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Engineering

DRUG EFFECT ON LEUKEMIA CELLS THROUGH SINGLE CELL ASSAY METHOD

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ABSTRACT Acute myeloid leukemia (AML) is a cancer of the myeloid line of blood cells that is characterized by accumulation of immature malignant cells in the bone marrow. Leukemia cells can hide in bone marrow niche or vascular niche. So,many of the chemical drugs cannot kill these cells. One of the greatest challenges in Acute Myeloid Leukemia (AML) treatment is to prevent relapsing. AMD3100 is used as one of the most common chemical drugs that can inhibit this signal. In the current study, the effect of AMD3100 on the adhesion property of leukemia cells on stromal cells can be investigated by using engineering tools, namely, Optical tweezers (OT) and Dielectrophoresis (DEP), to probe single cell property. AMD3100 not only inhibits the CXCR4/SDF-1α signal pathway but also reduces gene expression of CXCR4 and VLA4 on leukemia cells. This drug also softens leukemia cells. This work provides a new way to investigate cell behavior under drug treatment. The use of combined engineering tools will benefit drug discovery and assessment for leukemia treatment.

KEYWORDS : Myeloid leukemia, optical tweezers, dielectrophoresis

INTRODUCTION

Acute myeloid leukemia (AML) is a cancer of the myeloid line of blood cells that is characterized by accumulation of immature malignant cells in the bone marrow. The high relapsed rate of AML disease indicates persistence of leukemia stem cells, which hide in specific niches in the body. The constituent of niches, such as cells, extracellular matrix (ECM), and soluble factors, can interact with leukemia stem cells to maintain their self-renewal ability and to protect them from environmental stress. The components of niches are complicated, and their roles in regulating hematopoietic stem cells and leukemia stem cells are important. Although the details on the protection mechanism of niches are still unclear, the importance of investigating interactions between niches and leukemia cells are well recognized.

C-X-C chemokine receptor type 4 (CXCR4) is a chemokine receptor for stromal derived factor-1α (SDF-1α or CXCL-12), and the CXCR4/SDF-1α axis plays an important biological function. Very late antigens 4 (VLA-4) integrin binding and CXCR4 chemokine receptor activation are prerequisites for the migration of hematopoietic progenitors and AML cells. The CXCR4/SDF-1α with VLA-4/VCAM-1 (vascular cell adhesion molecule-1) signal pathway meditates interactions between leukemia cells and bone marrow stromal cells to protect leukemia cells from chemotherapy and radiation therapy..

AMD3100, also called Plerixafor, has been identified as a specific inhibitor of CXCR4. AMD3100 works as a slowly reversible antagonist of CXCR4. Furthermore, AMD3100 enhances the sensitivity of CXCR4-positive AMLs to chemotherapy in vitro. AMD3100 has also been used in chronic lymphocytic leukemia(CLL) to inhibit CLL cell trafficking and microenvironment mediated protective effects However, a specific study on the role of AMD3100 to leukemia cell adhesion has not been sufficiently reported.

Cell biomechanical properties are highly correlated with their biological functions. Previous study has shown that increased leukemia cell stiffness is associated with leucostasis in pediatric acute lymphoblastic leukemia. Chemotherapy-induced cell death increases the stiffness of leukemia cells, which may be the consequence of dynamic changes in the actin cytoskeleton. Meanwhile, normal leukemia cells become softer and the adhesion between the cells and the proteins become weaker after treatment with all-trans retinoic acid (ATRa), cytoxan (CTX) and dexamethasone (DEX). These cancer drugs could significantly decrease the stiffness of leukemia cells, and hence, might decrease the risk of leukostasis

Optical tweezers (OT) have been playing increasingly important role in single cell assay investigation. A highly-focused laser beam generated by OT can provide an attractive or repulsive force exerted on single cells. Because of advantages of non-contact and non-invasive properties for cell manipulation, high force resolution, and spatiotemporal resolution, optical tweezers have become one of the most powerful tools to manipulate and analyze single cells and molecules. Optical tweezers have been combined with fluorescence microscopy to study leukemia cell adhesion. Apart from OT, dielectrophoresis (DEP)-based manipulation has also been recognized as an important methodology to characterize biophysical properties of cells, such as cell stiffness, membrane capacitance, and cytoplasm conductivity .In the present study, we aimed to characterize the special effect of AMD3100 drug on leukemia cells.

