香港中文大學
The Chinese University of Hong Kong

Circulating Mesenchymal Stem Cells and Their Clinical Implications

循环间充质干细胞的生物学机制与临床意义

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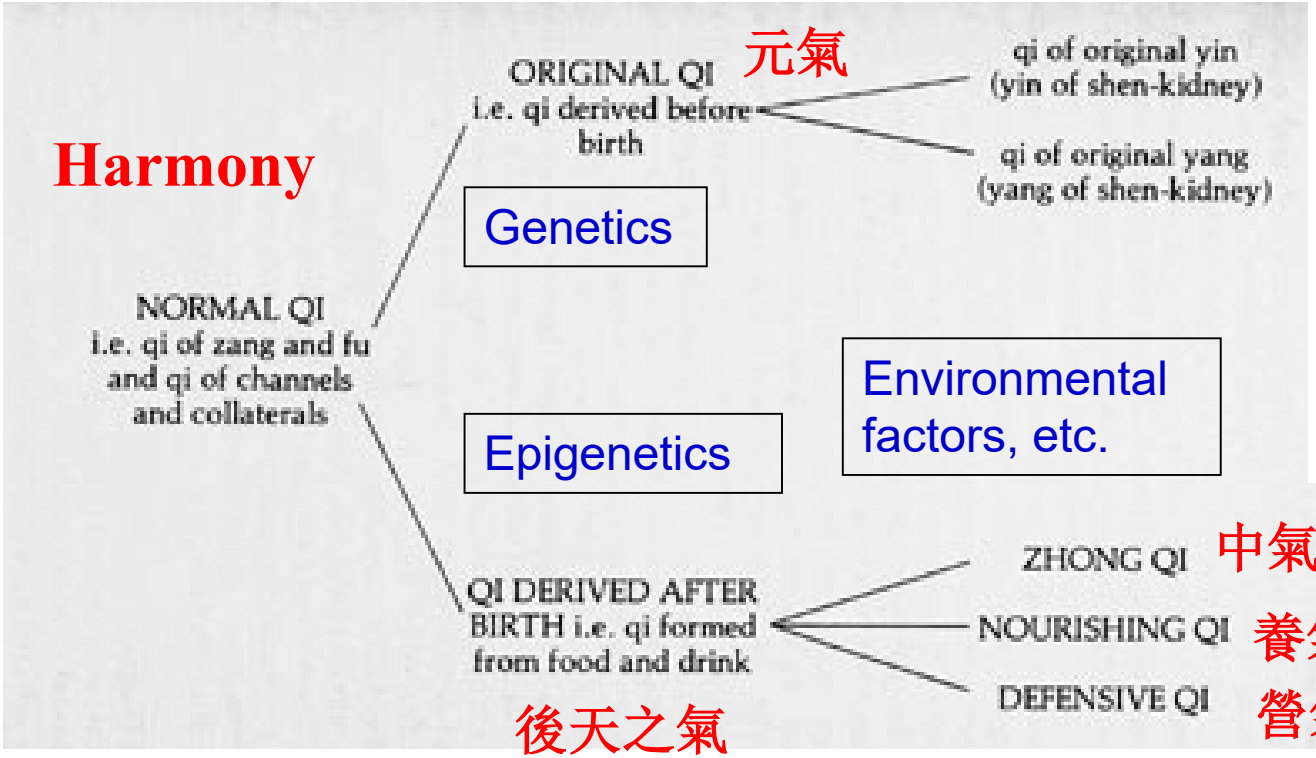
香港中文大学医学院创伤骨科

Traditional Chinese Medicine

氣血相生

Vital Energy (Qi)： The drive of blood

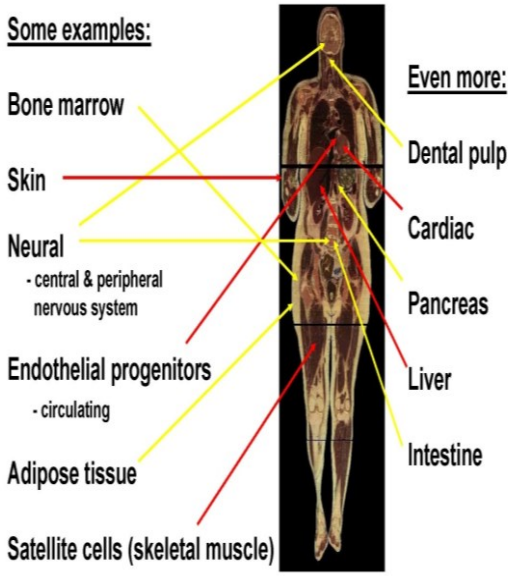
氣：血之動力



中氣--幹細胞？ Stem cells

養氣-- 食物、營養？

營氣-- 免疫？ Immunity



Blood (the carrier of Qi) ： 血： 氣之載體

- **Dependent on the liver, kidney & bone marrow**
- **Formation and circulation of blood and Qi is inter-dependent**

Blood borne MSCs - Questions

- **Is there MSCs in peripheral blood?**
- **When do they show up?**
- **Where do they come from?**
- **What can we learn from them?**
- **What are the clinical implications?**

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History

➢ *In search of blood borne MSCs*

Positive Findings

1928 ---- Maximow, A.: Cultures blood leukocytes to connective tissue. Arch. Exp. Zellforsch.5:169. 1928

1934 ---- Ehrich W. Die Leukocyten und ihre Entstehung. VII Die Leukocyten in der Gewebekultur. Ergeb Allg Pathol Pathol Anat 1934; 29: 1-

1956 ---- Hulliger L. Uber die unterschiedlichen Entwicklungsfahigkeiten der Zellen des Blutes und der Lymphe in vitro. Virchows Arch Pathol Anat Physiol Klin Med 1956; 329: 289-

1958 ---- Paul, J. :Establishment of permanent cell strains from human adult peripheral blood. **Nature 182: 808. 1958.**

1969 **----** Stirling GA, Kakkar VV. Cells in the circulating blood capable of producing connective tissue. Br J Exp Pathol 1969; 50: 51-.

Negative Findings

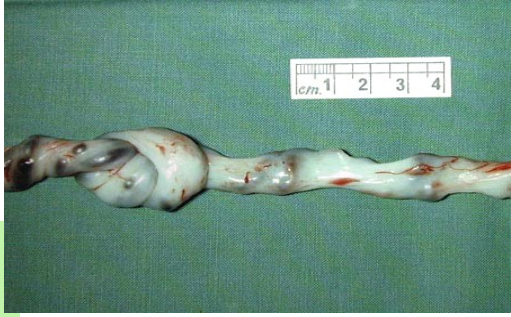
1965 ---- Ross R, Lillywhite IW. The fate of buffy coat cells grown in subcutaneously implanted diffuseion chambers. Lab. Invest 1965; 14: 1568-

1967 --- Rangan SRS. Origin of the fibroblastic growths in chicken buffy coat macrophage cultures. Exp. Cell Res 1967(3); 46:477-487.

- **Increased number of punctures during the collection of a given volume of blood did not lead to a higher numbers of fibroblastic progenitors.**

1971---Luria EA, Panasyuk AF, Friedenstein AJ. Fibroblast colony formation from monolayer culture of blood cells. **Transfusion;** 1971: 11(6):345-349.

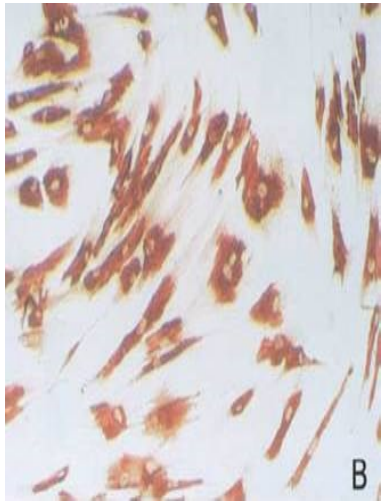
History

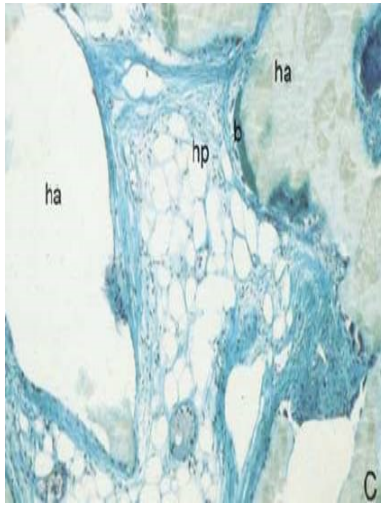


➢ *In search of blood borne MSCs*

Umbilical Cord Blood (UCB)

- **MSCs have been found in UCB**
- **More stroma tissue than bone formed when transplanted**





- **Slower to establish in culture**
- **Lower progenitor frequency than BM**
- **Source limited**
- **Allogeneic transplantation**

Evidence for MSCs migration

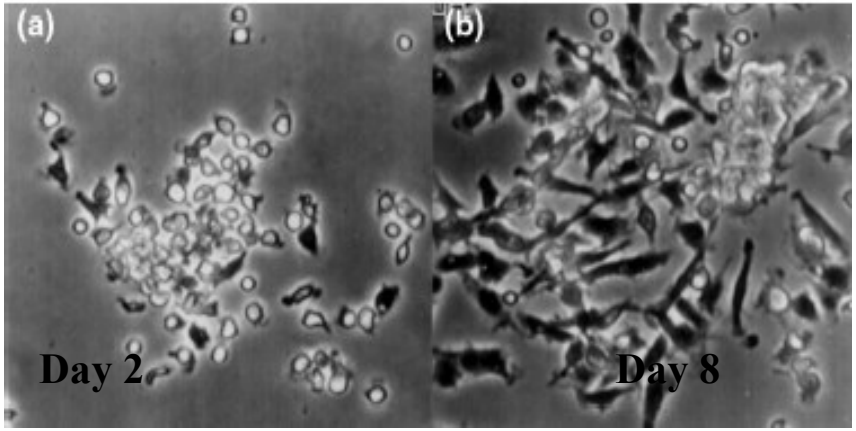
Primary research

Mesenchymal precursor cells in the blood of normal individuals

Nathan J Zvaifler, Lilla Marinova-Mutafchieva*, Gill Adams*,
Christopher J Edwards*, Jill Moss†, Jan A Burger and Ravinder N Maini*†

Department of Medicine, University of California, San Diego, CA, USA, *Kennedy Institute of Rheumatology, London, UK, and †Department of Pathology and Medicine, Imperial College School of Medicine, Charing Cross Hospital, London, UK

Arthriris Research, 2:477-488, 2000

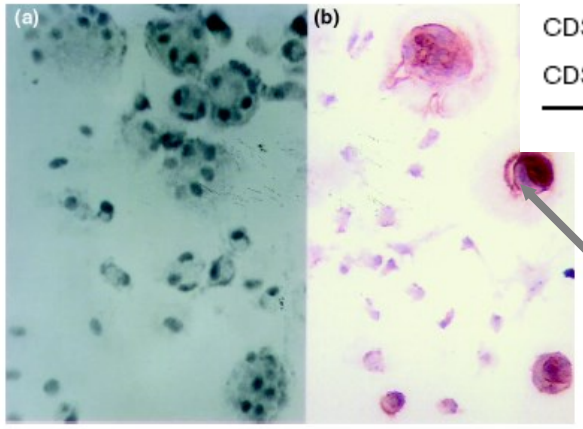


20% FCS DMEM

Table 2

Antibody profile of BMPCs (at days 7–10)

Antibody	Large cells	Fibroblastoid cells
Vimentin	+	+
Collagen type I	+	+
BMPR IA	+	+
IB	0	0
II	+	+
STRO-1	+	0
CD3, CD14, CD20	0	0
CD34, CD45, Class II	0	0



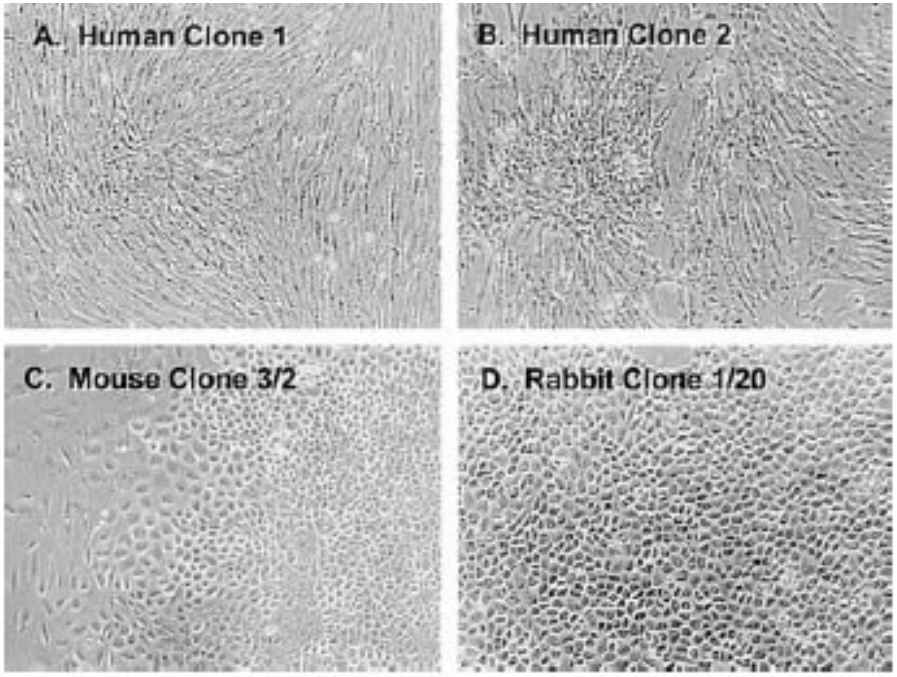
TRAP positive multinucleated cells

Journal of Cell Biology, 153: 1133-9, 2001

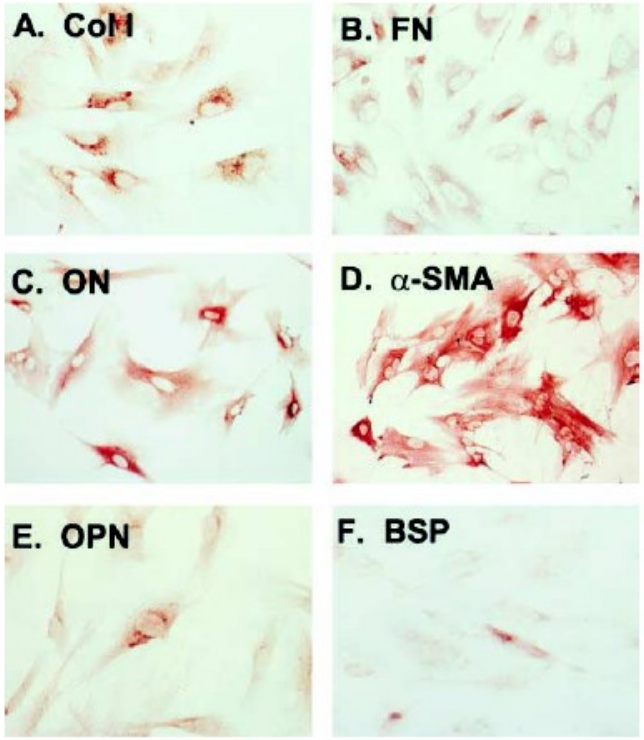
Circulating Skeletal Stem Cells

Sergei A. Kuznetsov,* Mahesh H. Mankani,* Stan Gronthos,* Kazuhito Satomura,‡
Paolo Bianco,§ and Pamela Gehron Robey*

*Craniofacial and Skeletal Diseases Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, Maryland 20892; ‡First Department of Oral and Maxillofacial Surgery, School of Dentistry, University of Tokushima, Tokushima 770-8504, Japan; and §Dipartmento di Medicina Sperimental e Patologia, Universita "La Sapienza," Rome 00161, Italy

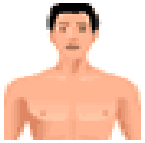


Fibroblastic morphology



ICC for blood-derived adherent cells

➢Progress to date



human

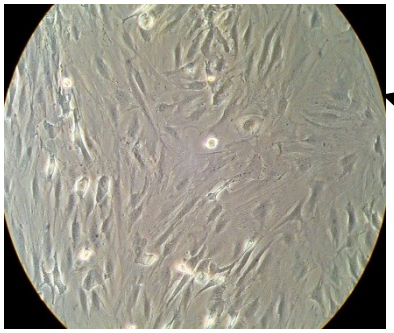
2001. J Cell Biol.

153:1133-1139

Osteogensesis in vivo; Adipogenesis in vitro

CD34-/CD45-/CD14-
CD44+/CD106+ / Type I collagen+
CD105-/Alkaline phosphatase -/ strol-1-

} human cells



PB-derived adherent, clonogenic, fibroblast-like cells

Circulating osteoblast-lineage cells in humans. New Engl. J. Med. May 12, 2005.

- **Sorted osteocalcin+ cells in children**
- **Formed bone in vivo**
- **Increased numbers in three adults with recent fractures.**

CD34 $^{-/low}$
Osteocalcin^{+}

2000. Stem cells

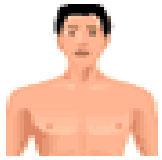
18:252-260

2000. Arthritis Res.

2:477-488

Osteogenesis in vitro

CD34^{-}/CD45^{-}/CD14^{-}/CD3^{-}
CD105^{+}/type I collagen^{+}



human

Stem Cells 2007; 25:69-77.

STEM CELLS

TISSUE-SPECIFIC STEM CELLS

Concise Review: Multipotent Mesenchymal Stromal Cells in Blood

QILING HE,[a] CHAO WAN,[b] GANG LI[a]

PB-MSCs are rare in the normal adult peripheral blood

- **1 MSC in ~ $2x10^9$ PB-MNCs *vs.* 1 MSC in $1x10^6$ BM-MNCs**
- **Numbers of PB-MSCs increased in patients with fracture and tumours**

ABSTRACT

Peripheral blood-derived multipotent mesenchymal stromal cells circulate in low number. They share, most although not all, of the surface markers with bone marrow-derived multipotent mesenchymal stromal cells, possess diverse and complicated gene expression characteristics, and are capable of differentiating along and even beyond mesenchymal lineages. Although their origin and physio-pathological function are still unclear, their presence in the adult peripheral blood might relate to some interesting but controversial subjects in the field of adult stem cell biology, such as systemic migration of bone marrow-derived multipotent mesenchymal stromal cells and the existence of common hematopoietic-mesenchymal precursors. In this review, current studies/knowledge about peripheral blood-derived multipotent mesenchymal stromal cells is summarized, and the above-mentioned topics are discussed.

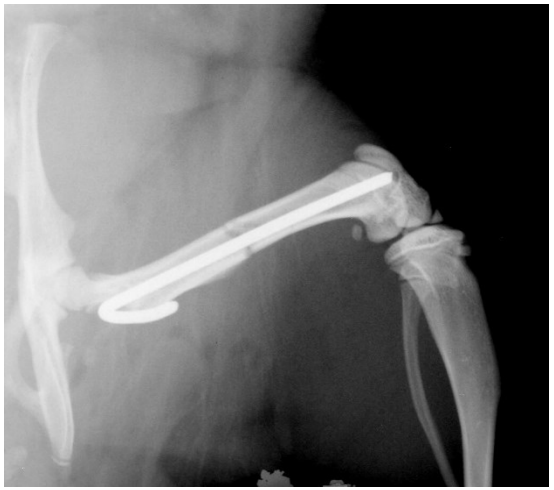
Blood borne MSCs - Questions

- **Is there MSCs in peripheral blood?**
- **When do they show up?**
- **Where do they come from?**
- **What can we learn from them?**
- **What are the clinical implications?**

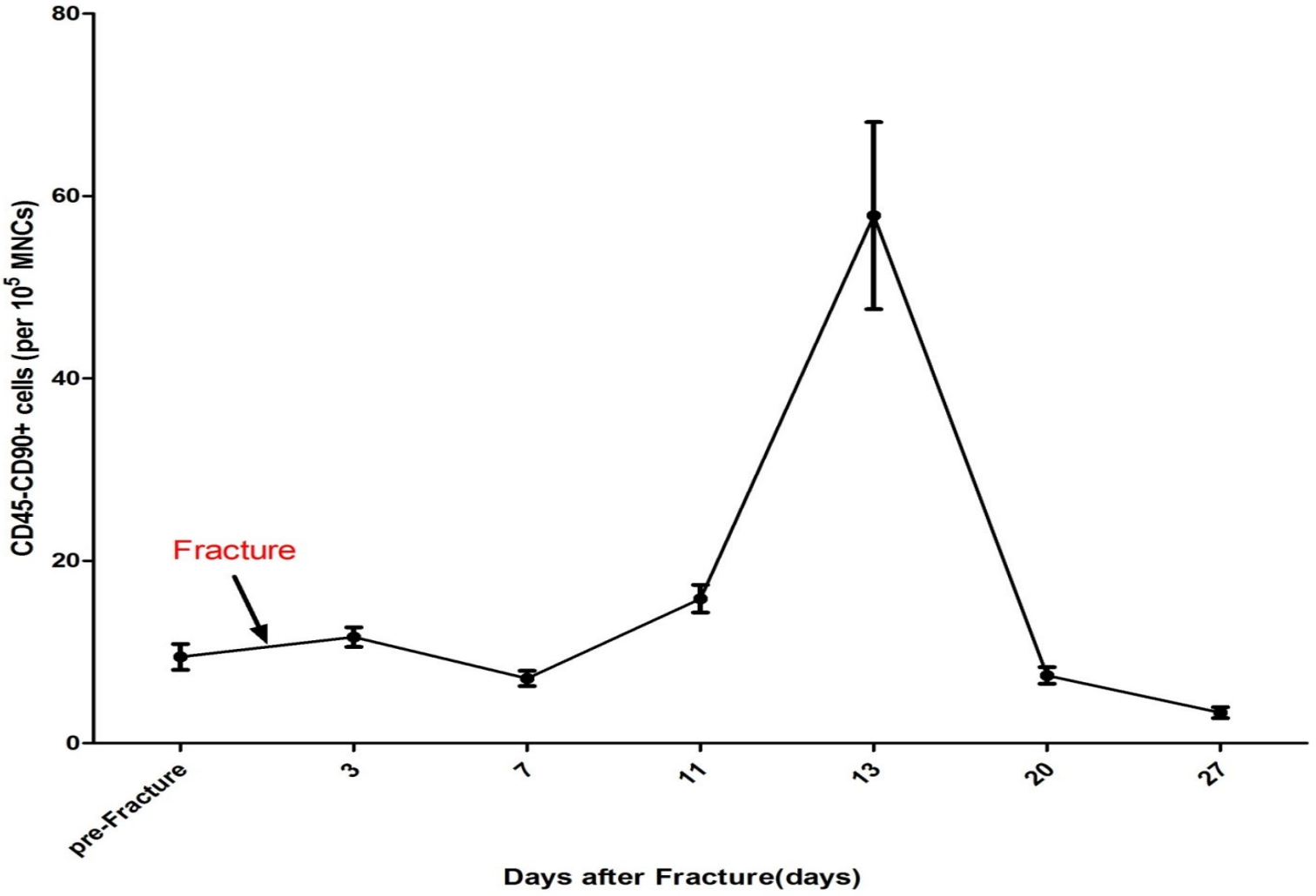
Changes of circulating MSCs during fracture healing in rats

