

(Transforming TGFbR1
 TGFbR2 growth factor-b Receptor1)
 (In vitro) [3] (Hematopoietic stem
 (In vivo) HSCs cell: HSCs)
 [4]
 (Autocrine)
 [5] (Paracrine) HLA (Alloantigen)
 [8-6] (Human Leukocyte Antigen)
 TGF-b
 ...
 (CD34_{high}) CD34 [1]
 CD34⁺CD38 HSC
 80 HSCs TGF-b
 90
 (Early Acting Cytokines) (Ex vivo) HSCs
 (FMS-like tyrosine kinase 3 Flt-3L SCF Expansion of HSCs)
 [9] (Thrombopoietin) Tpo Ligand
 HO/RO_{low} HSC
 (Hoechst 33342_{Low} and rhodamine-123_{Low})
 TGF-b
 [10]
 G1]
 [13-11 5] (Stem Cell Factor) SCF
 (Cyclin cdk [(Homeobox B4) HOXB4
 p21 p27 p15 Dependent Kinase)
 S/G2/M
 TGF-b (Tumor Necrosis Factor- α) TNF α]
 -14 12 6] [(Transforming growth factor-b)
 [17]
 TGF-b1 [2]
 [4] HSCs

[4] G1 HSCs [18] SCF [14] Tpo HSCs HSCs

19] HSCs HSCs .[20

HSCs HSCs G0 TGF-b

(mRNA) TGF-b .[4

G1/S

HSCs G0 G0 mRNA

CD34+ S G1 HSCs

HSC [19 13]

(Long-term Culture Initiating LT-CIC Cell) TGFbRI (TGFbRI-null mice) HSCs

[22 21]

-2

HSCs -1-2

[23]

mRNA [19 13] HSCs

TGF-b

(Hydroxyethyl 50 [24]

2 Starch) -1 TGF-b

-2 [19]

HSCs

24

TGFbR2	Stealth™ RNAi				
	Stealth™ RNAi Negative Control				
BLOCK-iT™					
SiRNA		Alexa Fluor Red			
(SiRNA Duplex)	SiRNA	6			
	Opti-MEM	50			
(Lipofectamine)	1				
	Opti-MEM	50			
		20	Miltenyi Biotech	CD34 ⁺ HSC	
SiRNA			(Bergisch Gladbach, Germany)	(Magnet)	
			(LS Separation LS		
			(Miltenyi Biotech) Columns)		
10		600	(Iscove's IMDM		
		SiRNA	FBS 10	Modified Dulbecco's Medium)	
		SiRNA		(Fetal Bovine Serum)	
			100 SCF	100	
5 CO2		37	Tpo	30 Flt-3L	
	6 4			30 IL-6	30
	HSCs				IL-3

-3-2

TGFbR2

SiRNA

-2-2

Real-Time PCR
(Quantitative Real-Time PCR: QRT-PCR)

(Stealth™ Select RNAi) TGFbR2 SiRNA
Stealth™ RNAi SiRNA
BLOCK-iT™ Alexa Negative Control
Fluor Red

72 48

RNA x-plus
RNA ()
(Fermentas) DNaseI cDNA
cDNA RNA
Invitrogen Lipofectamine™ RNAiMAX
50000
500 70000

HSCs (PARTEC Germany)	MMLV-RT (Random Hexamer)
6×10 ⁴	(Moloney Murine Leukemia Virus- Reverse Transcriptase)
2 FBS PBS	Fermentas
FITC	Rotor-Gene Real-Time PCR
(Fluorescein Isothiocyanate (FITC)- conjugated Antibodies)	Corrbet
FITC-mouse CD34	(Master Mix) 12/5
IgG1	10) (Primer) 1 Roche
(Gate)	(100) cDNA 1 (
HSCs (Low Side Scatter)	25
FloMax	: Oligo 6
	(Forward) TGFbR2:
	5'-TTTTCCACCTGTGACAACCA-3'
	(Reverse) TGFbR2:
	5'-GCTGATGCCTGTCACTTGAA-3'
	: PCR
	5 (Initial Denaturation)
	95 15 95
	56 (Annealing) 15
	72 (Extension) 25
	.(Melting) 45
	(Duplicate)
	Pfaffl (Relative Quantitation)
	(Threshold Cycle) Ct
	-
	Pfaffl
LT-CIC -6-2	
LT-CIC	
M2-	-4-2
10B4	

