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Biochemically Induced Biophysical Forces on Vascular Smooth Muscle Cell (VSMC) Nucleus Can Modify Cell Fate and the Onset of Vascular Calcification

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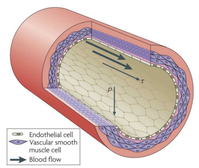
Abstract

Vascular calcification is the hardening of blood vessels into bone-like structures. Vascular smooth muscle cells (VSMCs) are capable of transdifferentiating into the osteogenic phenotype. Moreover, biophysical forces can alter chromatin structure and regulate cell fate for several cell lines. Here, we aim to study how biophysical forces can impact VSMC phenotype and the onset of vascular calcification. To that end, biochemically induced biophysical forces are applied to VSMCs via the vasodilator Sodium Nitroprusside (SNP) and the vasoconstrictor Phenylephrine (PE). Immunofluorescence of nuclear DNA confirm that the biochemical treatments generated the nuclear morphological changes expected from the corresponding biophysical forces. Changes in epigenetics and transcriptional activity will be evaluated via Acetyl H3 Staining and immunofluorescence imaging. VSMC cell fate will be determined via immunofluorescence imaging for myogenic versus osteogenic markers. Current data offer strong evidence that SNP- and PE-treated cells display the morphological changes expected with cellular dilation and contraction. Moreover, optimization experiments suggest that 48-hr treatment of VMSCs with 10μM SNP and 50μM PE induce the most significant results. Histone acetylation immunofluorescence imaging and VSMC protein expression analysis are due to follow.

Background

The Problem: Vascular Calcification

- Hardening of blood vessels due to deposition of calcium and phosphate in the form of hydroxyapatite in the VSMC extracellular matrix[1].
- Accompany cardiovascular complications such as atherosclerosis, diabetes, peripheral artery disease, coronary disease, etc[2].
- NO known treatment.

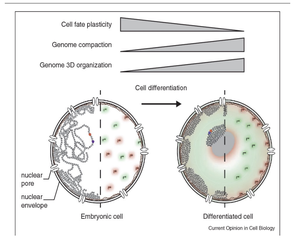


Role of VSMCs in Calcification

- *Healthy*: constricts/dilates to regulate blood flow and pressure; inhibits vascular calcification.
- *Diseased*: differentiate to osteogenic phenotype due to 1) senescence, 2) oxidative stress, 3) defects in nuclear lamina, 4) sympathetic denervation[1].

Role of Biophysical Forces in VSMC phenotype

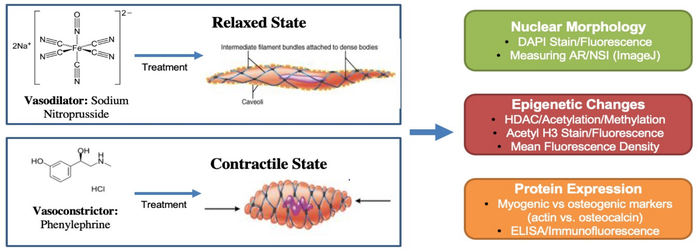
- Biophysical forces on nuclei alter chromatin structure and regulate cell fate for several cell lines: embryonic cells[3], mesenchymal stem cells[4], epithelial cells[5].



Hypothesis: Biophysical forces that cause VSMC contraction will facilitate heterochromatin formation over euchromatin in the nuclear genome, promoting histone methylation and reducing the transcriptional likelihood of osteogenic trans-differentiation (and vice versa).

Methods

1. **Prepare Myogenic VSMCs**: Prepare myogenic VSMCs exhibiting contractile markers from patient-derived HAOSMCs (exhibiting synthetic phenotype).
2. **Drug Treatment Optimization**: Administer SNP (10μM, 25μM, 50μM), PE (50μM, 100μM, 200μM), or no treatment to the myogenic VSMCs. Treat for 24hr or 48hr.
3. **Analysis**: Analyze the treated cells in the following three areas. Conduct ANOVA Single Factor test and Student's T-Test for post-hoc analysis to determine statistical significance between groups.



