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Cell-structure specific necrosis by optical-trap induced intracellular nuclear oscillation

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ABSTRACT

A drug-free procedure for killing malignant cells in a cell-type specific manner would represent a significant breakthrough for leukemia treatment. Here, we show that mechanically vibrating a cell in a specific oscillation condition can significantly promote necrosis. Specifically, oscillating the cell by a low-power laser trap at specific frequencies of a few Hz was found to result in increased death rate of 50% or above in different types of myelogenous leukemia cells, while normal leukocytes showed very little response to similar laser manipulations. The alteration of cell membrane permeability and cell volume, detected from ethidium bromide staining and measurement of intracellular sodium ion concentration, together with the observed membrane blebbing within 10 min, suggest cell necrosis. Mechanics modelling reveals severe distortion of the cytoskeleton cortex at frequencies in the same range for peaked cell death. The disruption of cell membrane leading to cell death is therefore due to the cortex distortion, and the frequency at which this becomes significant is cell-type specific. Our findings lay down a new concept for treating leukemia based on vibration induced disruption of membrane in targeted malignant cells.

1. Introduction

Acute myeloid leukemia (AML) is one of the most lethal cancers worldwide, and *untreated* chronic myeloid leukemia (*CML) can* lead to the blast crisis phase and be fatal (Yong et al., 2006). Intensive chemotherapy of myeloid leukemia and allogeneic haematopoietic stem cell transplantation (HSCT) are the mainstays of treatment. However, this approach has reached an impasse and leukemia relapse is the commonest cause of treatment failure. Developing drug- or reagent-free physiotherapeutic strategies based on purely physical disturbances to kill leukemia cells may provide a novel therapeutic strategy for this disease with hitherto dismal outcome. A possible approach along this direction can be understood from the membrane-cytoskeleton-nucleus structure shown in Fig. 1(C and D). Due to the discreteness of the intracellular components, a vibrating disturbance at a specific frequency and amplitude may set certain components into a higher motion, thus resulting in excessive stresses which may lead to cell damage. In particular, large forces generated from nuclear oscillations in certain cell types with particular structures and components may be imparted on the cell membrane via the interconnecting cytoskeleton, leading to structural damages such as membrane blebbing (Majno and Joris, 1995), or possibly upsetting of the trans-membrane ion channels (Lang et al., 2007). Since the structures of different cell types are different, the response of different cell types to vibratory disturbances may also be different.

Based on the above hypothesis, in the present study we focus on the cell cytoskeleton and the surrounding membrane. We employed an optical trap (Ashkin et al., 1986) to purposely set the nucleus in different cell types (Gallagher et al., 1979; Lange et al., 1987; Lozzio and Lozzio, 1975; Tsuchiya et al., 1980; Wang et al., 1989) (see Table 1) into oscillations at different frequencies. This approach is remarkably different from previous attempts in which laser-beam scanning was blindly done over the cell without specifically trapping any cellular component such as the nucleus (Ng et al., 2013), or batches of cells in culture medium were vibrated via a motion generator (Zhou et al., 2016). Here, the massive nucleus is specifically trapped and oscillated by the optical tweezers, Heto investigate the effects of the cytoskeleton on the vibration-induced cell death. Since in this work we do not specifically aim to examine the effects of the genetic difference between acute and chronic leukemia cells, chronic myeloid leukemia K562 cells, which are large leukemia cells with clearly observable subcellular structures, were chosen for in-depth analysis for the cell death mechanism under oscillations. Specifically, we applied a myosin II inhibitor Blebbistatin to modify the cytoskeletons of the K562 cells,