-5-2

-6-2

-4-2

				α MEM	3×10^4	M2-10B4		
	Methocult™ GF+ H4435			1		96		
							(Coat)	
						20	3	
		6					(Mitomycin) C	
		95			(Limiting Dilution)		LT-CIC	
15	CO ₂	5	37					
				18	5		M2-10B4	
					10^{-5} α MEM			
	LT-CIC				(Stemcell Technologies) (Hydrocortisone)			
Maximum					LT-CIC		20	
	(Fazekas de St. Groth, 1982) Likelihood							
					L-Calc™			
				-3				
					CO ₂	5	37	
	CD34+ HSC				3000	2000	1000	3
	10 FBS IMDM	HSCs			30			
						4	3	
		8						
HSCs		TGFbR2			5			
HSCs				LT-CIC				
							M2-10B4	
		TGFbR2	Stealth™ RNAi					
	Stealth™ RNAi Negative							
BLOCK-				Control				
				iT™ Alexa Fluor Red			-7-2	
8	6						LTC-IC	
							5	
CD34+ HSC								
		50	40				100	

(1 2)

72 48

HSCs

TGFbR2 (Transcript) RNA

8

QRT-PCR

HSCs

HSCs

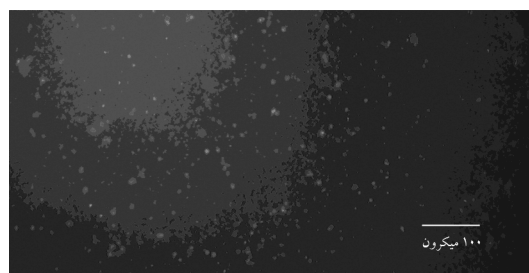
LT-CIC

QRT-PCR -2-3

RNA

72 48

TGFbR2



BLOCK-iT™ Alexa Fluor

HSCs

1

Red

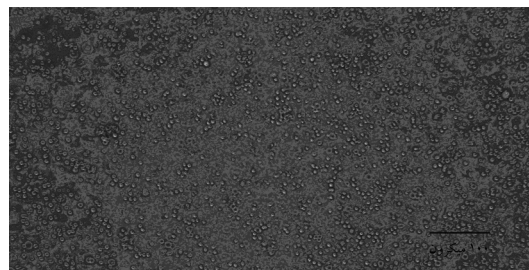
72

TGF-bR2

50

40

-3-3



BLOCK-iT™ Alexa Fluor Red

2

1

(Ex Vivo Expanded)

CD34

CD34

40000

FITC

CD34

HSC (Expansion)

-1-3

CD34+

CD34

(TGFbR2

Stealth™ RNAi

)

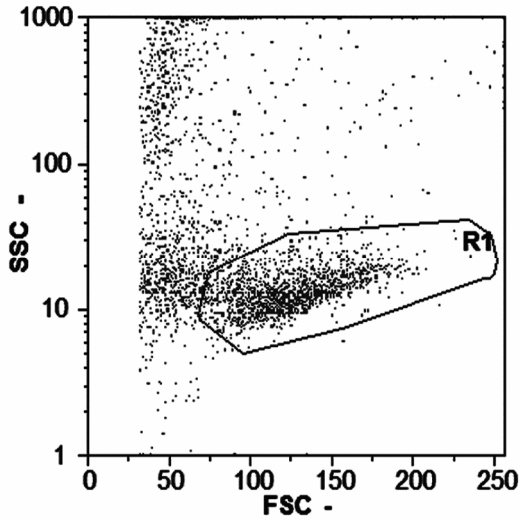
(Stealth™ RNAi Negative Control

HSCs

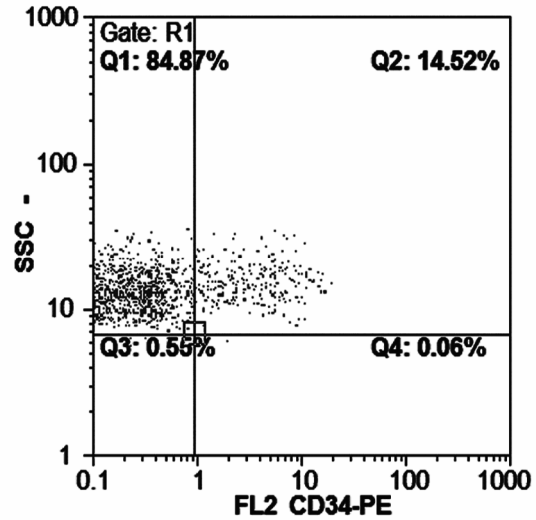
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85

