Mechanical Conditioning Using Magnetic Nanoparticles Bound to PDGF Receptors on HBMSCs Promotes the Smooth Muscle Alpha Actin (SMA) Expression

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Abstract— **Mechanotransduction is believe to have the potential to guide the cell fate by coupling mechanical cycles to biochemical signaling. This study investigated the possibility of controlling cell differentiation by mechanical stimulation of cell membrane surface receptor. Magnetic nanoparticle anchored multivalent ligands targeting platelet-derived growth factor receptor α and β (PDGFR α and β) of human bone marrow stromal cells were mechanically stimulated via a Lab-designed magnetic bioreactor. Smooth muscle α actin (SMA) expression was shown to be enhanced by immunofluorescence staining. Quantitative RT-PCR revealed that after 3 hrs magnetic stimulation via PDGFR α conjugated magnetic nanoparticles (MNPs), smooth muscle α actin mRNA expression levels were significantly upregulated both in 3 hrs and 24 hrs further culture groups.**

Keywords— **Magnetic conditioning, Mechanoresponsive, smooth muscle α actin.**

I. Introduction

Mechanical force has been shown to open ion channels, activate phosphorylation, and expose binding sites for other proteins [1]. Mechanical stimulation via magnetic particles has been employed in our group and succeeded in remote control of ion channels activation [2]. Platelet-derived growth factor receptor is known to be mechanoresponsive in vascular progenitor cells [3, 4]. PDGFR-α activation on HBMSC was demonstrated to upregulate smooth muscle cell α-actin (SMA) expression, whereas PDGFR-β activation stimulating SMA depolymerization [5]. The objective of this work is to identify whether magnetic fields applied to magnetic nanoparticle bound PDGF receptors can promote SMA up-regulation in HMBSC.

II. Materials and Methods

Cell Culture

Human bone marrow stromal cells (HBMSCs) were purchased from Lonza. Anti-human PDGFR-α and β antibodies (R&D Systems) were conjugated to the surface of 250 nm magnetic particles (Micromod) after the particles were functionalized with the Fc-specific secondary antibody (Sigma-Aldrich) according to the manufacturer's protocol.

After 48 hrs in serum free medium (DMEM, 1% L-glutamine, 1% antibiotic - antimyotic), HBMSCs (passage 4-6) were coated with antibody-immobilized magnetic particles (MP). Magnetic bioreactor described previously in our group (Fig 1) was used to apply translational force to receptors bound magnetic particles. Cells without magnetic conditioning were used as controls.

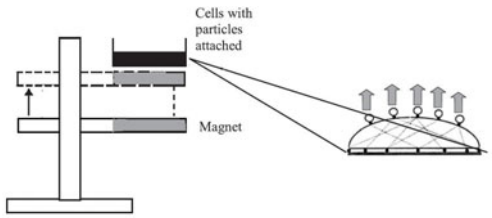

Fig. 1 Diagram of magnetic force bioreactor

Immunofluoresecnce Analysis

After 3 hrs and 24 hrs following culture, cells were washed with phosphate-buffered saline (PBS) and fixed in 95% ethanol for 10 min at room temperature. After washing with PBS, 3% Bovine serum albumin (BSA) in PBS was used to incubator cells for blocking non-specific binding. Then cells were incubated with a monoclonal mouse anti-human primary antibody (Dako) to smooth muscle α-actin clone 1A4. After washing with PBS, goat anti-mouse FITC-conjugated secondary antibody was incubated for 1hr at room temperature, then cells washed and stained nuclear with propidium iodide. Cell numbers were counted and expression levels quantified using confocal microscopy.

Quantitative Real Time RT-PCR

mRNA was isolated from 3 hrs and 24 hrs further cultured cells respectively using solution D, then using isopropanol to purify. mRNA (1 μg) was reverse transcriptted into cDNA using QuantiTect Reverse Transcription Kit (Qiagen). The efficiency-corrected relative quantitation method of relative quantification real-time PCR was performed using Applied Biosystems reagents in 25 μl reactions containing 50 ng cDNA, 12.5 μl 2 x Taqman Universal PCR Master Mix, 1.25 μl Probes and Primer Mix (SMA: Hs00909449_m1; 18s:

A. El Haj and D. Bader (Eds.): 8th International Conference on Cell & Stem Cell Engineering, IFMBE Proceedings 30, pp. 23–25, 2011.
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Hs99999901_m1;) and STRATGENE Mx3005P QPCR system was used. The cycle condition is set according to the manufacturer's protocol.

Statistical Analysis

One way ANOVA was used for results statistics analysis. A value of $P < 0.05$ was considered significant.

