

(Transforming TGFbR1

TGFbR2 growth factor-b Receptor1)

(In vitro)

[3]

(In vivo)

(Hematopoietic stem

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
(Magnet)
(Bergisch Gladbach, Germany)
(LS Separation LS
(Miltenyi Biotech) Columns)

10 600 (Iscove's IMDM
FBS 10 Modified Dulbecco's Medium)
SiRNA (Fetal Bovine Serum)
SiRNA
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

TGFbR2

(Quantitative Real-Time PCR: QRT-PCR)

72 48

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

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	

				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

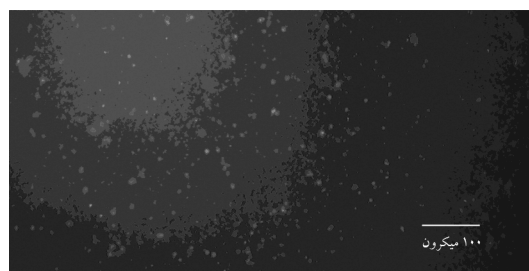
LT-CIC

QRT-PCR -2-3

RNA

72 48

TGFbR2



BLOCK-iT™ Alexa Fluor

HSCs

1

Red

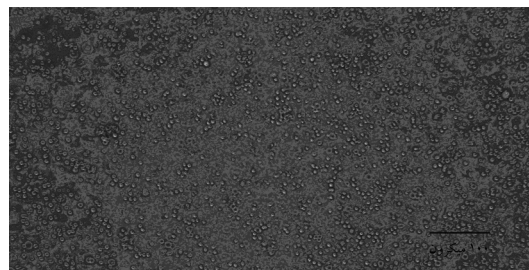
72

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+

HSCs

(Stealth™ RNAi Negative Control

CD34

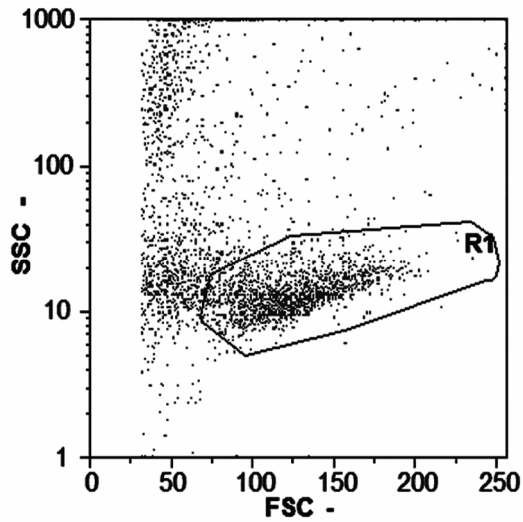
(TGFbR2

Stealth™ RNAi

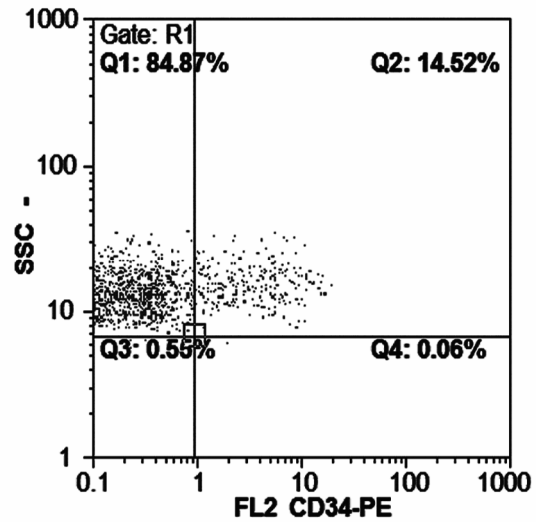
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85

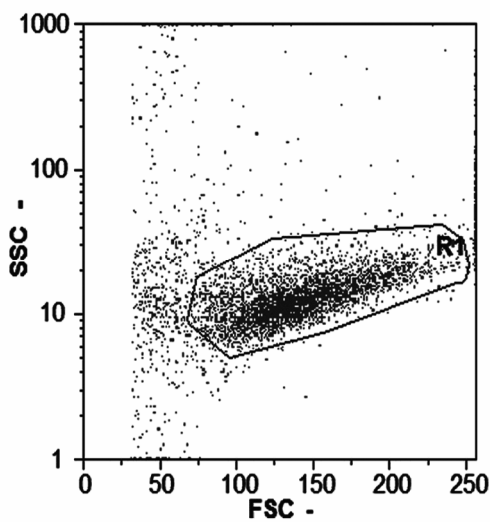
CD34+ (3) 14/2
 3/8) 19/6 SiRNA
 5/3 CD34+ . CD34+ HSC (4
 CD34+ CD34+
 (1)