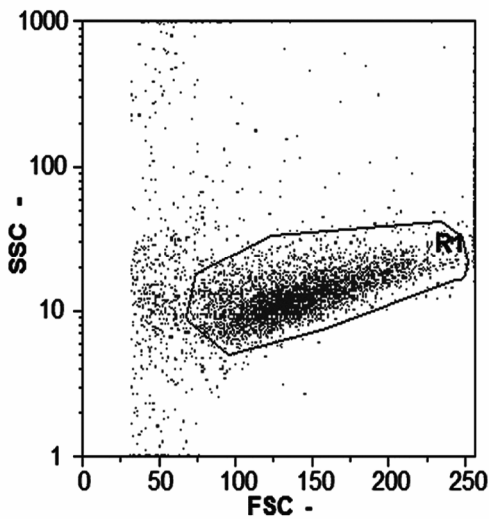
CD34+ (3) 14/2
 3/8) 19/6 SiRNA
 5/3 CD34+ . CD34+ HSC (4
 CD34+ CD34+
 (1)



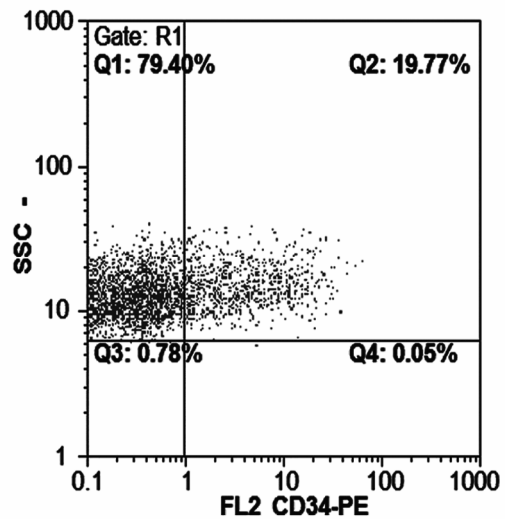
CD34



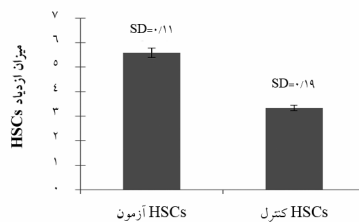
HSCs 3



CD34



HSCs 4



LT-CIC

HSCs 1

6 LT-CIC

-4-3

L-Calc™

LT-CIC

(Methocult) 8

SiRNA

P value=0/05 6024 1

P value=0/05 7344 1

2

1000

70±15

120±10

1/21

(2)

40

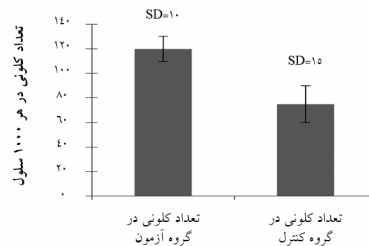
65

-4

TGF-b

HSCs

HSCs TGF-b RNA



HSC

[25]