Results

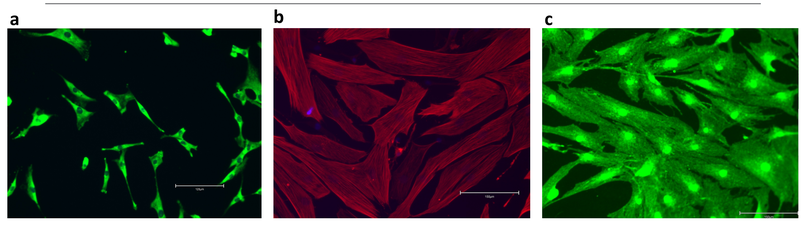


Fig 1: Expression of VSMC contractile markers confirm the successful preparation of myogenic VSMCs from synthetic-phenotype HAOSMCs

Preliminary studies (not shown here) suggest that commercially purchased HAOSMCs exhibit a synthetic phenotype, which dampens the dilating effect of SNP. Therefore, we cultured the commercial HAOSMCs in VSMC differentiation media (ThermoFisher, S0085) for 10 days to stimulate a contractile phenotype. Qualitative analysis of α-actin protein marker (1a, 1b) using immunofluorescence imaging confirm that the differentiation protocol resulted in VSMCs with the contractile phenotype. Compared to pre-treatment HAOSMCs with the synthetic phenotype (GFP, 1a), the post-treatment/contractile VSMCs exhibit organized actin fibers that strongly indicate contractility and the physiologically healthy, myogenic phenotype (RFP, 1b). In addition, smooth muscle myosin heavy chain (SM-MHC11), a contractile filament directly involved in contraction, is also detected in abundance in the post-treatment VSMCs (GFP, 1c). Antibodies were administered in 1:100 dilutions. Bar in a: 125μm. Bar in b-c: 150μm.

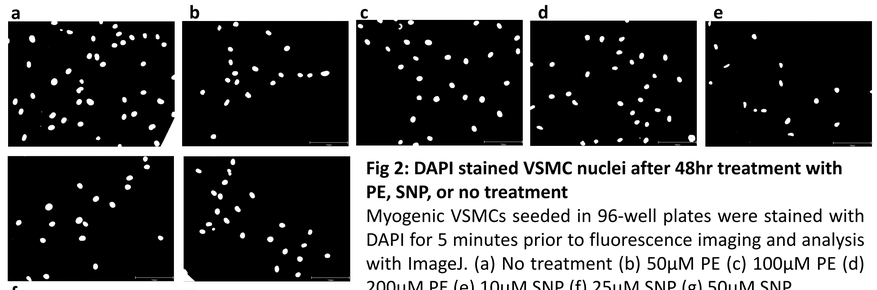


Fig 2: DAPI stained VSMC nuclei after 48hr treatment with PE, SNP, or no treatment

Myogenic VSMCs seeded in 96-well plates were stained with DAPI for 5 minutes prior to fluorescence imaging and analysis with ImageJ. (a) No treatment (b) 50μM PE (c) 100μM PE (d) 200μM PE (e) 10μM SNP (f) 25μM SNP (g) 50μM SNP

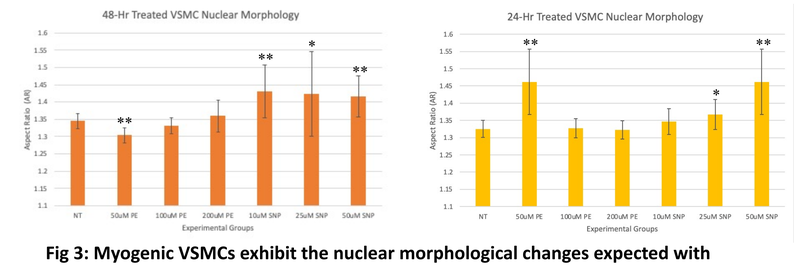


Fig 3: Myogenic VSMCs exhibit the nuclear morphological changes expected with biophysical force application

A stock solution of 10mM Sodium nitroprusside (Sigma-Aldrich, 71778) was made and diluted via serial dilution to 50μM, 25μM, and 10μM. For Phenylephrine (Sigma-Aldrich, P6126), the concentrations were 200μM, 100μM, and 50μM. All dilutions were added to 96-well containing myogenic VSMCs, and the cells were fixed and imaged at 24-hr or 48-hr. Treated VSMCs at 24-hr did not display the morphology expected (3b).

However, VSMCs treated for 48 hours adopted the morphological patterns expected from the biochemical indications for PE and SNP. Cells treated with 50μM PE displayed a more circular morphology compared to NT, while VSMCs treated at all three SNP concentrations display elongated morphology (3a). However, 10μM SNP was the most effective concentration. Key: ** indicates p-value < 0.001, * indicates –value < 0.01.

Conclusions and Future Directions

1. Myogenic VSMCs was successfully differentiated from commercial HAOSMCs with the synthetic phenotype.
2. VSMCs incubated with 10μM SNP and 50μM PE at 48-hr exhibit the nuclear morphological changes expected from vasodilation and vasoconstriction, respectively.
3. **Future Directions**: Finish optimizing the drug administration protocol, then conduct immunofluorescence imaging for histone acetylation and osteogenic/myogenic protein markers.

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