- **Femoral closed fracture was created in 12 male SD rats (age 12 weeks) with intramedullary nail fixation.**

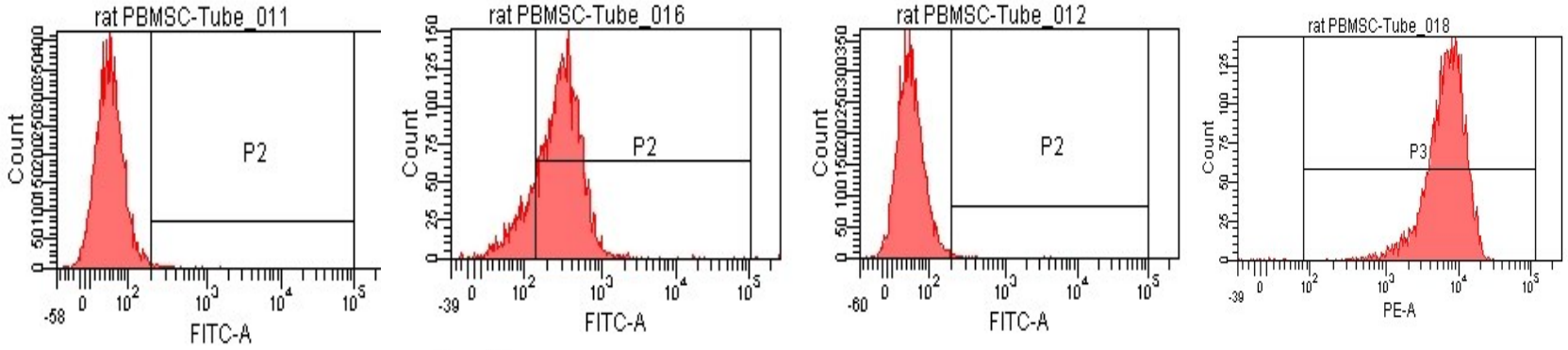
- **0.5 ml Peripheral blood was taken from the eye vein at day before fracture, 3, 7, 11, 13, 20, 27 post fracture; CD45 and CD 90 were used to labeled the cells as representative markers for circulating MSCs and subject to flowcytometry analysis.**



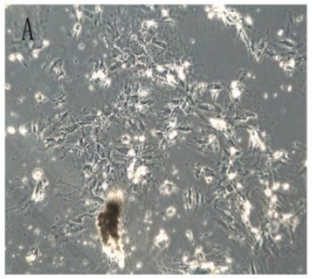
Results: Changes of blood MSCs (CD45-CD90+) during fracture process



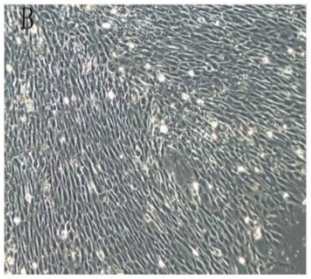
Results: Characterization and differentiation potentials of Circulating MSCs



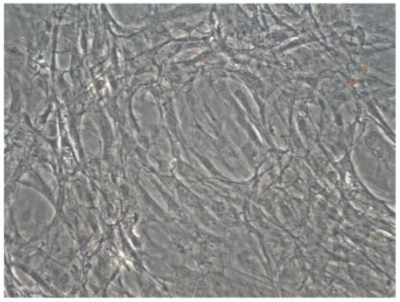
Day 8



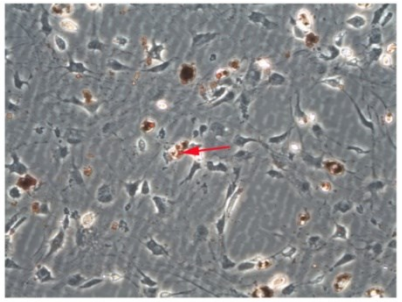
Day 11



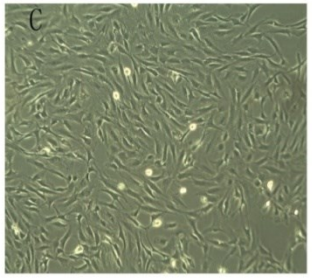
Control



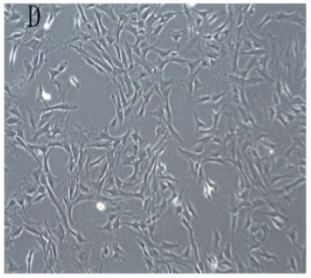
Adipogenesis



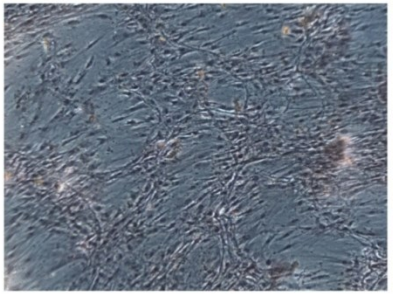
P1



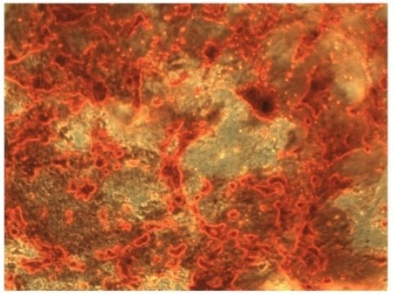
P3



Control



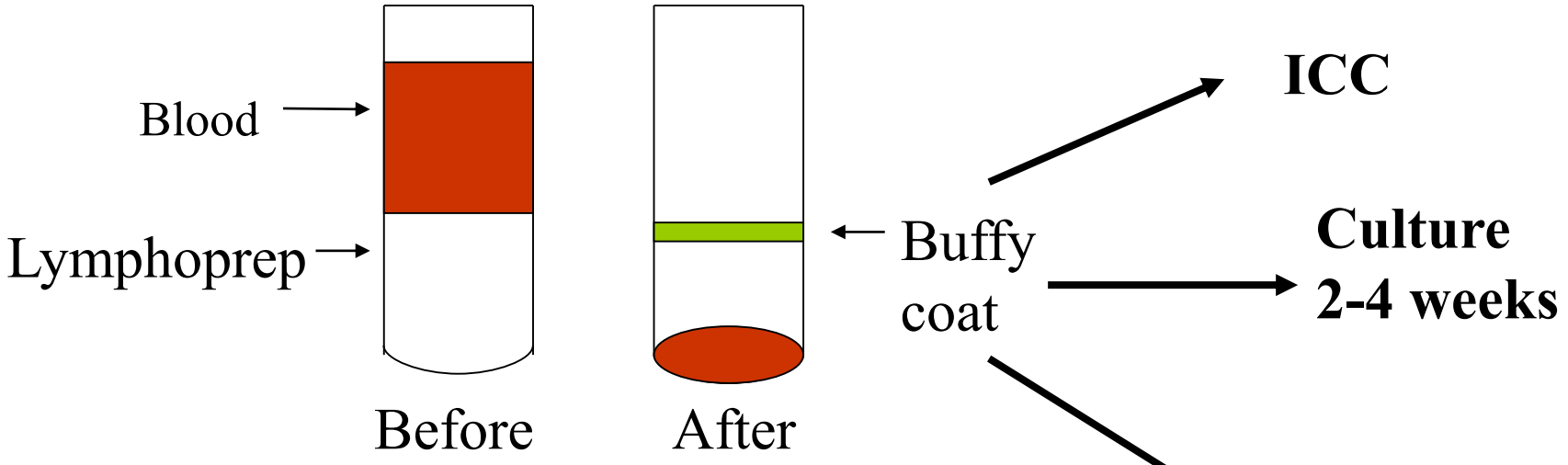
Osteogenesis



Study of Circulating MSCs in Fracture Patients

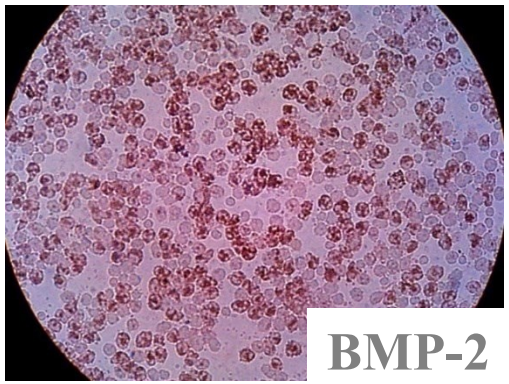
30 mls of peripheral blood collected from 8 fracture patients, at 3 time-points after fracture (days 1-3, 9-12 and 16-21) and also from 3 normal volunteers and 3 established non-union

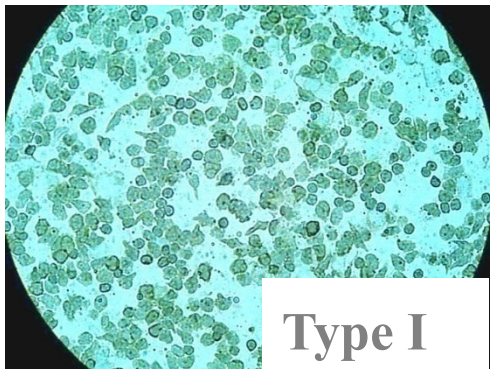
Peripheral blood mononuclear cells (PBMNCs) isolated using LymphoPrep™ density-gradient-centrifugation procedure.

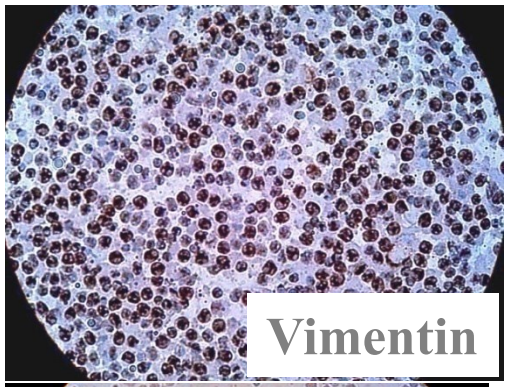


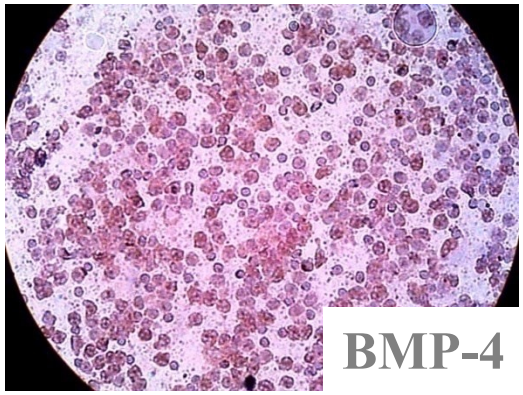
Centrifugation 400 g 20 min

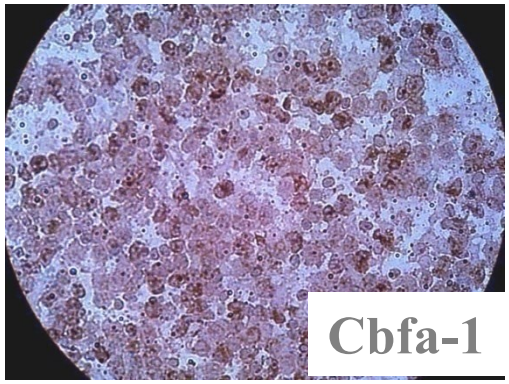
Immunostaining profile of the PBMNCs from a patient with tibial shaft fracture, at day 7 post- fracture

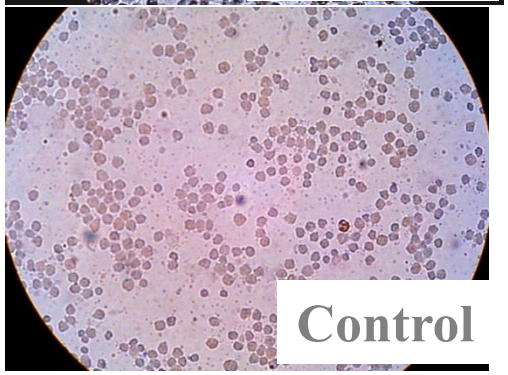


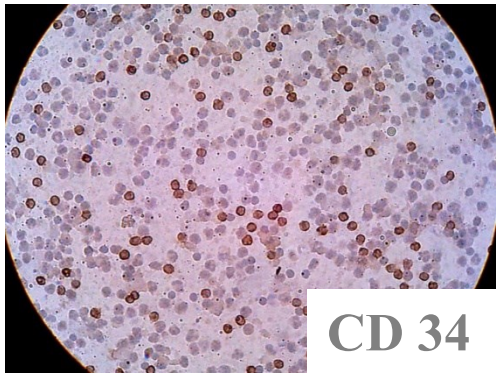


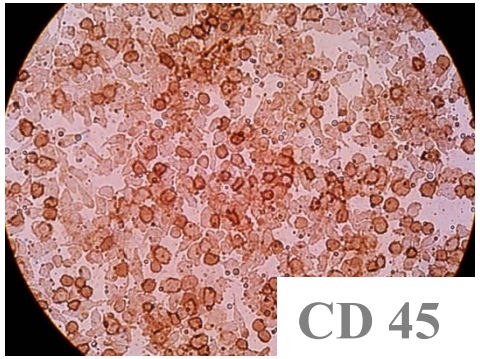


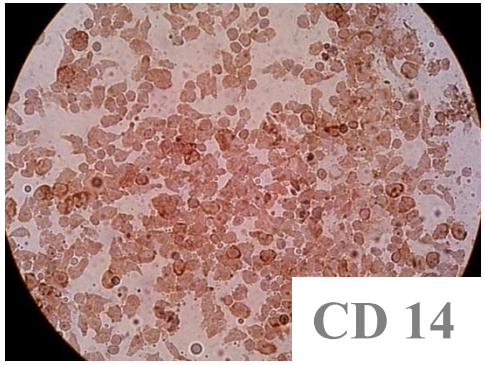




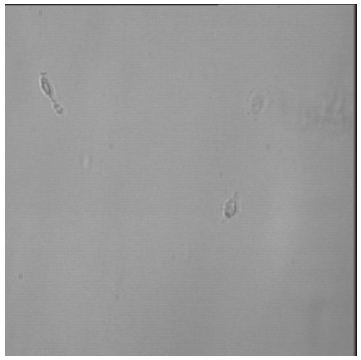
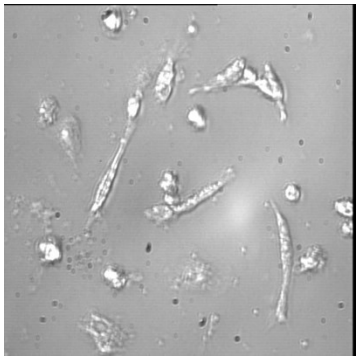
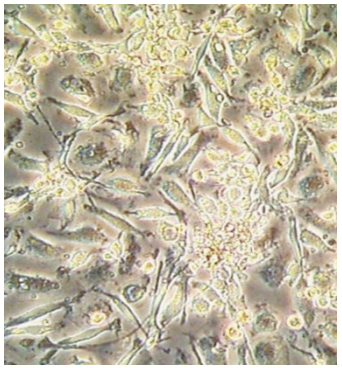
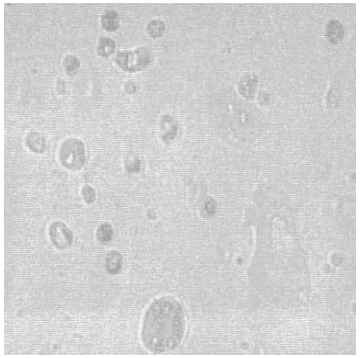




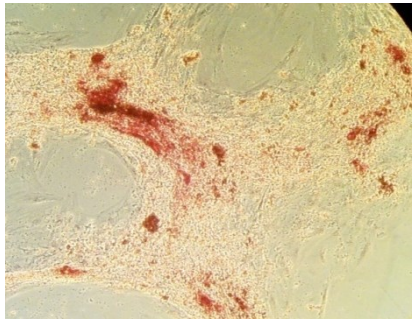




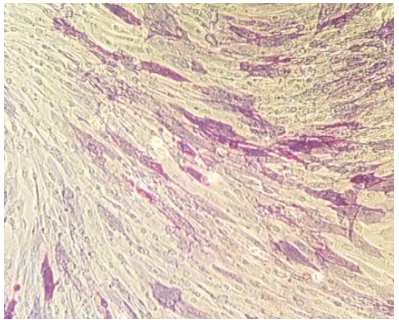
Summary of cell culture results

	< 4 days Post-fracture	> 14 days Post-fracture	Non-union patients	Control (normal)
cases	5	8	3	4
cells	few 	Some 	Many 	None/few 

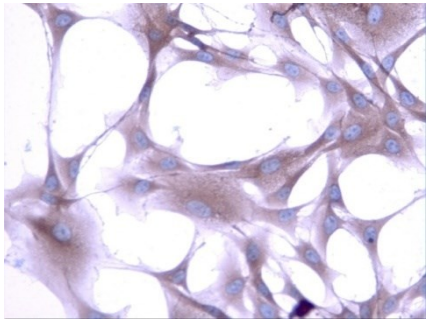
Differentiation potentials of human PBMSCs from Non-union patients



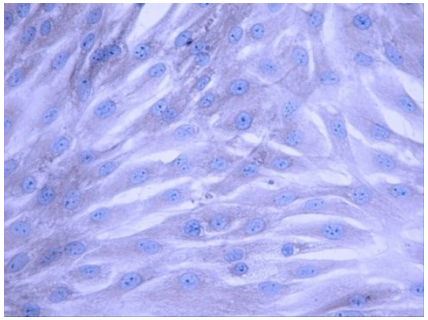
Alizarin red S d42 ×100



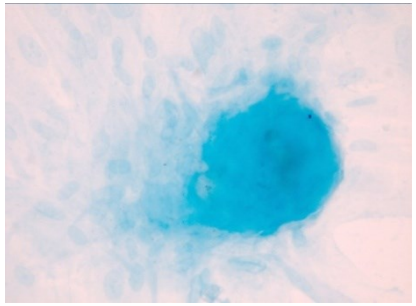
ALP d21 ×100



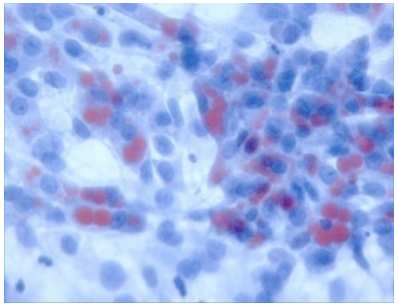
Osteocalcin d21 ×200



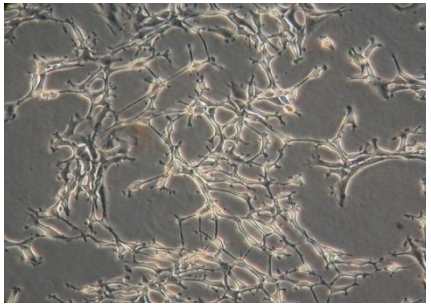
Collagen type I d21 ×200



Alcian blue d21

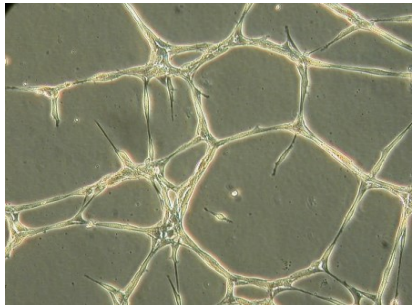


Oil red O d21 ×400

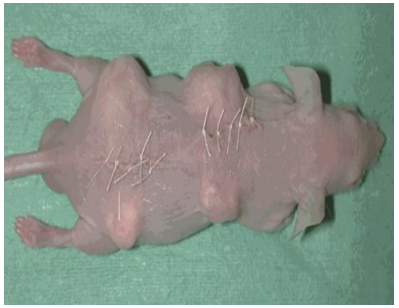


NGF x24 hr

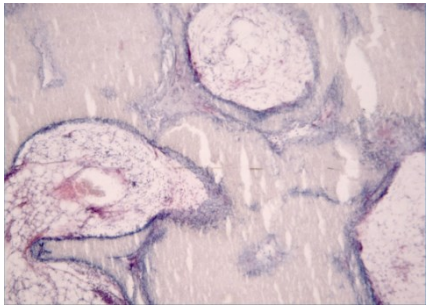
Neurofilament, ×200



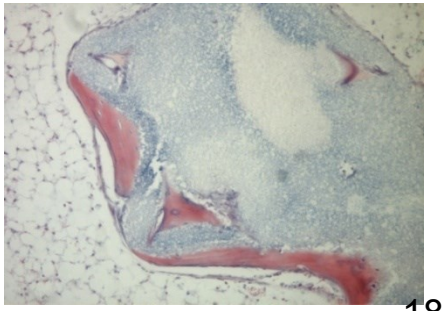
Matrigel 3D, 72h, x100



Nude Mice Implantation

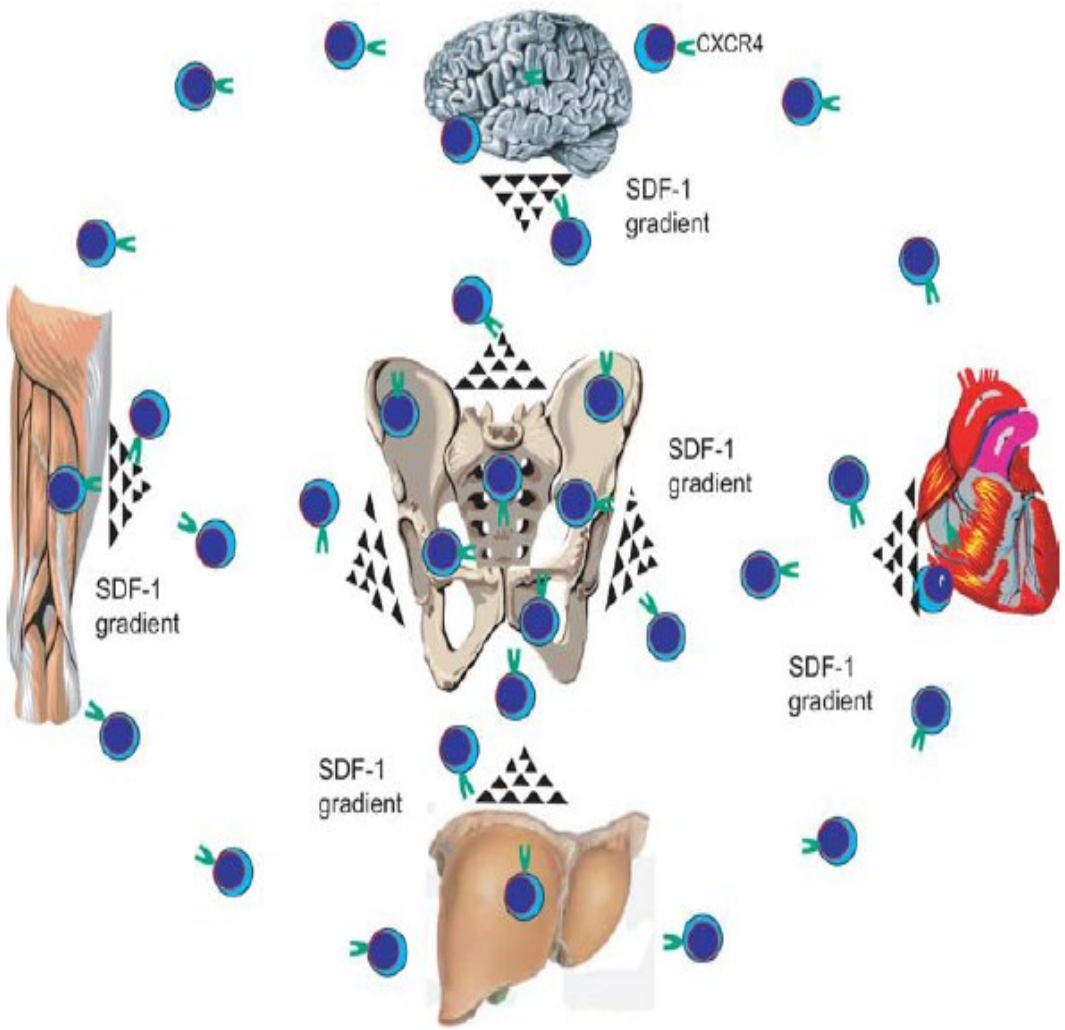


Non-cell Ca-P Block, 3M



PBMSCs Ca-P Block, 3M

MSCs Home to Injury Sites – Systemic Recruitment



MSCs home to a variety of tissues, particularly after tissue injury and ischemia.