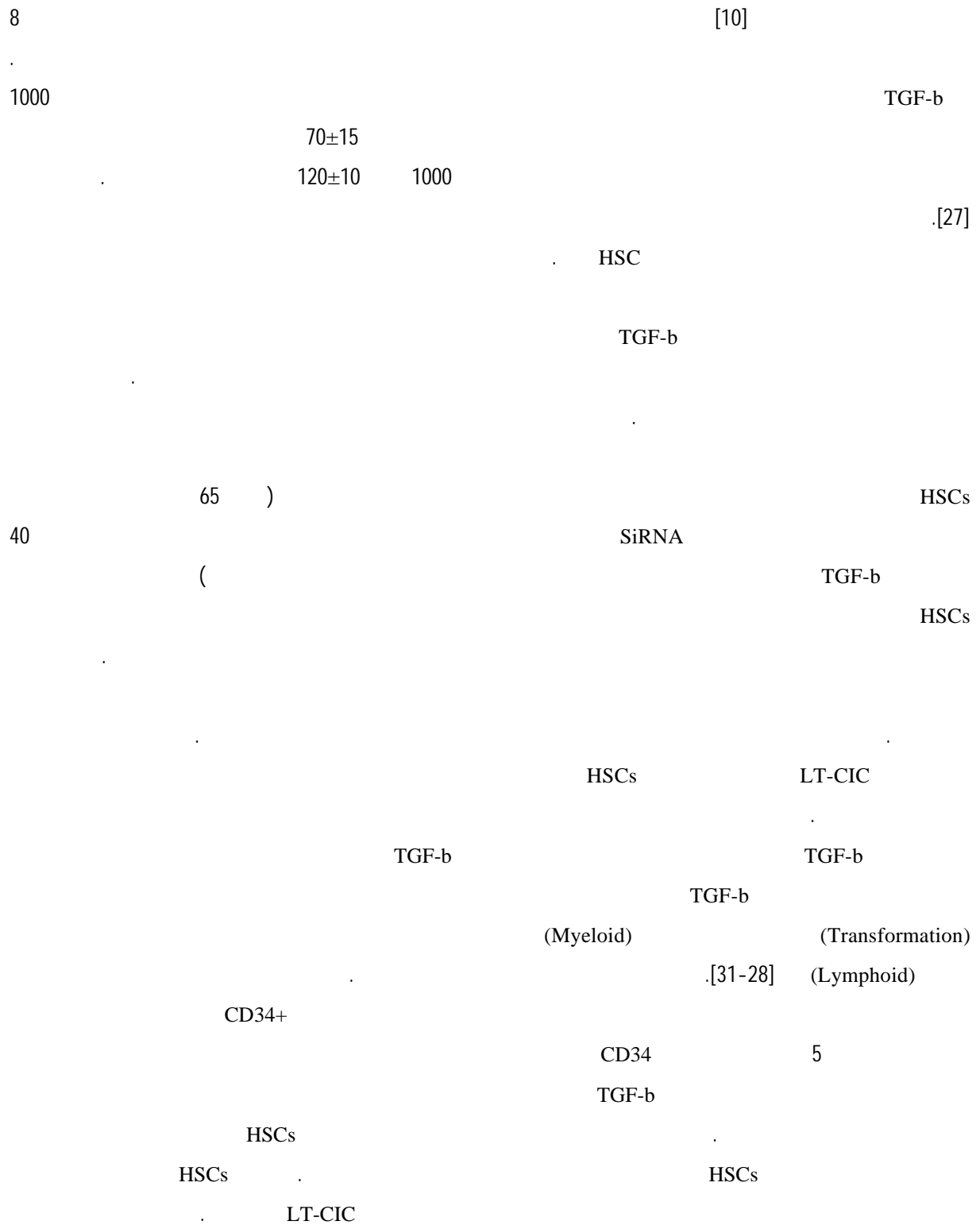
2

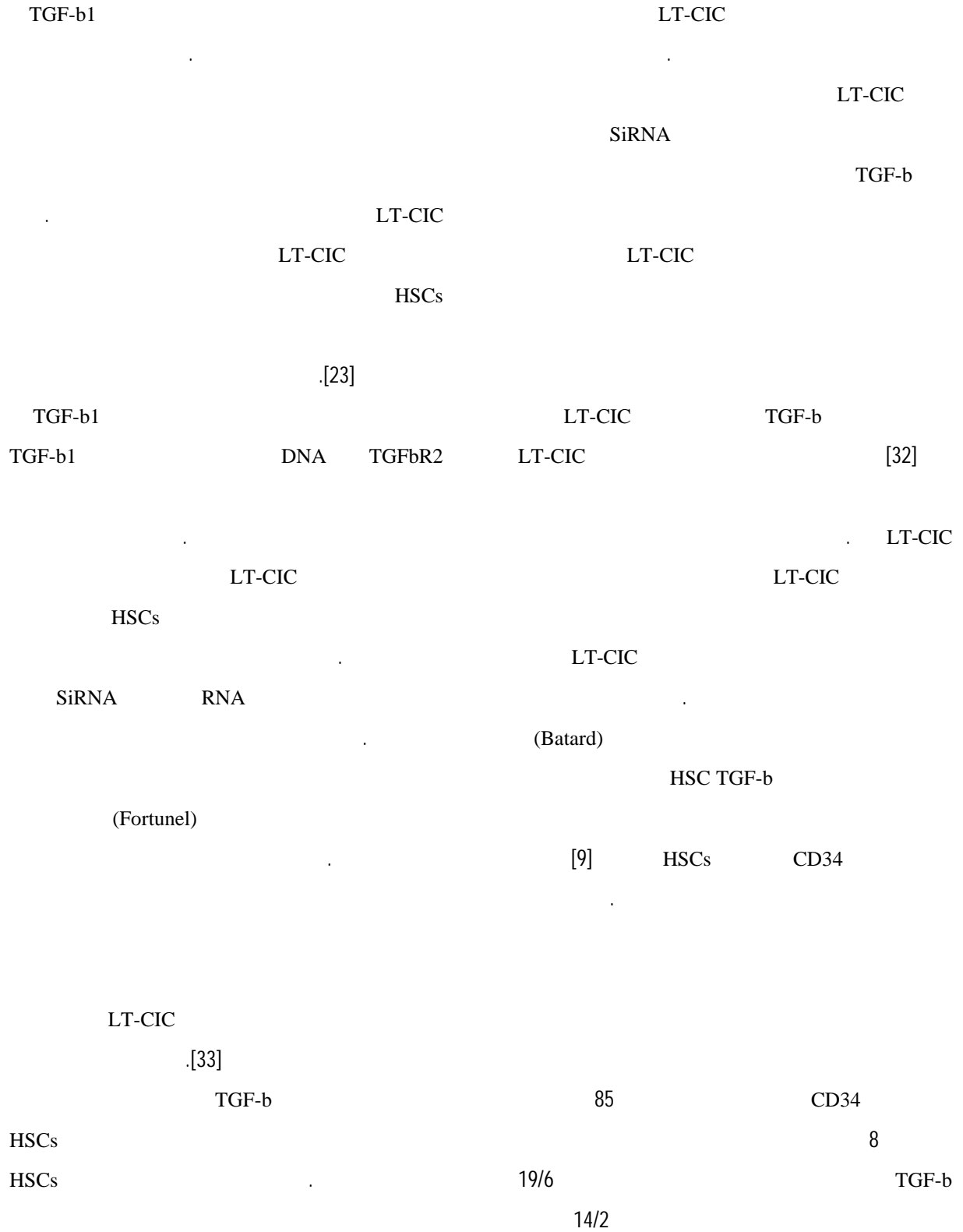
[26]

[23]

LT-CIC -5-3

TGFbR2





-5

HSCs

() HSCs
() HSCs

-6

- [1] To LB, Haylock DN, Simmons PJ, Juttner CA. The biology and clinical uses of blood stem cells. *Blood* 1997; 89(7): 2233-58.
- [2] Ogawa M. Differentiation and proliferation of hematopoietic stem cells. *Blood* 1993; 81(11): 2844-53.
- [3] Shi Y, Massagué J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. *Cell* 2003; 113(6): 685-700.
- [4] Fortunel NO, Hatzfeld A, Hatzfeld JA. Transforming growth factor-beta: pleiotropic role in the regulation of hematopoiesis. *Blood* 2000; 96(6): 2022-36.
- [5] Ohishi K, Katayama N, Itoh R, Mahmud N, Miwa H, Kita K, Minami N, Shirakawa S, Lyman SD, Shiku H. Accelerated cell-cycling of hematopoietic progenitors by the flt3 ligand that is modulated by transforming growth factor-beta. *Blood* 1996; 87(5): 1718-27.
- [6] Cashman JD, Eaves CJ, Sarris AH, Eaves AC. MCP-1, not MIP-1alpha, is the endogenous chemokine that cooperates with TGF-beta to inhibit the cycling of primitive normal but not leukemic (CML) progenitors in long-term human marrow cultures. *Blood* 1998; 92(7): 2338-44.
