

CELL EXPANSION GENES EXPRESSION BY THERAPEUTIC ULTRASOUND. PROS AND CONS.

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1. INTRODUCTION

Mesenchymal stem cells (MSCs) have the capabilities for self-renewal and differentiation into cells with the phenotypes of, among others, bone, cartilage, and fat. Because bone marrow derived cells, which are a main source of MSCs, are not always acceptable due to a significant drop in their cell number and proliferative/differentiation capacity with age, there is a need for a technique that is capable of expansion of these cells or finding an alternative stem cell source.

Nucleostemin is a newly discovered nucleolar protein present in both embryonic and adult rat central nervous system stem cells, and several human cancer cell lines [1]. It has been reported also that Nucleostemin gene is a marker of proliferating stromal stem cells in adult human bone marrow [2].

Ultrasound, an acoustic pressure wave at frequencies above the limit of human hearing, is transmitted into and through biological tissues and is being used widely in medicine as a therapeutic, operative, and diagnostic tool.[3, 4]. It has been reported that Low Intensity Pulsed Ultrasound (LIPUS) enhances different types of cell proliferation that includes chondrocytes [5]; skin fibroblasts [6] and increase VEGF vascular endothelial growth factor (VEGF), a growth factor associated with endothelial cell proliferation and migration [7]. The effect of LIPUS on stem cells is not fully studied or understood. It also has been shown that ultrasound stimulates tendon cell proliferation and upregulate of proliferating cell nuclear antigen (PCNA) [8]. Also, therapeutic ultrasound upregulate c-myc, a proto-oncogene, binds DNA through its basic helix-loop-helix/leucine zipper domain, which is essential to cell proliferation and differentiation [9].

Human Umbilical Cord PeriVascular (HUCPV) cells are a potential substitute for BMCs due to the immaturity of newborn cells [10]. Umbilical cord blood transplantation (UCBT) has become an established haematopoietic stem cell therapy for patients with no reported immune-rejection. [10]. Due to donor number limitation is a major constraint to bone marrow mesenchymal stem cell therapy, there is a high need for alternative cell sources for such cell-based therapies [11].

Taking the aforementioned information, we hypothesized that LIPUS can up-regulate the following genes Nucleostemin gene in rapidly proliferative and self renewal cells (Bone marrow stem cells, Human Umbilical perivascular endothelial cells..

2. METHOD

2.1 Cells

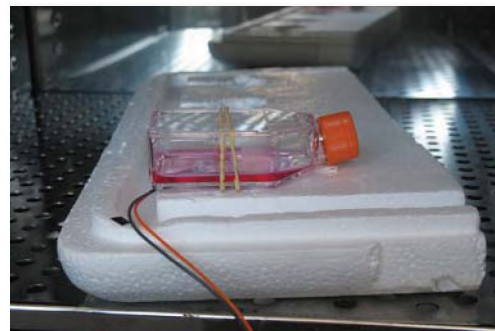
Bone marrow stem cells (BMSCs) were isolated from tibiae and femora of -3 month old male Sprague-Dawley rats (200gms). The femora will be extracted, and the bone marrow cells were flushed with basic media using 10-ml syringe from both sides. Then the cells were centrifuged for 6 min at 600 rpms and were resuspend in tissue culture media, and plated at 5×10^7 cell/mm culture dish (6-well plate), then were incubated in 95% and CO₂ at 37° C, with fresh media every 3 days. Cells were expanded and trypsenized and passaged after 2 weeks. Cells were seeded in 75 mm flasks. Cells were expanded in culture medium that contained (Dulbecco's Modified Eagle's Medmium-low Glucose medium (DMEM-LG), 10% fetal bovine serum (FBS), 1% streptomycin/penicillin, and ascorbic acid (50 µg/ml).

Human Umbilical Cord PeriVascular (HUCPV) were donated by Dr. JE Davis, UT, Toronto, ON, Canada. Details about isolation and behavior of these cells are reported previously [11].

