest PCR primers were designed in non-homology region at the 5' primer of the selected EST. These primers, combined with the SP6 promoter primer that are located in the upstream of the multiple cloning sites of the vector plasmid of the cDNA library, were used to extend the 5' side of the cDNA containing the EST. The amplified fragment was labeled with α -³²P and used for screening human bone marrow cDNA library. Several positive clones were picked up after the first round of screening. The positive clones were screened for two additional rounds to get single positive colonies. Several positive clones were acquired after three rounds of screening. The longest clone was sequenced.

1.2.4 DNA sequencing and cDNA sequence analysis: The clone with the longest insert was sequenced by Boyia Company in Shanghai. Nucleic acid and protein database searches were performed at the NCBI, EMBL, DDBJ, PDB server. Nucleic acid and protein sequences were sent to GenBank database to register. DNAMAN and DNASIS software were used to analysis the ORF and compare the cDNA sequence with the EST sequence.

1.2.5 Analysis of HZF2 transcript in tissues: The 0.8 kb fragment, which is located at the 3' side of HZF2 cDNA and is low homology with known DNA sequences, was labeled by ³²P. The Multiple Tissues

Northern (MTNTM) Blots and Human RNA Master Blot membrane were hybridized with the ³²P labeled probe. Prehybridize the blot in 10 ml of ExpressHyb with 1.0 mg sheared salmon testes DNA for 30 min at 65°C. Mix the labeled probe with 30 μ g of C₀t-1 DNA, 150 μ g of sheared salmon testes DNA, and 50 μ l of 20 \times SSC, in a total volume of 200 μ l. Add the probe in 5 ml of ExpressHyb with 0.5 mg sheared salmon testes DNA for 6 h with continuous agitation at 65°C. The blot was washed twice at 55°C for 30 min and exposed to X-ray film at -70°C.

1.2.6 Subcellular localization of HZF2-GFP fusion protein in transfected 3T3 cells: Full-length HZF2 ORF was inserted into pEGFP-N1 vector. 3T3 cells were transfected with the construct using LipofectamineTM 2000. Subcellular localization of the fusion protein was checked by fluorescence microscope 24 h after transfection.

2 Results

2.1 RT-PCR amplification of RNA regions encoding zinc finger domain

After the products of reverse transcription of bone marrow RNA were PCR amplification using the degenerated primers, electrophoresis was carried out through agarose gel. Six bands, with the lengths of 81 bp, 162 bp, 243 bp, 324 bp, 405 bp and 486 bp respectively, were identified and they stand for DNA corresponding 1 to 6 zinc finger motifs respectively.

2.2 Nest-PCR and human bone marrow cDNA library screening

Non-homology region of 5' end of one selected EST was amplified by nest-PCR method. The amplification fragment was cloned into T-vector easy and was used as a probe for screening human bone marrow cDNA library. Several positive clones were acquired after three rounds of screening. The length of inserts was identified by PCR. Several inserts with different length were observed, which stand for cDNA fragments derived from identical mRNA but with differentially truncated 5' end. The longest one was 3.9 kb.

2.3 Analysis of the cDNA sequence

The clone was sequenced. The length of the cDNA was 3 888 bp (Figure 1). Analysis of the sequence by DNAMAN software revealed a full read frame encoding 686 amino acids from 1 074 bp to 3 134 bp. Continuous 19 C2H2 zinc finger motifs including 17 typical and 2 atypical ones were identified. We designated it as human zinc finger protein 2 (HZF2). Its GenBank access number is AF246126.