Miyahara Y, Nagaya N, Kataoka M,et al . Monolayered mesenchymal stem cells repair scarred myocardium after myocardial infarction. Nat Med. 2006 Apr;12(4):459-65.

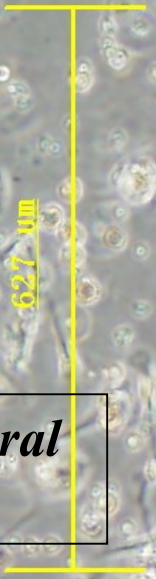
Carvalho KA, Guarita-Souza LC, Hansen P,et al. Cell Transplantation After The Coculture of Skeletal Myoblasts and Mesenchymal Stem Cells in the Regeneration of the Myocardium Scar: An Experimental Study in Rats. Transplant Proc. 2006 Jun;38(5):1596-1602.

Gnecchi M, He H, Noiseux N,et al. Evidence supporting paracrine hypothesis for Akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement. FASEB J. 2006 Apr;20(6):661-9.

Kraitchman DL, Tatsumi M, Gilson WD, et al. Dynamic imaging of allogeneic mesenchymal stem cells trafficking to myocardial infarction. Circulation. 2005 Sep 6;112(10):1451-61.

MSCs-like cells increased in patient with osteosarcoma, PBMNCs culture; 14 days; 40x

Flow cytometric analysis demonstrated an >9-fold increase in the number of cells with MSC-like phenotypes CD34(-)CD45(-)CD105(+) in patients with bone sarcomas compared with control subjects ($p<0.05$).



Bian, et al. Increased number of mesenchymal stem cell-like cells in peripheral blood of patients with bone sarcomas. Arch Med Res. 2009 Apr;40(3):163-8

Summary -1: PBMSCs

- **Is there MSCs in peripheral blood?**
  - ***Yes, they do exist.***
  - ***The number is significantly reduced with development/maturity, rare in normal adult.***
- **When do they show up?**
  - ***In conditions such as serious injuries, inflammation and cancer, et al.***

Where do the blood borne MSCs come from ? ***Bone marrow ?***

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Systemic recruitment of osteoblastic cells in fracture healing

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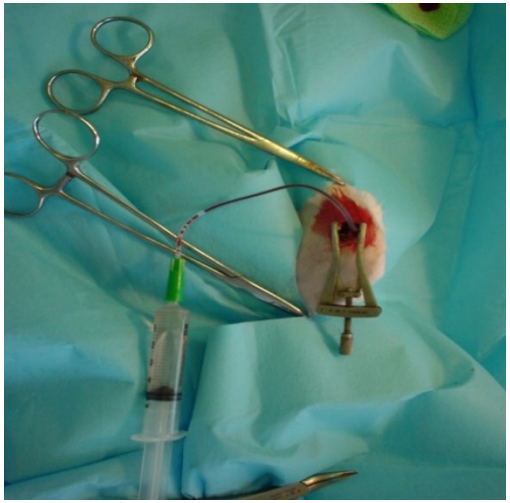
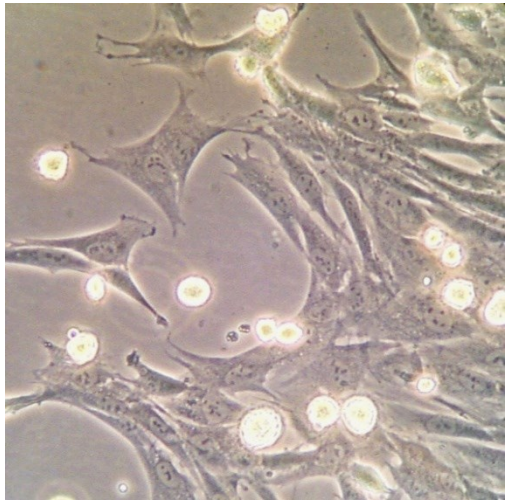
[b] *Department of Pathology, Royal Victoria Hospital, Belfast BT12 6BJ, UK*

Accepted 28 January 2005

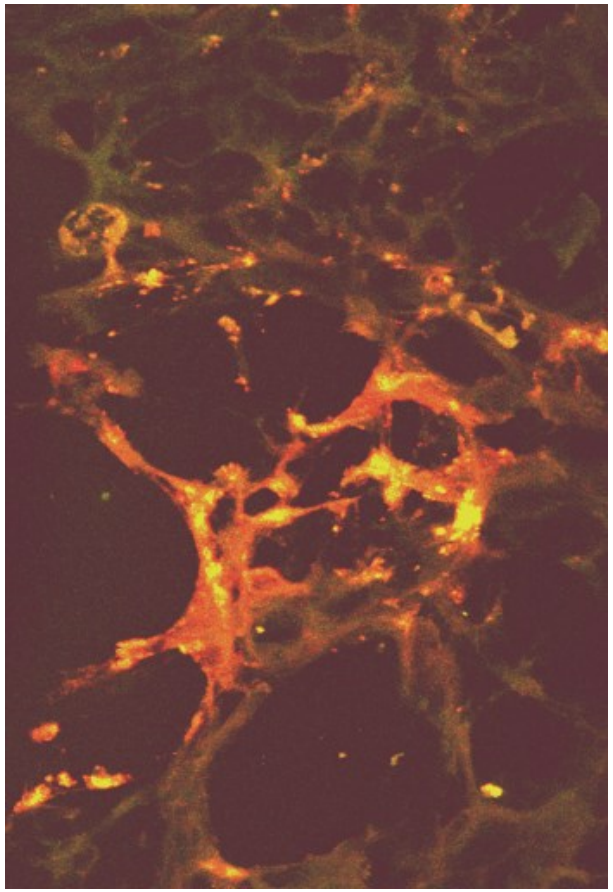
MSCs are recruited from bone marrow cavities and home to fracture sites through peripheral circulation.

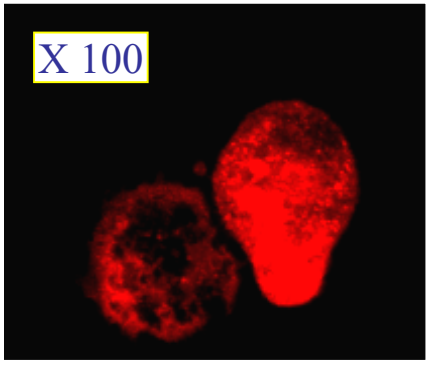


Bone marrow harvested



Rabbit bone marrow MSCs culture





Shirley D, et al, Journal of Orthopaedic Research, 2005, 23 (5): 1013-21.

Re-implantation of the labelled MSCs

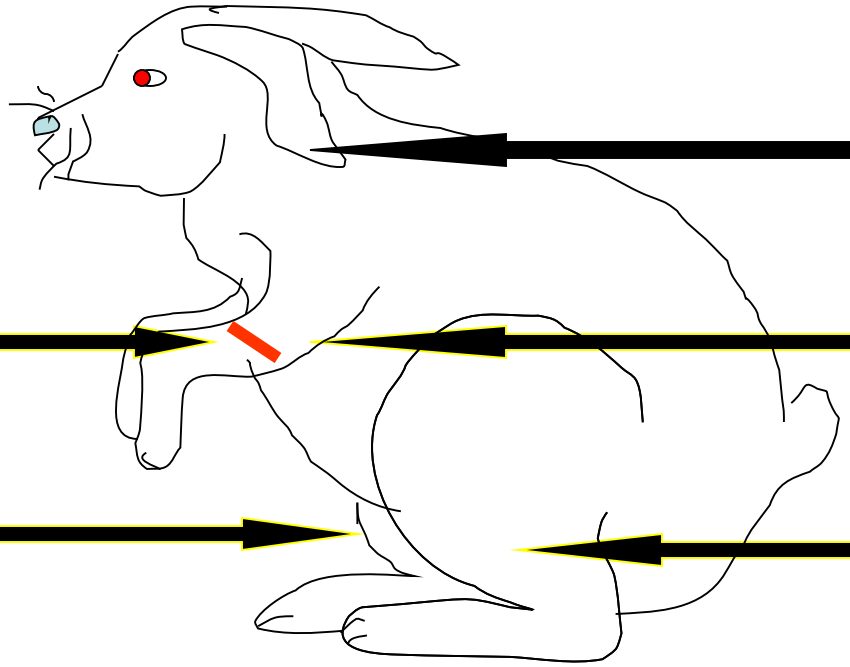
48 hours post Fx

In each group some animals were sacrificed at 3 & 12 weeks

Culture 3 weeks

Ulnar defect

BM harvest



Control E

Ear vein B

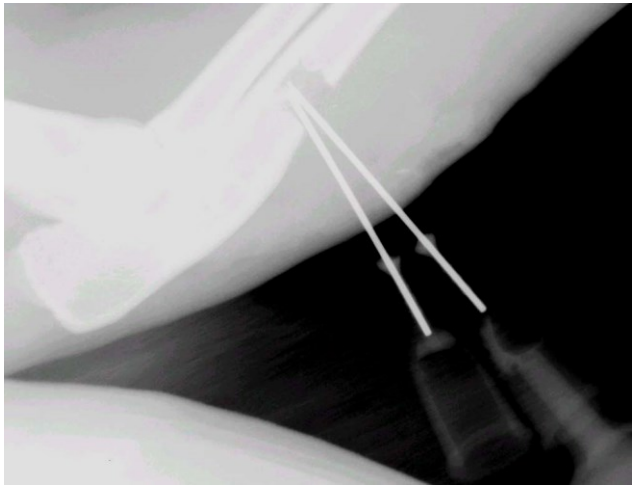
Fracture site A

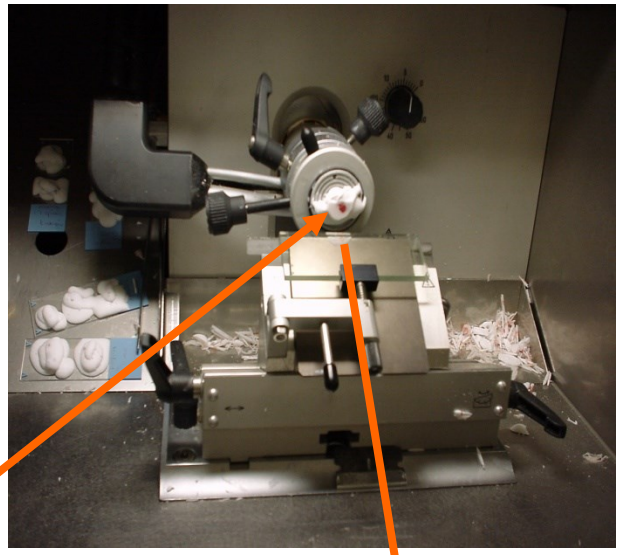
Remote BM site C

Shirley D, et al, Journal of Orthopaedic Research, 2005, 23 (5): 1013-21.

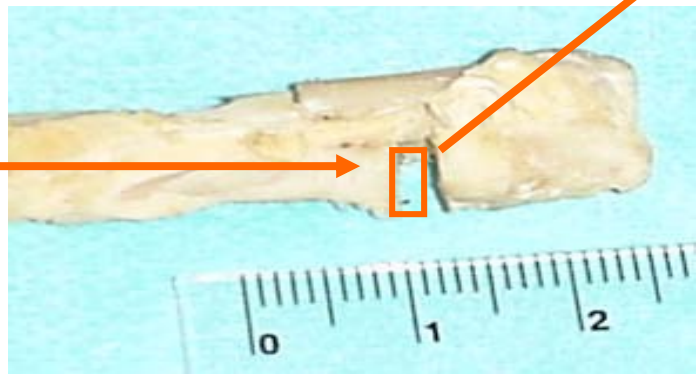
The tissues retrieved for frozen section – (5ųm)

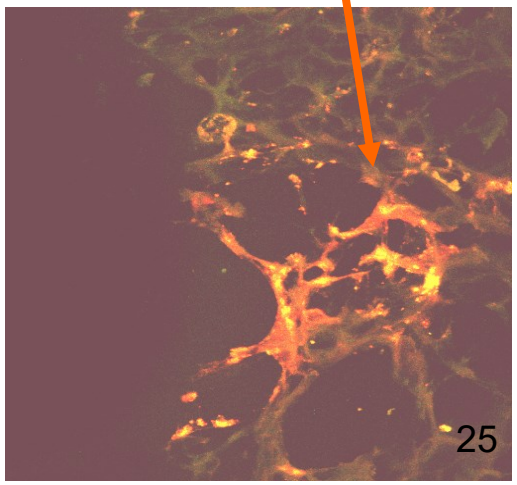
Animals were sacrificed at 3 and 12 weeks after cell implantation





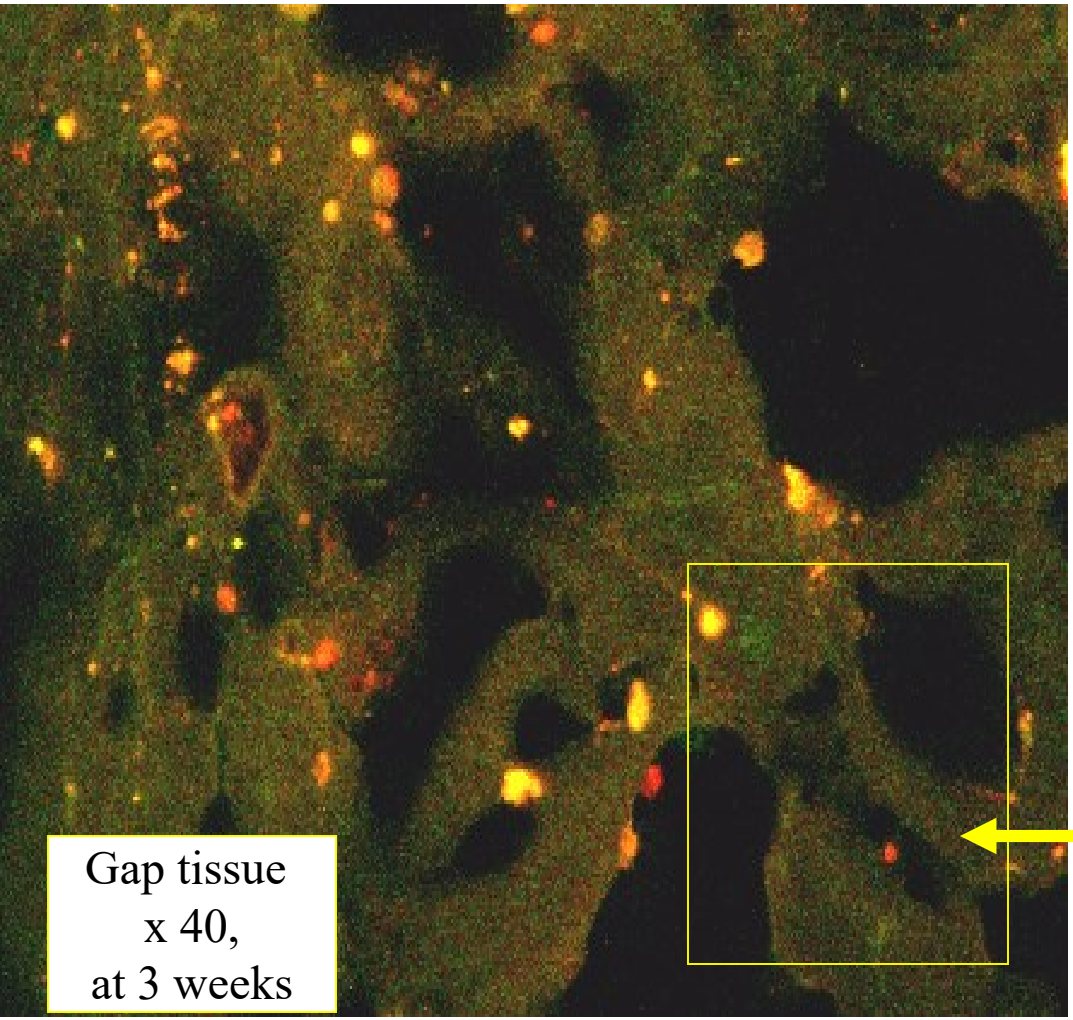
Gap tissue

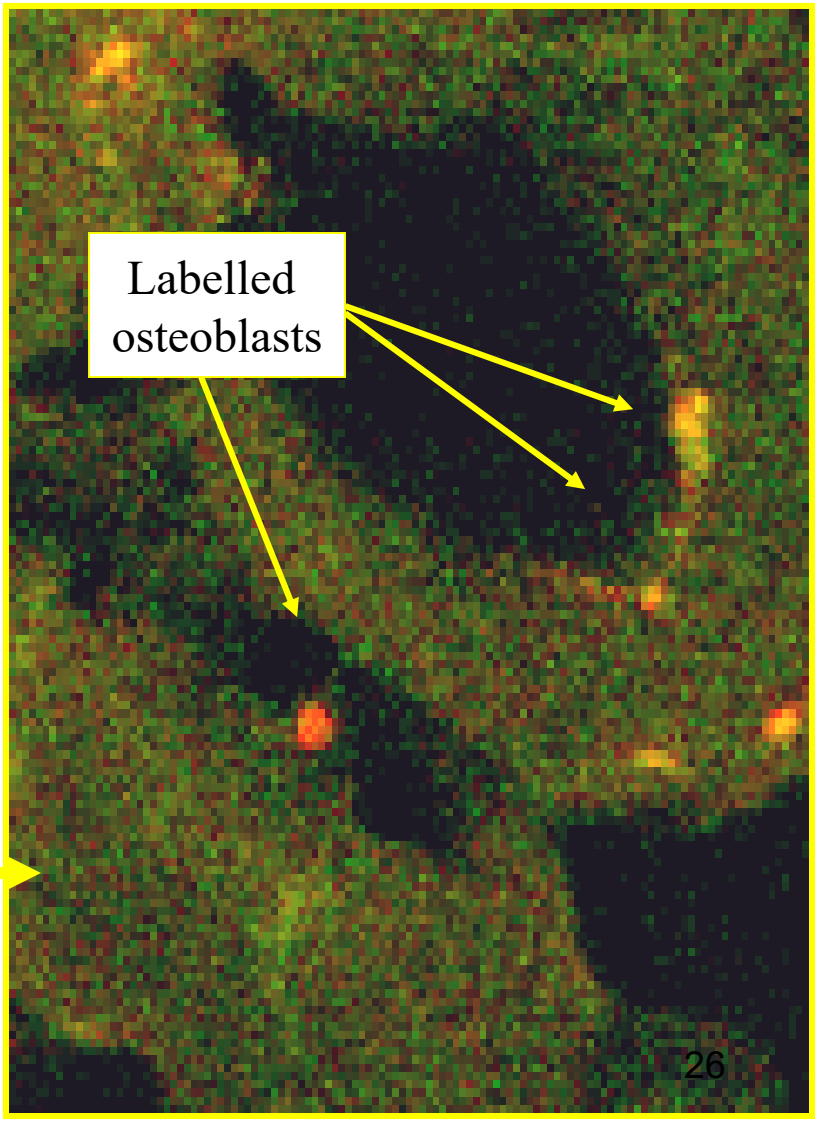




- Liver, lung, kidney, and spleen,
- Also cytospins of BM and blood
- (representative samples only)

Labelled cells from remote marrow were identified in fracture gap (Group C)

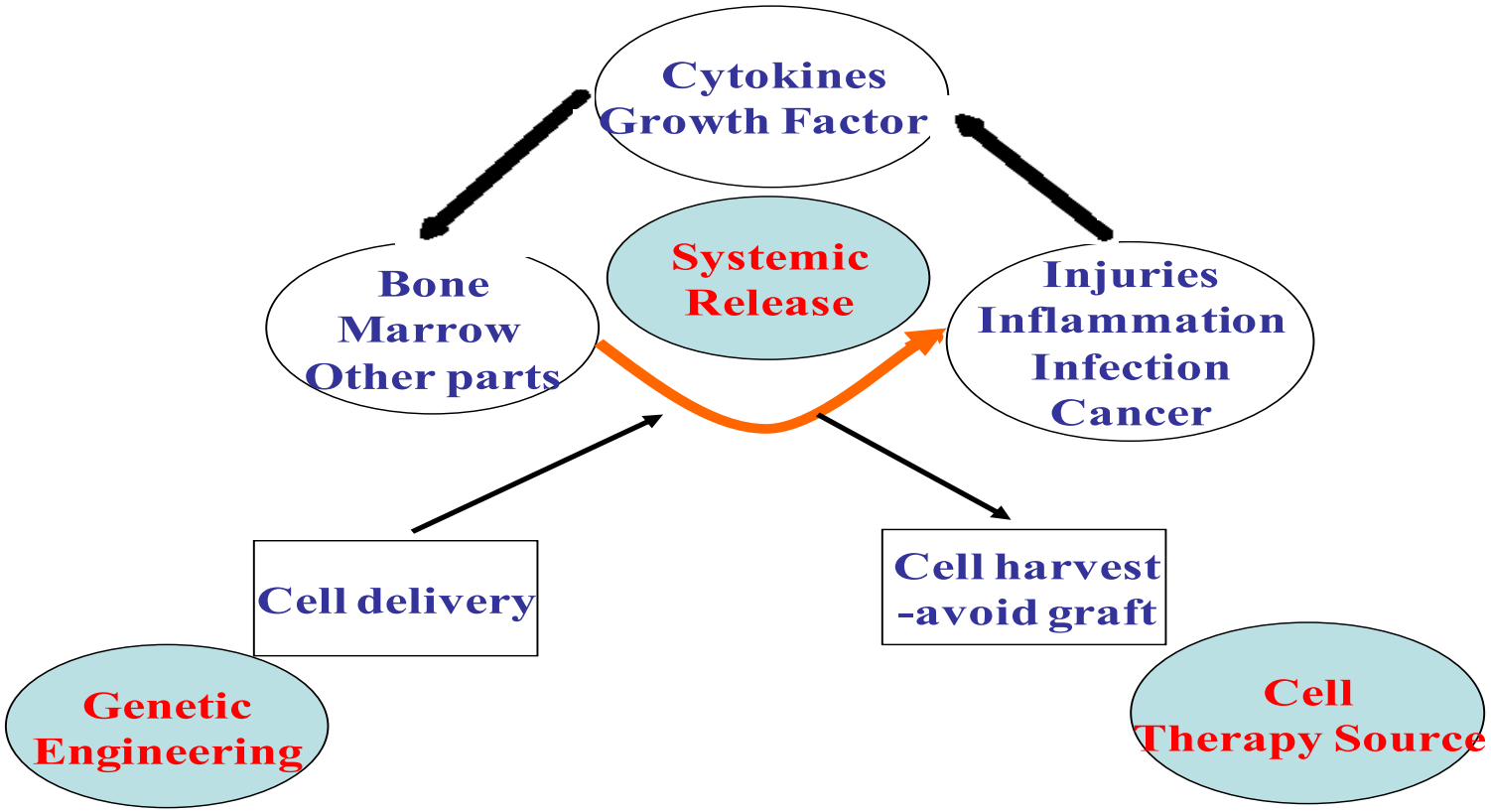




Shirley D, et al, JOR, 2005, 23 (5): 1013-21.

