Vimentin Knockdown Impairs Neo-Cartilage Formation in Human Mesenchymal Stem Cell Pellet Cultures

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DISCLOSURES: The authors have nothing to disclose.

INTRODUCTION: Vimentin intermediate filaments have recently been linked to cellular mechanosensing and chondrogenesis of hMSCs. Disruption of vimentin in chondrocytes has been shown to decrease cell stiffness and reduce type II collagen and aggrecan gene and protein expression.[1,2] Cyclic hydrostatic pressure (HP), used for chondrogenic stimulation, has also resulted in alterations in vimentin organization in chondrogenic mesenchymal stem cells (MSCs).[3] However, the specifics of vimentin's involvement in these processes are still unclear. Better understanding of how MSCs respond to mechanical stimulation can help illuminate strategies for developing cell therapies for diseases such as osteoarthritis or for the treatment of cartilage defects. Using a lentiviral shRNA expression vector targeting vimentin, we have sought to obtain a clearer picture of vimentin's role in cellular mechanosensing over long-term chondrogenesis and response to mechanical stimulation. The objectives of this study were to examine the effect of cyclic HP stimulation on vimentin production and the effect of vimentin knockdown on hMSC differentiation.

METHODS:

Hydrostatic pressure: hMSCs (RoosterBio) were embedded in 4% (w/v) agarose discs (Ø6mm x 3mm) and differentiated using chondrogenic media (high glucose DMEM with 4mM L-glutamine supplemented with 10% FBS, 4mM L-glutamine, 50µg/mL L-ascorbic acid, 40µg/mL L-proline, 1mg/mL insulin, 0.55mg/mL transferrin, 0.5µg/mL sodium selenite, 50mg/mL bovine serum albumin, 470µg/mL linoleic acid, 100IU penicillin/streptomycin,10µg/mL $TGFB_3$) and stimulated using cyclic hydrostatic pressure from 2-10MPa at 1Hz for 4hr/day, 5days/week for 14 days. Select discs were used as free swelling controls. Discs were fixed using 4% paraformaldehyde, infiltrated with 30% sucrose, and embedded in Optimal Cutting Temperature (OCT) medium. The OCT blocks were cryosectioned (20µm), fluorescently immunostained with a primary antibody targeting vimentin, and visualized using confocal microscopy. Corrected Total Cell Fluorescence (CTCF) values were determined using ImageJ, and statistical comparisons were performed using a Mann-Whitney test (mean ± StDev), n>15-25.

Vimentin Knockdown: hMSCs were transduced with doxycycline-inducible lentiviral shRNA vectors (MOI 15) targeting vimentin (shVim) or LacZ (shLacZ, control). Pure populations were selected using 12ng/mL blasticidin for 4 days. Following selection, shRNA expression was induced using 1µg/ml doxycycline. Transduced cells were stimulated with doxycycline for 14 days prior to initiating differentiation.

Chondrogenesis: 2.5 x 10^5 transduced and stimulated MSCs were centrifuged into pellets and differentiated using chondrogenic media (high glucose DMEM with 4mM L-glutamine and 1mM sodium pyruvate supplemented with 10% FBS, 50µg/mL L-ascorbic acid, 40µg/mL L-proline, 1.0mg/mL insulin, 0.55mg/mL transferrin, 0.5µg/mL sodium selenite, 50mg/mL bovine serum albumin, 470µg/mL linoleic acid, 10nM dexamethasone, 100IU penicillin/streptomycin, 10µg/mL $TGFB_3$) over 14 or 21 days. Pellets were fixed with 4% paraformaldehyde, infiltrated with 30% sucrose, and embedded in OCT medium. The OCT blocks were cryosectioned (8µm) and immunostained with a primary antibody targeting type II collagen. Collagen content was visualized using DAB and glycosaminoglycans visualized using 0.1% Safranin-O (Saf-O). Samples were imaged using bright field microscopy.

