STEM CELLS®

STEM CELL GENETICS AND GENOMICS

Higher Expression of Transcription Targets and Components of the Nuclear Factor-κB Pathway Is a Distinctive Feature of Umbilical Cord Blood CD34$^+$ Precursors

RODRIGO ALEXANDRE PANEPUCCI,[a] RODRIGO TOCANTINS CALADO,[a] VANDERSON ROCHA,[b] RODRIGO PROTO-SIQUEIRA,[a] WILSON ARAUJO SILVA, JR.,[a] MARCO ANTONIO ZAGO[a]

[a]Center for Cell Therapy and Regional Blood Center, Department of Clinical Medicine, Faculty of Medicine, University of São Paulo, Ribeirão Preto, Brazil; [b]Bone Marrow Transplantation Unit, Hôpital Saint-Louis, Paris, France

Key Words. Hematopoiesis • Stem cells • Nuclear factor-κB • Umbilical cord blood • Bone marrow

ABSTRACT

Delayed engraftment, better reconstitution of progenitors, higher thymic function, and a lower incidence of the graft-versus-host disease are characteristics associated with umbilical cord blood (UCB) transplants, compared with bone marrow (BM). To understand the molecular mechanisms causing these intrinsic differences, we analyzed the differentially expressed genes between BM and UCB hematopoietic stem and progenitor cells (HSPCs). The expressions of approximately 10,000 genes were compared by serial analysis of gene expression of magnetically sorted CD34$^+$ cells from BM and UCB. Differential expression of selected genes was evaluated by real-time polymerase chain reaction on additional CD34$^+$ samples from BM (n = 22), UCB (n = 9), and granulocyte colony stimulating factor-mobilized peripheral blood (n = 6). The overrepresentation of nuclear factor-κB (NF-κB) pathway components and targets was found to be a major characteristic of UCB HSPCs. Additional promoter analysis of 41 UCB-overrepresented genes revealed a significantly higher number of NF-κB *cis*-regulatory elements (present in 22 genes) than would be expected by chance. Our results point to an important role of the NF-κB pathway on the molecular and functional differences observed between BM and UCB HSPCs. Our study forms the basis for future studies and potentially for new strategies to stem cell graft manipulation, by specific NF-κB pathway modulation on stem cells, prior to transplant. STEM CELLS *2007;25:189–196*

INTRODUCTION

The appearance of hematopoietic stem and progenitor cells (HSPCs) in the early embryo occurs at a site called aorta-gonad-mesonephros. Later, HSPCs from this site home to secondary sites in the fetus such as the liver, and finally into the bone marrow (BM), where definitive hematopoiesis takes place in adults [1]. In addition to the BM, HSPCs also home to organs such as the thymus to generate T cells, a process much more active in children [2]. These cells still circulate in high numbers at birth, so that the umbilical cord blood (UCB) is enriched with HSPCs. The use of UCB as a source of stem cells for transplants has proved beneficial both in children and adults; the outcomes of patients transplanted with UCB differ from those receiving bone marrow, with a lower incidence of the graft-versus-host disease (GVHD) but with a delayed engraftment [3]. UCB provides a better reconstitution of early and committed progenitors compared with BM as the source of HSPCs, indicating that UCB-derived HSPCs would privilege self-renewal at the expense of differentiation and maturation [4]. UCB CD34$^+$ cells also show a superior overall engraftment in non-obese diabetic/severe combined immunodeficient mice [5, 6]. Furthermore, thymic function and T cell receptor (TCR) diversity are higher in UCB recipients than in BM recipients [7]. In vitro, UCB CD34$^+$ HSPCs show higher migration across fibronectin coated and uncoated filters [8], and the ability to generate T lymphocytes on fetal thymic organ cultures without prestimulation, unlike BM-derived cells [9]. The number and generative potential of B-lymphocyte progenitors is also higher in UCB HSPCs [10]. Although the basis for the differences observed between BM and UCB transplants is not well-studied, some of these differences can be partially explained by differences in graft cell composition, as well as intrinsic molecular features of HSPCs from both sources. For instance, the reduced incidence of GVHD on UCB transplants may be partially explained by the reduced T cell number in these graft sources or by a reduced immune response of those cells, whereas delayed neutrophil engraftment may result from the reduced number of total cells infused [11]. Furthermore, the more primitive CD38$^-$ subset of CD34$^+$ HSPCs is more abundant in UCB [12], and although this may partially explain the better reconstitution of early and committed progenitors in UCB transplants, even this subpopulation has distinct intrinsic properties depending on the ontological age [13], with UCB cells showing a higher generative potential [6] and a higher in vitro migration compared with BM and mobilized peripheral blood (MPB) [8]. In fact, differences between CD38$^+$ and CD38$^-$ subsets are less pronounced in CD34 cells from UCB than BM [12]. The later observations highlight an intrinsic molecular feature that would partially

Correspondence: Marco Antonio Zago, M.D., Ph.D., Hemocentro, Rua Tenente Catão Roxo 2501, 14051-140 Ribeirão Preto, Brazil. Telephone: 55-16-2101-9361; Fax: 55-16-2101-9309; e-mail: marazago@usp.br Received May 30, 2006; accepted for publication September 7, 2006; first published online in STEM CELLS *EXPRESS* September 14, 2006. 1066-5099/2007/$20.00/0 doi: 10.1634/stemcells.2006-0328