- [7] Atkins GJ, Haynes DR, Geary SM, Loric M, Crotti TN, Findlay DM. Coordinated cytokine expression by stromal and hematopoietic cells during human osteoclast formation. *Bone* 2000; 26(6): 653-61.
- [8] Liesveld JL, Harbol AW, Belanger T, Rosell KE, Abboud CN. MIP-1alpha and TGF-beta production in CD34+ progenitor-stromal cell coculture systems: effects of progenitor isolation method and cell-cell contact. *Blood Cells Mol Dis* 2000; 26(4): 261-75.
- [9] Batard P, Monier MN, Fortunel N, Ducos K, Sansilvestri-Morel P, Phan T, Hatzfeld A, Hatzfeld JA. TGF-(beta) 1 maintains hematopoietic immaturity by a reversible negative control of cell cycle and induces CD34 antigen up-modulation. *J Cell Sci* 2000; 113(Pt 3): 383-90.
- [10] Wiesmann A, Kim M, Georgelas A, Searles AE, Cooper DD, Green WF, Spangrude GJ. Modulation of hematopoietic stem/progenitor cell engraftment by transforming growth factor beta. *Exp Hematol* 2000; 28(2): 128-39.
- [11] Tanaka R, Katayama N, Ohishi K, Mahmud N,

