

Low-intensity vibration (LIV) enhances the anti-tumor capability of MSCs

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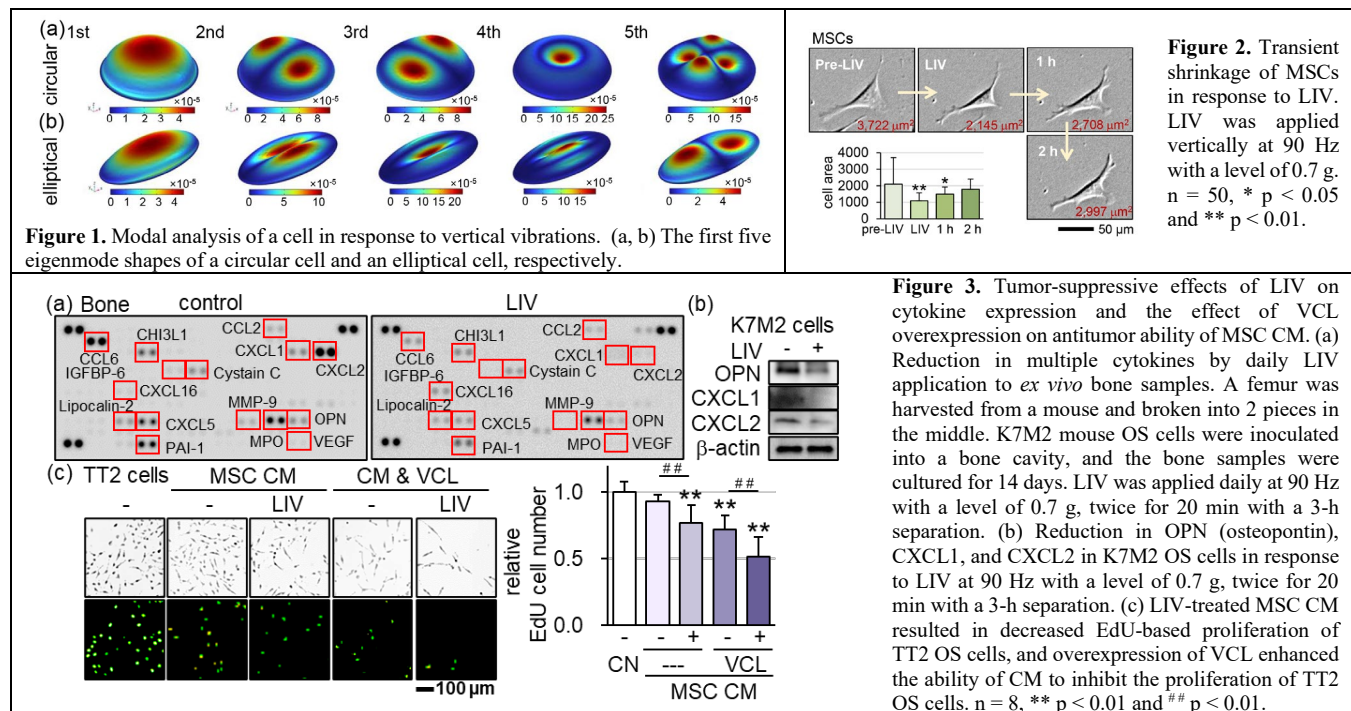
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INTRODUCTION: Osteosarcoma (OS) denotes a malignant bone tumor originating from mesenchymal tissues. However, the efficacy of targeted treatments remains constrained (1). A technique involving low-intensity vibration (LIV) emerges as a promising avenue, delivering exercise-like mechanical stimulation to uphold bone health (2, 3). Notably, the impact of LIV extends beyond bone health, influencing cancer cells (4). Furthermore, LIV treatment of mesenchymal stem cells (MSCs) has demonstrated the capability to redirect their developmental path, favoring osteoblasts over adipogenic cells (5). In this context, we introduce an innovative approach utilizing LIV to generate induced tumor suppressor cells (iTSCs) and cultivate tumor-suppressing conditioned medium (CM). Additionally, we investigate the involvement of vinculin (VCL) in MSCs' responses to LIV (6, 7). Our findings indicate that LIV exhibits significant potential as a safe, effective, and straightforward intervention or adjunctive therapy for OS.

METHODS: Application of LIV: LIV was administered using a specifically designed vibration apparatus. Cells cultured in a 35 × 10 mm dish were subjected to vertical vibrations with frequencies at 90 Hz and a gravitational acceleration level of 0.7 g. The LIV exposure consisted of two 20-min sessions, each separated by a 3-h interval. Numerical Simulation: Finite element analysis was conducted using COMSOL Multiphysics. The assumption was made that the nucleus maintained solidity, while the cytoplasm and outer fluid possessed respective densities of 1,080 kg/m³ and a speed of sound measuring 1,500 m/s. Cell Imaging: Morphological changes in MSCs before and after LIV application were captured using a Celleyte X live cell analyzer. Cytokine Array: A mouse XL cytokine array was employed to evaluate the levels of 111 cytokines and chemokines in ex vivo bone cultures with and without LIV application. Western Blotting and EdU Assay: Protein levels were examined through Western blotting, while metabolic activities were assessed using the EdU assay (8).

RESULTS SECTION: In response to vertical vibrations, we carried out a modal analysis employing circular and elliptical cell models. The natural frequencies were depicted (Fig. 1). To scrutinize how MSCs respond to vibration, we employed vertical LIV on MSCs, monitoring alterations in cell shape and surface contact area before and after exposing them to LIV at 90 Hz with an acceleration of 0.7 g for 20 min. The outcomes indicated a notable reduction in the surface contact area of MSCs following LIV application (Fig. 2). To probe the influence of LIV within the bone tumor microenvironment, *ex vivo* bone assays were conducted. Pairs of femurs were extracted from healthy female mice and introduced to K7M2 OS cells within the bone marrow cavity. Through analysis of cytokines and Western blotting, we revealed a reduction in various chemokine ligands and osteopontin levels in bone samples subjected to a 2-week LIV treatment. This underscored the tumor-suppressing impact of LIV within the intricate bone tumor microenvironment (Fig. 3a & b). CMs derived from MSCs overexpressing VCL demonstrated significantly heightened anti-tumor efficacy in comparison to CMs from MSCs subjected only to LIV. These findings propose that VCL overexpression serves as an enhancer of the inherent anti-tumor potential within LIV-treated MSC CM (Fig. 3c).

DISCUSSION: In this study, we present a unique approach for prompting the conversion of MSCs into iTSCs utilizing LIV. Remarkably, LIV exhibited a dual functionality against OS, simultaneously impeding the progression of OS cells and facilitating the conversion of MSCs into iTSCs. We demonstrated that LIV at a frequency of 90 Hz at 0.7 g, triggered a remarkable morphological shift in MSCs. Of particular note, iTSCs induced through LIV demonstrated robust proficiency in suppressing the proliferation of OS cells. Moreover, the augmentation of VCL expression heightened MSCs' responsiveness to LIV, thereby significantly amplifying the potency of CM in its capacity to quell OS cells. These revelations underscore the feasibility of generating LIV-induced tumor-suppressing iTSCs and emphasize the pivotal role of VCL in initiating receptiveness to LIV.



SIGNIFICANCE/CLINICAL RELEVANCE: This study presents a secure, efficient, and straightforward approach to provoke the generation of iTSCs using LIV, suggesting its potential as a novel intervention or supplementary therapy against OS.

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