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1  ctggtccgga attcccggt cgaccacgc gtcgctttt atgtaatgac accattactg
61  cttgtgatta ttctgatatg atactttatc tgtttttttt tcttaaatat cacaagctat
121 taaattttat tcacaattga ctagtagacc actttgtcaa ttggaaaac attctgttta
181 aagacaaaac tatctacatc cagaaacttt ctataccttg ctttcaaaga gacagcattc
241 tttgctagcc ttccaatttt gactcagtcg aaaaacctta gaaggatcca gtgcataggt
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481 gctcaaacct aactctggga ttgaagtaga gaagaaatgt tggaaagtc ttaatataga
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601 ggcagtaact tctgggctgt ggttgaaac tgtgagcact acaaaatgtt ttctctattt
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721 gtgtttttta atgggtttca ttagtgctta gcaattggga gcttgggtga ccatctcttg
781 gttttggacc atctcttggt ttctgtcagt atgtaaaacca gaaacttcaa atgtgtcaca
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901 atcttaaatc ttgtgaacaaa aaaattcact atctctctga atcctttgtc cttacaggag
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1 021 gttggcaaat ctggcaacaa attgcaaatg acttaaccag gtgtcaagac tccATGatca
1 081 aaaaattcga gtgtcaacaa caagggtgatt tcccttacca ggtagggaca gaactgtcta
1 141 ttcaaatctt tgaagatgag aactatatag taaataaagc agatgggtccc aataaactgt
1 201 ggaatccaga gtttctctatc ttgagaaccc aggattcttg gaggaaaaca ttctgactg
1 261 agtcacagag attgaacaga gatcagcaaa ttccataaaa aaataaatta tgtcaatgta
1 321 agaagggtgt tgaatccatc ggttggattt cacatcatga tgggtcataga gtacacaaaa
1 381 gtgaaaaatc ttatagacc aatgattatg aaaaagacaa catgaagatt ttgacatttg
1 441 atcacaatag catgattcac acaggacaga aatogtacca gtgtaattgag tgaataaaac
1 501 cttcagtaga tctctccagc ttgatctctc atcagcagtt acaatcagga gagaagcttc
1 561 ttacatgtgt tgagcgttga aaaggcttct gttacagccc agttcttctc gttcatcaga
1 621 aagtacatgt gggagaaaaa cttaatgtgt atgagtgtgg taaggaaatc agtcaggcgg
1 681 ctcatctaca gaccctcag aaagtccacg tgatagagaa accatacaaa tgaagcaat
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1 801 agaaacctta taattgtgag gagtgtggga gggccttcag tcaggcctct catcttcagg
1 861 accatcagag actccacact ggggagaagc cattcaaatg tgatgcattg ggtaaggagt
1 921 tcagtcggaa ttcatctctt caatcccatc aaagagttca tacaggagag aaacatcata
1 981 aatgtgagga gtgtgttaag ggcttcattt gtatgtcaaa tctttacatt catcagaggt
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2 461 tccatacagg ttgagaacca tacaatgtgt aagagtgtgg caagggtatt agtcgtagag
2 521 cagatcttaa aattcactgt aggtccacac caggagagaa accatataat tgtgaggagt
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2 641 aaaaaccatt caaatgtgaa gaatgtggga agagtttcgg tcggagtcca catcttcaag
2 701 cccatcaaaa agtccacact ggagataagc catcaaatg tgatgagtgt ggggaaggct
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2 821 aatgtgggga gtgtgttaag tacttcagtc aggcctcaag tcttcaactt catcagagt
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3 241 aatctttatg attagcatag caagggtctt tttagtccga acctgacctt ttataaaatg
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3 661 tctctcttta gccctactaa tcaagtgtgg tcttatcttc ccagatgtct agttttctac
3 721 tggttttata ccatatagat tcaattatct tttgtaacag ataatttgtt tcatcttgta
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3 841 gtgaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaa

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MINNSQCHKQGDFFPYQVGTLSIQISEDENYIVNKADGPNNTGNPEPILRTQDSWRKFTLTESQRLNRDQQISIKNKLQCKKGVDPIGWISHDHGRVHKSEKSYRPN
DYEDKNMKILTFDHNMSIHTGQKSYQ[Q]NE[Q]KPPFDLSSFDL[H]QQL[S]GSEKSLT[C]VER[G]KGFCYSPVLPV[H]QKV[H]VGEKLA[C]DE[C]GKEFSQGAHLQ[T]H[Q]KV[H]VIEKP
YK[Q]KQ[G]KGFSRRSALNV[H]CKV[H]TAEKPYN[C]E[C]GGAFFSQAHLQD[H]QRL[H]TGEKPPK[C]DAC[G]KSFPSRNSHLQSH[Q]RV[H]TGEKPYK[C]E[C]GKGFCSSNLYI[H]QRV
[H]TGEKPYK[C]E[C]GKGFSRPSLQA[H]QGV[H]TGEKSYT[C]TVC[G]KGFTLSSNLQA[H]QV[H]TGEKPYK[C]NE[C]GKSFRNSHYQVHLV[H]TGEKPYK[C]E[C]GKGFSQSSYL
Q[H]QKAH[S]IEKPPK[C]E[C]GQGFNQSSRLQI[H]QL[H]TGEKPYK[C]E[C]GKGFSRRADLKI[H]CRI[H]TGEKPYN[C]E[C]GKVFRQASNLHA[H]QV[H]SGBKPPK[C]E[C]GKSF
GRSAHLQA[H]QV[H]TGDKPYK[C]E[C]GKGFKWSLNDM[H]QV[H]TGEKPYK[C]E[C]GKYFSQASSLQI[H]QSV[H]TGEKPYK[C]DVC[G]KVFSRSSQLQSH[Q]RV[H]TGEKPYK[C]E
ICGKSFWSRNSLT[H]HRI[H]VGDKSYKSNRGGKNIRESQEKKSIIK

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Fig.1 Sequence of HZF2 cDNA and the deduced amino acids

ATG:start code, TGA:stop code. The amino acids of zinc finger motifs are underlined. [Q] and [H]: cysteine and histidine by which a Zn ion is liganded from the zinc finger motif.

2.4 Analysis of HZF2 transcript in tissues

A 0.8 kb fragment that locates in 3' side of the cDNA and low homology with the known DNA sequences was used as a probe for hybridization with the multiple tissues Northern blot mRNA transfer membrane (Figure 2). The result showed that HZF2's transcription in lymph node and thymus is pretty high. Its transcription in spleen and fetal liver is lower than that in lymph node and thymus. The transcription is very low in bone marrow and it is hardly detectable in peripheral blood leukocyte.

Human RNA Master Blot membrane was also used for analysis of HZF2 transcript in 60 adult tissues, 7 fetal tissues and 8 tumor cell lines. The results for adult lymph node, thymus, spleen, bone marrow, fetal liver and peripheral blood leukocyte were consistent with the results by using the Northern blot mRNA transfer membrane. In addition HZF2 transcript was also highly in some portions of brain, placenta and adrenal gland while it was very low in other tissues. HZF2 transcript in 8 tumor cell lines including B lymphocyte tumor cell lines Raji and Daudi and T lymph cell leukemia cell line MOLT-4 was also very low (Figure 3).

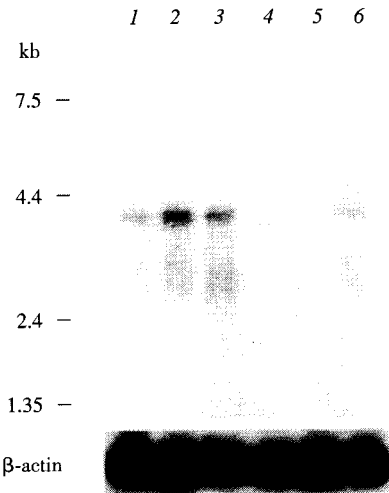


Fig. 2 Analysis of HZF2 transcript in human tissues associated with blood cells
1: spleen; 2: lymph node; 3: thymus; 4: peripheral blood leukocyte; 5: bone marrow; 6: fetal liver.