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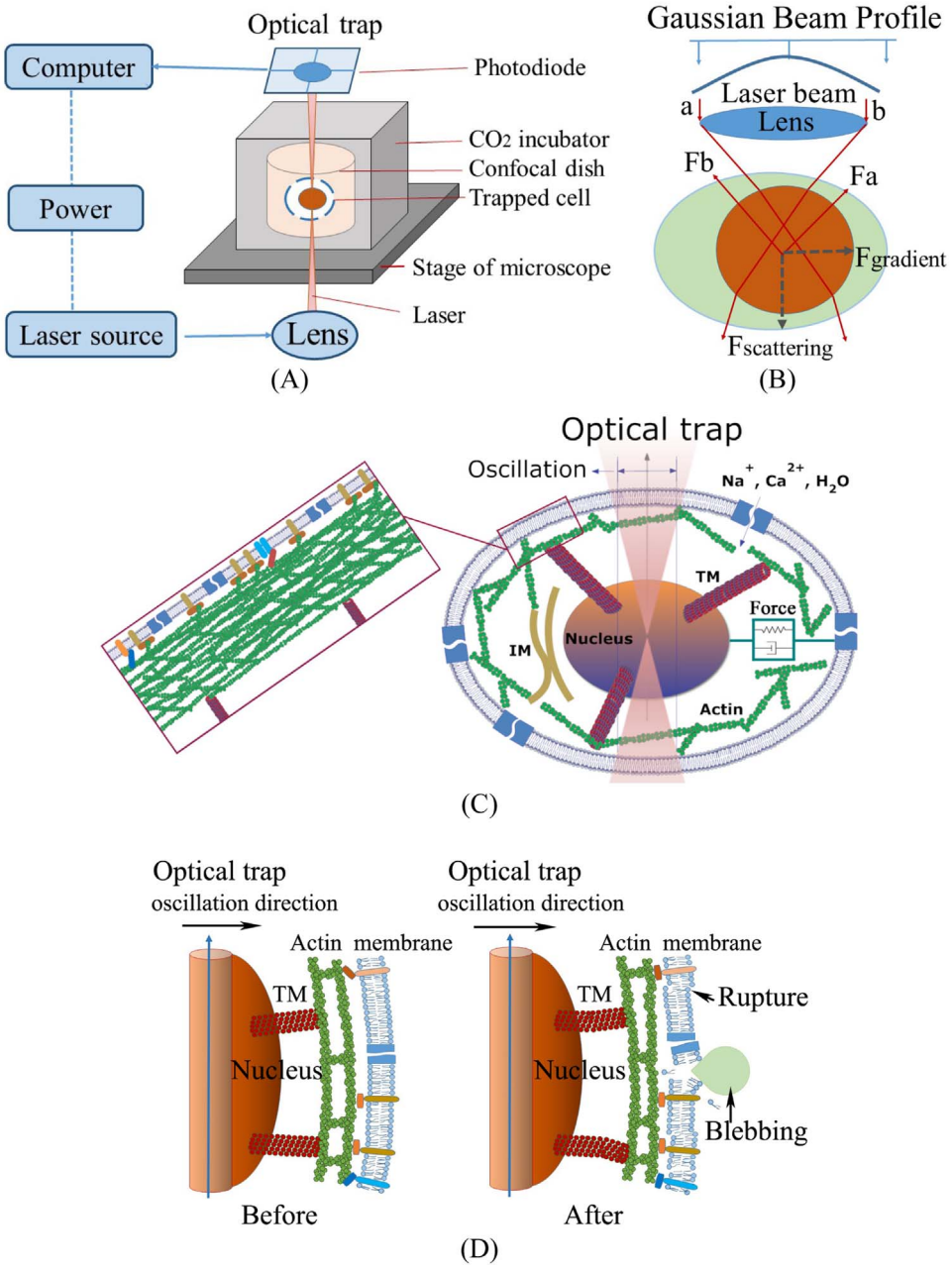

Fig. 1. (A) Schematic showing the setup used for cell oscillation by optical trap. (B) Schematic showing the mechanism of a cell nucleus being trapped by a laser beam. (C) Schematic showing nuclear oscillation by optical trap. The oscillatory motion of the trapped nucleus is resisted by the cytoskeleton network comprising tubulin microfilaments (TM), intermediate microfilaments (IM) and actin microfilaments, which in turn transmit forces to upset the cell membrane. (D) Schematic showing a cell oscillated by a laser trap undergoing membrane rupture and blebbing.

prior to the laser manipulation experiments. Blebbistatin is known for its inhibiting effects on actin polymerization, kinetochore microtubule formation, mitotic spindle assembly (Kovacs et al., 2004; Limouze et al., 2004; Straight et al., 2003), and movement of actin filaments and microtubules leading to their unbundling (Burnette et al., 2008; Schaefer et al., 2008). In addition, the alteration of cell membrane permeability and cell volume were evaluated using staining by ethidium bromide (EB) and a Na^+ fluorescent dye Coro Na™ Green AM (Invitrogen) respectively, during the optical oscillations. Finally, the distortion of the cytoskeleton and the membrane cortex due to vibrational disturbances at frequencies ranging from 1 Hz to about 100 Hz is analyzed by finite-element modeling.

2. Materials and methods

2.1. Cell preparation

Myelogenous leukemia cell lines K562,OCI-AML-3, THP-1, HL60 and MV-4–11 (Lam et al., 2016), with a concentration of ~10^5 cells/ml were cultured in a suspension manner in confocal dishes with fresh GIBCO RPMI 1640 media containing 10% fetal bovine serum and 1% penicillin and streptomycin solution (Sigma-aldrich, St. Louis, USA) inside a CO_2 incubator (Bionex, Model-VS-9160C, Seoul, South Korea), at a humidity of 95% and a temperature of 37 °C for ~24 to 48 h, and were then transferred into human blood plasma, pre-filtered by a 0.22 μm filter, in confocal dishes. The cell passage numbers of all the leukemia cell lines used for the optical trap experiments are no more than 15, and those used for cell death determination and cytoskeleton