Myosin-Driven Intercellular Transportation of Wheat Germ Agglutinin Mediated by Membrane Nanotubes between Human Lung Cancer Cells

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ABSTRACT Membrane nanotubes can facilitate direct intercellular communication between cells and provide a unique channel for intercellular transfer of cellular contents. However, the transport mechanisms of membrane nanotubes remain poorly understood between cancer cells. Also largely unknown is the transport pattern mediated by membrane nanotubes. In this work, wheat germ agglutinin (WGA), a widely used drug carrier and potential antineoplastic drug, was labeled with quantum dots (QDs-WGA) as a model for exploring the intercellular transportation via membrane nanotubes. We found that membrane nanotubes allowed effective transfer of QDs-WGA. Long-term single-particle tracking indicated that the movements of QDs-WGA exhibited a slow and directed motion pattern in nanotubes. Significantly, the transport of QDs-WGA was driven by myosin molecular motors in an active and unidirectional manner. These results contribute to a better understanding of cell-to-cell communication for cancer research.

KEYWORDS: membrane nanotubes cancer cells wheat germ agglutinin quantum dots intercellular transportation myosin motors

Intercellular communication can regulate the balance between proliferation and apoptosis of cells, which plays a crucial role in cancer cell coordination and tumor invasion. To date, most researches have focused on the gap junctional intercellular communication between cancer cells.1,2 Membrane nanotubes, as recently discovered membranous tethers between cells, attract keen interest due to their ability to facilitate direct intercellular communication, signaling and the spread of pathogens.3–8 These tubular structures have been found between various cell types, including neuronal cells, immune cells, and cancer cells as well as other types of cells, both in vivo and in vitro.9–12 More recently, some researchers have reported that the cellular contents, such as endosomes, mitochondria, and Golgi vesicles, could transfer between cancer cells through membrane nanotubes.12,13 However, many questions regarding the transport pattern of biomolecules via nanotubes remain poorly understood, and little is known about the transport mechanism involved in the nanotubes between cancer cells.

Herein, we chose wheat germ agglutinin (WGA) as a model for exploring the intercellular transportation via membrane nanotubes between human lung cancer A549 cells. WGA can specifically recognize N-acetylglucosamine and sialic acid moieties, and has been widely used as a drug carrier and potential antineoplastic drugs in biology and medicine.14–16 Researching on intercellular transportation of WGA not only helps to reveal the transport mechanism in membrane nanotubes, but also provides insights into drug delivery between cancer cells.

In this work, we labeled WGA with quantum dots (QDs) for live-cell imaging. Combining with the high brightness and excellent...
Microinjection. An Eppendorf TransferMan NK2 micromanipulator and an Eppendorf FemtotJet injection system (Eppendorf AG, Germany) were utilized for microinjection. Capillaries were prepared with a model P-2000 capillary puller (Sutter Instruments Co., USA). The microinjected samples were monitored using a fluorescence microscope (Axiovert 200M, Carl Zeiss) and microinjected with ~20 fL mouse monoclonal to myosin (0.1 mg/mL) based on standard procedures. FITC was used to denote the microinjected cells. Next, the cells were cultured for 30 min at 37 °C and then imaged by confocal microscopy.

Fluorescence Imaging. Fluorescent images were acquired using a spinning disk confocal microscope (Andor Revolution XD) with an Olympus IX 81 microscope, a Nikpok disk-type confocal unit (CSU 22, Yokogawa), a cell culture system (INUBG2-PI), and an EMCCD (Andor iXon DV885K). DiO/Dylight 488, QDs and Dylight 649 were excited at 488 nm, 561 and 640 nm by DPSS lasers, respectively. Using 525/50 nm, 617/73 nm, and 685/40 nm band-pass emission filters, the emission was split into different channels. For simultaneous multiple-color imaging, the fluorescence was detected separately with the EMCCD by appropriate channels.

Imaging Analysis. Each frame of the movies was denoised by using a Gauss filter, and the orthogonal slice view was obtained by Andor IQ software (Andor Technology). Mean squared displacement was calculated for each time interval over a trajectory with the user-plexed Biological Detection and Imaging. PLoS ONE 2012, 7.


Wang, E. C.; Schi, K.; Schaf, J.; Mckenna, M. G. A Novel Micromanipulator for Statistical Tests. Avnon or Two-Sample T-Test was utilized for statistical tests.

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Conflict of Interest: The authors declare no competing financial interest.

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Supporting Information Available: Figures S1–S4 and Movies S1–S8 as described in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES AND NOTES