Summary -2: PBMSCs

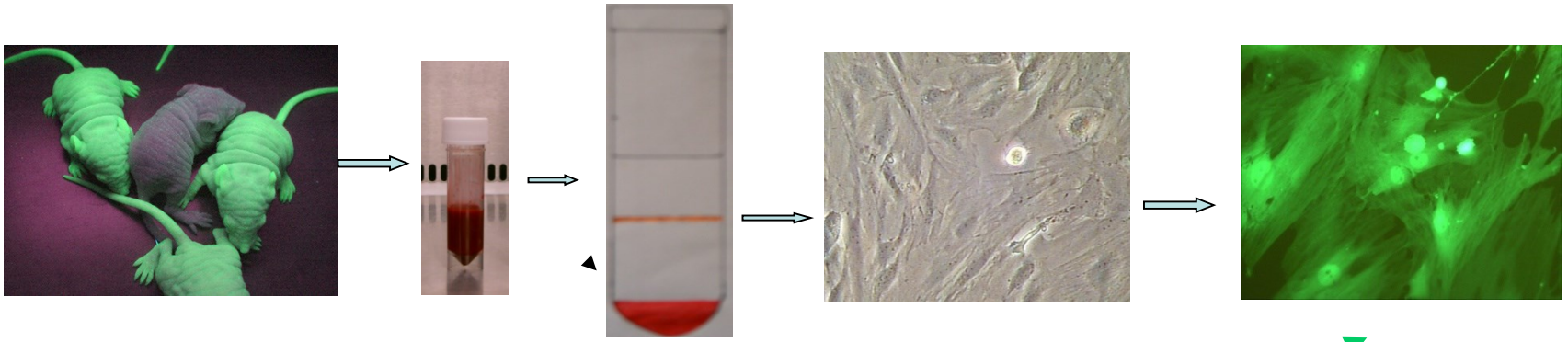
- ***Some osteoblasts integral in fracture repair come from remote bone marrow.***
- ***They are actively recruited through the peripheral circulation.***

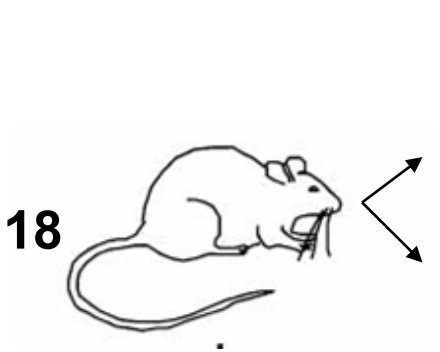


Blood borne MSCs - Questions

- **Is there MSCs in peripheral blood?**
- **When do they show up?**
- **Where do they come from?**
- **What can we learn from them?**
- **What are the clinical implications?**

➢ ***Compare*** *the biological characteristics of the MSCs derived from peripheral blood and bone marrow in the GFP rats.*





18

male

3 ~ 6 month old

PB samples

BM samples

7/18

18/18

3

3

1

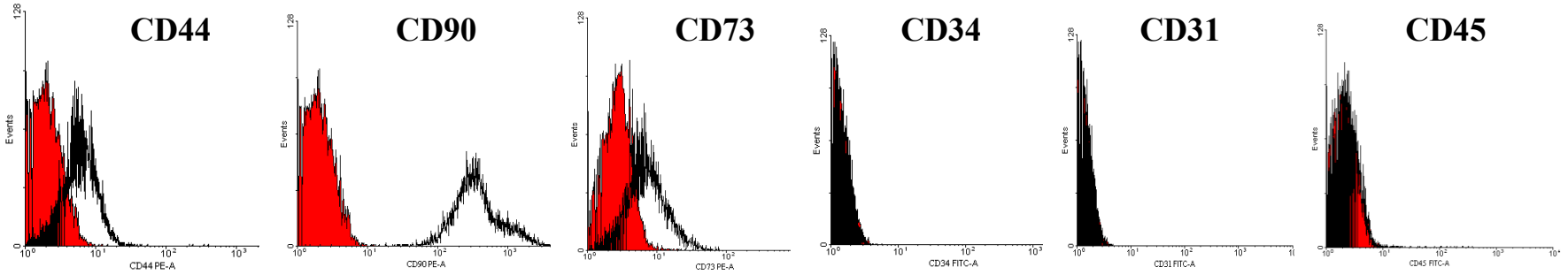
ICC, Flow Cytometry

Microarray

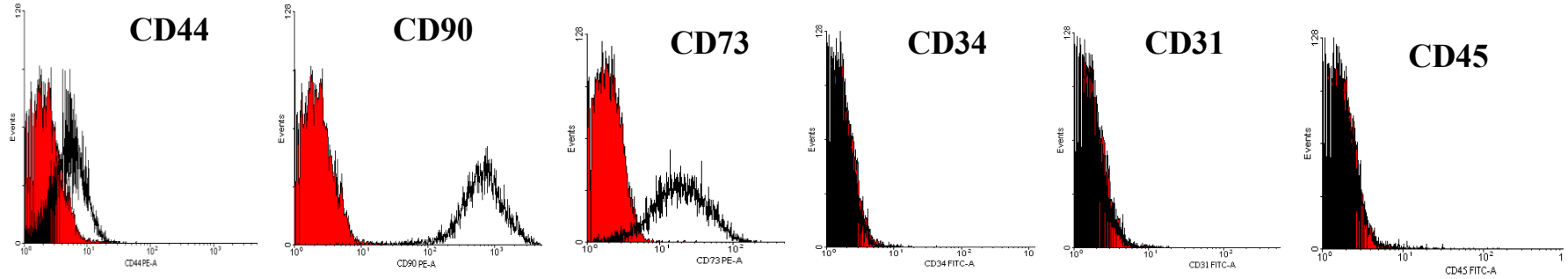
Single colony derived strains

Surface Markers of BM-MSCs and PB-MSCs

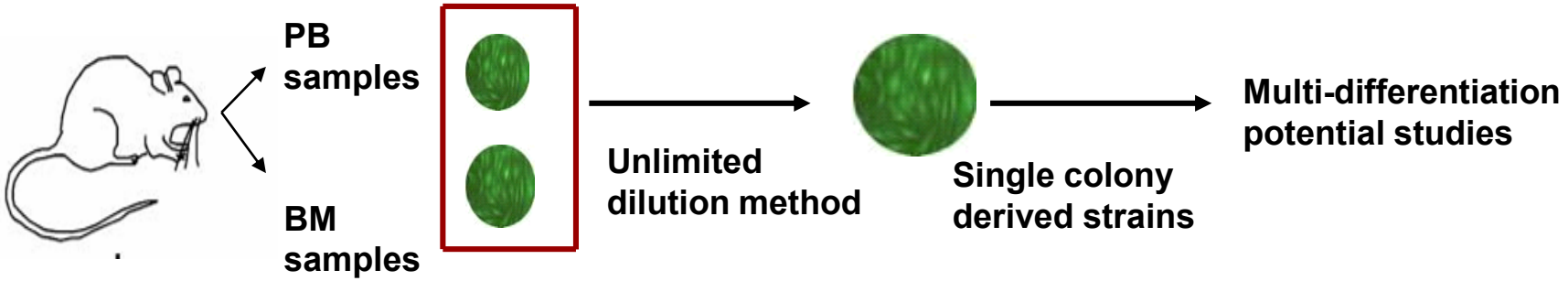
BM-MSCs



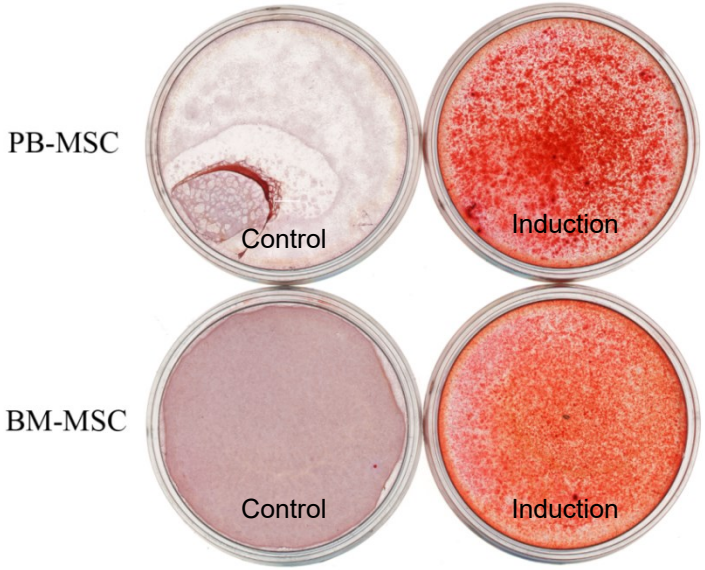
PB-MSCs



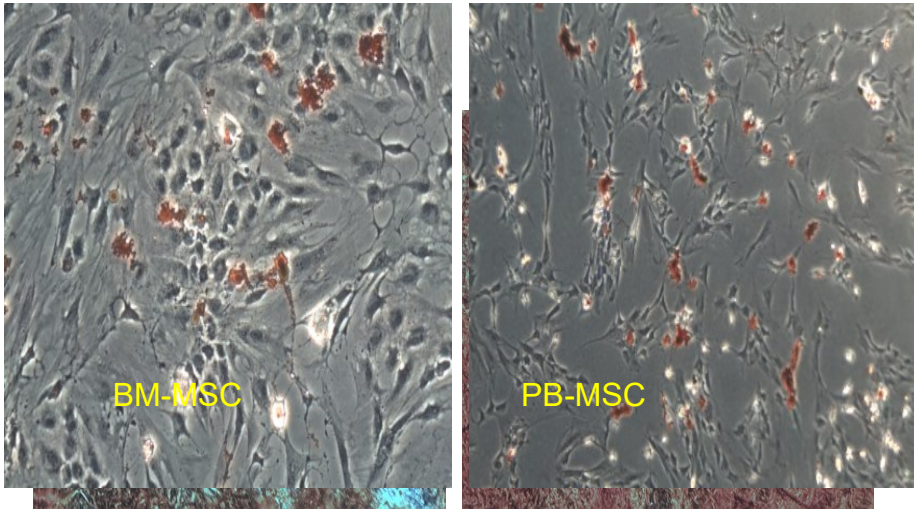
➢ *Results – Studies of multi-differentiation potentials*



Osteogenic inductive condition



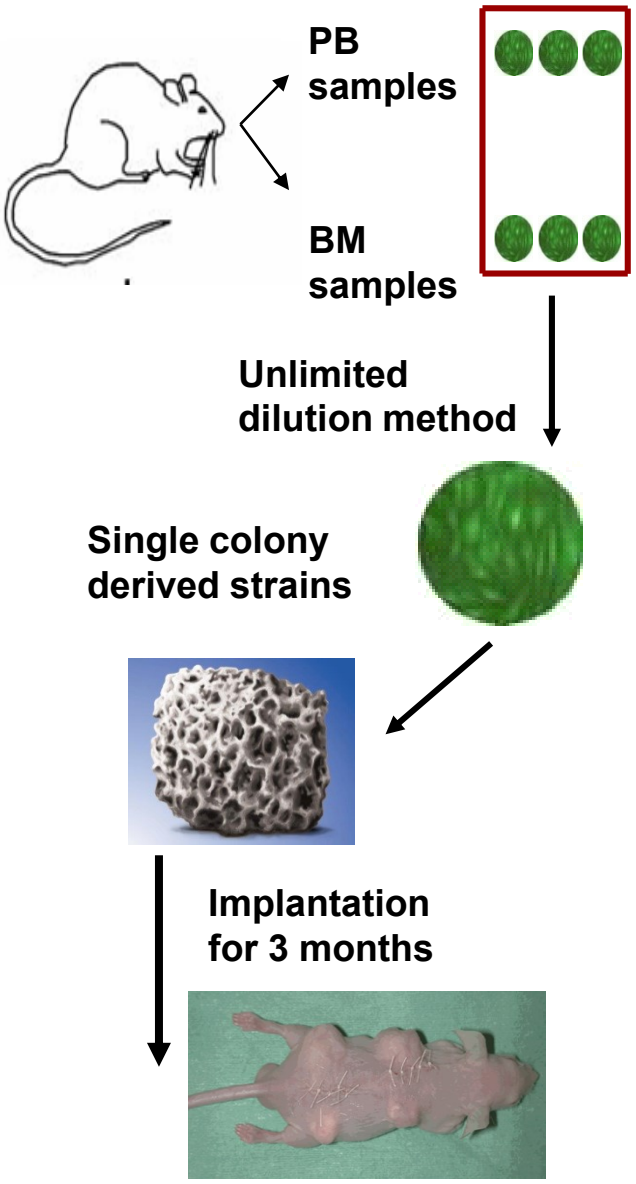
Adipogenic inductive condition

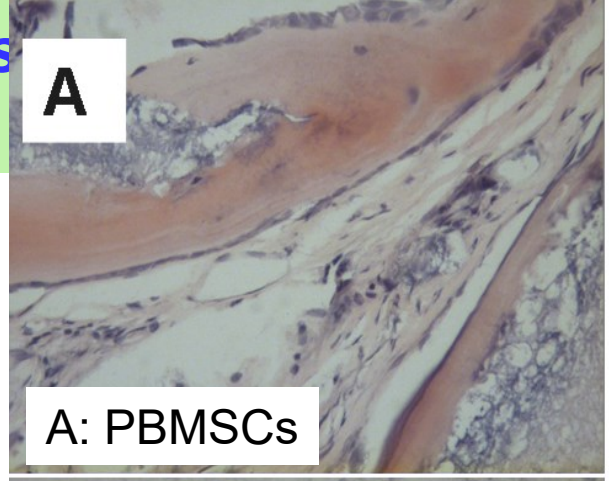


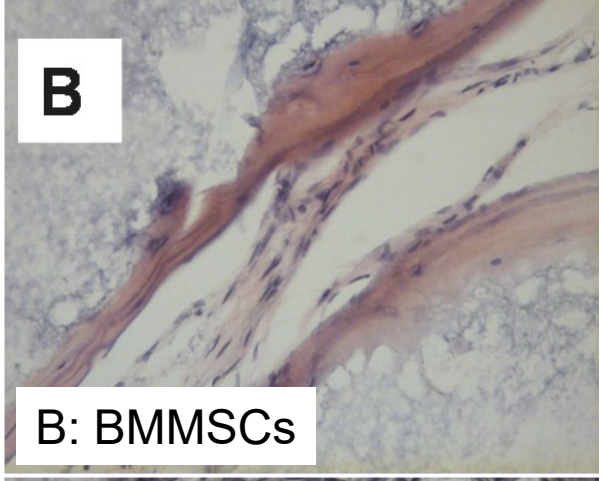
Adipogenesis – staining with Oil Red O

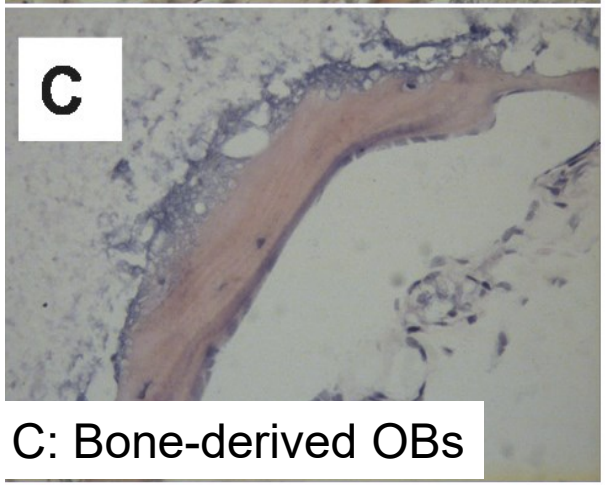
Osteogenesis – staining with Alizarin Red

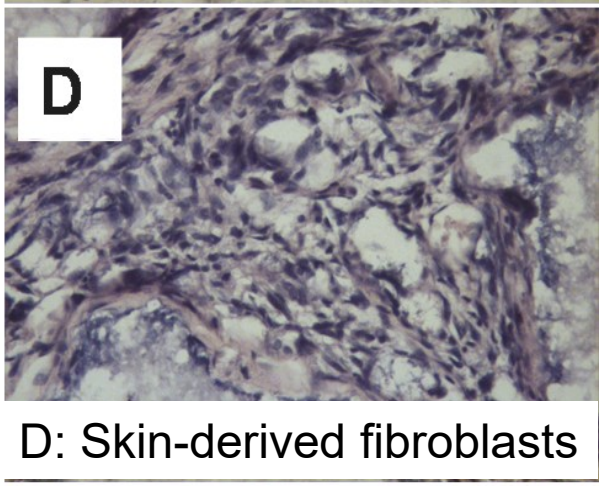
➢ *In vivo osteogenesis of PB-MSCs*

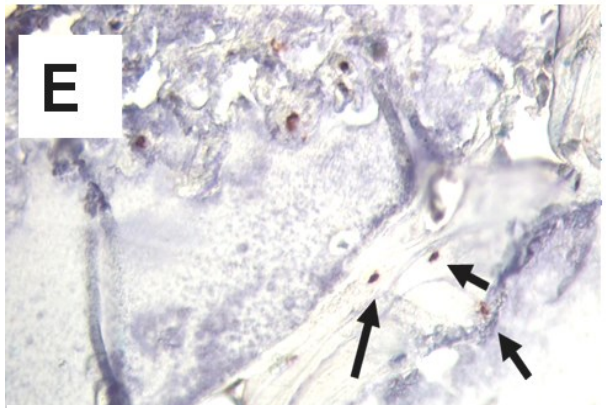


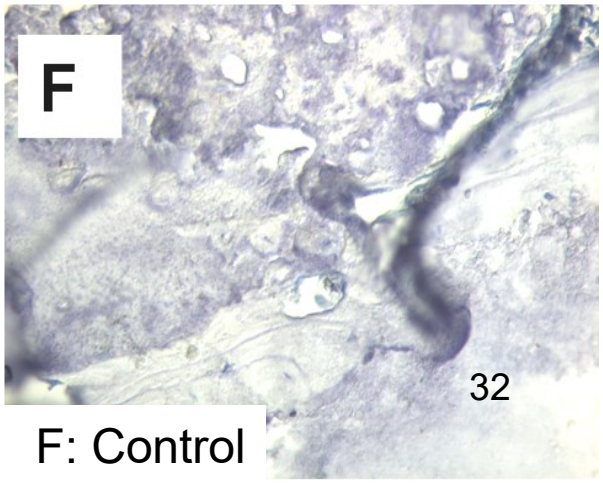












Microarray Results

Clustering analysis

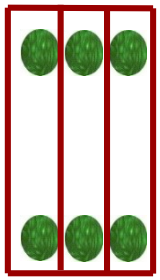
PB vs. BM (>2fold, E-B/B-E>100, p<0.05, 167 genes)

83 genes are up regulated

84 genes are down regulated

PB samples

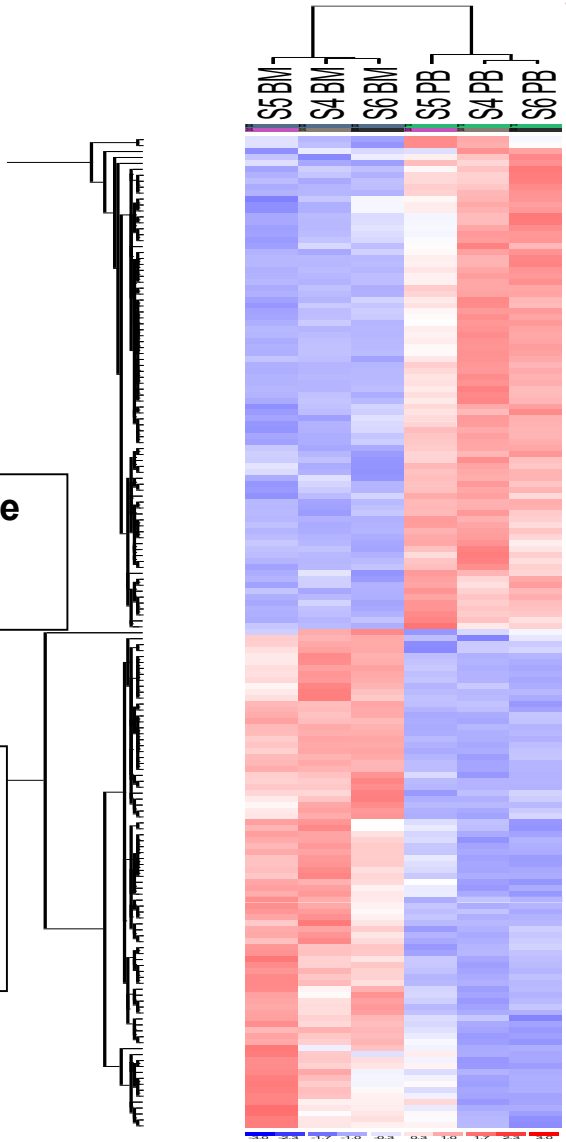
BM samples



3 paired RNA samples - cDNA

Microarray analysis using gene chip Affymetrix rat expression Array 230A

Data acquisition by Affymetrix Gene Chip Scanner 3000. The initial quantification by the GCOS (Affymetrix) and followed by DNA-Chip Analyzer 1.3.