Table 1. Differentially expressed transcripts between UCB and BM HSPCs

Tag	UniGene	Symbol	Description	UCB	BM	Fold
Tags overrepresented in UCB HSPCs						
ATGCAGAGCT	Hs.530049	HBG1[a,b]	Hemoglobin γA	151	1	151
ATTCAGAGCT	Hs.530049	HBG1[a,b]	Hemoglobin γA	61	—	61
GATAACACAT	Hs.75703	CCL4[c]	Chemokine (C-C motif) ligand 4	49	1	49
CTTCTTGCCC	Hs.398636	HBA2[b]	Hemoglobin α2	215	7	30.7
CCCAACGCGC	Hs.398636	HBA2[b]	Hemoglobin α2	118	6	19.7
ACTCAGCCCG	Hs.525607	TNFAIP2	Tumor necrosis factor, α-induced protein 2	17	1	17
GAAAGATGCT	Hs.398989	BEX2	Brain expressed X-linked 2	17	1	17
GCACCAAAGC	Hs.512304	MGC12815	Chemokine (C-C motif) ligand 3-like, centromeric	66	4	16.5
CAATTTGTGT	Hs.126256	IL1B[c]	Interleukin 1β	32	2	16
CTTCTGCCCC	Hs.76480	UBL4	Ubiquitin-like 4	14	1	14
TTGAAGCTTT	Hs.75765	CXCL2	Chemokine (C-X-C motif) ligand 2	14	1	14
CCTGTAATCT	Hs.148584	LOC51333	Mesenchymal stem cell protein DSC43	13	1	13
GAATTAACAT	Hs.513851	YWHAE	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide	13	1	13
ACATTTCCAA	Hs.432132	G0S2[a]	Putative lymphocyte G0/G1 switch gene	12	—	12
ATAATAAAAG	Hs.89690	CXCL3	Chemokine (C-X-C motif) ligand 3	12	—	12
GGAAGGGGAG	Hs.73090	NFKB2[c]	Nuclear factor of κ light polypeptide gene enhancer in B-cells 2 (p49/p100)	94	8	11.8
ACACTAAAAT	Hs.458276	NFKBIE	Nuclear factor of κ light polypeptide gene enhancer in B-cell inhibitor, epsilon	11	—	11
GTTCACTGCA	Hs.515126	ICAM1[a,c]	Intercellular adhesion molecule 1 (CD54), human rhinovirus receptor	22	2	11
CTAAACTTTT	Hs.180919	ID2	Inhibitor of DNA binding 2, dominant-negative helix-loop-helix protein	21	2	10.5
TCCTTGCTAC	Hs.75256	RGS1	Regulator of G-protein signaling 1	21	2	10.5
AATGAGCAAC	Hs.386567	GBP2	Guanylate binding protein 2, interferon-inducible	10	—	10
GCCACCATCA	Hs.284244	FGF2	Fibroblast growth factor 2 (basic)	10	—	10
GGAAAAGTGG	Hs.525557	SERPINA1	Serine (or cysteine) proteinase inhibitor, clade A (α-1 antiproteinase, antitrypsin), member 1	10	—	10
TAGTTGGAAA	Hs.524430	NR4A1[a]	Nuclear receptor subfamily 4, group A, member 1	19	2	9.5
CTGAGGTGTG	Hs.170019	RUNX3	Runt-related transcription factor 3	24	3	8
TGGAAGCACT	Hs.624	IL8[a,c]	Interleukin 8	68	9	7.6
TGGGGGCACC	Hs.307905	RELB[c]	V-rel reticuloendotheliosis viral oncogene homolog B, nuclear factor of κ light polypeptide gene enhancer in B-cells 3 (avian)	26	4	6.5
TGGGGTTTCC	Hs.448223		CDNA FLJ34941 fis, clone NT2RP7007480	32	5	6.4
AAGGGAGGGT	Hs.529892	SQSTM1	Sequestosome 1	25	4	6.3
TCCGTGGTTG	Hs.201641	BASP1[a]	Brain-abundant, membrane-attached signal protein 1	18	3	6
TGTTTTCATA	Hs.512305	CCL4L[c]	Chemokine (C-C motif) ligand 4-like	18	3	6
CCAGGCCGGG	Hs.119302	C1QTNF4	C1q and tumor necrosis factor-related protein 4	23	4	5.8
CTTCTGGGGA	Hs.501728	RHOG	Ras homolog gene family, member G (rho G)	22	4	5.5
CTGCATCTTA	Hs.73797	GNA15	Guanine nucleotide binding protein (G protein), α15 (G_q class)	32	6	5.3
GTCATAGCTG	Hs.466759		Transcribed locus, weakly similar to XP_510104.1 similar to hypothetical protein FLJ25224 [Pan troglodytes]	20	4	5
CGACGAGGAG	Hs.9999	EMP3	Epithelial membrane protein 3	39	8	4.9
TTGCGTGTGT	Hs.1183	DUSP2	Dual specificity phosphatase 2	25	6	4.2
TAAAAAAAAA	Hs.381126	RPS14	Ribosomal protein S14	28	8	3.5
CAGTTCTCTG	Hs.521487	MGC8721	Hypothetical protein MGC8721	40	12	3.3
CTGCCAAGTT	Hs.490415	ZYX	Zyxin	31	10	3.1
GGGGCTGTAT	Hs.1103	TGFB1[c]	Transforming growth factor β1 (Camurati-Engelmann disease)	37	12	3.1
GCAAGAAAGT	Hs.523443	HBB[a]	Hemoglobin β	101	34	3
GGCTTTACCC	Hs.534314	EIF5A	Eukaryotic translation initiation factor 5A	35	12	2.9
TCAGATCTTG	Hs.550542	MED12L	Mediator of RNA polymerase II transcription, subunit 12 homolog (yeast)-like	51	18	2.8
GCAGTGGGAA	Hs.376208	LTB	Lymphotoxin β (TNF superfamily, member 3)	62	23	2.7
Tags overrepresented in BM HSPCs						
AGCATCTCCA	Hs.535791		LOC441069	1	30	−30
AAGATTGGTG	Hs.114286	CD9	CD9 antigen (p24)	1	29	−29
TCTGCAAAGG	Hs.434953	HMGB2	High-mobility group box 2	1	19	−19
ATCACGAAGG	Hs.369921	VAV2	Vav 2 oncogene	1	16	−16
TTTATGACTG	Hs.534206	DNTT	Deoxynucleotidyltransferase, terminal	2	30	−15
GCCTGCTATT	Hs.380781	DEFA1	Defensin, α1, myeloid-related sequence	1	15	−15
GGCTGGGGCC	Hs.99863	ELA2[a]	Elastase 2, neutrophil	2	22	−11
TGTTTGTGTG	Hs.440494	CKLFSF7	Chemokine-like factor super family 7	2	19	−9.5
GAAGAGGGTG	Hs.247979	VPREB1	Pre-B lymphocyte gene 1	7	61	−8.7
CTGGCCCGAG	Hs.504877	ARHGDIB	Rho GDP dissociation inhibitor (GDI) β	6	39	−6.5
CACGAAGGGA	Hs.348935	IGLL1	Immunoglobulin λ-like polypeptide 1	20	121	−6.1
GACCCAACTG	Hs.89575	CD79B	CD79B antigen (immunoglobulin-associated β)	11	61	−5.5
GTGCGCTGAG	Hs.534125	HLA−C	Major histocompatibility complex class I C	12	61	−5.1
GCTCCCCTTT	Hs.458272	MPO	Myeloperoxidase	15	61	−4.1
GTAATCCTGC	Hs.443609	SLCO5A1	Solute carrier organic anion transporter family, member 5A1	19	76	−4
AAGGTCGAGC	Hs.477028	RPL24	Ribosomal protein L24	12	38	−3.2

Expression profiles of CD34+ HSPCs from BM and UCB were generated by serial analysis of gene expression (SAGE) and compared. Differentially expressed transcripts were selected based on experimental significance ($p < .001$) and fold differences (>2.5). The SAGE technique generates a specific 10-base pair sequence (Tag) from each mRNA transcript expressed which are counted (UCB and BM columns) and linked to a specific gene cluster (UniGene). Fold values were calculated based on actual *tag* counts; in the case of absent tags (indicated by dashes), fold was calculated by dividing tags by 1, yielding an approximate fold value.

Abbreviations: BM, bone marrow; HSPC, hematopoietic stem and progenitor cell; UCB, umbilical cord blood.

[a]Genes validated on independent studies.

[b]Genes represented by two distinct tags.

[c]Genes validated by real-time polymerase chain reaction.