REQUIREMENTS

In this experimental method,there are two Engineering Tools required:

- Optical Tweezers
- Dielectrophoresis System

2.1 OPTICAL TWEEZERS

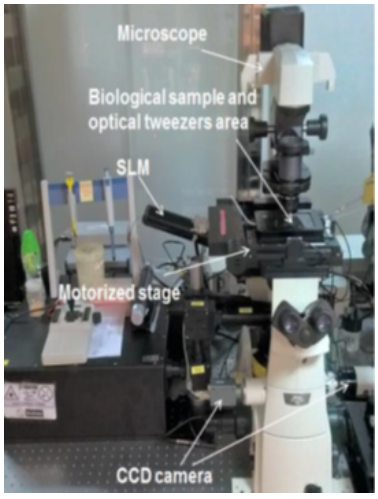

Fig 1:Optical Tweezer

Optical tweezers (originally called "single-beam gradient force trap") are scientific instruments that use a highly focused laser beam to provide an attractive or repulsive force (typically on the order of piconewtons), depending on the relative refractive index between particle and surrounding medium, to physically hold and move microscopic objects similar to tweezers. They are able to trap and manipulate small particles, typically order of micron in size, including dielectric and absorbing particles. Optical tweezers have been particularly successful in studying a variety of biological systems in recent years.

Optical tweezers are capable of manipulating nanometer and micron-sized dielectric particles by exerting extremely small forces via a highly focused laser beam. The beam is typically focused by sending it through a microscope objective. The narrowest point of the focused beam, known as the beam waist, contains a very strong electric field gradient. Dielectric particles are attracted along the gradient to the region of strongest electric field, which is the center of the beam. The laser light also tends to apply a force on particles in the beam along the

direction of beam propagation. This is due to conservation of momentum: photons that are absorbed or scattered by the tiny dielectric particle impart momentum to the dielectric particle. This is known as the scattering force and results in the particle being displaced slightly downstream from the exact position of the beam waist, as seen in the figure.

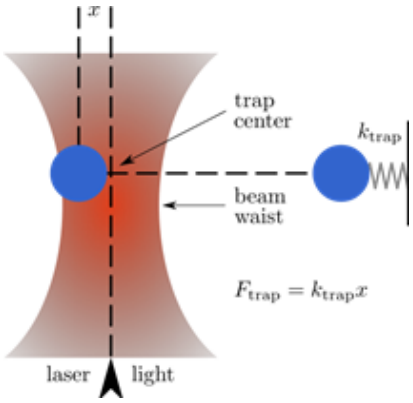

Fig 2: Optical trapping

Optical traps are very sensitive instruments and are capable of the manipulation and detection of sub-nanometer displacements for sub-micron dielectric particles. For this reason, they are often used to manipulate and study single molecules by interacting with a bead that has been attached to that molecule. DNA and the proteins and enzymes that interact with it are commonly studied in this way.For quantitative scientific measurements, most optical traps are operated in such a way that the dielectric particle rarely moves far from the trap center. The reason for this is that the force applied to the particle is linear with respect to its displacement from the center of the trap as long as the displacement is small. In this way, an optical trap can be compared to a simple spring, which follows Hooke's law

2.2 DEP SYSTEM

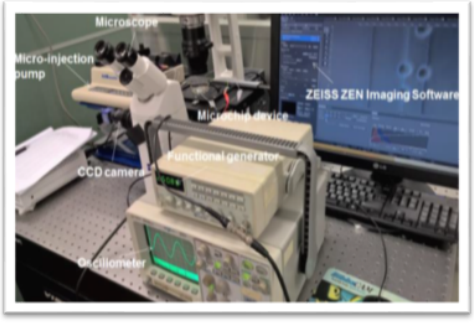

Fig 3: DEP system.