Difference in expression of selected genes between PBMSCs and BMMSCs determined by Microarray and real-time PCR

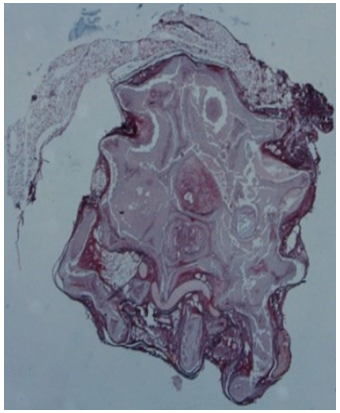
Gene name	PBMSCS vs BMMSCs	Microarray		Real-time PCR	
		Fold change	P value	Fold change	P value
Retinol-binding protein 1	up	496.54	0.042	574.00	0.035
Cadherin 2	up	51.11	0.037	101.00	0.013
Bone morphogenetic protein 6	up	21.35	0.012	7.17	0.003
SRY-box containing gene 11	up	17.15	0.040	39.50	0.009
Chloride intracellular channel 5	up	10.68	0.007	3.07	0.023
Small nuclear ribonucleoparticle-associated protein (snRNP) mRNA, clone Sm51	up	10.35	0.007	3.89	0.000
Aquaporin 1	down	-94.01	0.048	-75.70	0.011
Arginine vasopressin receptor 1A	down	-15.31	0.030	-11.60	0.011
Prostaglandin E receptor 4 (subtype EP4)	down	-13.13	0.048	-4.56	0.000
Collagen, type XVIII, alpha 1	down	-10.23	0.000	-2.23	0.004

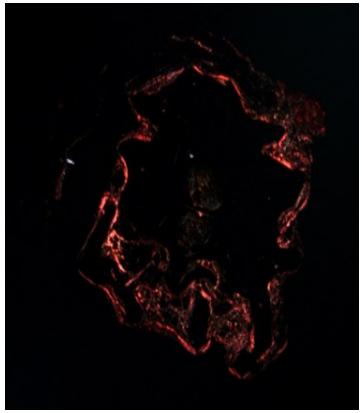
Function of genes with >10 fold change (PBMSCs *vs.* BMMSCs)

PBMSCs vs BMMSCs	Gene	Function
up	**Retinol-binding protein 1 (+496 fold)**	**Embryonal development, vision, epithelial differentiation, immune function, reproduction**
	Cadherin 2 (+51 fold)	**Calcium dependent cell-cell adhesion glycoprotein, gastrulation, asymmetry, synapses, cancer development and stem cell homing**
	Bone morphogenetic protein 6	Osteogenesis, early development
	SRY-box containing gene 11	**Sox11, oligodendrocyte development, DNA binding, transcription factor function**
	Chloride intracellular channel 5	Choloride channel activity, remains unclear, no in gene bank
	Small nuclear ribonucleoparticle-associated protein (snRNP) mRNA, clone Sm51	**May be involved in tissue-specific alternative RNA processing events**
down	**Aquaporin 1 (-94 fold)**	**Integral membrane protein that is a major water transport molecule in the kidney proximal tubule and red blood cells, membrane permeability**
	Arginine vasopressin receptor 1A (-15 fold)	Encodes a receptor for arginine vasopressin, G-coupled receptor activity
	Prostaglandin E receptor 4 (subtype EP4)	Binds prostaglandin estradiol (PGE(2)) and induces cAMP-dependent bone resorption

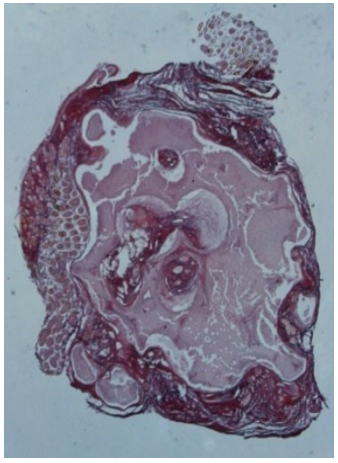
CRBP1 Over-expression promotes osteogenic differentiation of BM-MSCs

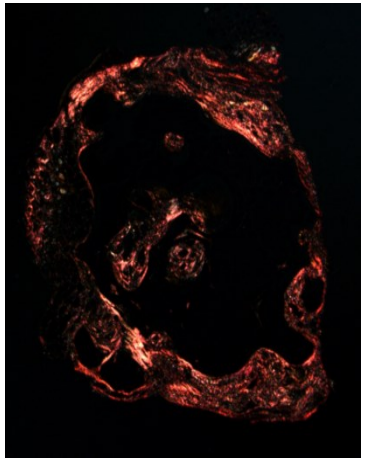
MSCs-dsRed

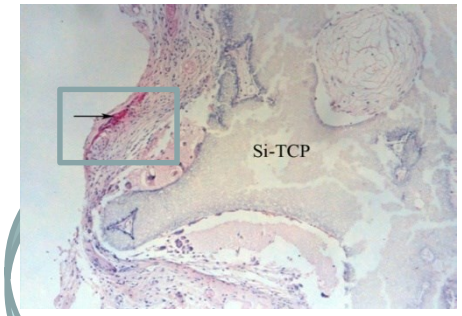




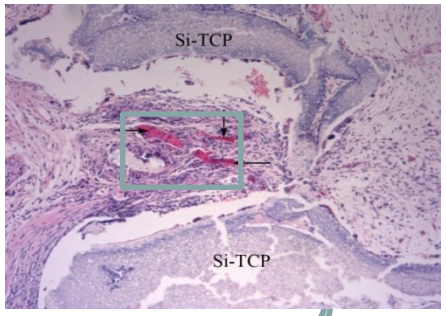
MSCs-CRBP1



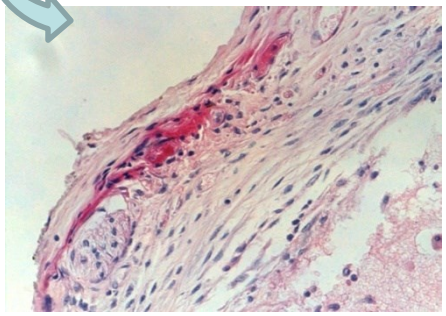




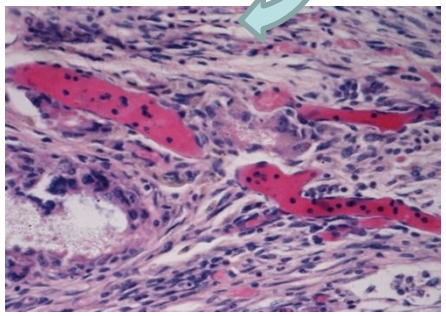
MSCs-dsRed, x10



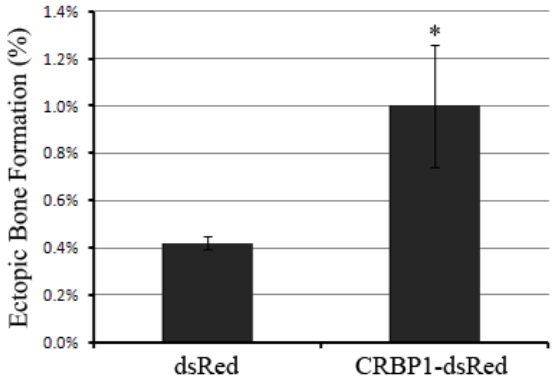
MSCs-CRBP1, x10



MSCs-dsRed, x40



MSCs-CRBP1, x40



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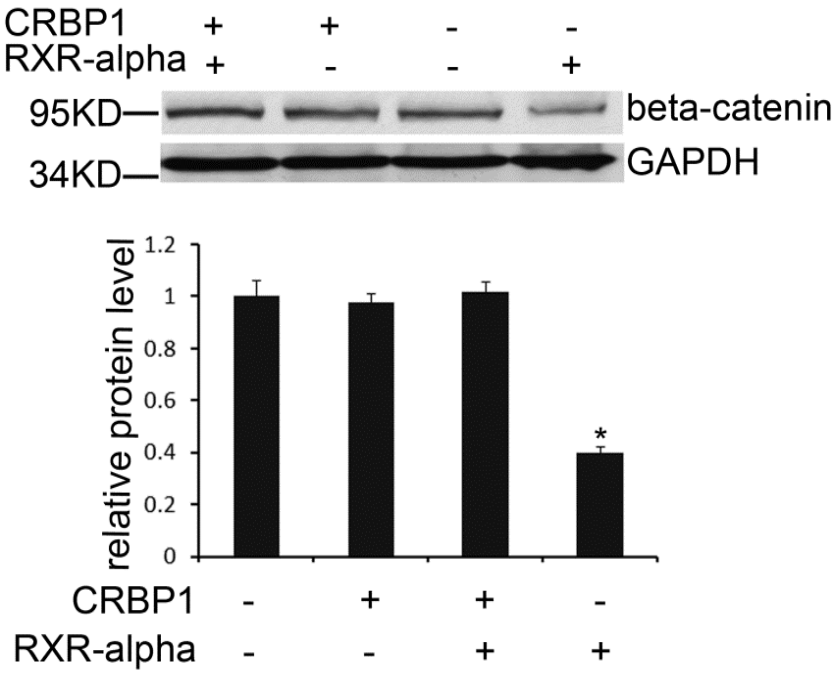
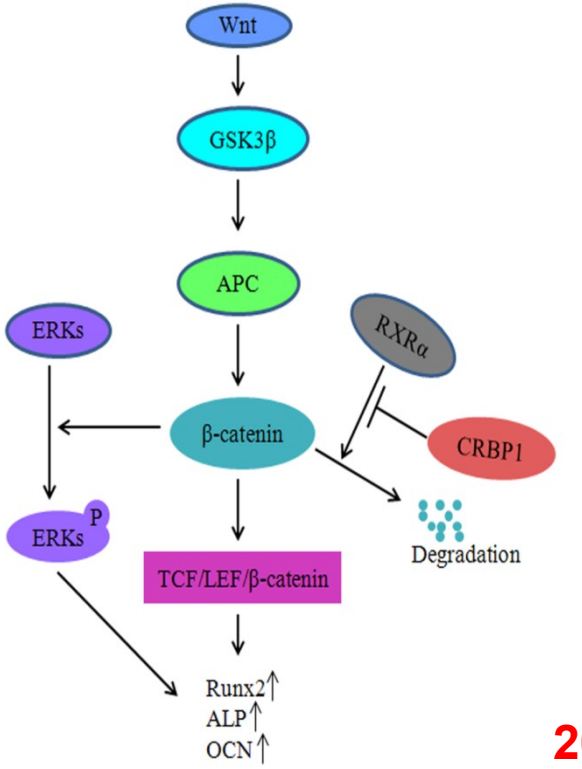
Cellular retinol-binding protein 1 (CRBP-1) regulates osteogenenesis and adipogenesis of mesenchymal stem cells through inhibiting RXRα-induced β-catenin degradation

Liangliang Xu[a,b], Chao Song[b,c], Ming Ni[b], Fanbiao Meng[a,b], Huiqi Xie[e], Gang Li[a,b,d,e,*]

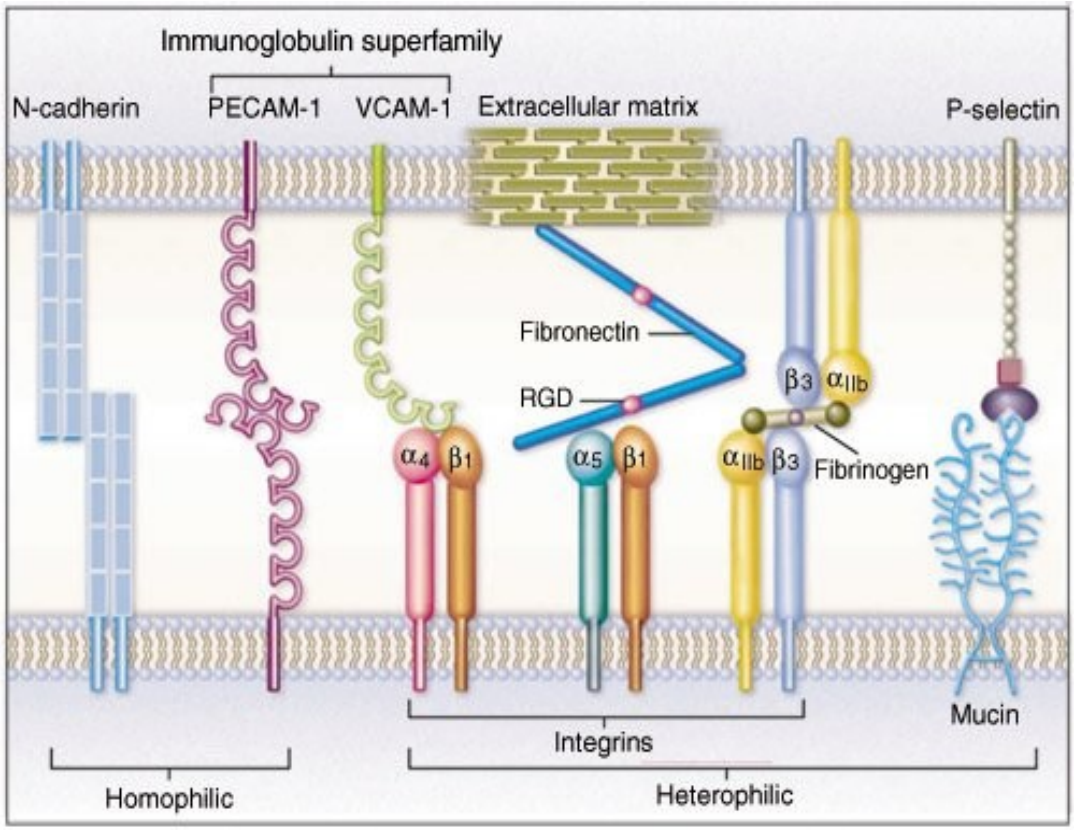
[a] Stem Cells and Regeneration Program, School of Biomedical Sciences, Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong, China





2012, June,
IF:4.96

N-cadherin: calcium dependent cell adhesion glycoprotein



Structure of N-cadherin and other major classes of adhesion receptors.
Frenette and Wagner, N Engl J Med, 1996

✓ **N-cadherin plays a role in mediating signal transduction events during bone development (Guntur et al., 2011).**

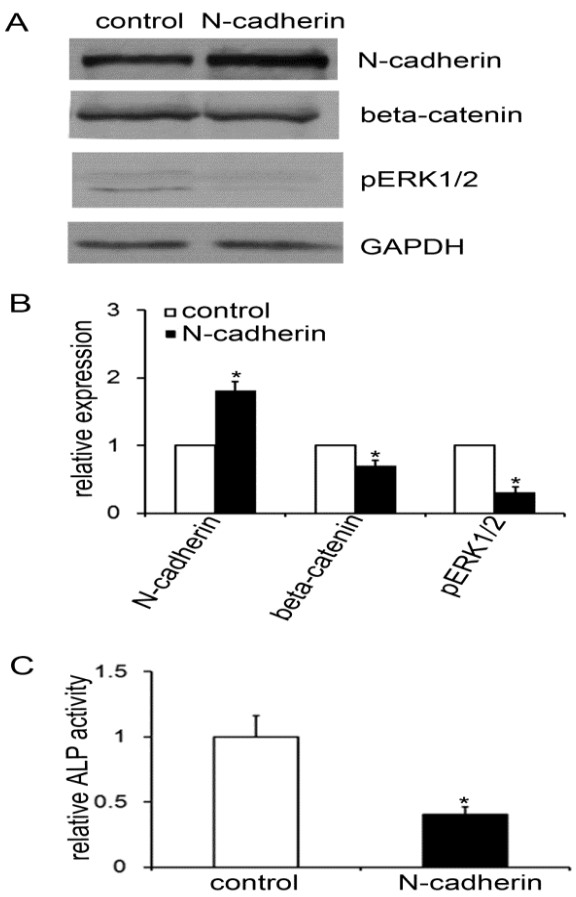
✓ **N-cadherin has been shown to interact with Wnt receptor LRP5 to negatively regulate Wnt/β-catenin signaling (Hay et al., 2009).**

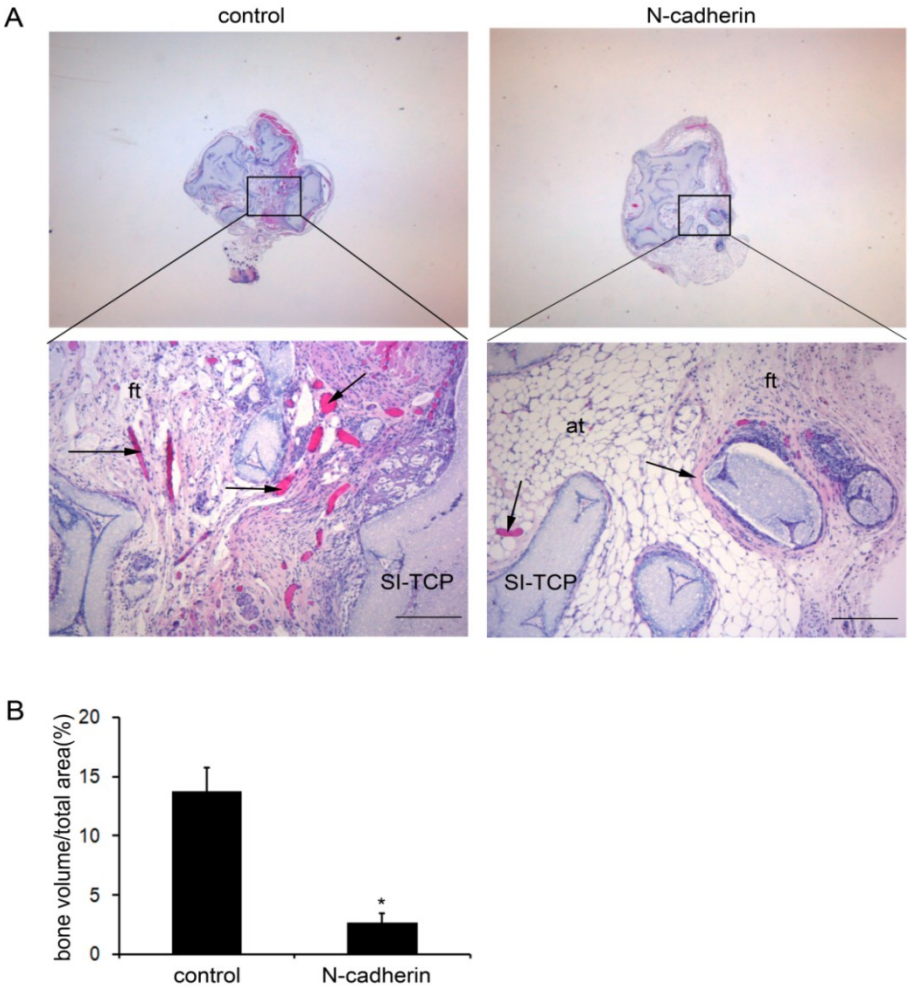
Mol Biol Rep
DOI 10.1007/s11033-012-2334-0

2012, November, IF: 3.4

N-cadherin regulates osteogenesis and migration of bone marrow-derived mesenchymal stem cells

Liangliang Xu · Fanbiao Meng · Ming Ni · Yukwai Lee · Gang Li

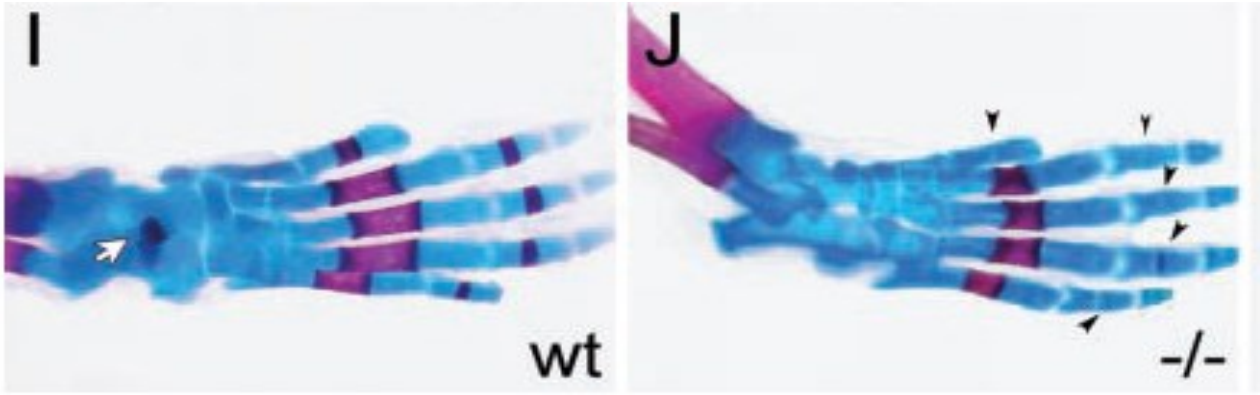




SOX11: Group C HMG (high mobility group) transcription factor

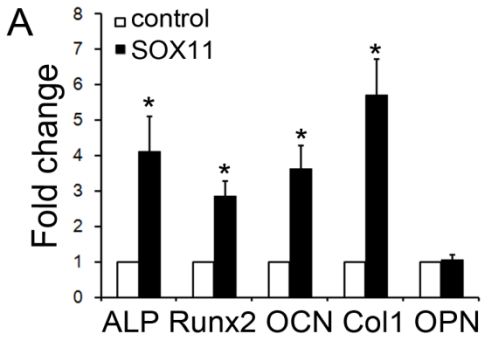
- **Sox11 is expressed at high levels in developing sensory neurons and is hypothesized to regulate neuronal maturation (Hargrave et al., 1997).**

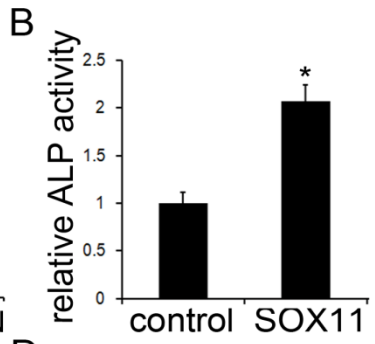
- **Sox11 knockdown suppressed the self-renewal capacity, reduced the osteogenic and adipogenic differentiation potential in MSCs (Kubo, Shimizu et al., 2009).**

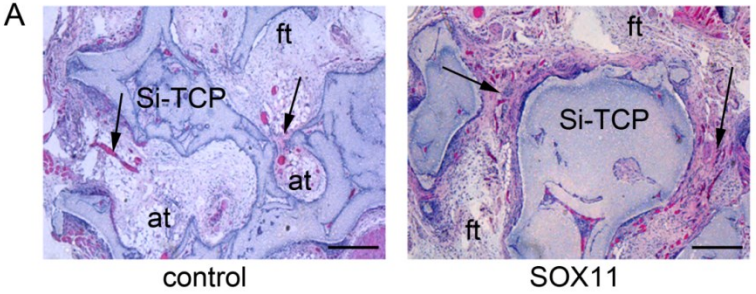


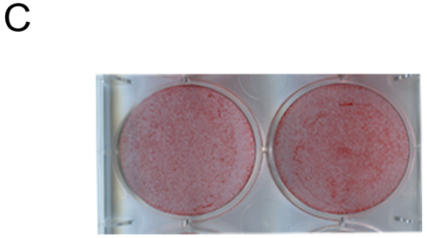
Sox11-deficient embryos at 18.5 dpc, Elisabeth Sock, et al., 2004