Adipogenesis: Transduced MSCs were seeded at 35 x 10^3 cls/cm^2 and differentiated using adipogenic media (high glucose DMEM with 4mM L-glutamine supplemented with 10% FBS, 100 IU penicillin/streptomycin, 10 µM dexamethasone, 10 mg/mL insulin, 0.5 mM IBMX, and 200 mM indomethacin) for 14 or 28 days. Samples were stained with Oil Red O and imaged using bright field microscopy.

RESULTS: Hydrostatic pressure stimulation of hMSCs resulted in a statistically significant increase in the fluorescence intensity of vimentin compared to free swelling controls (p=0.032) (Fig.1). On day 14, chondrogenic differentiation of shVim-hMSCs revealed no noticeable differences in collagen and GAG content compared to shLacZ-hMSCs. However, by day 21, shVim-hMSCs appeared to have decreased GAGs and a looser, less organized type II collagen matrix (Fig. 2). Vimentin knockdown did not appear to affect adipogenic differentiation, as no visible differences were discernible in lipid droplet formation between shLacZ-hMSCs and shVim-hMSCs at both 14 and 28 days (Fig. 3).

DISCUSSION: In this study, we show that vimentin cytoskeleton is involved in hMSC mechanosensing and differentiation. We found that the combination of chondrogenic differentiation and cyclic HP lead to an increase in vimentin expression in hMSCs. Chondrogenic differentiation of shVim-hMSCs resulted in changes to the quality and organization of extracellular matrix components of neocartilage in pellet cultures. Vimentin expression may be related to the mechanosensing of hydrostatic pressure and decreasing vimentin expression may cause an impairment of extracellular matrix deposition. In the future, we hope to examine the relationship between decreased vimentin and chondrogenesis of hMSCs stimulated by hydrostatic pressure by assessing changes in gene expression, cytoskeletal elements, and mechanical stability of pellet cultures.

SIGNIFICANCE: Engineering of MSCs to understand the role of mechanosensing cellular elements in differentiation, e.g. chondrogenesis, will ultimately improve methods of designing and improving cell therapies and tissue engineering strategies for diseases such as osteoarthritis.

REFERENCES: 1. E.J. Blain+. Matrix Biology. 2006. 25:398-408; 2. D.R. Haudenschild+. J Orthop Res. 2011. 29:20-25; 3.A.J. Steward+. Eur Cell Mater. 2013. 25:167-178

ACKNOWLEDGEMENTS: This work was funded by the National Science Foundation (CMMI 1563721, DRW; CBET 0845754, AHH)

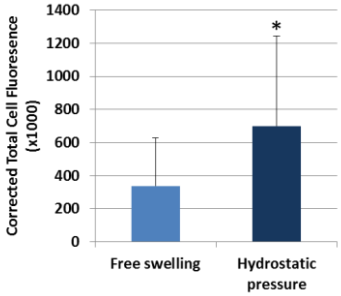

Fig. 1 *– Corrected total cell fluorescence measure-ments of the vimentin cytoskeleton in chondrogenic hMSCs immunostained for vimentin with or without hydrostatic pressure stimulation. Data represented as mean±SD (n=15-25 cells per condition). *p<0.05 between samples.*

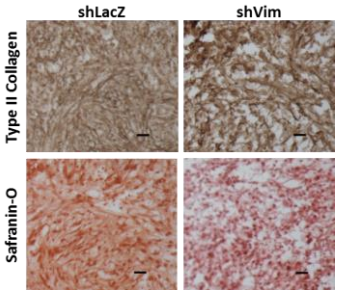

Fig. 2 *– Cryosections of shLacZ- or shVim-hMSC pellet cultures at Day 21 of chondrogenic differentiation. Immunostaining for type II collagen and Safranin-O show a looser, less organized matrix in vimentin-deficient cells, indicating an impaired ability to form neocartilage. Scale bar: 25µm*

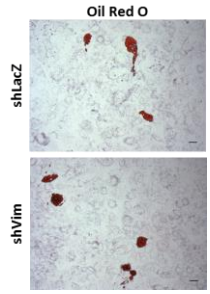

Fig. 3 *– Oil Red O staining of shLacZ- and shVim-hMSCs showed no difference in lipid droplet formation after 28 days of adipogenic differentiation. Scale bar: 50 µm*