Functional roles of microRNA 489 and 148b in hMSCs osteogenesis depend on microenvironment elasticity

Chun Yang[1,2], Kristi S Anseth[2,3]

[1]Department of Chemistry & Biochemistry, the [2]BioFrontiers Institute, and the [3]Howard Hughes Medical Institute, University of Colorado, Boulder, CO 80309

Statement of Purpose: MicroRNAs (miRNA) are small, non-coding RNAs that are found to control gene expression globally. In stem cell differentiation, microRNAs regulate protein expression levels by inhibiting the translation of mRNAs, whose 3' untranslated regions are complementary to the miRNAs sequence[1]. Previously, transfecting human mesenchymal stem cells (hMSCs) with miRNA 489 inhibitor and 148b mimic was shown to synergize with soluble osteogenic factors to enhance hMSCs osteogenesis [2]. However, the differentiation of hMSCs is also known to be affected by the elasticity of the extracellular microenvironments[3]. Thus, the differentiation of hMSCs is likely a complex, combined result of endogenous genetic control and extracellular microenvironment regulation, and we sought to investigate the functional roles of miRNAs in hMSCs osteogenesis using hydrogels with tunable elasticities. Preliminary results indicated that, while miRNA 489 mimic and 148b inhibitor were quiescence when hMSCs were seeded on stiff substrates (plastic tissue culture plates); they were activated when hMSCs were seeded on comparatively soft substrates (hydrogels with modulus of 100kPa or 10kPa), thus increasing osteogenic gene expression at early time points.

Methods: 8-Armed 10K or 20K poly(ethylene glycol) (PEG) was coupled with norbornene acid to yield PEG-norbornene monomer as described in [4]. A peptide cross-linker, KCGPQG(d-)IAGQCK, and integrin binding motif, CRGDS, were synthesized using standard solid phase peptide synthesis (Tribute®). Peptides were purified by reverse phase HPLC (Waters Delta Prep 4000).

Hydrogels were formed by thiol-ene reaction between PEG-norbornene and KCGPQG(d-)IAGQCK and CRGDS at stoichiometric ene to thiol ratios. Briefly, monomer solutions containing 1.7mM photoinitiator lithium phenyl-2,4,6 trimethyl benzoylphosphinate were exposed to 365 nm light at 5 mW/cm^2 for 3 min to form hydrogels. The elasticity of the gels was controlled from 10 to 100kPa by varying the wt % of the initial macromer solution. 5mM CRGDS was included in every hydrogel formulation to promote hMSCs attachment.

hMSCs were transfected with miRNA mimics and inhibitors (miRIDIAN, Dharmacon/ThermoFisher) using a human MSC Nucleofection Kit (Lonza). Plated hMSCs were trypsinized and pelleted. Cells were suspended in the nucleofection buffer at a concentration of 2.5x10^6 cells per 100µl transfection. miRNA mimics and inhibitors were added to the solution at a final concentration of 5µM. hMSCs were seeded on top of hydrogels at 10,000 cells/cm^2 after transfection.

Quantitative RT-PCR was conducted to determine the relative expression of osteogenic genes, CBFA1and ALP, and normalized to GAPDH.

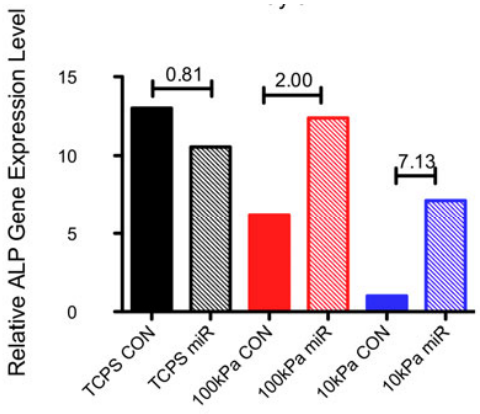

Figure 1: ALP expression profile of hMSCs transfected with control miRNA and osteogenic miRNAs at Day 3. The transfected hMSCs were seeded on TCPS and hydrogels with modulus of 100kPa and 10kPa, respectively.

Results: As shown in Figure 1, when hMSCs were seeded on TCPS, transfecting them with miRNA 489 mimic and miRNA 148b inhibitor (denoted as condition miR in figure) did not promote a higher ALP expression level than transfecting them with control miRNAs (denoted as condition CON in figure). As the substrates became softer, more significant differences in ALP expression were observed between the miR and CON condition. Specifically, ALP expression was 2.00 fold higher for the miR condition compared to the CON condition when hMSCs were seeded on hydrogels of 100kPa. When hMSCs were seeded on hydrogels of 10kPa, the miR condition promoted a 7.13 fold increase in ALP expression compared to the CON condition. Overall, these results indicated that the activities of miRNAs, such as the 489 mimic and 148b inhibitor, in promoting differentiation of hMSCs may depend on the microenvironmental stiffness. Here, we observed that softer substrates, promoted higher levels of ALP expression in hMSCs.

Acknowledgements: The authors are grateful for financial support of this work from NIH (1R21 AR057904) and HHMI.

References: [1]B. Zhang, J Cell Physiol 209(2) (2006) 266-269.
[2]A. Schoolmeesters, PLoS One 4(5) (2009) e5605.
[3]A.J. Engler, S. Sen, Cell 126(4) (2006) 677-689.
[4]B.D. Fairbanks, Advanced Materials 21(48) (2009) 5005-5010.