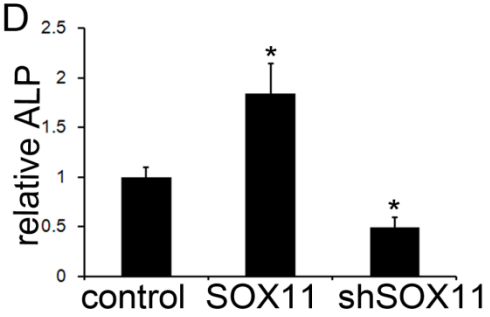
SOX11 promotes osteogenesis and migration of MSCs

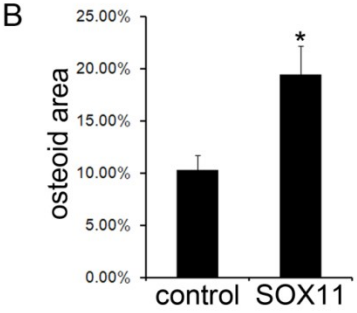


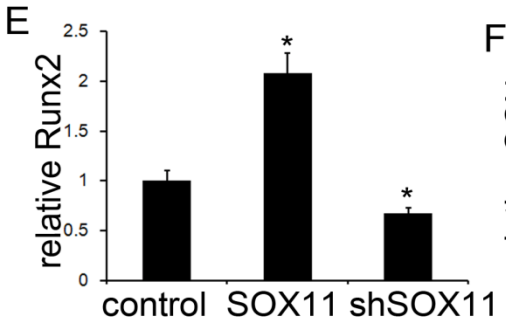


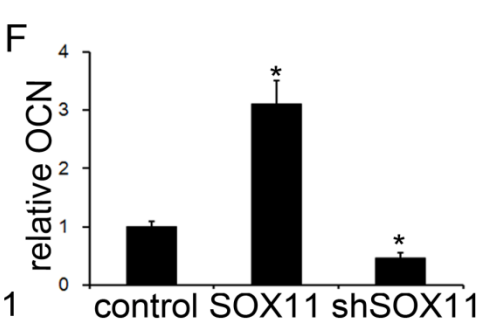


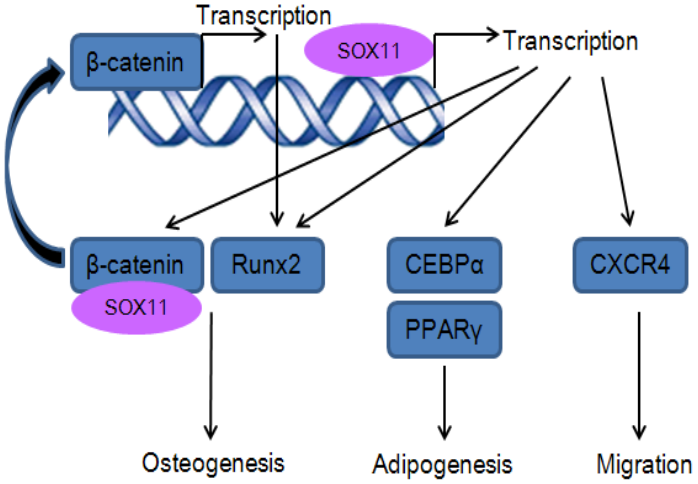












FASEB Journal, 2015; 29(4):1143-52

FASEB Journal, 2015; 29(4):1143-52

Sox11-modified mesenchymal stem cells (MSCs) accelerate bone fracture healing: Sox11 regulates differentiation and migration of MSCs

Liangliang Xu,*,†,1 Shuo Huang,*,1 Yonghui Hou,†,1 Yang Liu,* Ming Ni,* Fanbiao Meng,* Kuixing Wang,* Yunfeng Rui,* Xiaohua Jiang,‡,2 and Gang Li*,†,§,¶,2

*Department of Orthopaedics & Traumatology and †Stem Cell and Regeneration Theme, School of Biomedical Sciences and Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kon Prince of Wales Hospital, Shatin, Hong Kong, People's Republic of China; ‡Epithelial Cell Biology Research Center and §MOE Key Laboratory of Regenerative Medicine, School of Biomedical Science The Chinese University of Hong Kong, Shatin, Hong Kong, People's Republic of China; and ¶The CUHK-ACC Space Medicine Centre on Health Maintenance of Musculoskeletal System, Sh Research Institute, The Chinese University of Hong Kong, Shenzhen, People's Republic of

Figure 6

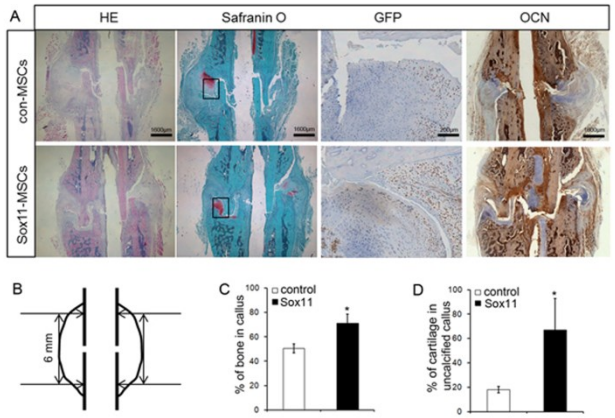
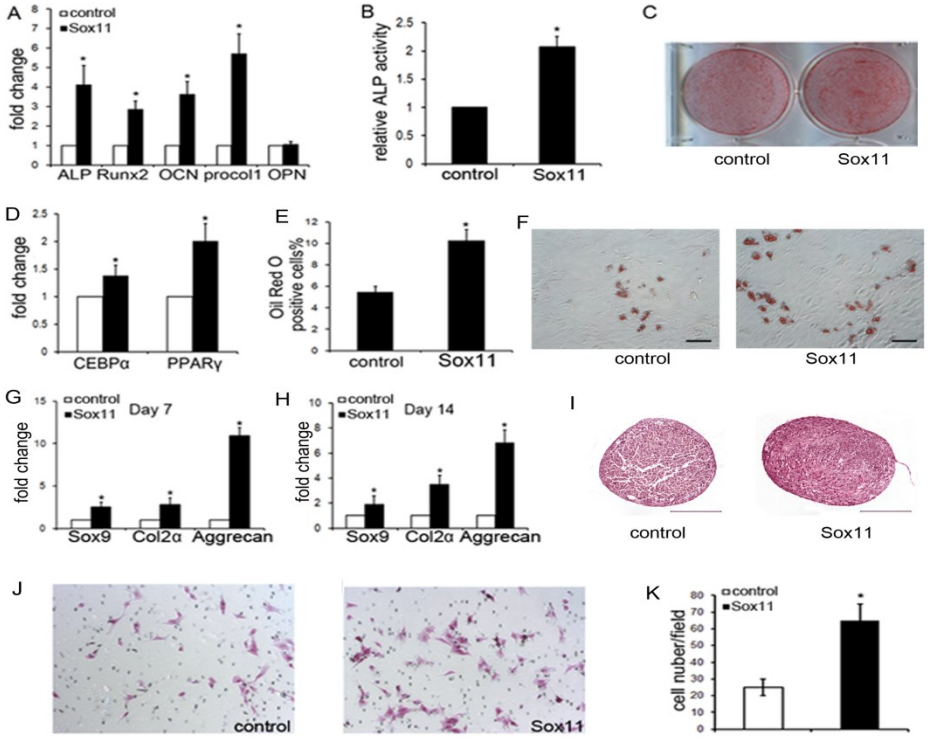
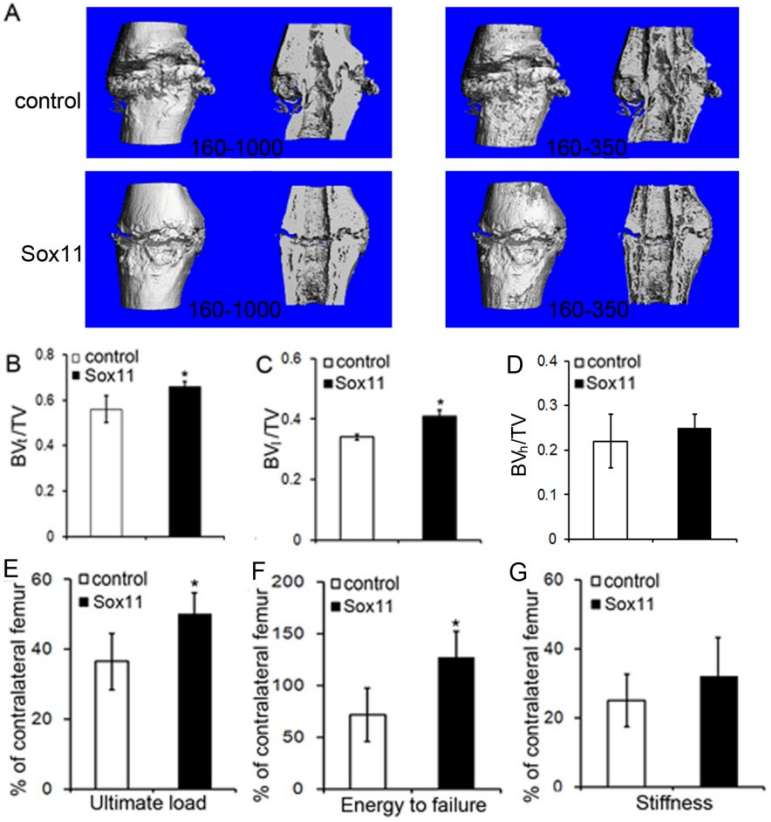


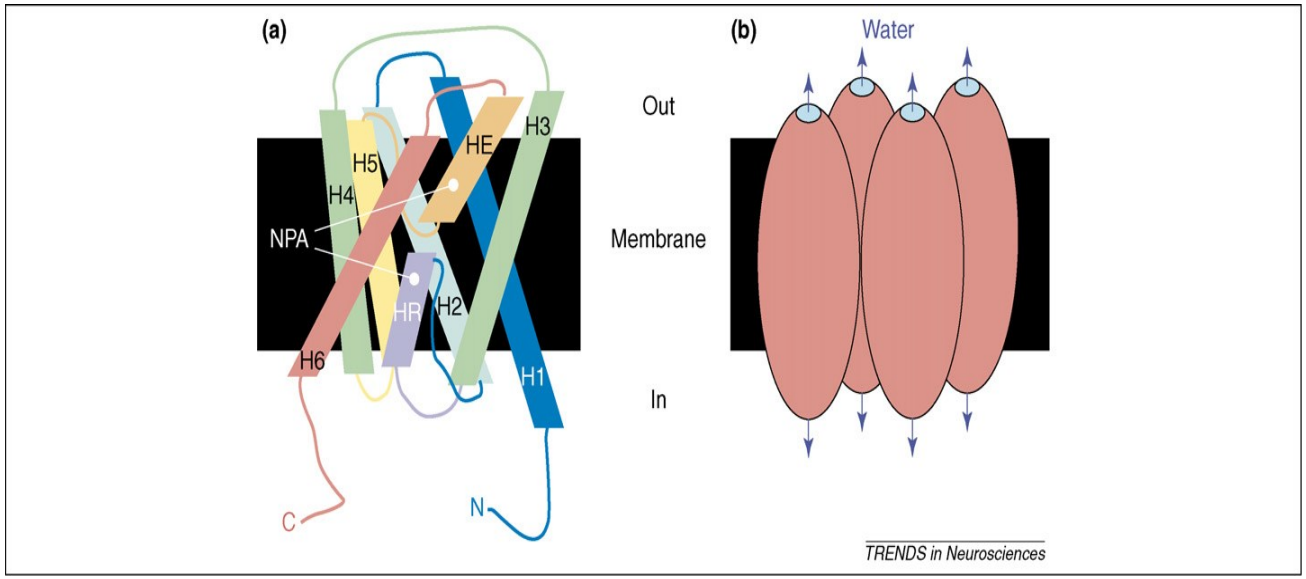
Figure 5





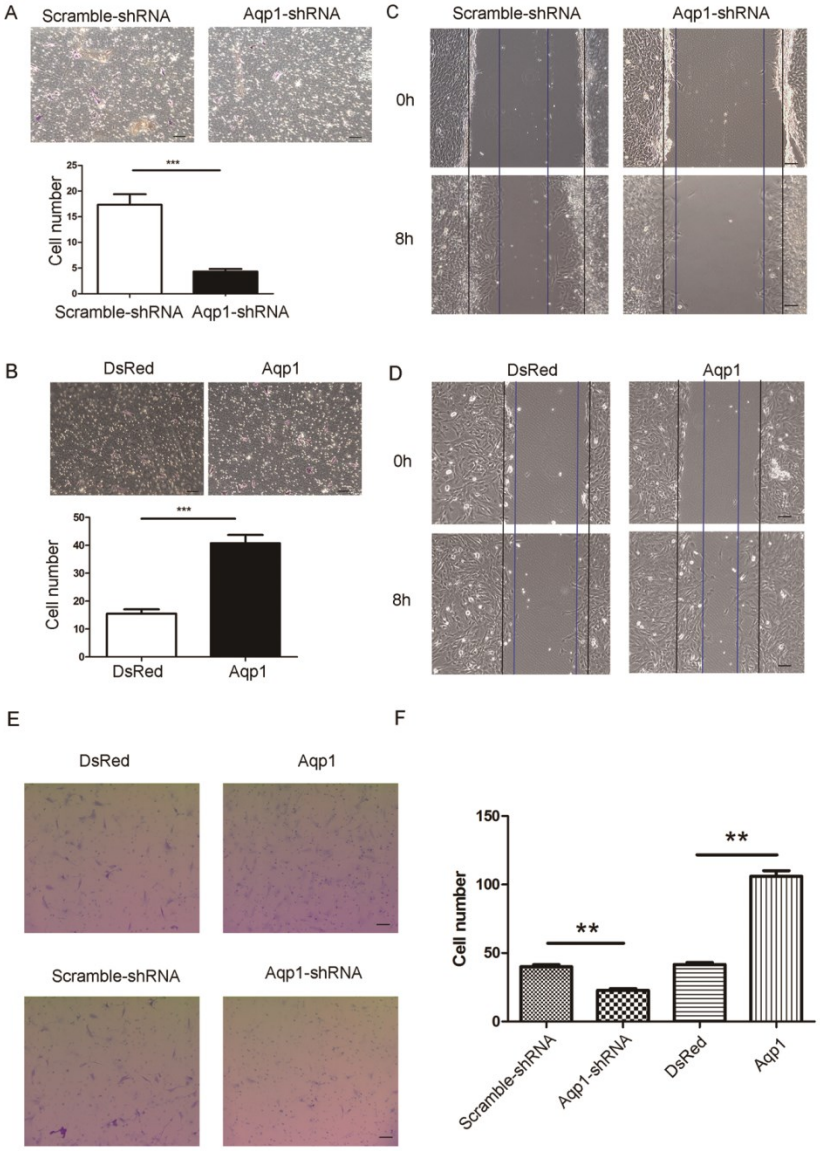
What is Aqp1?

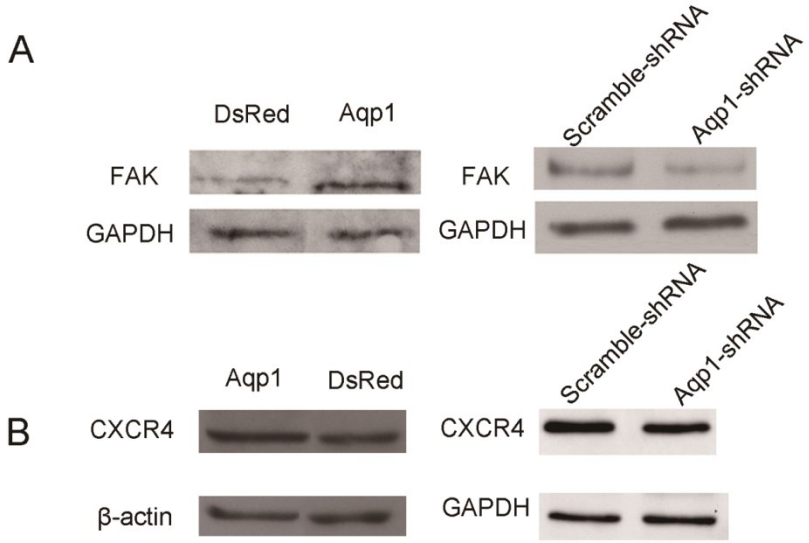
- **AQP1 is the first molecularly identified aquaporin as water channel, which assemble in cell membranes as tetramers.**
- **Aqp1 was shown to promote tumor angiogenesis and endothelial cells migration(*Saadoun*, *Nature* 2005,*434,786–792*).**

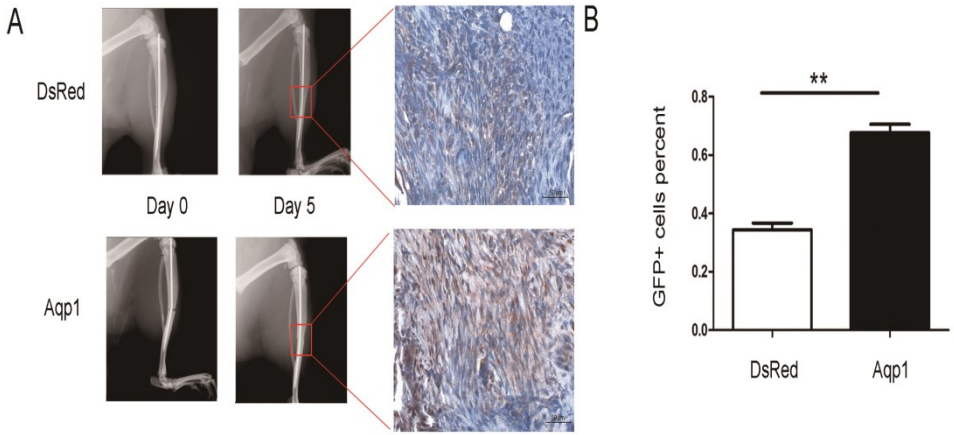


Tait MJ Trends Neurosci. 2008 Jan;31(1):37-43.

Aqp1 enhances migration of B-MSCs through regulation of FAK and β-catenin







Stem Cells and Development, 2013

STEM CELLS AND DEVELOPMENT
Volume 00, Number 00, 2013

DOI: 10.1089/scd.2013.0185

ORIGINAL RESEARCH REPORT

Aqp1 Enhances Migration of Bone Marrow Mesenchymal Stem Cells Through Regulation of FAK and β-Catenin

Fanbiao Meng,[1–3] Yunfeng Rui,[1,4] Liangliang Xu,[1,3] Chao Wan,[2] Xiaohua Jiang,[2] and Gang Li[1,2,3,5]

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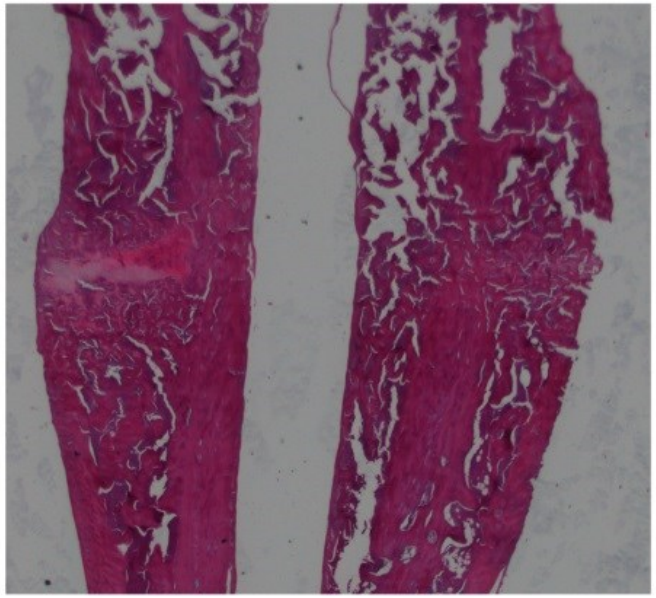
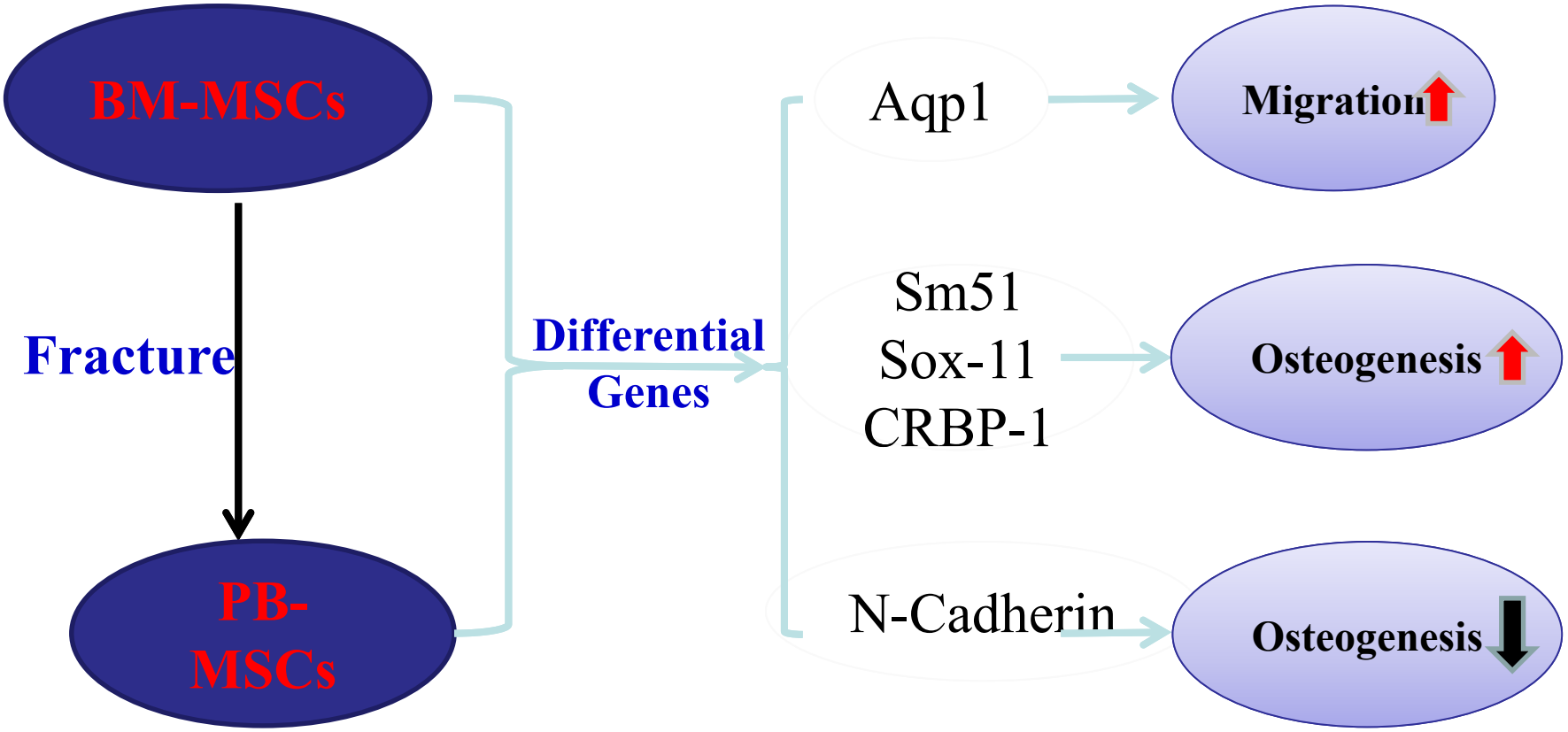


Aqp1

The use of Aqp-1-MSCs administration enhanced fracture healing.