- Itoh R, Tanaka Y, Komada Y, Minami N, Sakurai M, Shirakawa S, et al. Accelerated cell-cycling of hematopoietic progenitor cells by growth factors. *Blood* 1995; 86(1): 73-9.
- [12] Sitnicka E, Ruscetti FW, Priestley GV, Wolf NS, Bartelmez SH. Transforming growth factor beta 1 directly and reversibly inhibits the initial cell divisions of long-term repopulating hematopoietic stem cells. *Blood* 1996; 88(1): 82-8.
- [13] Ruscetti FW, Bartelmez SH. Transforming growth factor beta, pleiotropic regulator of hematopoietic stem cells: potential physiological and clinical relevance. *Int J Hematol* 2001; 74(1): 18-25.
- [14] Ramsfjell V, Borge OJ, Cui L, Jacobsen SE. Thrombopoietin directly and potently stimulates multilineage growth and progenitor cell expansion from primitive (CD34+ CD38-) human bone marrow progenitor cells: distinct and key interactions with the ligands for c-kit and flt3, and inhibitory effects of TGF-beta and TNF-alpha. *J Immunol* 1997; 158(11): 5169-77.
- [15] Fortunel N, Batard P, Hatzfeld A, Monier MN, Panterne B, Lebkowski J, Hatzfeld J. High proliferative potential-quiescent cells: a working model to study primitive quiescent hematopoietic cells. *J Cell Sci* 1998; 111(Pt 13): 1867-75.
- [16] Pierelli L, Marone M, Bonanno G, Mozzetti S, Rutella S, Morosetti R, Rumi C, Mancuso S, Leone G, Scambia G. Modulation of bcl-2 and p27 in human primitive proliferating hematopoietic progenitors by autocrine TGF-beta1 is a cell cycle-independent effect and influences their hematopoietic potential. *Blood* 2000; 95(10): 3001-9.
- [17] Keller JR, Mcniece IK, Sill KT, Ellingsworth LR, Quesenberry PJ, Sing GK, Ruscetti FW. Transforming growth factor beta directly regulates primitive murine hematopoietic cell proliferation. *Blood* 1990; 75(3): 596-602.
- [18] Heinrich MC, Dooley DC, Keeble WW. Transforming growth factor beta 1 inhibits expression of the gene products for steel factor and its receptor (c-kit). *Blood* 1995; 85(7): 1769-80.
- [19] Sitnicka E, Ruscetti FW, Priestley GV, Wolf NS, Bartelmez SH. Transforming growth factor beta 1 directly and reversibly inhibits the initial cell divisions of long-term repopulating hematopoietic stem cells. *Blood* 1996; 88(1): 82-8.
- [20] Fan X, Valdimarsdottir G, Larsson J, Brun A, Magnusson M, Jacobsen SE, ten Dijke P, Karlsson S. Transient disruption of autocrine TGF-beta signaling leads to enhanced survival and proliferation potential in single primitive human hemopoietic progenitor cells. *J Immunol* 2002; 168(2): 755-62.
- [21] Larsson J, Blank U, Helgadottir H, Björnsson JM, Ehinger M, Goumans MJ, Fan X, Levéen P, Karlsson S. TGF-beta signaling-deficient hematopoietic stem cells have normal self-renewal and regenerative ability in vivo despite increased proliferative capacity in vitro. *Blood* 2003; 102(9): 3129-35.
- [22] Larsson J, Goumans MJ, Sjöstrand LJ, van Rooijen MA, Ward D, Levéen P, Xu X, ten Dijke P, Mummery CL, Karlsson S. Abnormal angiogenesis but intact hematopoietic potential in TGF-beta type I receptor-deficient mice.

