Vibration, a treatment for childhood cerebral palsy, induces changes in cell shape

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Abstract

Understanding how a particular cell type reacts to vibration is essential to understanding why vibration platforms help children with cerebral palsy. We previously showed that vibration increased calpain, which is one of several changes that happen when a cell transitions to a mobile cell (EMT). We hypothesize that vibration induces other calpain-related changes associated with EMT, particularly effects on cell shape and on expression of biomarkers. HeLa, an epithelial cell line, were vibrated and assessed by microscopy, cytoskeletal staining and quantitative RT-PCR. Here, we report that vibration led to a calcium-associated increase in rounded cells associated with actin filament bundling and repair and altered gene expression of biomarkers.

Introduction

Cerebral palsy is a disorder that impacts a child's muscle function[1]. Although cerebral palsy is not curable, supportive treatments can often help children lead more productive lives. Recent studies have shown that treatment with a whole body vibration table results in children with cerebral palsy having less muscle spasms[2,3].

At a cellular level, external vibration forces have been shown to trigger cell shape changes indicative of an epithelial-mesenchymal transition (EMT)[4]. EMT describes a process whereby epithelial cells (cells that line the surface of body cavities) morph into a more mobile and 'healing' type of cell (mesenchymal cell). Biomarkers of EMT include loss of E-cadherin (an epithelial cell marker), increase in FSP-1 (a mesenchymal cell marker)[5] and changes in calpain (a regulator of actin filament bundling)[6]. Our lab previously showed that vibration led to an increase in calpain protein. In this regard, the **overarching hypothesis for this study is that vibration of a cell induces calpain-related changes associated with EMT.**

By influencing cell shape and inducing an EMT, cells in the vicinity of a vibrating force would have enhanced mobility. Nomadic cells with a mesenchymal phenotype are often involved in repair mechanisms, which may explain why treatment with vibration tends to relieve the symptoms of children with cerebral palsy.

Materials & Methods

Cells. HeLa (ATCC-CCL-165), a human cervical epithelial carcinoma cell line was grown in Dulbecco's Modified Eagle Media (DMEM) supplemented with 10% Fetal Bovine Serum and antibiotics at 37˚C with 5% CO_2.

Vibration. HeLa were washed with PBS and either placed on a vibration resistant table [vibration (-)] or exposed to vibration with an analog vortex mixer (VWR) for 15 minutes at 1,200 rpm [vibration (+)].

Microscope. Micrographs were documented using CellSens imaging software using an inverted, Olympus CKX41 outfitted with phase contrast, fluorescence (excitation 470/40; emission 525/50) and an InfinityHD digital camera.

Actin Filament Bundling (cytoskeleton). Cells were fixed with 3.7% formaldehyde, permeabilized with 0.1% Triton X-100 and stained for actin filaments with 0.165 µM phalloidin-AlexaFluor488. NIH ImageJ software was used to quantify intensity density of fluorescence staining normalized to cell area.

Statistics. GraphPad prism freeware used to conduct two-tailed t-test.

Real-Time Reverse-Transcription Polymerase Chain Reaction (qRT-PCR). SurePrep™ TrueTotal™ RNA Purification Kit (Fisher) used to extract total RNA. RNA quantification at A_{260} used to standardize amount RNA loaded into High-Capacity RNA to cDNA kit cDNA for reverse transcription. TaqMan® Fast Advance Master Mix (Applied Biosystems) was used for real-time PCR in conjunction with primer probes (ThermoFisher): human calpain-1 (Hs00559804_m1), E-cadherin (Hs01013958_m1),FSP1 (Hs00243202_m1) and GAPDH (Hs0392907_g1) and a QuantStudio 5 Real-Time PCR instrument. The $\Delta\Delta C_t$ method calculation was used for comparing expression levels between vibration (-) and (+) cells.

