CD49b inhibits osteogenic differentiation and plays an important role in osteosarcoma progression

SUPPLEMENTARY MATERIALS



Supplementary Figure 1: Differential expression of six candidate genes were validated by quantitative real-time PCR, using GAPDH as endogenous control.



Supplementary Figure 2: Mutilineage differentiation capacity of MSCs were confirmed by (A) Alizarin red staining (day 18): Osteogenesis identification, (B) Oil-red O staining (day 21): Adipogenesis identification, and (C) IHC and western blot detection of collagen II at day 28: Chondrogenesis identification.



Supplementary Figure 3: Expression of CDCP1 during the differentiation of MSCs into osteoblasts, adipocytes, and chondrocytes. (A) western blot analysis, (B) densitometric analysis. ***indicates significant p-values.



Supplementary Figure 4: Expression of CDH6 during the differentiation of MSCs into osteoblasts, adipocytes, and chondrocytes. (A) western blot analysis, (B) densitometric analysis. ***indicates significant p-values.

Cell Proliferation results



Supplementary Figure 5: Cell proliferation was examined by MTT assay. No significant difference in cell proliferation was seen in osteosarcoma cells with decreased CD49b expression.

Supplementary Table 1: Microarray - MSC and OB

genename	symbol	P.Value		
Endoglin	CD105	0.005061		
CUB domain containing protein 1	CDCP1	0.005073		
cadherin 6, type 2, K-cadherin	CDH6	0.005315		
prostate stem cell antigen	PSCA	0.006426		
frizzled homolog 3	FZD3	0.00767		
epidermal growth factor receptor	EGFR	0.009175		
cadherin 2, type 1, N-cadherin (CD325)	CDH2/CD325	0.014005		
CD72 molecule	CD72	0.019666		
integrin, alpha 2 (CD49b)	ITGA2/CD49b	0.020014		
CD200 molecule	CD200	0.022439		
chemokine (C-X-C motif) ligand 16	CXCL16	0.022514		
CD6 molecule	CD6	0.023729		
colony stimulating factor 2 receptor, beta, low- affinity (CD131)	CSF2RB/CD131	0.024141		
Rh blood group, D antigen	RHD	0.025365		
tumor necrosis factor receptor superfamily, member 10d	TNFRSF10D	0.030534		
CD1c molecule	CD1c	0.036153		
CD8a molecule	CD8a	0.04123		
coagulation factor II receptor-like 1	F2RL1	0.041743		
intercellular adhesion molecule 1 (CD54)	ICAM1/CD54	0.041873		
integrin, alpha 9	ITGA9	0.042456		
CD177 molecule	CD177	0.045178		
syndecan 1 (CD138)	SDC1/CD138	0.04839		
dipeptidyl-peptidase 4 (CD26)	DPP4/CD26	0.048982		
chemokine (C-C motif) receptor 9	CCR9	0.04952		

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Supplementary Table 2: Frequency of CD49b measured

Tissue	0	1+	2+	3+	Negative	Positive
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Osteosarcoma	22	22	11	2	22	35
	(38.6)	(38.6)	(19.3)	(3.5)	(38.6)	(61.4)