Dielectrophoresis (DEP) is a phenomenon in which a force is exerted on a dielectric particle when it is subjected to a non-uniform electric field .This force does not require the particle to be charged. All particles exhibit dielectrophoretic activity in the presence of electric fields. However, the strength of the force depends strongly on the medium and particles electrical properties, on the particles shape and size, as well as on the frequency of the electric field. Consequently, fields of a particular frequency can manipulate particles with great selectivity. This has allowed, for example, the separation of cells or the orientation and manipulation of nanoparticles and nanowires. Furthermore, a study of the change in DEP force as a function of frequency can allow the electrical (or electrophysiological in the case of cells) properties of the particle to be elucidated.

The DEP stretching system as shown in Fig.3, consists of a micro injection pump, a function generator (GFG-8255A, GWinstek), an oscilloscope (54622A, Agilent), and an inverted microscope (Axio Vert.A1, Zeiss) with a CCD visual detection system (Axiocam 105 color, Zeiss).

PROPOSED METHODOLOGY

1) Cell Culture and Materials

Leukemia cell line OCI-AML3 and stromal cell line MG63 were obtained from ATCC. HUVEC was provided by The University of Hong Kong. OCI-AML3, MG63, and HUVEC were respectively maintained in MEM α, DMEM, and EBM-2 supplemented with 10% (v/v) fetal bovine serum (FBS, Invitrogen). All cells were cultured at 37 ℃ in 5% CO2 in a humidified incubator. AMD3100, a widely used drug that can selectively antagonize the binding of SDF-1 to CXCR4 and preferentially mobilize leukemic blasts into the peripheral circulation, was chosen to treat leukemia cells. SDF-1α

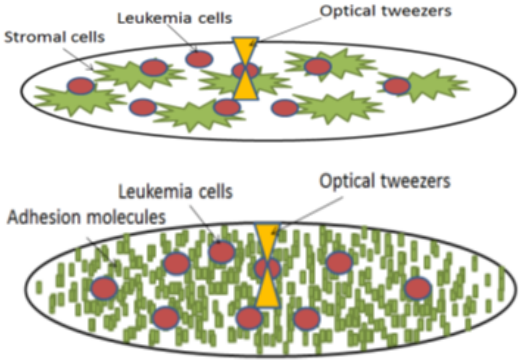

Fig. 4. Schematic of optical tweezers manipulation. (a) Optical tweezers system. (b) Use of optical tweezers to trap leukemia cells adhered on the stromal cells layer

2) Optical tweezers manipulation

Application of optical tweezers to study cell adhesion at the single cell level has been reported in previous works [34, 35]. The optical tweezers system, as shown in Fig. 1(a), consists of holographic optical tweezers and fluorescence microscope. A 35 mm Petri dish with a thin glass bottom (Catalog #D35-10-0-N, Cellvis) was used to culture the OCI-AML3 cells. At 1 day before the experiments, a dish was used to culture stromal cells to obtain the adhesive cell layers, as shown in Fig. 1(b), or coated with fibronectin or VCAM-1 (1μg/cm2) overnight at 4 ℃ to obtain protein layers [Fig. 1(c)]. As for the co-coating experiments, the ratio of SDF-1α to the other proteins was set as 1:2. This is because that the force between CXCR4 and SDF-1α does not play a main role for VLA4/FN/VCAM-1adhesion. The test here aimed to examine how the CXCR4/SDF-1a pathway could improve the VLA4/FN/VCAM-1 adhesion. Therefore, the 1:2 ratio of SDF-1α with respect to the other proteins in the co-coating step should be good enough. The protein was aspirated the next day, and the dish was rinsed with phosphate buffered saline (PBS)