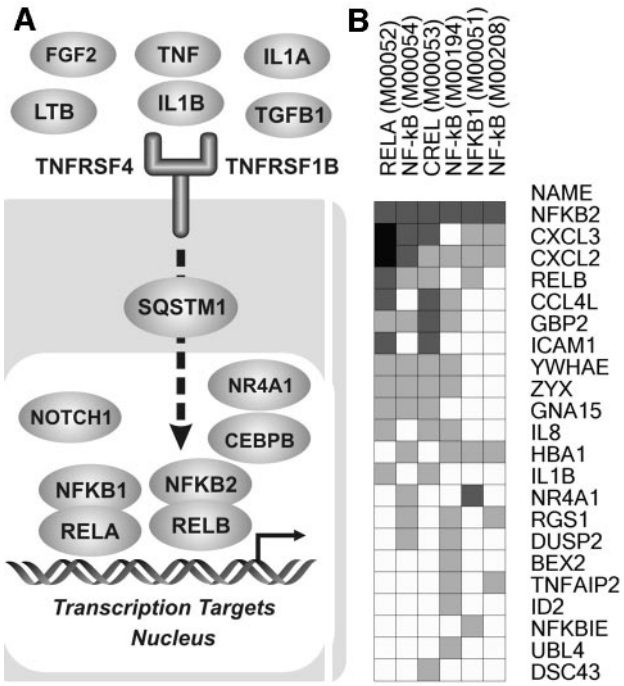

Figure 1. NF-κB signaling and transcriptional targets in umbilical cord blood (UCB) hematopoietic stem and progenitor cells (HSPCs). **(A):** Schematic illustration of NF-κB signaling components overrepresented on UCB HSPCs. **(B):** Heatmap illustrating the number of NF-κB binding sites on known or potentially new transcription targets. A promoter analysis carried out on UCB-overexpressed genes highlighted five NF-κB binding sites (BSs) overrepresented along 22 of 43 gene promoters. Numbers of NF-κB BS are shown as follows: 0, white; 1, light gray; 2, dark gray; 3, black. BSs are shown in decreasing order of significance from left to right. Transfac accession numbers are shown in parentheses. M00208 was not among the overrepresented BSs found. Abbreviations: CEBPB, CCAAT/enhancer-binding protein B; FGF, fibroblast growth factor; ICAM, intercellular adhesion molecule; IL, interleukin; LTB, lymphotoxin-β; NF-κB, nuclear factor-κB; TGF, transforming growth factor; TNF, tumor necrosis factor.

explain the differences between cells from the two sources, in addition to graft cell composition. To uncover the molecular basis of these functional differences, we used serial analysis of gene expression (SAGE) as a gene expression profiling technique to compare $CD34^+$ HSPCs originated from BM and UCB. This high throughput technique generates small specific tags (10 base pairs [bp]) from each of the transcripts present on the initial mRNA sample. These tags can be concatemerized, cloned, and sequenced, and the number of times that each tag is found reflects the initial distribution of mRNA transcripts [14].

We demonstrate that transcripts enriched in UCB HSPCs included activators, mediators, regulators, and transcription targets of nuclear factor-κB (NF-κB) signaling. The promoter analysis of these transcript genes further corroborated the importance of NF-κB transcription factors (TFs) by showing that NF-κB binding sites (BSs) were significantly overrepresented in these promoters.

Materials and Methods

$CD34^+$ Cells

UCB from full-term deliveries, BM iliac crest aspirates from healthy adult donors, and granulocyte colony stimulating factor MPB were collected after informed consent was obtained, approved by the local Institutional Review Board. Magnetic cell sorting was carried out using the MACS Direct CD34 Progenitor Cell Isolation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany, http://www.miltenyibiotec.com), following the manufacturer's instructions, except that after gradient centrifugation separation, mononuclear cells were incubated for 1 hour in culture flask at 37°C (RPMI; 5% bovine serum albumin) to remove adherent cells before magnetic labeling. Expression of selected genes was evaluated in additional $CD34^+$ cell samples from BM ($n = 22$), UCB ($n = 9$), and MPB ($n = 6$). Percentage of CD34 cells (purity) was determined by flow cytometry using anti-CD34-PE and anti-CD45-PerCP (BD Pharmingen, San Diego, http://www.bdbiosciences.com/pharmingen).

SAGE Transcriptomes

Total RNA of $CD34^+$ cells from nine BM samples (mean purity of 94%) and seven UCB samples (mean purity of 89%) were pooled to yield two pools of 15 μg of RNA, which were used to generate the SAGE libraries. RNA extraction, library construction, and data analysis were done as previously described [15].

Promoter Analysis

Promoter analysis of differentially expressed genes, comparing BM and UCB HSPCs, was carried out by the Toucan software [16] in an approach similar to that used by Mayer et al. [17]. Promoter regions up to 600 bp upstream of the first exon were retrieved and subjected to a transcription factor binding site (TFBS) search, followed by a statistical analysis to identify significantly overrepresented TFBS, compared with the overall TFBS expected frequencies on the human promoter set of the Eukaryotic Promoter Database. The stringency level (prior value) of the TFBS search was set to 0.02.

Quantitative Polymerase Chain Reaction

Total RNA from $CD34^+$ HSPCs, isolated from 22 BM, nine UCB, and six MPB samples, was reverse transcribed to cDNA using the High Capacity cDNA Archive Kit (Applied BioSystems, Foster City, CA, http://www.appliedbiosystems.com), following the manufacturer's instructions. Real-time polymerase chain reaction (PCR) (in duplicate) for CCL4, NFKB2, interleukin 8 [IL8], RELB, and TGFB1 were carried out with TaqMan probes and MasterMix, whereas IL1B, ICAM1, RELA, and TNFA were assayed with SYBr Green Mix (Applied BioSystems). The 5′ to 3′ sequences of the forward (f) and reverse (r) primers used in conjunction with the SYBr Green Mix were as follows: IL1B-f, TCAGCCAATCTTCATTGCA; IL1B-r, TGGCGAGCTCAGGTACTTCT; ICAM1-f, GCCAACCAATGTGCTATTCA; ICAM1-r, GCCAGTTCCACCCGTTCT; RELA-f, CCACGAGCTTGTAGGAAAGG; RELA-r, CTGGATGCGCTGACTGATAG; TNFA-f, CTCTTCTGCCTGCTGCACTT; and TNFA-r, GCCAGAGGGCTGATTAGAGA. To normalize sample loading, the differences of threshold cycles (ΔCt) were derived by subtracting the Ct value for the internal reference (glyceraldehyde-3-phosphate dehydrogenase) from the Ct values of the evaluated genes. The relative fold value was obtained by the formula $2^{-\Delta\Delta Ct}$ using the median ΔCt value of BM samples as a reference; ΔΔCt was calculated by subtracting the reference ΔCt from the ΔCt values of the samples. Expression of all samples was measured in a single plate for each gene evaluated. The Kruskal-Wallis test with Dunn's post hoc test was performed using Prism 4 (GraphPad Software, Inc., San Diego, http://www.graphpad.com).