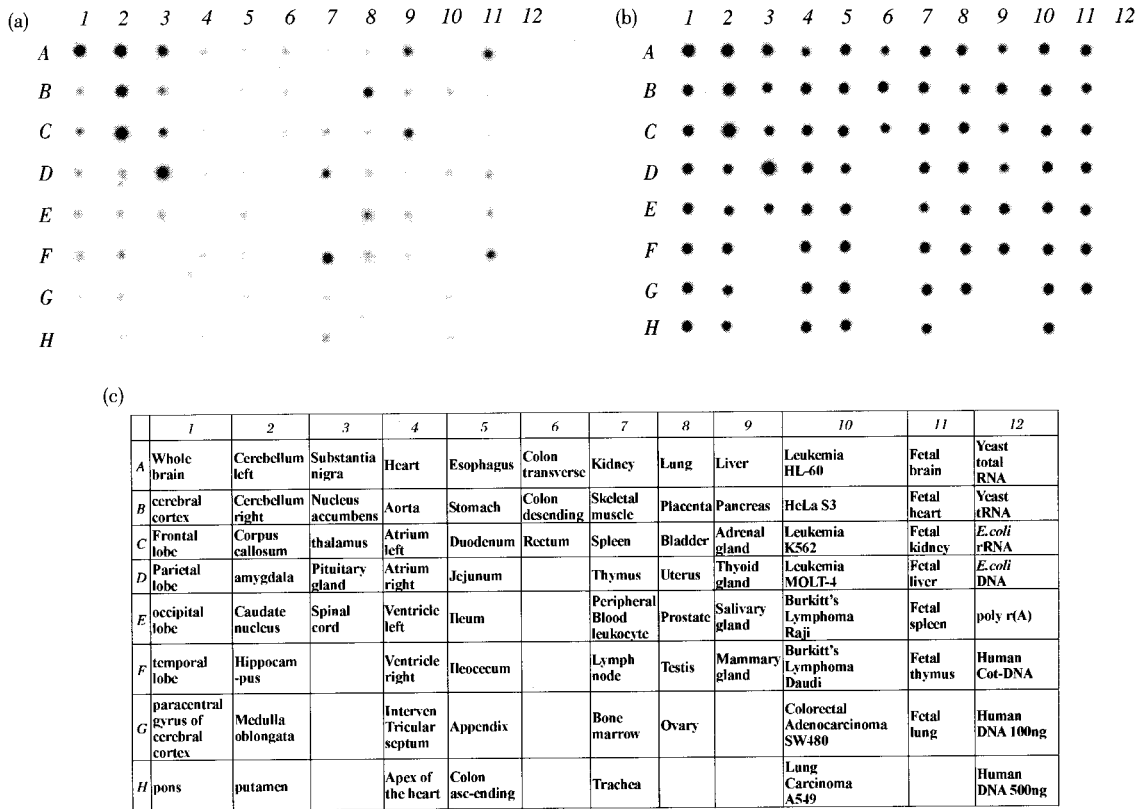


Fig. 3 Analysis of HZF2 transcript in human tissues using Human RNA Master Blot membrane
(a) Hybridization with 3' end of HZF2 cDNA as a probe; (b) Hybridization with β-actin cDNA as a probe; (c) 60 human tissues, 7 fetal tissues and 8 tumor cell lines mRNA blotted on Human RNA Master Blot membrane.

2.5 Subcellular localization of HZF2-GFP fusion protein in 3T3 cells

3T3 cells were transfected with the pEGFP-N1-HZF2 construct. HZF2-GFP fusion protein is located in the nuclei while the EGFP in the whole cells (Figure 4).

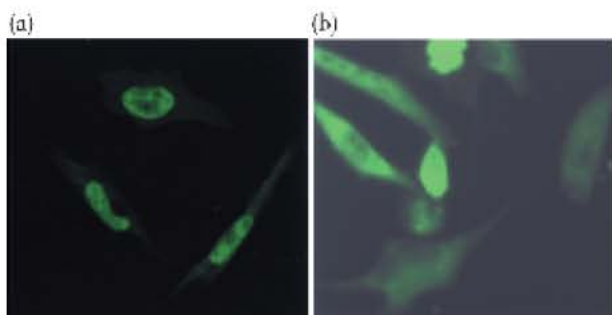


Fig. 4 Subcellular localization of HZF2-GFP fusion protein in 3T3 cells

(a) pEGFP-N1-HZF2 construct; (b) pEGFP-N1 control.

3 Discussion

Zinc finger motif is an important DNA-binding domain in transcription factors. Hundreds of zinc finger proteins have been identified and characterized up to now. There are several zinc finger proteins related to hematopoiesis, such as GATA family, FOG^[3], EKLF and FKL-2, which play roles in red cell and megakaryocyte differentiation, WT1 and Egr-1^[4] in macrophage differentiation, as well as MZF-1 in the differentiation, proliferation and apoptosis in earlier myeloid cells^[5,6].

