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The effect of low-intensity ultrasound and met signaling on cellular motility and morphology

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ABSTRACT

During the past decade, the potential of low-intensity ultrasound (LIUS) was demonstrated in medical research including microvascular remodeling, wound repair, blood flow restoration, angiogenesis and activation of mechanosensitive signaling pathways. However, the current clinical application of LIUS hasn't been extended beyond physiotherapy. In cancer therapy, LIUS related research is focused on ultrasound-mediated techniques for chemotherapeutic drug activation, drug delivery enhancement and gene transfection improvement. While clinical trials show highly promising results, the ambiguity regarding the mechanism of ultrasound-cell interaction is a major limitation in the road to clinical applications. In 2011, the bilayer sonophore (BLS) model suggested that cell intramembrane cavitation is the underlying mechanism for ultrasound-induced bio-effects. According to the BLS model, ultrasound affects cell shape and functionality by inducing intramembrane gas bubbles that deform the cell membrane. This mechanical effect might involve alterations in the bio-chemical signaling pathways. Many biological processes such as cell motility, proliferation and angiogenesis are triggered by MET tyrosine kinase growth factor receptor and its ligand HGF/SF (Hepatocyte growth factor/Scatter Factor). The activation of MET receptor increases cancer cell motility via membrane alterations, leading to epithelial to mesenchymal transformation (EMT), invasion and metastasis. The aim of this work is to examine the crosstalk between MET-activation and LIUS under the assumption that both induce cell membrane alterations whereby affecting cell morphology and motility. In this paper, we present preliminary observations regarding the effect of MET-activation, LIUS exposure and their combined effect on living cells. Measurements of morphokinetic parameters using single cell time-lapse analysis are presented as well. The analysis demonstrates that LIUS inhibits the motility and modulates the morphology of MET-activated cells. These findings demonstrate the balance between the inner cellular processes (MET signaling pathways) and the external forces (LIUS exposure). Understanding the mechanism of LIUS on motile cells might improve the performance of existing LIUS-based cancer treatment modalities and help develop new ones.

1. Introduction

1.1. Low-intensity ultrasound for cancer therapy research

Low-intensity ultrasound (LIUS) is featured in several areas of cancer therapy research such as chemotherapy and gene delivery. Many studies, including *in vitro* observations and *in vivo* treatments of small animals, have indicated the beneficial effect of LIUS-based therapies [1–10]. The ongoing research and clinical trials for such applications show highly promising results [11–14] while understanding the mechanism of ultrasound-cell interaction is far from being complete. So far, ultrasound is known to remodel the cell cytoskeleton [15]. This remodeling is modulated either by cell signaling and energy metabolism levels [16,17] or by discrete forces or distributed stresses [18–20]. Cytoskeletal fluidization induced by LIUS is one example for such a remodeling [21].

1.2. The bilayer sonophore model

The bilayer sonophore (BLS) model is the first comprehensive explanation for the effects of ultrasound on cells. According to this model, the direct interaction between the fluctuating acoustic pressure and the cell bilayer membrane originate in an

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intra-membrane space, which is filled with gasses [22]. The hydrophobic lipid leaflets of the BLS alternately inflate and deflate in response to negative and positive acoustic pressure respectively. The cyclic inflation and deflation of the BLS is followed by cycles of stretch and release in the cell membranes, which change membrane permeability, activate mechano- and electro-sensitive proteins and have direct effect on cell shape and dynamics. An advanced version which combine the BLS model together with the biophysical models of Hodgkin-Huxley, denoted by the Neuronal Intra-membrane Cavitation Excitation (NICE) model [23]. Predictions of the NICE model for a single neuron and for a neural network show close agreement with *in vivo* experimental results [24,25].

1.3. The MET receptor and its HGF/SF ligand

The MET tyrosine kinase receptor is involved in many biological processes such as cell motility, proliferation, angiogenesis, wound healing and tissue regeneration [26]. The ligand for the MET receptor is the HGF/SF (hepatocyte growth factor (HGF)/scatter factor (SF)). MET binding to its ligand HGF/SF activates a wide range of cellular signaling pathways, denoted as MET signaling pathways. In cancer, an activated MET pathway contributes to the tumor progression, invasion and metastasis by promoting cell survival, proliferation, migration and angiogenesis [27,28]. In response to MET activation, Epithelial cells, particularly Madin-Darby Canine Kidney (MDCK) cell-line, undergo colony dispersal (scattering), epithelial-mesenchymal transition (EMT), and increase their motility [26]. Therefore, the scattering model of the activated MDCK cells by HGF/SF is used to study many cell mechanisms such as motility and EMT [29]. It was previously shown that MET activation by HGF/SF, increases cell motility via membrane ruffling and membrane blebbing [30,31]. Ruffling is a formation of polymerized actin filaments on the cell surface. Blebs are membrane-based structures induced by round protrusions of the cell surface, which are driven by detachment of structural proteins and the actin cortex within the cell membrane. MET regulates the machinery of ruffling and blebbing through Rho-ROCK pathway, which controls the actin–myosin contractile force [32]. It is believed that polymerization of the actin cytoskeleton, located adjacent to the cell membrane, has a significant role in halting and retracting the MET induced ruffling and blebbing [33]. Cell shape and membrane curvature are controlled by the physical properties of the membrane and the actin cortex [34]. Moreover, MET induced branching morphogenesis was shown to be dependent on a mechanical feature where cells induce traction forces motion [35].