Results

A total of 61,302 and 60,745 tags from BM and UCB CD34 HSPC SAGE libraries were sequenced and corresponded to, respectively, 15,398 and 14,518 unique tags that could be mapped to 10,439 and 9,973 distinct UniGene clusters (full data available at http://gdm.fmrp.usp.br).

A direct comparison of the SAGE transcriptomes from BM and UCB revealed a large overall similarity. Only 61 differentially expressed tags ($p < .001$) with 2.5 or greater fold differ-

Table 2. Additional genes related to the nuclear factor κ B (NF-κ B) pathway overrepresented on UCB hematopoietic stem and progenitor cells (HSPCs)

Tag	UniGene	Symbol	Description	*p*	UCB	BM
GTTACAATCA	Hs.431926	NFKB1	Nuclear factor of κ light polypeptide gene enhancer in B-cell 1 (p105)	.012	11	2
AGACCACTGT	Hs.1722	IL1A	Interleukin 1α	.014	6	—
TAGCCCCCTG	Hs.241570	TNF[a]	Tumor necrosis factor (TNF superfamily, member 2)	.08	9	3
ATGGAGCGCA	Hs.256278	TNFRSF1B	Tumor necrosis factor receptor superfamily, member 1B	.025	5	—
CATACCTCCT	Hs.129780	TNFRSF4	Tumor necrosis factor receptor superfamily, member 4	.008	7	—
CCTGACTTCC	Hs.510528	TRAF3	TNF receptor-associated factor 3	.045	4	—
GACATAAATC	Hs.355141	TNIP1	TNFAIP3-interacting protein 1	.05	10	3
CAGAAATGAA	Hs.81424	SUMO1	SMT3 suppressor of mif two 3 homolog 1 (yeast)	.033	7	1
GGGCCCCTGG	Hs.165950	FGFR4	Fibroblast growth factor receptor 4	.017	19	7
CCTTCTGTGG	Hs.279522	NR4A3	Nuclear receptor subfamily 4, group A, member 3	.002	21	5
GCTGAACGCG	Hs.517106	CEBPB	CCAAT/enhancer binding protein (C/EBP) β	.025	5	—
AGGAACTGTA	Hs.495473	NOTCH1	Notch homolog 1, translocation-associated (*Drosophila*)	.007	23	8

Additional tags of genes related to NF-κ B signaling, overexpressed in UCB HSPCs, were selected from our serial analysis of gene expression data. All these genes were found to be differentially expressed with a less stringent *p* value ($p \leq .05$). —, absent tag.
Abbreviations: BM, bone marrow; UCB, umbilical cord blood.
[a] $p > .05$.

ences were found (Table 1): 45 of these tags (corresponding to 43 genes) were overrepresented in UCB, and many were related to NF-κB signaling (Fig. 1A), a pathway with important roles in immune cell biology [18]. Other genes, such as NFKB1, IL1A, tumor necrosis factor α (TNFα), TNF receptors (TNFRSF1B and TNFRSF4), and NOTCH1, known to induce and sustain NF-κB signaling, were also found to be overexpressed in UCB HSPCs, albeit with a higher *p* value ($p < .05$) (Table 2; Fig. 1A).

A promoter analysis on 41 of these 43 genes revealed that five NF-κB binding sites were among the top significantly overrepresented TFBSs, distributed along the promoters of 22 genes (Fig. 1B). Many known NF-κB transcription targets were among these genes, including CXCL2 [19], CXCL3 [20], ICAM1 [21], IL8 [22], IL1B [23], NFKB2 [24], RELB [25], and others.

To evaluate the significance of this findings on additional CD34+ cell samples, we selected a set of genes to evaluate by real-time PCR, including activators (IL1B, TNF, and TGFB1), effectors (NFKB2, RELA, and RELB) and transcriptional targets (ICAM1, IL8, and CCL4L) of NF-κB signaling.

The RELA (p65) subunit of the NF-κB TF was not detected by our SAGE analysis, but we demonstrated a significant higher expression of this transcript on UCB HSPCs by real-time PCR (Fig. 2). SAGE tags for TNF were present in higher numbers in UCB HSPCs (nine tags) than in BM HSPCs (three tags), and although this difference was not statistically significant ($p = .08$), the difference obtained by real-time PCR was highly significant (Fig. 2).

Differences observed between UCB and BM HSPCs, for all nine selected genes, were validated by real-time PCR (Fig. 2), supporting our assumption that a higher constitutive NF-κB signaling is a distinctive feature of UCB CD34+ HSPCs. In addition, results for MPB were similar to those obtained for BM samples, except for TGFB1, which was similar to UCB (Fig. 2).

DISCUSSION

The overrepresentation of the central components of the NF-κB pathway is a major characteristic of UCB HSPCs (Figs. 1, 2), and inhibition of constitutive NF-κB activity in BM CD34+ HSPCs causes loss of clonogenic function and induces apoptosis [26], probably by inhibiting the elimination of reactive oxygen species [27], among other mechanisms.

The large number of known and new potential NF-κB transcription targets among the UCB-overrepresented genes (as detected by our promoter analysis) is a strong evidence of NF-κB signaling. The role of this pathway becomes clearer when our set of UCB-overrepresented genes are compared with NF-κB genomic targets identified by two independent large scale studies, using TNF [28] and IL1 [17] as activators. The large number of common genes further corroborates our conclusion.

NF-κB TF complexes are composed of regulatory (NFKB1 or NFKB2) and transcriptionally active (RELA, RELB, or REL) subunits. NF-κB signaling (Fig. 1A) acts through two pathways: the classic or canonical pathway (mediated by RELA and NFKB1), and the noncanonical or constitutive pathway (mediated by RELB and NFKB2). Although the latter is responsible for the sustained activation of NF-κB signaling, the former may influence its duration and amplitude [18]. Upon binding of specific factors (such as IL1A, IL1B, TNF, lymphotoxin-β [LTB], fibroblast growth factor 2 [FGF2], or TGFB1) to receptors (such as TNFRSF4 or TNFRSF1B), cytoplasmic proteins (including SQSTM1) allow the activation of the NF-κB TF complex, which translocates to the nucleus. In the nucleus, the NF-κB complex binds to specific cis-regulatory elements (BS) on the promoters of target genes, activating their transcription. In addition, proteins such as NOTCH1, C/EBPB, and NR4A1 positively regulate NFKB activity [22, 29–35].

Although gene expression studies of HSPCs from different sources have been carried by others [36–40], only Ng et al. [38] and we directly compared CD34+ HSPCs from BM and UCB. From a total of 51 genes selected by Ng et al. [38], 15 were found on our analysis with statistically significant ($p < .05$) differential tag counts, allowing a direct comparison with our data; all but one were highly concordant (Table 3).