HZF2 is a cDNA we cloned from human bone marrow cDNA library. It is 3 888bp long and contains an open reading frame of 686 amino acids. The deduced protein contains 17 typical and 2 atypical C2H2 zinc fingers continuously. Further analysis showed that there were 5 bases different between HZF2 cDNA and the EST sequence while these differences did not change amino acids, suggesting that there are single nucleotide polymorphisms within the gene in different individuals. Variation of nucleotides but conservation of amino acids coded is a character of many genes that are crucial for cell normal functions.

Northern blot and human RNA Master Blot analysis showed HZF2 transcript with a high levels in lymph node, thymus, placenta, adrenal gland and several portions of brain, with a moderate level in spleen, fetal liver, with a low level in bone marrow

and hardly detectable in peripheral leukocyte. Thymus is a central immune organ. In adults progenitors of T cells initiate from bone marrow and then immigrate into thymus where they differentiate and develop. Then the matured T lymphocytes leave thymus and habit in peripheral immune organs. Lymph node and spleen are important peripheral immune organs where there are large numbers of T and B lymphocyte as well as cells that support and promote their development and amplification. Bone marrow is the major organ where B cells develop. From the high expression of HZF2 in thymus and lymph nodes we can speculate that HZF2 is associated with T and B lymphocyte. From the low expression of HZF2 in bone marrow where B cells develop the relation between HZF2 and B cell development could be excluded. Moreover, from the hardly detectable HZF2 transcript in peripheral leukocyte with abundant T cells and in T lymphocyte leukemia line MOLT-4, we can conclude that HZF2 is transcribed in stoma cells that are associated with T cell development. Additionally, the relatively high-level transcription of HZF2 gene in some portions of brain, placenta, adrenal gland suggested that it may play roles in maintaining essential functions in these tissues. Since several zinc finger proteins have been identified to function as DNA-binding proteins to regulate the gene transcription, the localization of HZF2 in nuclei is accordant with its character as a zinc finger protein.

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一个可能与 T 细胞发育相关的新的 C2H2 型锌指蛋白基因的克隆*

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摘要 一些在组织和细胞分化中起重要作用的蛋白质包含锌指结构域。为了克隆分离和研究与造血细胞分化和发育成熟相关的蛋白基因, 利用编码 C2H2 型锌指蛋白结构域中部分保守氨基酸序列设计简并引物, 以骨髓 cDNA 为模板, 进行 PCR 扩增, 得到若干新的锌指蛋白基因 EST。用其中一条为探针筛选人骨髓 cDNA 文库, 获得了一个新的锌指蛋白基因全长 cDNA, GenBank 收录号为 AF246126, 长 3 888 bp, 包括一个完整阅读框, 编码 686 个氨基酸, 包括 17 个典型的和 2 个非典型的 C2H2 模体, 命名为 HZF2。RNA 印迹、人多组织 mRNA 斑点杂交分析结果显示, 其在 T 淋巴细胞发育和定居的器官组织胸腺、淋巴结中有较高表达, 在脾脏、胎肝有中度表达, 在 B 淋巴细胞发育的骨髓中表达很低, 在外周血几个淋巴细胞系中仅有极微量的表达, 提示 HZF2 可能对于 T 淋巴细胞发育和增殖有重要功能。该基因也在脑组织的若干部位、胎盘及肾上腺有较高表达, 在多种其他组织细胞有微量表达, 说明其可能对维持这些组织细胞的生理功能也起一定作用。将编码 HZF2 读框的 DNA 顺序克隆到 pEGFP-N1 载体中, 转染 3T3 细胞, 证明表达的 HZF2-GFP 融合蛋白定位于细胞核, 这与根据 HZF2 蛋白结构推测其可能作为 DNA 结合蛋白行使调节基因转录的功能是一致的。

关键词 锌指蛋白, 基因克隆, cDNA 文库筛选, 表达谱, T 细胞发育

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