Summary -3: PBMSCs

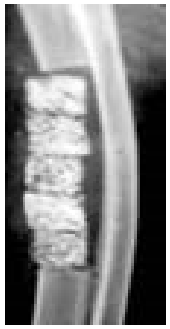
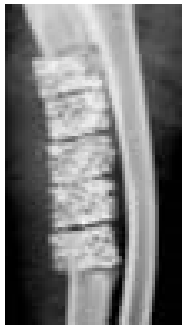
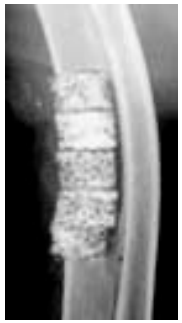
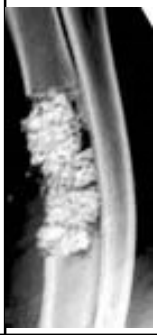
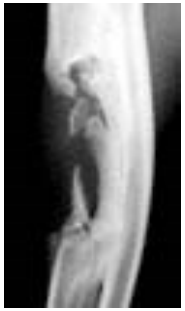
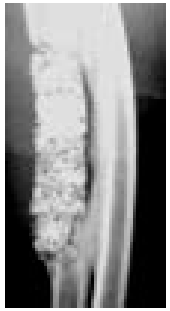
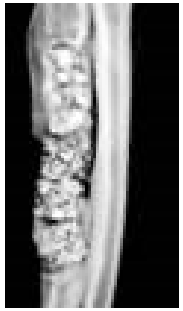
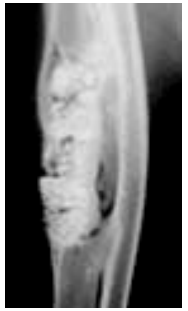
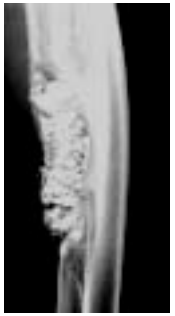
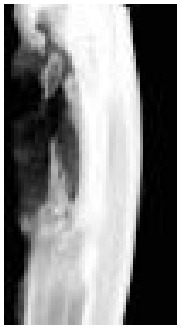
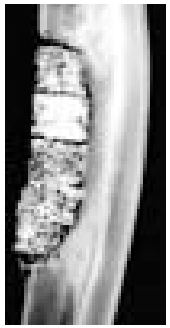
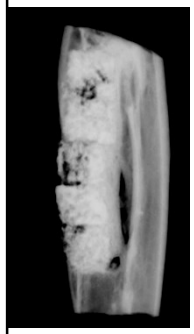
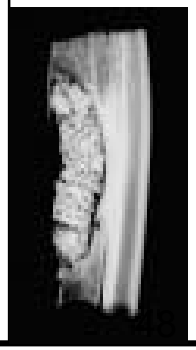
Many novel genes were identified by comparison of PB-MSCs and BM-MSCs, some of these genes appeared to be important in regulating MSCs differentiation and migration potentials.



Blood borne MSCs - Questions

- **Is there MSCs in peripheral blood?**
- **When do they show up?**
- **Where do they come from?**
- **What can we learn from them?**
- **What are the clinical implications?**

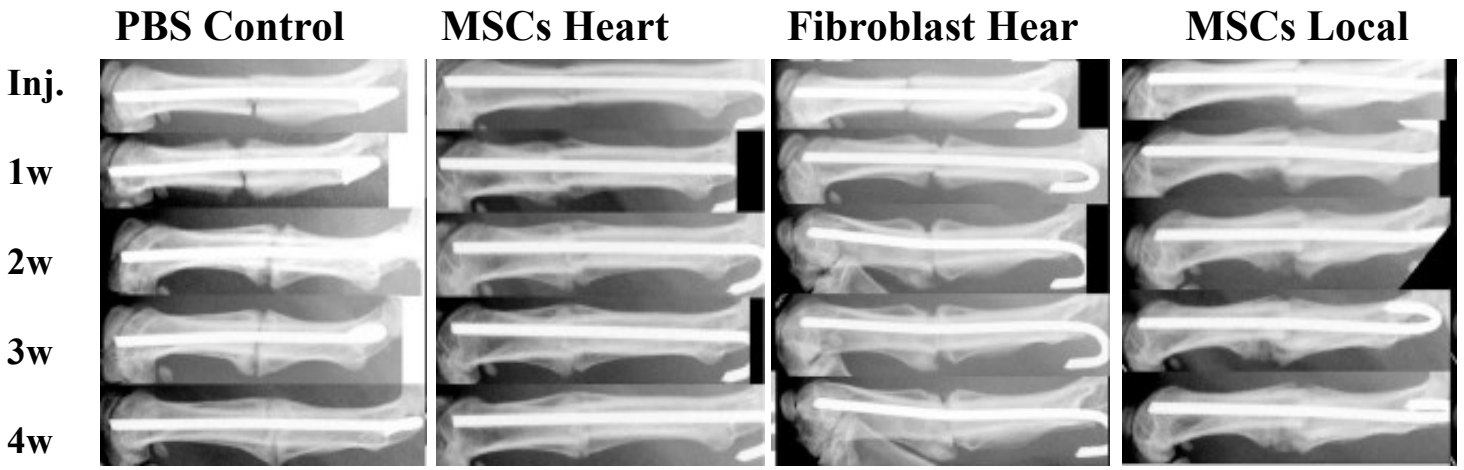
- **Rabbit**
- **PBMSCs**
- **Repair cortical-sized bone defect**

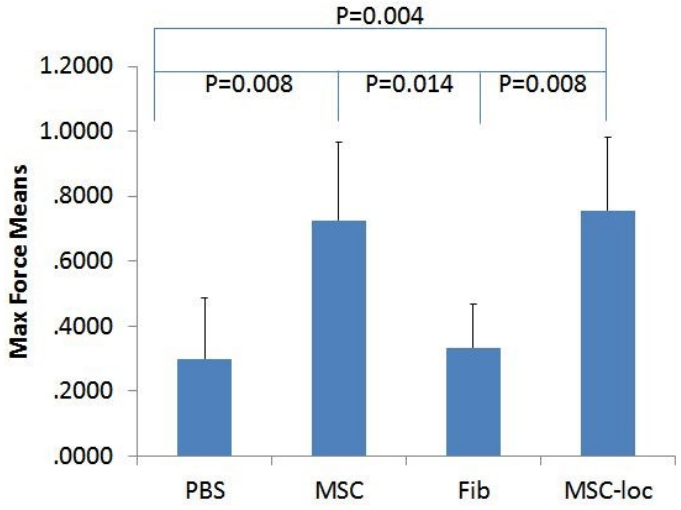
Groups	Empty Control	Skelite Alone	PBMSC Skelite	BMMSC Skelite	PBMNC Skelite
Day 0					
Week 8					
Week 12					

Wan C, He Q, Li G. Allogenic peripheral blood derived mesenchymal stem Cells (MSCs) enhance bone regeneration in Rabbit ulna critical sized bone defect model.

Journal of Orthopaedic Research; 2006; 24(4):610-8.

Systemic administration of allogenic BM-MSCs promoted fracture healing in rats (Cell Transplantation, 2015, in press)





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Systemic and Local Administration of Allogeneic Bone Marrow-Derived Mesenchymal Stem Cells Promotes Fracture Healing in Rats

Shuo Huang,*†‡[1] Liangliang Xu,*†‡[1] Yifeng Zhang,*† Yuxin Sun,*†‡ and Gang Li*†‡§

*Department of Orthopaedics and Traumatology, Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong, PR China
†Lui Che Woo Institute of Innovative Medicine, Faculty of Medicine, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong, PR China
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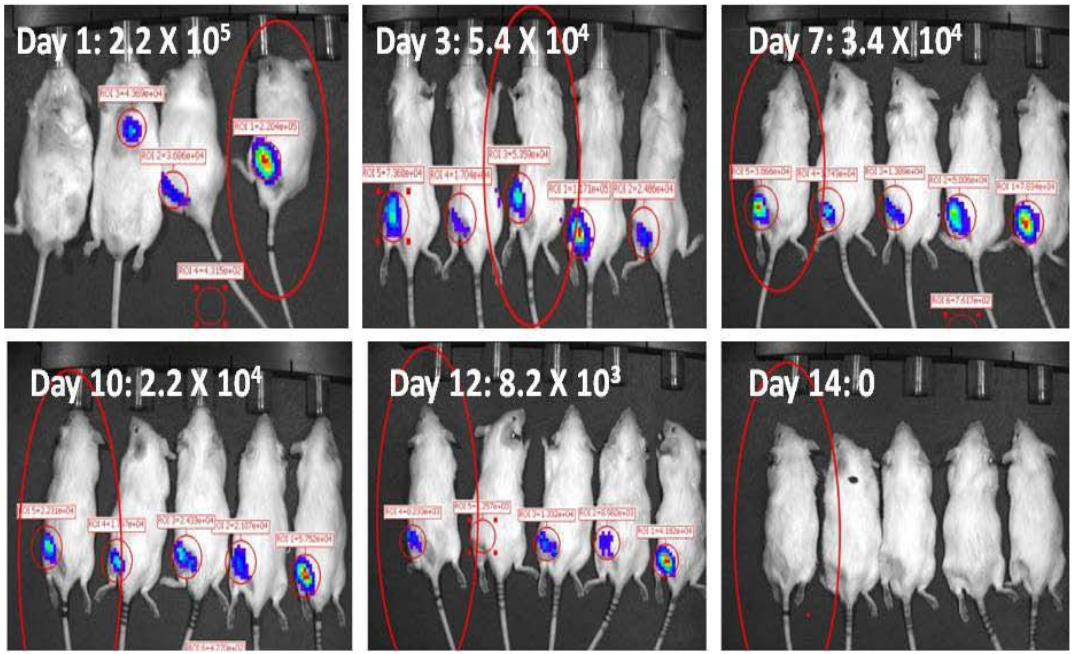
§Key Laboratory for Regenerative Medicine, Ministry of Education, School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China

Mesenchymal stem cells (MSCs) are immune privileged and a cell source for tissue repair. Previous studies showed that there is systemic mobilization of osteoblastic precursors to the fracture site. We hypothesized that both systemic and local administration of allogeneic MSCs may promote fracture healing. Bone marrow-derived MSCs and skin fibroblasts were isolated from GFP Sprague–Dawley rats, cultured, and characterized. Closed transverse femoral fracture with internal fixation was established in 48 adult male Sprague–Dawley rats, which were randomly assigned into four groups receiving PBS injection, MSC systemic injection, fibroblast systemic injection, and MSC fracture site injection; 2×10^6 cells were injected at 4 days after fracture. All animals were sacrificed at 5 weeks after fracture; examinations included weekly radiograph, micro-CT, mechanical testing, histology, immunohistochemistry, and double immunofluorescence. The callus size of MSC injection groups was significantly larger among all the groups. Radiographs and 3D-reconstruction images showed that the fracture gaps naked in the MSC injected groups, while gaps were still seen in the fibroblast and PBS injection groups. The mechanical properties were significantly higher in the MSC injection groups than those in the fibroblast and PBS groups, but no difference was found between the MSC local and systemic injection groups. Immunohistochemistry and double immunofluorescence demonstrated that GFP-positive MSCs were present in the callus in the MSC injection groups at 5 weeks after fracture, and some differentiated into osteoblasts. Quantitative analysis revealed the number of GFP-positive cells in the callus in the MSC systemic injection group was significantly lower than that of the MSC local injection group. The proportion of GFP-osteoblasts in GFP-positive cells in the MSC systemic injection group was significantly lower than that of the MSC local injection group. These findings provide critical insight for developing MSC-based therapies and systemic injection of allogeneic MSCs may be a novel treatment method for promoting fracture repair.

Key words: Allogeneic mesenchymal stem cells (MSCs); Systemic injection; Local injection; Fracture healing

Study of survival of allogenic MSCs in-vivo



Huang et al. Stem Cell Research & Therapy (2015) 6:206
DOI 10.1186/s13287-015-0198-7

stem cell research&therapy

RESEARCH Open Access

The fate of systemically administrated allogeneic mesenchymal stem cells in mouse femoral fracture healing

Shuo Huang[1,2†], Liangliang Xu[1,2†], Yuxin Sun[1,2], Yifeng Zhang[1] and Gang Li[1,2,3,4*]

Abstract

Introduction: The fate and whereabouts of the allogenic mesenchymal stem cells (MSCs) following their transplantation are not well understood. The present study investigated the fate of systemically administrated allogeneic MSCs in mouse fracture healing by using *in vivo* imaging and immunohistochemistry methods.

Methods: Open femoral fracture with internal fixation was established in 30 FVB mice, which were assigned to three groups receiving phosphate-buffered saline (PBS) injection, MSC systemic injection, or MSC local injection. Luc-MSCs (5×10^5) isolated from the luciferase transgenic mice with FVB background were injected at 4 days after fracture. All animals were terminated at 5 weeks after fracture; examinations included bioluminescence-based *in vivo* imaging, micro-computer tomography, mechanical testing, histology, immunohistochemistry, and double immunofluorescence staining.

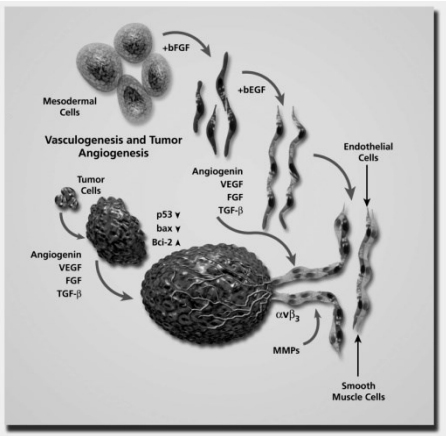
Results: The bioluminescence signals of the Luc-MSCs at the fracture site could be detected for 12–14 days following their injection in the Luc-MSC local injection group, whereas in the Luc-MSC systemic injection group, Luc-MSCs were initially trapped in lungs for about 8–9 days and then gradually redistributed to the fracture site. Bone mineral density, bone volume/tissue volume, ultimate load, and E-modulus in the MSC injection groups were significantly higher than those in the PBS group. Double immunostaining demonstrated that the MSC local injection group had more Luc-positive cells, and there was a higher apoptotic rate at the fracture site than the MSC systemic injection group. Both Luciferase-positive MSCs and osteoblasts were present in the callus in the MSC injection groups at 5 weeks after fracture, suggesting that some of allogenic Luc-MSCs contributed to the new bone formation. Only less than 3 % of injected Luc-MSCs remained at the fracture site in the MSC injection groups at 5 weeks following the fracture, and the rest of the injected Luc-MSCs disappeared.

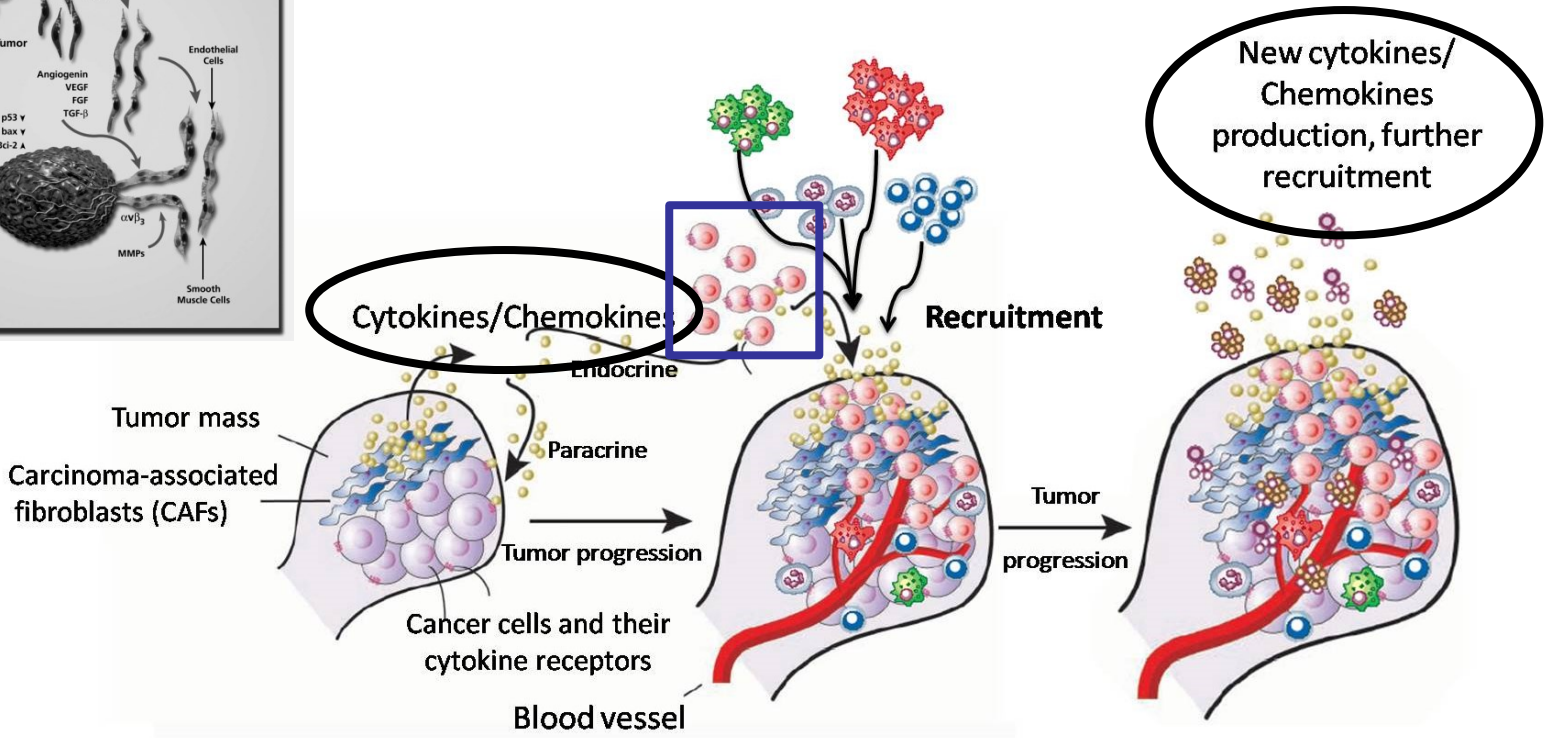
Conclusions: Our data showed that both systemic and local injection of allogeneic MSCs promoted fracture healing through enhancing biomechanical properties, bone content, and enlarged callus sizes. Immunohistochemistry confirmed that the injected MSCs are still present in the fracture site and can differentiate into osteoblasts to participate in fracture healing even at 5 weeks following the fracture. These findings provide useful information for the use of allogenic MSCs for cell therapy applications.

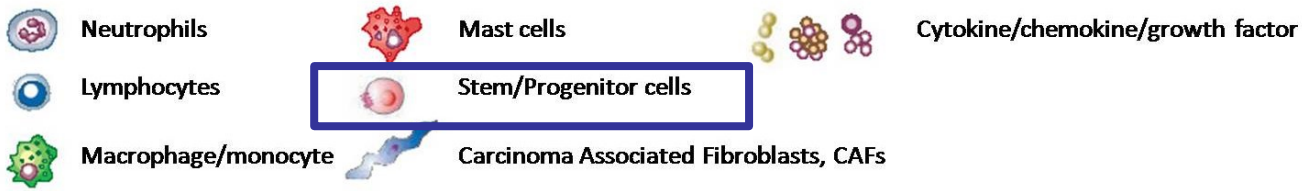
Keywords: Allogeneic mesenchymal stem cells (MSCs), systemic injection, local injection, fracture healing

Allogenic Luc-MSCs injected into the fracture site in mice, and were monitored using in vivo imaging system. Allogenic MSCs became undectable 14 days after injection. All animals did not show obvious adverse side effects. ***Stem Cell Research and Therapy, 2015, 6: 206***

The tumour environment recruits MSCs



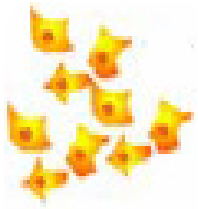




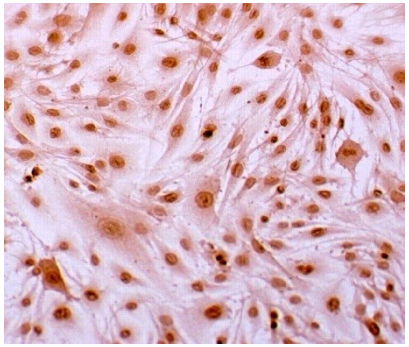
Study MSCs Homing to Tumours

TUMOUR CELLS SUBCUTANEOUS IMPLANTATION

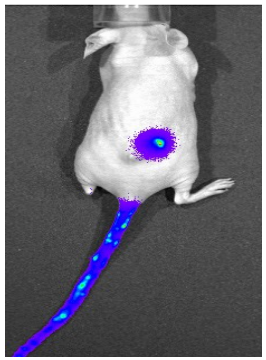
Luciferase labelled MSCs

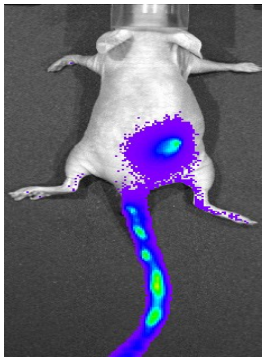


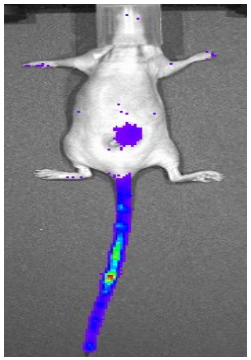
Tumour xenograft model

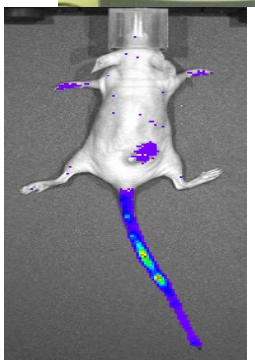


Luciferin injection
In-vivo imaging









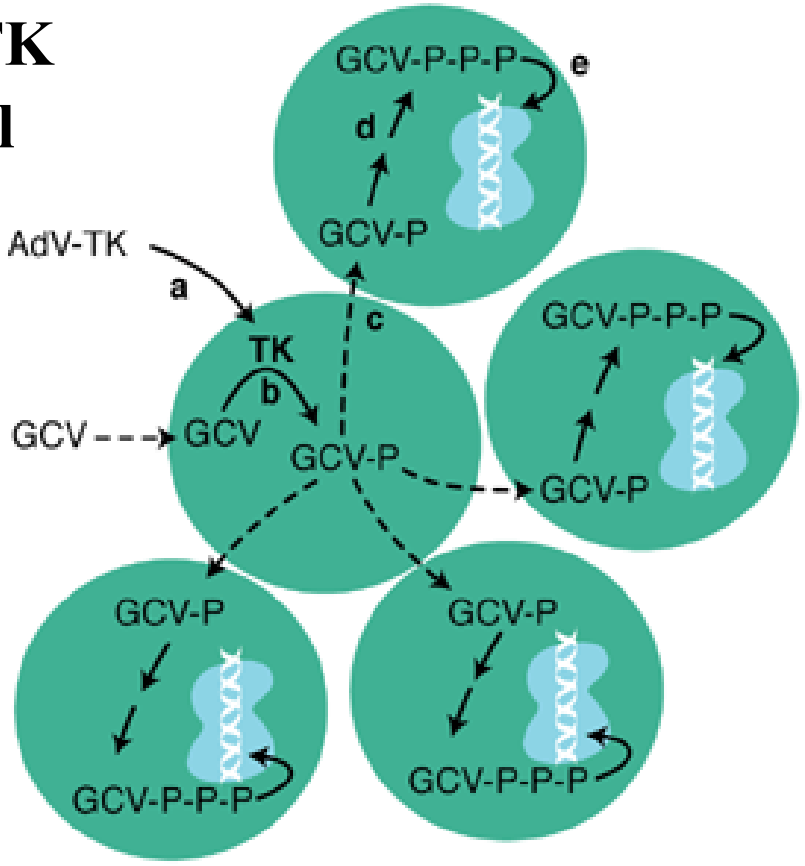
Day 3 Day 6 Day 9 Day 12

TK (Thymidine Kinase)/GCV (Ganciclovir) System

Herpes simplex virus 1 (HSV-1) TK enzyme is not expressed in normal cells.