Many of the genes identified in common in both studies, such as elastase, myeloperoxidase, and cathepsin G, which are overrepresented on BM CD34+ HSPCs, are discussed

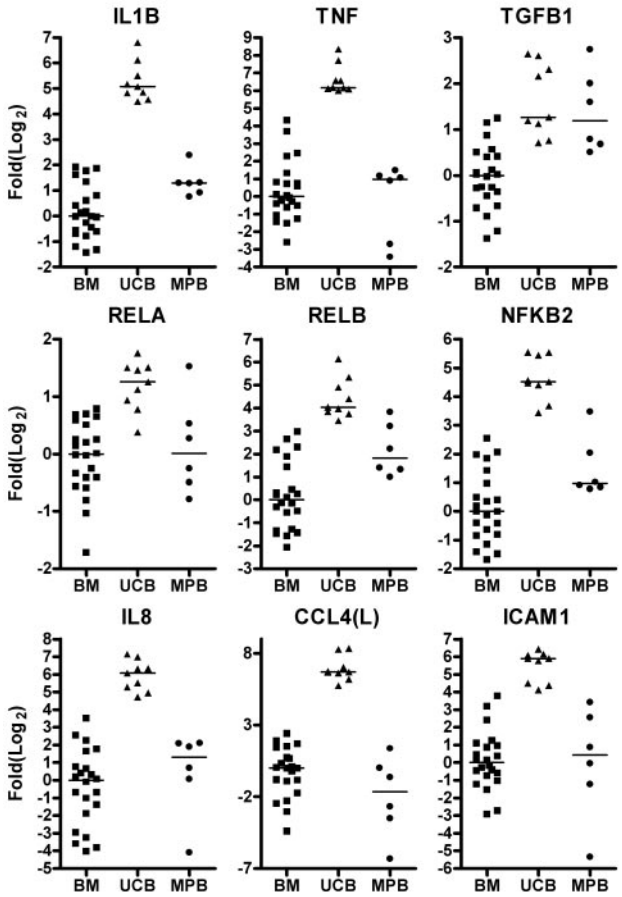

Figure 2. Validation of nuclear factor-κB (NF-κB)-related genes by real-time polymerase chain reaction (PCR). Quantitative PCR was carried out on $CD34^+$ hematopoietic stem and progenitor cells isolated from 22 BM, nine UCB, and six MPB samples. Gene expression is shown as the fold relative to the median gene expression of the BM samples. Differences between BM and UCB were all significant ($p < .001$). MPB did not differ from BM, except for TGB1 ($p < .01$). UCB and MPB differed significantly for RELA and IL8 ($p < .05$), TNF and ICAM1 ($p < .01$), and CCL4(L) ($p < .001$). The assay used for CCL4 does not distinguish it from CCL4L. Abbreviations: BM, bone marrow; ICAM, intercellular adhesion molecule; IL, interleukin; MPB, mobilized peripheral blood; TGF, transforming growth factor; TNF, tumor necrosis factor; UCB, umbilical cord blood.

elsewhere [38]. The overrepresentation (on UCB $CD34^+$ HSPCs) of the transcription factor ID2 (found in our study [Table 1]) and ID1 (found by Ng et al. [38]) indicates the importance of these factors in the maintenance of an undifferentiated stem cell state, to specific characteristics of UCB-derived HSPCs [41].

Despite the agreement of the two studies in relation to this limited set of genes, our work differs from that of Ng et al. [38] because we point out the possible underlying mechanism responsible for the most significant molecular differences observed in the UCB $CD34^+$ cells: the overrepresentation of NF-κB pathway components and transcription targets. Thus, a higher expression of two NF-κB transcription targets, IL8 and NR4A1 (NUR77), was also detected by Ng et al. [38], although the finding was not emphasized.

Although IL8 has long been known to be a transcription target of NF-κB signaling [22], NR4A1 has only recently been shown to be a potential new NF-κB target [42], although its functions are not well-defined and it may play ambiguous roles in apoptosis [30, 43, 44]. Interestingly, NR4A1 antiapoptotic function may act through NF-κB, and its overexpression may protect the cell from many apoptotic stresses [30].

In addition, the proapoptotic function of NR4A1 on T cells at the $CD4^-$ $CD8^-$ stage may be inhibited by the interaction with NOTCH1 [45, 46]. The overrepresentation of NOTCH1 on UCB $CD34^+$ HSPCs would also regulate positively the NF-κB activity in hematopoietic progenitors by controlling the transcription of its subunits [29] and by facilitating its nuclear retention [33]. NOTCH1 has a central role on thymopoiesis, directing CD34 HSPCs to a T-cell fate [47]. In addition to NOTCH1, other genes overrepresented on UCB HSPCs also play important roles on T-cell development and may explain the higher thymic function and TCR diversity observed on UCB recipients [7], and the ability of UCB HSPCs to generate T lymphocytes on fetal thymic organ culture (FTOC) without prestimulation [9], compared with BM recipients and HSPCs, respectively.

For instance, BM HSPCs differentiation into T lymphocytes in FTOC depends on the prestimulation with TNF [9]. TNF and IL1 are both overrepresented in UCB HSPCs and necessary to T-cell development [48, 49]. Thus, it is likely that UCB HSPCs bypass the need for a prestimulation on FTOC because of an autocrine effect of these factors, known to activate NF-κB signaling. The higher generative potential of B-lymphocyte progenitors present in UCB HSPCs [10] may also be related to NF-κB, an important player in B lymphocyte differentiation [18].

Moreover, other characteristics of UCB HSPCs, such as a higher in vitro migration [8], may result from factors such as TNF and IL8 [50, 51], or CXCL2 (GROB) [52], and the privilege of self-renewal at the expense of differentiation and maturation [4] may be influenced by TGF-β [53] and by NOTCH1 [54].

Although the expression profiles of NF-κB components and targets of MPB and BM HSPCs are similar, they differ in relation to TGFB1, because its expression is significantly higher both in MPB and in UCB HSPCs, compared with BM (Fig. 2). This high expression may be related to specific needs of these in-transit cells, as for instance by its ability to modulate the responsiveness of $CD34^+$ cells [55].

Many additional genes overrepresented in UCB HSPCs (Table 1) may be involved in the mechanism of higher constitutive NF-κB signaling in these cells. For instance, LTB, which has a crucial role in the formation of peripheral lymphoid organs, promotes the nuclear translocation of p52/RELB dimmers, activating the noncanonical constitutive NF-κB pathway [18]. Moreover, NFKBIE may collaborate with the sustained activation of this alternate pathway [56], whereas SQSTM1 (p62) intermediates the activation of NF-κB by TNF [57] and by IL1 [32]. FGF2 is another antiapoptotic factor capable of activating NF-κB [34] while preserving long-term repopulating ability of HSPCs [58]. Even TGFB1 may activate NF-κB [35]. In addition to NF-κB, the transcription factor RUNX3 (AML2) may have additional roles on hematopoiesis, equivalent to AML1 [59].