Results

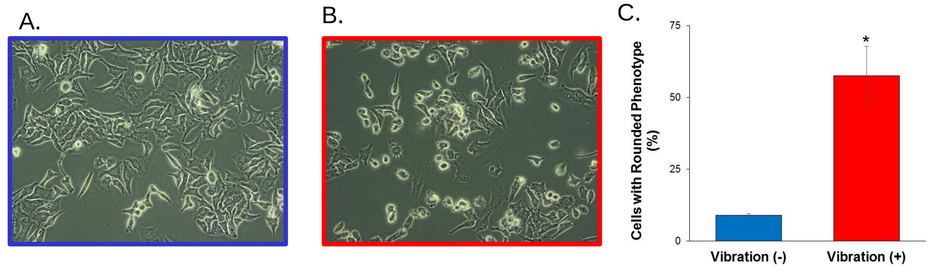

Figure 1: Vibration induces a cell shape change indicative of enhanced migratory ability. Representative phase contrast micrographs of cells without vibration (A) and with vibration (B) at 200x total magnification. Cells (≥250) were counted as either round or not. Rounded phenotype indicates a cell transitioning to a mesenchymal phenotype, or a more mobile cell. Data shown as average percentage round cells (±sd) (C). * = $p < 0.05$ Vibration (+) vs. Vibration (-).

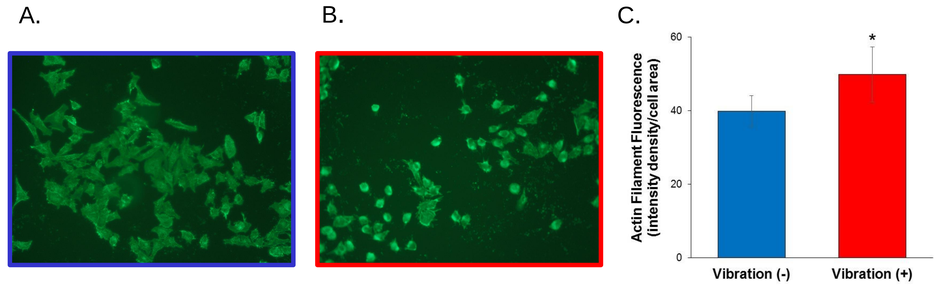

Figure 2: Vibration induces calpain-related actin filament bundling. Representative fluorescence micrographs of cells without vibration (A) and with vibration (B) stained for actin filaments. Actin filament fluorescence calculated using ImageJ image analysis software. Cells (≥10) were outlined for particle analysis. Data shown as average intensity density of fluorescence normalized to cell area (±sd) (C). * = $p < 0.05$ Vibration (+) vs. Vibration (-).

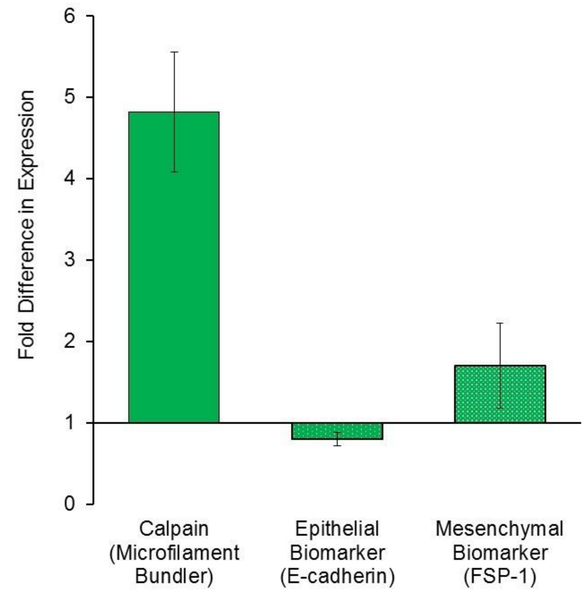

Figure 3: qRT-PCR indicates altered gene expression of biomarkers necessary for a switch to a more mobile cell (EMT). PCR was performed using primers for Calpain (actin microfilament bundler), E-cadherin (epithelial marker), and Fibroblast Specific Protein (FSP-1, a mesenchymal maker). Threshold cycles were normalized to GAPDH, a loading control. A fold difference of expression comparing vibration to no vibration of HeLa was calculated with the $\Delta\Delta C_T$ method where no change is indicated by "1". No reverse transcriptase and no template controls showed no product.

Conclusions

The study presented here shows that epithelial cells exposed to vibration 1) are more likely to have a round shape indicative of a cell transitioning to a more mobile mesenchymal cell, 2) display increased actin filament bundling, a calpain-related process associated with a cell having enhanced mobility, and 3) have altered gene expression of biomarkers specific to an epithelial cell (HeLa) transitioning to a mesenchymal cell. In this regard, epithelial cells exposed to vibration have traits necessary for a transition to a mobile, mesenchymal cell.

We, therefore, supported our hypothesis that vibration induced calpain-related changes associated with an epithelial to mesenchymal cell transition (EMT).

The data presented here also provides a probable mechanism for how vibrational treatment helps cerebral palsy patients.

References

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