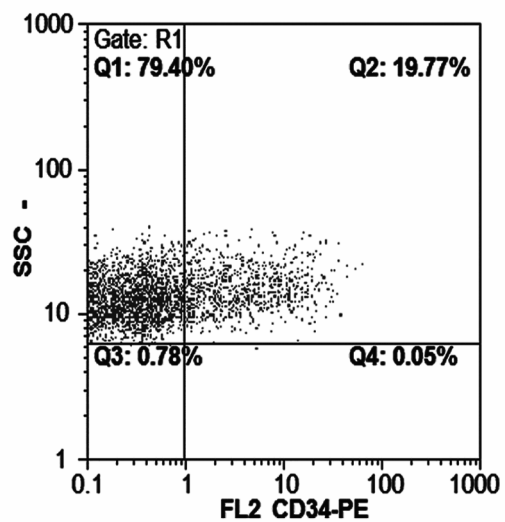
CD34



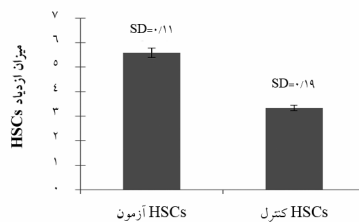
HSCs 3



CD34



HSCs 4



CD34+
HSCs

LT-CIC

HSCs 1

6 LT-CIC

-4-3

L-CalcTM

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

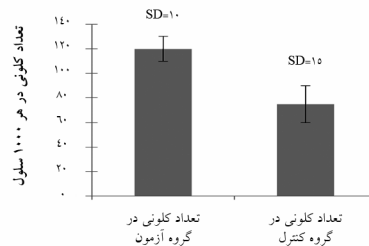
65

-4

TGF-b

HSCs

HSCs TGF-b RNA



HSC

[25]