III. Results and Analysis

A. Immnuofluorescence Staining Images

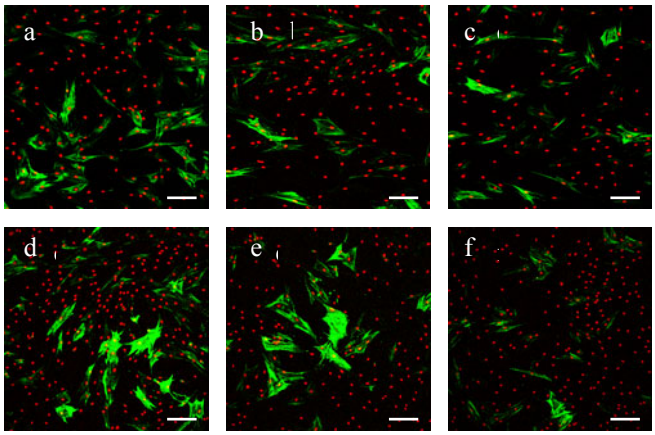
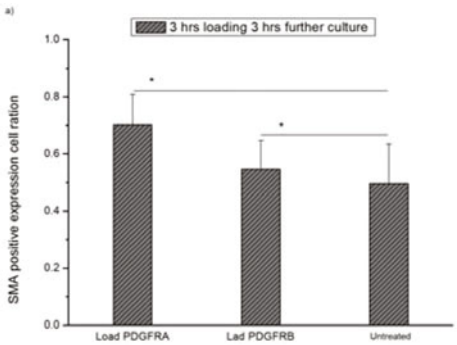


Fig. 2 Smooth muscle α-actin expression of HBMSCs. a: cells after 3hrs loading via PDGFRα conjugated MNPs and 3 hrs further culture; b: upper middle: cells after 3hrs loading via PDGFRβ conjugated MNPs and 3 hrs further culture; c: control cells without treatment; d: cells after 3hrs loading via PDGFRα conjugated MNPs and 24 hrs further culture; e: bottom middle: cells after 3hrs loading via PDGFRβ conjugated MNPs and 24 hrs further culture; f: control cells without treatment. Scale bar: 200 μm

B. Semi-quantification of SMA Expression



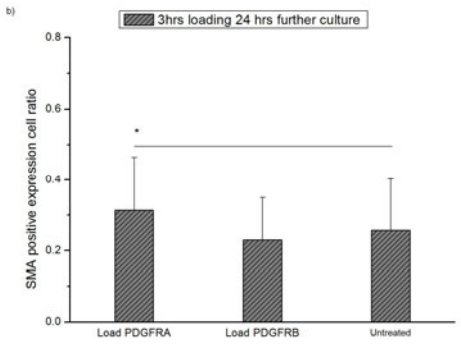


Fig. 3 a) SMA expression significantly changed after 3 hrs magnetic stimulation and 3 hrs further culture (n=5, *p<0.05). b) SMA expression significantly changed after 3 hrs magnetic stimulation and 24 hrs further culture (n=5, *p<0.05)

C. Real Time RT-PCR

Compared with untreated cells, after 3 hrs further culture, mRNA of cells stimulated via PDGFRα conjugated MNPs were upregulated; after 24 hrs further culture, mRNA of cells stimulated via both PDGFRα and PDGFRβ conjugated MNPs were upregulated.

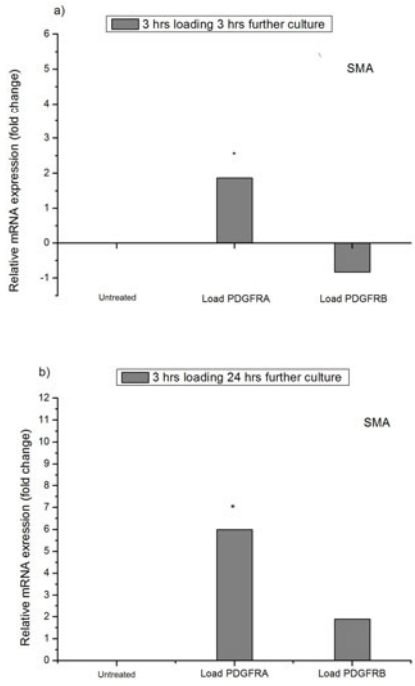


Fig. 4 Relative mRNA expression of SMA in HBMSCs at untreated control group and magnetic stimulated groups, all data have been normalized to 18s and to 0 for control cells without treatment (ANOVA, n=4, *p<0.05)

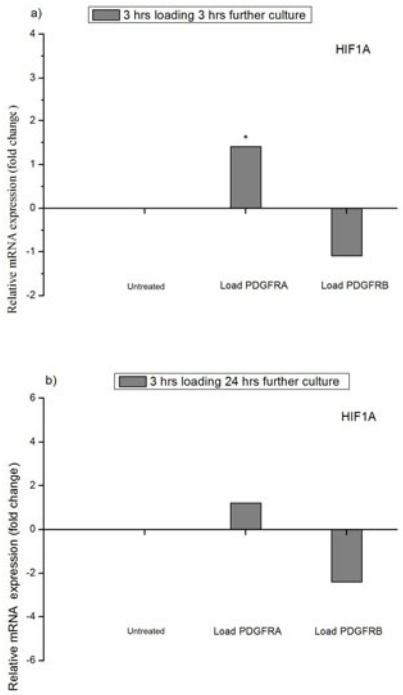


Fig. 5 Relative mRNA expression of HIF1A in HBMSCs at untreated control group and magnetic stimulated groups, all data have been normalized to 18s and to 0 for control cells without treatment (ANOVA, n=4, *p<0.05)

IV. Conclusions

Our results demonstrated that magnetic stimulation could activate cell membrane surface receptors and holds the promise to be a potential tool for remote control of stem cells differentiation.

Acknowledgment

EXPERTISSUES.

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