As mentioned, additional components of the NF-κB signaling machinery (and other potential players) can be found in Table 2, as, for instance, TNIP1 (NAF1/ABIN-1), TRAF3/LAP1, and SUMO-1 act on NF-κB signaling regulation [31, 60, 61]. The TNF receptors TNFRSF4 and TNFRSF1B are both capable of activating the noncanonical NF-κB pathway [31], and C/EBPβ can act synergistically with NF-κB, activating the transcription of IL8 [22]. Finally, fibroblast growth factor receptor 4 and the other member of the NUR77 family NR4A3 are also overrepresented on UCB HSPCs.

Table 3. Agreement between our data (based on SAGE) and that of Ng et al. [38] (using microarray) in relation to differentially expressed genes between BM and UCB hematopoietic stem and progenitor cell (HSPCs)

UniGene identification	Transcript	Best tag	Fold[a]	SAGE		Description
				UCB	BM	
Hs.99863	ELA2	GGCTGGGGCC	−10	2	22	Elastase 2, neutrophil
Hs.728	RNASE2	TGACAACAGA	−4.4	1	8	Ribonuclease, RNase A family, 2 (liver, eosinophil-derived neurotoxin)
Hs.421724	CTSG	AGGAGGGGAA	−4.2		4	Cathepsin G
Hs.515122	TK1	CTCCCTCCTC	−2.9		4	Thymidine kinase 1, soluble
Hs.409065	FEN1	CGCTGTTTTT	−2.8		4	Flap structure-specific endonuclease 1
Hs.512680	SCGF	GGGCTCGGGG	−2.8	2	12	Stem cell growth factor; lymphocyte secreted C-type lectin
Hs.201641	BASP1	TCCGTGGTTG	2.7	18	3	Brain-abundant, membrane-attached signal protein 1
Hs.78824	TIE1	CCCTGTTCAG	3.0	5		Tyrosine kinase with immunoglobulin-like and EGF-like domains 1
Hs.315177	IFRD2	GGGTGGGTAG	3.0	5		Interferon-related developmental regulator 2
Hs.524430	NR4A1	TAGTTGGAAA	3.6	19	2	Nuclear receptor subfamily 4, group A, member 1
Hs.432132	G0S2	ACATTTCCAA	5.3	12		Putative lymphocyte G_0/G_1 switch gene
Hs.128433	PGDS	CTCCCTCCCC	6.8	4		Prostaglandin D2 synthase, hematopoietic
Hs.282376	RPS4Y1	TGAAGGATGC	6.9	1	10	Ribosomal protein S4, Y-linked 1
Hs.112405	S100A9	GTGGCCACGG	6.9	63	29	S100 calcium binding protein A9 (calgranulin B)
Hs.624	IL8	TGGAAGCACT	10.3	68	9	Interleukin 8

The description of the differentially expressed genes in Ng et al. [38] was used to identify the corresponding UniGene cluster. The best tag representing this cluster (obtained through CGAP SAGE Genie) was used to directly compare this set of genes to our SAGE data. The negative fold value from the work of Ng et al. [38] indicates a lower expression on UCB HSPCs. All the genes (except RPS4Y1) were concordant.
Abbreviations: BM, bone marrow; EGF, epidermal growth factor; SAGE, serial analysis of gene expression; UCB, umbilical cord blood.
[a]Based on Ng et al. [38].

It is also tempting to speculate about the role of NF-κB signaling and the reduced risk of GVHD on UCB transplants in comparison to BM transplants. For instance, whereas TNF plays an important role in the immune regulatory activity of CD34$^+$ HSPCs [62], TGFB1 plays an important role on the development and maintenance of tolerogenic CD4$^+$CD25$^+$ regulatory T cells [63].

The chemokine known as MIP-1β is derived from two paralogous genes, CCL4 (ACT2) and CCL4L (LAG1) [64]. Transcripts for both were found to be overrepresented in UCB HSPCs by our SAGE analysis (Table 1) and confirmed by real-time PCR, although the assay used could not distinguish CCL4 from CCL4L (Fig. 2). It is interesting that our promoter analysis detected NF-κB BS only in the promoter of CCL4L (Fig. 1), a finding that may explain their different regulation in monocytes and in B lymphocytes [65].

Increased constitutive phosphorylation of the NF-κB inhibitor IkBα and an increased percentage of UCB-derived CD34$^+$ cells (90%) showing nuclear RELA in relation to BM (50%) and MPB-derived cells (not detected) strongly corroborate our observations [66]. Finally, the large percentages of CD34$^+$ cells showing nuclear RELA indicates that this is not a specific feature of the CD38$^-$ subset, since CD38$^-$ corresponds only to a small subset of the CD34$^+$ cells, even in UCB [12]. Our conclusion is also supported by the demonstration that membrane receptor ICAM1 (CD54) is more frequently expressed on UCB CD34$^+$ HSPCs as compared with BM [67]. Nevertheless, a higher expression of NFKB1 found on CD34$^+$CD38$^-$ HSPCs from early gestational fetal blood, compared with CD38$^+$ cells or MPB CD34$^+$CD38$^-$ HSPCs, indicates that higher NF-κB levels are related to a somehow more primitive state [13].

In conclusion, increased NF-κB constitutive signaling, indicated by our gene expression and promoter study, is a major hallmark of UCB HSPCs. This would not be associated with late transient events, but rather with specific needs of HSPCs for the development of immune system, which are ultimately reflected on their in vitro and in vivo behavior.

Understanding the molecular mechanisms involved in these differences may contribute to improve the outcomes of transplantation (reducing the risk of GVHD or the time to engraftment) or the in vitro behavior of the graft (e.g., favoring self renewal and inhibiting differentiation of expanded cells). The functional roles of NF-κB pathway components and targets in UCB CD34$^+$ precursors, proposed by us, are based on the information from the literature. Thus, the specific participation of the NF-κB pathway on some of the distinct in vivo and in vitro characteristics observed on BM and UCB CD34$^+$ cells should be experimentally addressed.

ACKNOWLEDGMENTS

We thank Aglair B. Garcia, Anemari D. Santos, Amélia G. Araujo, Marli H. Tavella, and Maristela Orellana for expert help with the laboratory techniques and Carlos A. Scrideli and Rita C.V. Carrara for providing primers and samples. We also thank Mair P. Souza and Vergilio Colturato from the Hospital Amaral de Carvalho de Jaú, Carmino A. Souza from the Universidade Estadual de Campinas, and Milton Ruiz from Faculdade de Medicina de S. José do Rio Preto for obtaining bone marrow samples for this study. This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo, Conselho Nacional de Desenvolvimento Científico e Tecnológico, and Financiadora de Estudos e Projetos.

DISCLOSURES

The authors indicate no potential conflicts of interest.