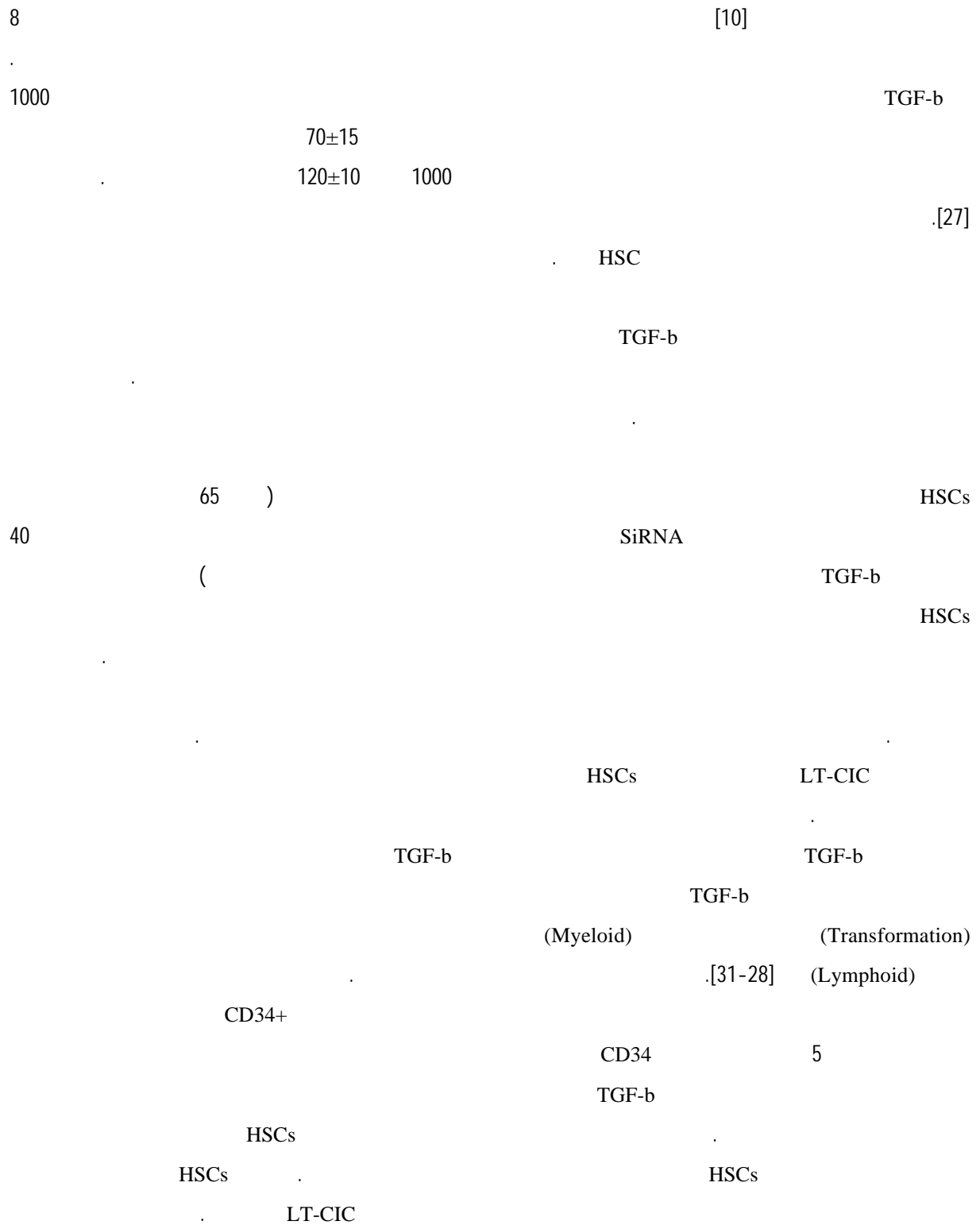
2

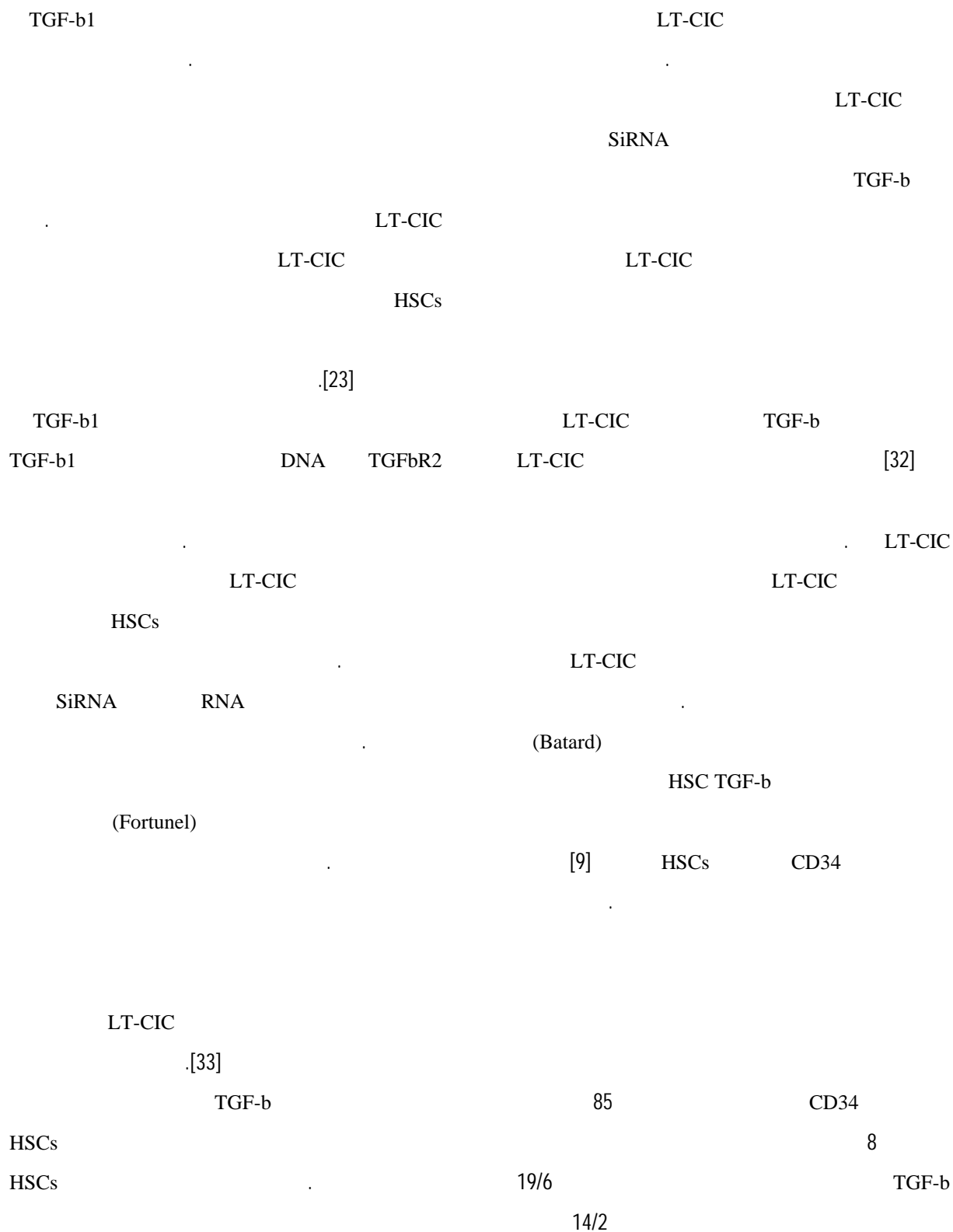
[26]

[23]

LT-CIC -5-3

TGFbR2





-5

HSCs

() HSCs
() HSCs

-6

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