2.2 LIPUS

LIPUS was applied for twenty minutes per day to the base of the cell culture flasks. LIPUS was applied using a 2.5 cm² transducer that delivers 1.5 MHz with a duty cycle of ¼ and delivers SATA intensity of 30 mW/cm². The power output was calibrated weekly to ensure that the output is maintained throughout the experiment. Ultrasound was applied to the bottom of each cell flask for 20 minutes per day for 7 and 20 days (Figure 1).

Fig. 1. Application of LIPUS to the cell culture flasks.



2.3 RT-PCR

Primers for the Nucleostemin were as follows: Forward: TCCGAAGTCCAGCAAGTATTG; and reverse: AATGAGGCACCTGTCCACTC. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a control. Samples were amplified for 22 - 30 cycles in an Eppendorf Master-cycler thermal cycler. PCR reactions were electrophoresed on 2% agarose gels and

visualized under UV light after staining with Ethidium Bromide using image analyzer.

3. RESULTS

3.1 Cell response to LIPUS.

Figures 1 and 2 show PCR reactions gel electrophoresis. It appears that LIPUS has increased Nucleostemin expression in both types of stem cells.

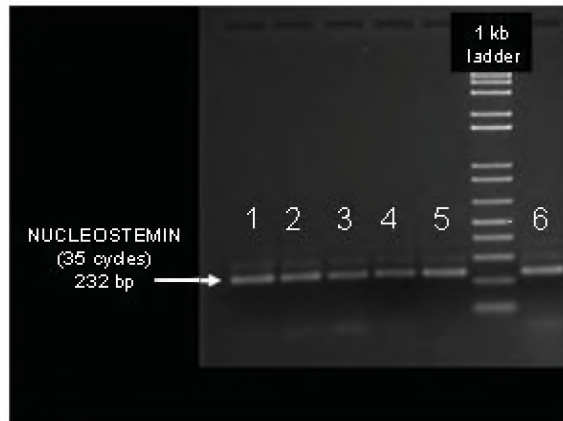


Fig. 1. Nucleostemin up-regulation by LIPUS after 7 days in rat BMSCs. RT-PCR of mRNA from BMCs in Culture. 1,2 (T0), 3,4 (Control), 5,6 (LIPUS).

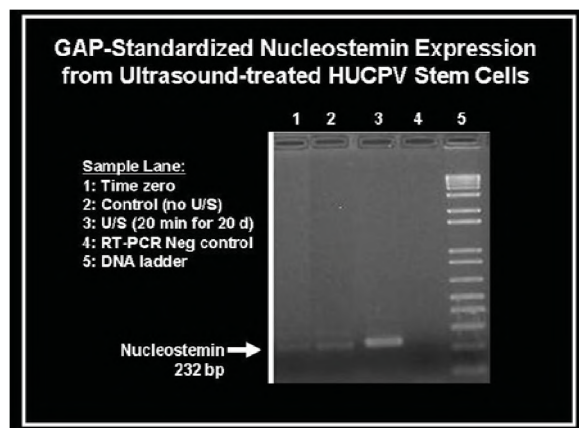


Fig. 2. Nucleostemin up-regulation by LIPUS after 20 days (3) compared to control (2) lanes in HUCPVSCs.

4. DISCUSSION

Our results support previous findings that LIPUS can increase gene expression by different cells. The increased gene expression of Nucleostemin is in accordance with increased cell proliferation. Rat bone marrow stem cells responded positively to LIPUS by increased Nucleostemin expression after 7 days, while HUCPVSCs responded to LIPUS by increased expression of Nucleostemin expression by LIPUS after 20 days. This preliminary study suggests that optimization experiment to test expression of Nucleostemin by each type of stem cells be conducted.

Our data suggests that Therapeutic ultrasound might be beneficial in enhancing proliferation of stem cells and maintaining their pluripotent characteristic by continuing expression of Nucleostemin. However this might be a negative effect on neoplastic cells. Since Nucleostemin is also a marker of cancer cells [1], until a study is conducted on the effect of LIPUS on cancer cell proliferation and associated gene expression, it should be recommended that caution must be taken before application of therapeutic application should cancerous cells are expected to be in the vicinity of the application area until further investigation might shows it is safe in such a condition.

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