3) DEP system setup and stretching experiments

The DEP stretching system as shown in Fig. 3, consists of a micro injection pump, a function generator (GFG-8255A, Gwinstek), an oscilloscope (54622A, Agilent), and an inverted microscope with a CCD visual detection system (Axiocam 105 color, Zeiss). Before the stretching experiment, different groups of OCI-AML3 were centrifugated at 300 g for 5 min. After removing the supernatant, the cells were resuspended in sucrose medium for further experiments. The sucrose medium was an isotonic buffer medium, which contained 0.3% dextrose, 8.5% sucrose, and 20 mgL−1 CaCl2, thereby allowing positive DEP force on cells. The electrical conductivity and the relative permittivity of the medium were approximately 10 mSm−1 and 78, respectively.

After treatment, cells were loaded into the microfluidic chip with fluid flow control system. Then, a sinusoidal signal with 250 kHz frequency and 1.15 Vpp voltage was applied to the ITO electrodes to attract cells. When cells were aggregated near the electrodes, the voltage was increased to 3.5 V to stretch the cells. Finally, the deformation information of cells was collected by CCD camera.

4) Fabrication of the microfluidic chip

A microfluidic chip was designed for the DEP experiments, as shown in Fig. 5 The chip had three layers. The bottom layer was glass slide coated with electrically conductive material. The middle layer was an optically transparent indium tin oxide (ITO). The cover layer was PDMS. The average diameter of OCI-AML3 cells was approximately 18 μm. Thus, the channel was designed to have a width of 25 μm. The detailed fabrication was reported in the previous work.

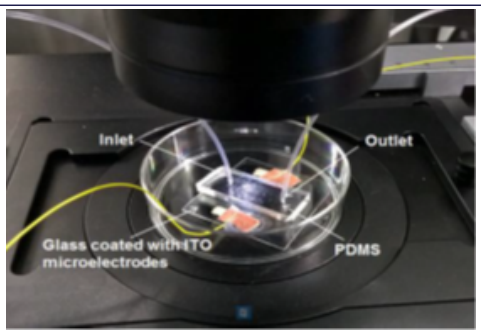

Fig 5: Microfluidic chip design. (a) Schematic of microfluidic chip. (b) Photograph of microfluidic chip**.**

5) CXCR4 expression flow cytometry

For CXCR4 expression studies, leukemia cancer cell lines were adjusted to a density of 0.5 × 106/ml in the culture medium. Cells were washed with a 20-fold volume of ice-cold buffer without FBS, stained at 4 °C with saturating concentrations of phycoerythrin-conjugated anti-CXCR4 antibody \and analyzed by flow cytometry

6) Cell viability

Cells were harvested and aliquoted up to 1 × 106 cells/100 μL into 1.5 ml tubes. After washing twice by adding 1 mL of PBS, the cells were resuspended to 1 mL of absolute ethanol at –20 °C. After ethanol treatment at –20 °C for 10 min, the cells were washed again by adding 1 ml of PBS. Then, 1 mL of diluted propidium iodide (PI) was added to staining buffer (50 μg/ml). Finally, the cells were incubated for 15 min at room temperature and analyzed by flow cytometry in the presence of the dye.

CONCLUSION

This paper presents a single cell assay method to characterize the effect of the drug AMD3100 on leukemia cells. Optical tweezers are used to study the adhesion between leukemia cells and stromal cells, as well as the interaction between leukemia cells and adhesion molecules. CXCR4/SDF-1α signal pathway could promote the binding of VLA-4 and FN/VCAM-1. Meanwhile, AMD3100 could destroy this promotion. DEP force is further applied to deform the leukemia cells, thereby showing the change in the stiffness before and after AMD3100 treatment. AMD3100 could decrease the stiffness of leukemia cells. Both engineering tools of optical tweezers and DEP are useful for drug assessment at the single cell level, as shown in this study. The proposed single cell assay method not only confirmed some results proposed in previous studies but also obtained new results that have not been reported yet in the literature.

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