REFERENCES

1 Dzierzak E. Embryonic beginnings of definitive hematopoietic stem cells. Ann N Y Acad Sci 1999;872:256–262.
2 Spits H. Development of alphabeta T cells in the human thymus. Nat Rev Immunol 2002;2:760–772.
3 Rocha V, Sanz G, Gluckman E. Umbilical cord blood transplantation. Curr Opin Hematol 2004;11:375–385.
4 Frassoni F, Podesta M, Maccario R et al. Cord blood transplantation provides better reconstitution of hematopoietic reservoir compared with bone marrow transplantation. Blood 2003;102:1138–1141.
5 Noort WA, Wilpshaar J, Hertogh CD et al. Similar myeloid recovery despite superior overall engraftment in NOD/SCID mice after transplantation of human CD34(+) cells from umbilical cord blood as compared to adult sources. Bone Marrow Transplant 2001;28:163–171.
6 Theunissen K, Verfaillie CM. A multifactorial analysis of umbilical cord blood, adult bone marrow and mobilized peripheral blood progenitors using the improved ML-IC assay. Exp Hematol 2005;33:165–172.
7 Talvensaari K, Clave E, Douay C et al. A broad T-cell repertoire diversity and an efficient thymic function indicate a favorable long-term immune reconstitution after cord blood stem cell transplantation. Blood 2002;99:1458–1464.
8 Voermans C, Gerritsen WR, von dem Borne AE et al. Increased migration of cord blood-derived CD34+ cells, as compared to bone marrow and mobilized peripheral blood CD34+ cells across uncoated or fibronectin-coated filters. Exp Hematol 1999;27:1806–1814.
9 Weekx SF, Snoeck HW, Offner F et al. Generation of T cells from adult human hematopoietic stem cells and progenitors in a fetal thymic organ culture system: Stimulation by tumor necrosis factor-alpha. Blood 2000; 95:2806–2812.
10 Arakawa-Hoyt J, Dao MA, Thiemann F et al. The number and generative capacity of human B lymphocyte progenitors, measured in vitro and in vivo, is higher in umbilical cord blood than in adult or pediatric bone marrow. Bone Marrow Transplant 1999;24:1167–1176.
11 Bradley MB, Cairo MS. Cord blood immunology and stem cell transplantation. Hum Immunol 2005;66:431–446.
12 Weekx SF, Van Bockstaele DR, Plum J et al. CD34++ CD38- and CD34+ CD38+ human hematopoietic progenitors from fetal liver, cord blood, and adult bone marrow respond differently to hematopoietic cytokines depending on the ontogenic source. Exp Hematol 1998;26: 1034–1042.
13 Shojaei F, Gallacher L, Bhatia M. Differential gene expression of human stem progenitor cells derived from early stages of in utero human hematopoiesis. Blood 2004;103:2530–2540.
14 Velculescu VE, Zhang L, Vogelstein B et al. Serial analysis of gene expression. Science 1995;270:484–487.
15 Panepucci RA, Siufi JL, Silva WA Jr. et al. Comparison of gene expression of umbilical cord vein and bone marrow-derived mesenchymal stem cells. STEM CELLS 2004;22:1263–1278.
16 Aerts S, Van LP, Thijs G et al. TOUCAN 2: the all-inclusive open source workbench for regulatory sequence analysis. Nucleic Acids Res 2005; 33:W393–W396.
17 Mayer H, Bilban M, Kurtev V et al. Deciphering regulatory patterns of inflammatory gene expression from interleukin-1-stimulated human endothelial cells. Arterioscler Thromb Vasc Biol 2004;24:1192–1198.
18 Beinke S, Ley SC. Functions of NF-kappaB1 and NF-kappaB2 in immune cell biology. Biochem J 2004;382:393–409.
19 Jaramillo M, Godbout M, Olivier M. Hemozoin induces macrophage chemokine expression through oxidative stress-dependent and -independent mechanisms. J Immunol 2005;174:475–484.
20 Anisowicz A, Messineo M, Lee SW et al. An NF-kappa B-like transcription factor mediates IL-1/TNF-alpha induction of gro in human fibroblasts. J Immunol 1991;147:520–527.
21 Collins T, Read MA, Neish AS et al. Transcriptional regulation of endothelial cell adhesion molecules: NF-kappa B and cytokine-inducible enhancers. FASEB J 1995;9:899–909.
22 Stein B, Baldwin AS Jr. Distinct mechanisms for regulation of the interleukin-8 gene involve synergism and cooperativity between C/EBP and NF-kappa B. Mol Cell Biol 1993;13:7191–7198.
23 Stylianou E, Saklatvala J. Interleukin-1. Int J Biochem Cell Biol 1998; 30:1075–1079.
24 Liptay S, Schmid RM, Nabel EG et al. Transcriptional regulation of NF-kappa B2: Evidence for kappa B-mediated positive and negative autoregulation. Mol Cell Biol 1994;14:7695–7703.
25 Bren GD, Solan NJ, Miyoshi H et al. Transcription of the RelB gene is regulated by NF-kappaB. Oncogene 2001;20:7722–7733.
26 Pyatt DW, Stillman WS, Yang Y et al. An essential role for NF-kappaB in human CD34(+) bone marrow cell survival. Blood 1999;93:3302–3308.
27 Nakata S, Matsumura I, Tanaka H et al. NF-kappaB family proteins participate in multiple steps of hematopoiesis through elimination of reactive oxygen species. J Biol Chem 2004;279:55578–55586.
28 Tian B, Nowak DE, Jamaluddin M et al. Identification of direct genomic targets downstream of the NF-kappa B transcription factor mediating TNF signaling. J Biol Chem 2005;280:17435–17448.
29 Cheng P, Zlobin A, Volgina V et al. Notch-1 regulates NF-kappaB activity in hemopoietic progenitor cells. J Immunol 2001;167:4458–4467.
30 de Leseleuc L, Denis F. Inhibition of apoptosis by Nur77 through NF-kappaB activity modulation. Cell Death Differ 2006;13:293–300.
31 Hauer J, Puschner S, Ramakrishnan P et al. TNF receptor (TNFR)-associated factor (TRAF) 3 serves as an inhibitor of TRAF2/5-mediated activation of the noncanonical NF-kappaB pathway by TRAF-binding TNFRs. Proc Natl Acad Sci U S A 2005;102:2874–2879.
32 Sanz L, az-Meco MT, Nakano H et al. The atypical PKC-interacting protein p62 channels NF-kappaB activation by the IL-1-TRAF6 pathway. EMBO J 2000;19:1576–1586.
33 Shin HM, Minter LM, Cho OH et al. Notch1 augments NF-kappaB activity by facilitating its nuclear retention. EMBO J 2006;25:129–138.
34 Vandermoere F, El Yazidi-Belkoura I, Adriaenssens E et al. The antiapoptotic effect of fibroblast growth factor-2 is mediated through nuclear factor-kappaB activation induced via interaction between Akt and IkappaB kinase-beta in breast cancer cells. Oncogene 2005;24: 5482–5491.
35 Zhu Y, Culmsee C, Klumpp S et al. Neuroprotection by transforming growth factor-beta1 involves activation of nuclear factor-kappaB through phosphatidylinositol-3-OH kinase/Akt and mitogen-activated protein kinase-extracellular-signal regulated kinase1,2 signaling pathways. Neuroscience 2004;123:897–906.
36 Georgantas RW, III, Tanadve V, Malehorn M et al. Microarray and serial analysis of gene expression analyses identify known and novel transcripts overexpressed in hematopoietic stem cells. Cancer Res 2004;64: 4434–4441.
37 Graf L, Heimfeld S, Torok-Storb B. Comparison of gene expression in CD34+ cells from bone marrow and G-CSF-mobilized peripheral blood by high-density oligonucleotide array analysis. Biol Blood Marrow Transplant 2001;7:486–494.
38 Ng YY, van Kessel B, Lokhorst HM et al. Gene-expression profiling of CD34+ cells from various hematopoietic stem-cell sources reveals functional differences in stem-cell activity. J Leukoc Biol 2004;75:314–323.
39 Steidl U, Kronenwett R, Rohr UP et al. Gene expression profiling identifies significant differences between the molecular phenotypes of bone marrow-derived and circulating human CD34+ hematopoietic stem cells. Blood 2002;99:2037–2044.
40 Steidl U, Kronenwett R, Haas R. Differential gene expression underlying the functional distinctions of primary human CD34+ hematopoietic stem and progenitor cells from peripheral blood and bone marrow. Ann N Y Acad Sci 2003;996:89–100.
41 Cooper CL, Brady G, Bilia F et al. Expression of the Id family helix-loop-helix regulators during growth and development in the hematopoietic system. Blood 1997;89:3155–3165.
42 Pei L, Castrillo A, Chen M et al. Induction of NR4A orphan nuclear receptor expression in macrophages in response to inflammatory stimuli. J Biol Chem 2005;280:29256–29262.
43 Suzuki S, Suzuki N, Mirtsos C et al. Nur77 as a survival factor in tumor necrosis factor signaling. Proc Natl Acad Sci U S A 2003;100:8276–8280.
44 Liu ZG, Smith SW, McLaughlin KA et al. Apoptotic signals delivered through the T-cell receptor of a T-cell hybrid require the immediate-early gene nur77. Nature 1994;367:281–284.
45 Cohen LY, Bourbonniere M, Sabbagh L et al. Notch1 antiapoptotic activity is abrogated by caspase cleavage in dying T lymphocytes. Cell Death Differ 2005;12:243–254.
46 Jehn BM, Bielke W, Pear WS et al. Cutting edge: Protective effects of notch-1 on TCR-induced apoptosis. J Immunol 1999;162:635–638.
47 De Smedt M, Reynvoet K, Kerre T et al. Active form of Notch imposes T cell fate in human progenitor cells. J Immunol 2002;169:3021–3029.
48 Samira S, Ferrand C, Peled A et al. Tumor necrosis factor promotes human T-cell development in nonobese diabetic/severe combined immunodeficient mice. STEM CELLS 2004;22:1085–1100.
49 Zuniga-Pflucker JC, Di J, Lenardo MJ. Requirement for TNF-alpha and IL-1 alpha in fetal thymocyte commitment and differentiation. Science 1995;268:1906–1909.
50 Janowska-Wieczorek A, Marquez LA, Dobrowsky A et al. Differential MMP and TIMP production by human marrow and peripheral blood CD34(+) cells in response to chemokines. Exp Hematol 2000; 28:1274–1285.
51 Zheng Y, Watanabe N, Nagamura-Inoue T et al. Ex vivo manipulation of umbilical cord blood-derived hematopoietic stem/progenitor cells with recombinant human stem cell factor can upregulate levels of homing-essential molecules to increase their transmigratory potential. Exp Hematol 2003;31:1237–1246.
52 Pelus LM, Bian H, King AG et al. Neutrophil-derived MMP-9 mediates synergistic mobilization of hematopoietic stem and progenitor cells by the combination of G-CSF and the chemokines GRObeta/CXCL2 and GRObetaT/CXCL2delta4. Blood 2004;103:110–119.