1.4. Rationale of the study

In this work we present the interplay between MET activation and LIUS exposure and their effect on living cells. We hypothesize that both HGF/SF and LIUS affect the cell membrane and cytoskeleton. HGF/SF results in cell cytoskeleton remodeling through MET signaling pathways whereas LIUS does that through intra-membrane gas accumulation (according to the BLS model). Fig. 1 presents a scheme describing the activation path of HGF/SF treatment (blue arrows) and the hypothesized activation path of LIUS (red arrows). HGF/SF activates the MET receptor resulting in cell cytoskeleton remodeling (by MET signaling pathways) and membrane modifications. For HGF/SF, membrane modifications such as ruffling and blebbing are caused by actomyoin cortex alterations. For LIUS, we hypothesize that cell membrane modifications are caused by the BLS and it causes cell cytoskeleton remodeling in an unknown way.

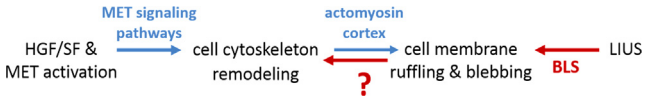

Fig. 1. A scheme presenting the effect of HGF/SF and the hypothesized effect of LIUS on a cell membrane.

2. Material & methods

2.1. Fluorescently-tagged cells

A viral infection was performed on MDCK cells with pmCherry to label the cytoplasm (pQC.mCherry vector, pQCXIP Retroviral Vector (500 ng/μl), Takara Bio USA Inc., Mountain View, CA, USA). A second transfection was performed with pEYFP to label the cell membrane using Lipofectine reagent (cat. No. 18292-011), as described in the protocol provided by the manufacturer (pEYFP-Mem, Takara Bio USA Inc., Mountain View, CA, USA). The fluorescently tagged MDCK cells (pEYFP-mem & pmCherry) were seeded on a glass-coverslip bottomed 35 mm Petri dish (10 mm microwell #1.5 cover glass, catalog number D35-10-1.5-N, Cellvis), 24 h before experiment, using 2*ml* culture medium (high glucose Dulbecco's Modified Eagle Medium (DMEM) + 10% fetal calf serum (FCS) + 1% 200 mM L-glutamine + 0.2% Pen-strep). 5×10^5 K cells were seeded and cell confluency rate at experiment was 80%. For Met-activated cells, 100uM (HGF/SF) was added to the cell sample culture medium, one hour before experiment.

2.2. Ultrasound field and ring transducer

A ring-shaped transducer, consisted of a Pz26 (OD32 ID28 L14) piezoceramic tube of lead zirconate titanate (PZT) (Ferroperm Piezoceramics, Kvistgård, Denmark), with a resonance frequency at 960 KHz. The transducer was connected to an AFG3021B arbitrary waveform generator (Tektronix Company, Beaverton, Oregon, USA). The generated signal was amplified by a Broadband Power amplifier with 50 dB gain (2100L, Electronics and Innovation Company, Rochester, NY, USA). A 2D scan of the pressure field at the bottom center of the ring transducer (scan area = [−0.4:0.4,−0.4:0.4]; z = 0; step size = 0.05 mm), was performed using a micromanipulator system (MP-285, Sutter Instruments) and a calibrated needle hydrophone (HNR-1000, ONDA Technologies ltd). The generated signal consisted of a continuous 960 kHz sine wave with 60 mV peak-to-peak amplitude. The calculated pressure at the bottom center of the ring transducer is 200 kPa.

2.3. Experimental setup and time-lapse microscopy

The ring transducer was placed in the middle of a cell-cultured glass-cover-slip bottomed 35 mm dish and mounted on the microscope stage (Fig. 2). Live cell imaging was performed on a Leica TCS SP5 II inverted confocal microscope (Leica Microsystems Inc., Wetzlar, Germany), equipped with a 63x oil objective lens (Leica HCX PL APO 63×/1.40-0.60NA OIL). A live-cell environmental incubator box was used to maintain constant temperature (37 °C), CO2 concentration (8%) and humidity for the duration of the experiment. VIS lasers were used for excitation, at 514 nm and 567 nm for pEYFP and mCherry respectively. The emission spectrum was collected by a spectral detector (SP). The selected emission band was 520–570 nm for pEYFP and 580–630 nm for pmCherry. The field of view was determined at the center of the ring transducer. Time-lapse videos of 0.5 sec resolution and 5 min. duration were acquired. Experiments were conducted at the Sackler Cellular & Molecular Imaging Center (SCMIC), Tel Aviv University, Tel Aviv, Israel.