- EMBO J 2001; 20(7): 1663-73.
- [23] Yamazaki S, Iwama A, Takayanagi S, Eto K, Ema H, Nakauchi H. TGF-beta as a candidate bone marrow niche signal to induce hematopoietic stem cell hibernation. *Blood* 2009; 113(6): 1250-6.
- [24] Eaves CJ, Cashman JD, Kay RJ, Dougherty GJ, Otsuka T, Gaboury LA, Hogge DE, Lansdorp PM, Eaves AC, Humphries RK. Mechanisms that regulate the cell cycle status of very primitive hematopoietic cells in long-term human marrow cultures. II. Analysis of positive and negative regulators produced by stromal cells within the adherent layer. *Blood* 1991; 78(1): 110-7.
- [25] Smiler DG, Soltan M, Soltan C, Matthews C. Growth factors and gene expression of stem cells: bone marrow compared with peripheral blood. *Implant Dent* 2010; 19(3): 229-40.
- [26] Larsson J, Blank U, Klintman J, Magnusson M, Karlsson S. Quiescence of hematopoietic stem cells and maintenance of the stem cell pool is not dependent on TGF-beta signaling in vivo. *Exp Hematol* 2005; 33(5): 592-6.
- [27] Capron C, Lacout C, Lécluse Y, Jalbert V, Chagraoui H, Charrier S, Galy A, Bennaceur-Griscelli A, Cramer-Bordé E, Vainchenker W. A major role of TGF-beta1 in the homing capacities of murine hematopoietic stem cell/progenitors. *Blood* 2010; 116(8): 1244-53.
- [28] Le Bousse-Kerdilès MC, Chevillard S, Charpentier A, Romquin N, Clay D, Smadja-Joffe F, Praloran V, Dupriez B, Demory JL, Jasmin C, Martyré MC. Differential expression of transforming growth factor-beta, basic fibroblast growth factor, and their receptors in CD34+ hematopoietic progenitor cells from patients with myelofibrosis and myeloid metaplasia. *Blood* 1996; 88(12): 4534-46.
- [29] Rooke HM, Vitas MR, Crosier PS, Crosier KE. The TGF-beta type II receptor in chronic myeloid leukemia: analysis of microsatellite regions and gene expression. *Leukemia* 1999; 13(4): 535-41.
- [30] DeCoteau JF, Knaus PI, Yankelev H, Reis MD, Lowsky R, Lodish HF, Kadin ME. Loss of functional cell surface transforming growth factor beta (TGF-beta) type 1 receptor correlates with insensitivity to TGF-beta in chronic lymphocytic leukemia. *Proc Natl Acad Sci USA* 1997; 94(11): 5877-81.
- [31] Lagneaux L, Delforge A, Bron D, Massy M, Bernier M, Stryckmans P. Heterogenous response of B lymphocytes to transforming growth factor-beta in B-cell chronic lymphocytic leukaemia: correlation with the expression of TGF-beta receptors. *Br J Haematol* 1997; 97(3): 612-20.
- [32] Eaves CJ, Cashman JD, Kay RJ, Dougherty GJ, Otsuka T, Gaboury LA, Hogge DE, Lansdorp PM, Eaves AC, Humphries RK. Mechanisms that regulate the cell cycle status of very primitive hematopoietic cells in long-term human marrow cultures. II. Analysis of positive and negative regulators produced by stromal cells within the adherent layer. *Blood* 1991; 78(1): 110-7.
- [33] Fortunel N, Hatzfeld J, Kisselev S, Monier MN, Ducos K, Cardoso A, Batard P, Hatzfeld A. Release from quiescence of primitive

دوره ۱۴ شماره ۱ بهار ۱۳۹۰

مجله علوم پزشکی مدرس: آسیب‌شناسی زیستی

human hematopoietic stem/progenitor cells by
blocking their cell-surface TGF-beta type II

receptor in a short-term in vitro assay. Stem
Cells 2000; 18(2): 102-11.