53 Mishra L, Derynck R, Mishra B. Transforming growth factor-beta signaling in stem cells and cancer. Science 2005;310:68–71.
54 Duncan AW, Rattis FM, DiMascio LN et al. Integration of Notch and Wnt signaling in hematopoietic stem cell maintenance. Nat Immunol 2005;6:314–322.
55 Basu S, Broxmeyer HE. Transforming growth factor-beta1 modulates responses of CD34+ cord blood cells to stromal cell derived factor-1/CXCL12. Blood 2005;106:485–493.
56 Hoffmann A, Levchenko A, Scott ML et al. The IkappaB-NF-kappaB signaling module: Temporal control and selective gene activation. Science 2002;298:1241–1245.
57 Sanz L, Sanchez P, Lallena MJ et al. The interaction of p62 with RIP links the atypical PKCs to NF-kappaB activation. EMBO J 1999;18:3044–3053.
58 Yeoh JS, van Os R, Weersing E et al. Fibroblast growth factor-1 and 2 preserve long-term repopulating ability of hematopoietic stem cells in serum-free cultures. STEM CELLS 2006;24:1564–1572.
59 Goyama S, Yamaguchi Y, Imai Y et al. The transcriptionally active form of AML1 is required for hematopoietic rescue of the AML1-deficient embryonic para-aortic splanchnopleural (P-Sp) region. Blood 2004;104:3558–3564.
60 Hay RT, Vuillard L, Desterro JM et al. Control of NF-kappa B transcriptional activation by signal induced proteolysis of I kappa B alpha. Philos Trans R Soc Lond B Biol Sci 1999;354:1601–1609.
61 Mauro C, Pacifico F, Lavorgna A et al. ABIN-1 binds to NEMO/IKKgamma and co-operates with A20 in inhibiting NF-kappa B. J Biol Chem 2006;281:18482–18488.
62 Gur H, Krauthgamer R, Bachar-Lustig E et al. Immune regulatory activity of CD34+ progenitor cells: Evidence for a deletion-based mechanism mediated by TNF-alpha. Blood 2005;105:2585–2593.
63 Schramm C, Huber S, Protschka M et al. TGFbeta regulates the CD4+CD25+ T-cell pool and the expression of Foxp3 in vivo. Int Immunol 2004;16:1241–1249.
64 Modi WS, Bergeron J, Sanford M. The human MIP-1beta chemokine is encoded by two paralogous genes, ACT-2 and LAG-1. Immunogenetics 2001;53:543–549.
65 Lu J, Honczarenko M, Sloan SR. Independent expression of the two paralogous CCL4 genes in monocytes and B lymphocytes. Immunogenetics 2004;55:706–711.
66 Giron-Michel J, Caignard A, Fogli M et al. Differential STAT3, STAT5, and NF-kappaB activation in human hematopoietic progenitors by endogenous interleukin-15: implications in the expression of functional molecules. Blood 2003;102:109–117.
67 Gigant C, Latger-Cannard V, Bensoussan D et al. Quantitative expression of adhesion molecules on granulocyte colony-stimulating factor-mobilized peripheral blood, bone marrow, and cord blood CD34+ cells. J Hematother Stem Cell Res 2001;10:807–814.