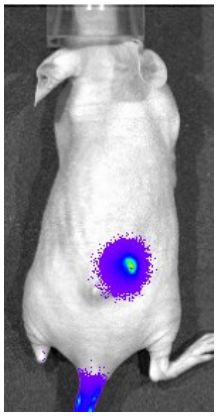
HSV-TK converts GCV to triphosphate GCV (GCV-TP), which is cytotoxic.

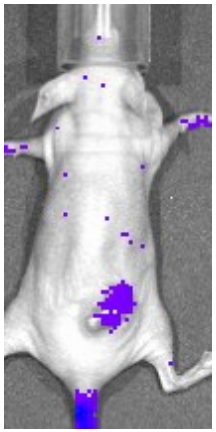
GCV-TP causes cell death by apoptosis

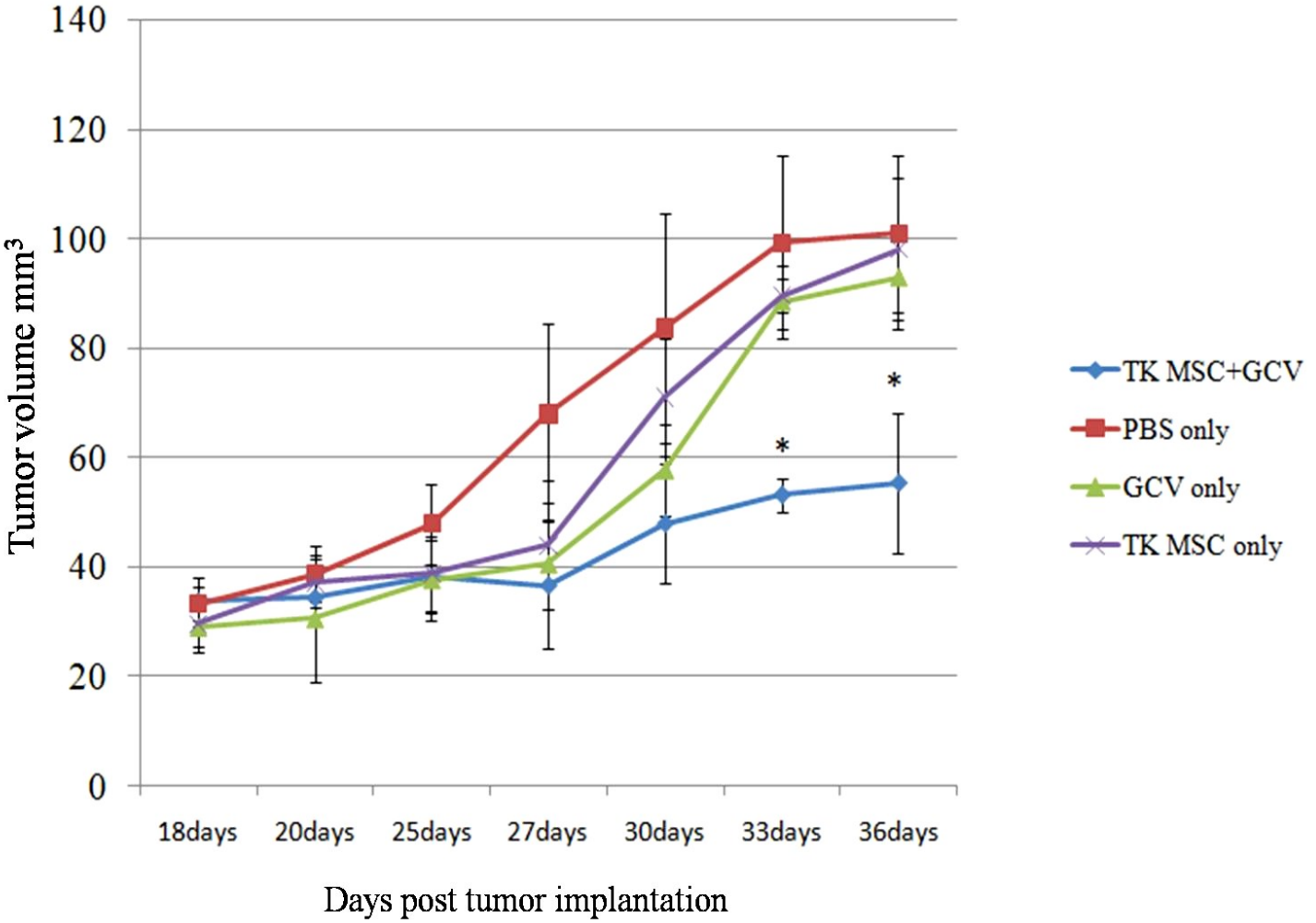


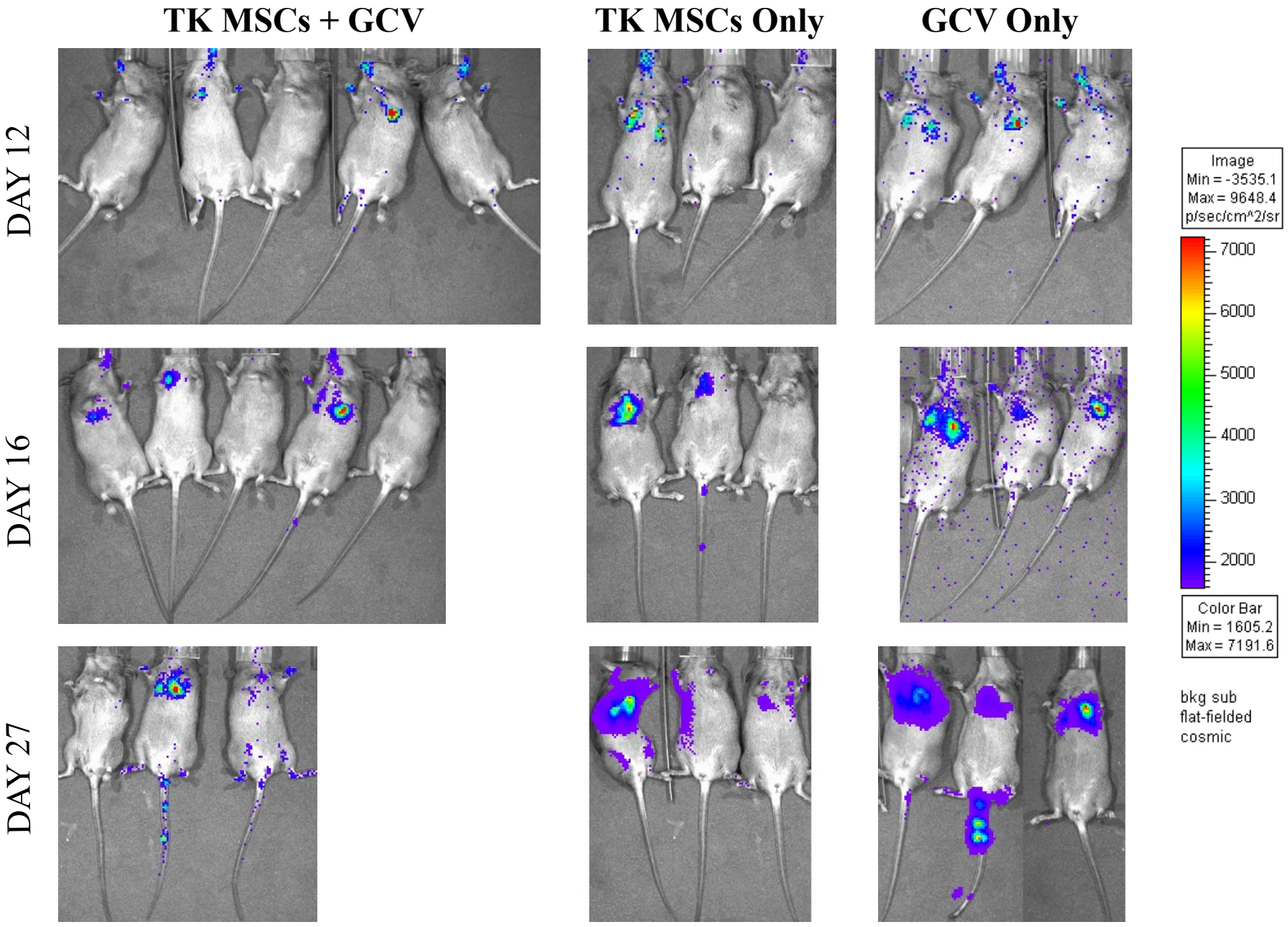
Suicide gene therapy

Anti-tumour effect of systemically administered TK-MSCs in the presence of GCV in tumour bearing mice.

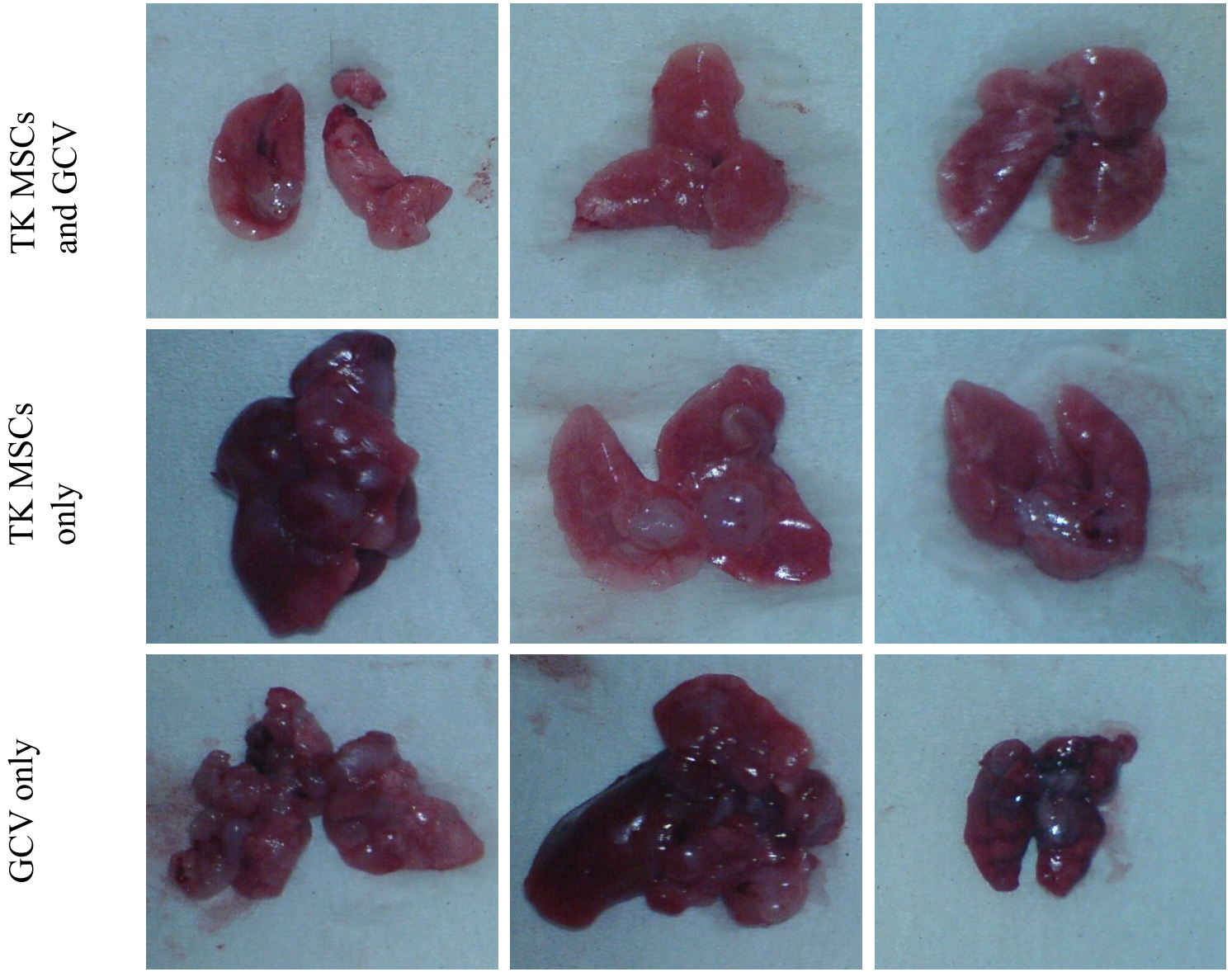







TK MSCs + GCV
TK MSCs Only
GCV Only
DAY 12
DAY 16
DAY 27
Image
Min = -3535.1
Max = 9648.4
p/sec/cm^2/sr
7000
6000
5000
4000
3000
2000
Color Bar
Min = 1605.2
Max = 7191.6
bkg sub
flat-fielded
cosmic

Gross Sample of RIF-1 tumor in lung day 27



HUMAN GENE THERAPY 22:1–11 (April 2011)

DOI: 10.1089/hum.2010.116

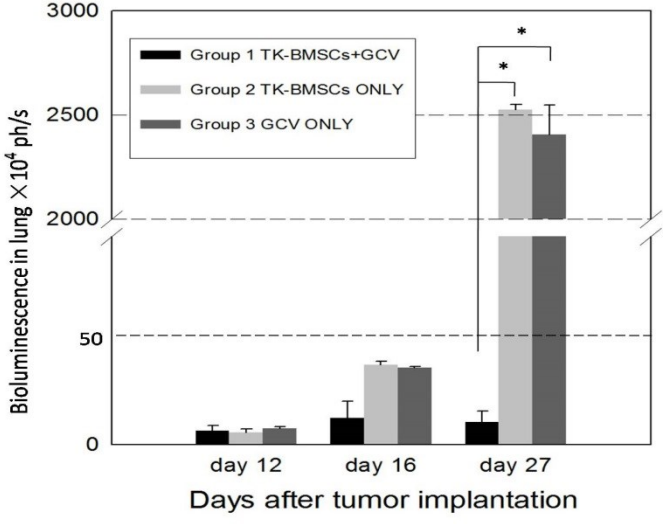
Thymidine Kinase Gene Modified Bone Marrow Mesenchymal Stem Cells as Vehicles for Antitumor Therapy

Chao Song,[1,2,3] Juanjuan Xiang,[4] Jingqun Tang,[5] David G. Hirst,[6] Junwei Zhou,[1,2] Kai-Ming Chan,[2,7] and Gang Li[1,2,7,8]

Abstract

Bone marrow mesenchymal stem cells (BMSCs) represent an important source of cells for tissue repair. The tropism of these cells to the sites of injury and tumors has been well established. Their tumor-homing properties make BMSCs good candidates as antitumor agent delivery vehicles. In this study, we showed that BMSCs have the ability to migrate toward various cancer cells, including prostate cancer cells *in vitro* and *in vivo* and incorporating into the tumor mass. When infected with herpes simplex virus thymidine kinase (TK) gene by lentiviral transduction, TK-BMSCs maintained their tumor tropism capabilities and significantly inhibited the growth of subcutaneous PC3 prostate cancer xenografts in nude mice, in the presence of prodrug ganciclovir (GCV). Xenogenic TK-BMSCs also survived and exerted a significant antitumor effect in an animal model bearing metastastic RIF-1 (fibrosarcoma) tumor in the presence of prodrug GCV. The present study demonstrated that overexpression of TK in BMSCs did not affect their multidifferentiation potentials and their specific homing capacities toward the tumor mass, and the TK-BMSCs alone did not cause any harmful side effects *in vivo*. The use of TK-BMSCs as tumor-specific delivery vehicles together with controlled prodrug treatment may be a safe and novel anticancer therapy approach.

Human Gene Therapy, 2011; 22: 1-11. IF:4.8



Introduction

CANCER GENE THERAPY using the suicide gene(s) has been established. The so-called suicide genes encode enzymes such as herpes simplex virus thymidine kinase (TK), which can convert prodrugs [*e.g.*, ganciclovir (GCV)] with low inherent toxicity into toxic compounds and thus lead to apoptosis of the target cells as a result of the production of ganciclovir phosphates (Fillat *et al.*, 2003). However, suicide gene therapy is limited by the delivery methods currently available.

A cell-based delivery strategy that exploits the tumor-homing property of bone marrow-derived mesenchymal stem cells (BMSCs) has the potential to solve inherent gene therapy delivery problems. Intravenous/systematic delivery of BMSCs resulted in their specific migration to sites of injury and improved recovery in animal models of skin wounds (Sasaki *et al.*, 2008), stroke, and myocardial infarction (Kawada *et al.*, 2004). Tumor/cancer is considered as wounds that never heal (Dvorak, 1986); tumor microenvironments have many similarities to the tissue repair processes that attract specific homing of mesenchymal stem cells (MSCs) (Dwyer *et al.*, 2007; Menon *et al.*, 2007). Stem/progenitor cells of human or murine origin have been demonstrated to migrate to multiple tumor types, including glioblastoma, melanoma, pancreatic and breast carcinoma, and neuroblastoma

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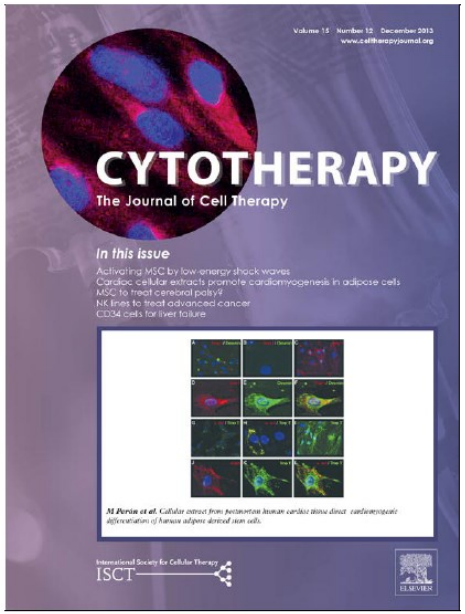
Cytotherapy, 2013; 15: 1484—1497

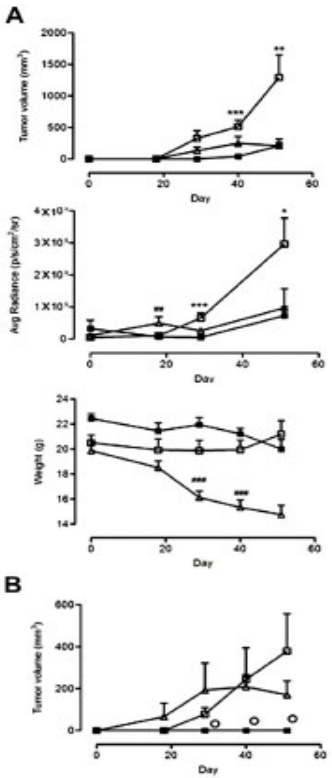
International Society for Cellular Therapy
ISCT

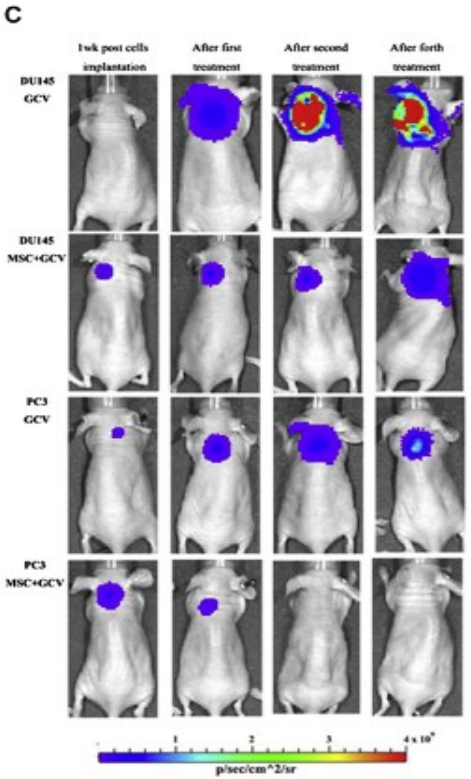
Immortalized human fetal bone marrow-derived mesenchymal stromal cell expressing suicide gene for anti-tumor therapy *in vitro* and *in vivo*

WAYNE Y. W. LEE[1], TING ZHANG[1], CAROL P. Y. LAU[1], C. C. WANG[2], KAI-MING CHAN[1] & GANG LI[1,3,4]

[1]Departments of Orthopaedics and Traumatology and [2]Obstetrics and Gynaecology and [3]Stem Cells and Regeneration Program, School of Biomedical Sciences, Li Ka Shing Institute of Health Sciences, and [4]MOE Key Laboratory of Regenerative Medicine, School of Biomedical Sciences, The Chinese University of Hong Kong, Shatin, Hong Kong SAR, China







Cytotherapy, 2013; 15: 1484-1497.

Conclusions:

1. **Circulating MSCs are important in development and diseases.**
2. **Their release and homing are tightly controlled by genes and local environmental factors, yet to be defined.**
3. **Potential diagnostic and prognostic markers.**
4. **Potential therapeutic benefits of using MSCs through systemic administration for promoting regeneration and cell/gene therapies.**

CUHK LiHS-ORT Stem Cell and Regeneration Lab Members

香港中文大学医学院-骨科干细胞与再生医学组-李刚团队

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