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Probing for chemotherapy-induced peripheral neuropathy in live dorsal root ganglion neurons with atomic force microscopy

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Abstract

Chemotherapy-induced peripheral neuropathy (CIPN) remains a major reason for cancer patients to withdraw from their lifesaving therapy. CIPN results in irreversible sensory and motor impairments; however, the epidemiology is largely unknown. Here, we report for the first time that chemotherapy drug vincristine not only reduced axonal regeneration in primary dorsal root ganglion neuron but also induced substantial changes in cell mechanical properties detected by atomic force microscopy (AFM). Confocal imaging analysis revealed vincristine-induced microtubule depolymerization. By using AFM for high-resolution live cell imaging and quantitative analysis, we observed significant changes in cell surface roughness and stiffness of vincristine-treated neurons. Elastic modulus was decreased (21-45%) with increasing dosage of vincristine. Further study with paclitaxel, another well-known CIPN drug, confirmed the link between cell mechanics and cytoskeleton organization. These data support that our system can be used for probing potential CIPN drugs that are of enormous benefit to new chemotherapy drug development.

Key words: AFM live cell imaging; Chemotherapy-induced peripheral neuropathy; Dorsal root ganglion neurons; Cell mechanic; Nanoindentation

Background

Chemotherapy refers to the treatment of cancer using antineoplastic drugs (which prevent growth and proliferation of malignant cells) that mainly target proliferative cells (i.e., cancer cells); however, for some unknown reasons, such drugs can also harm healthy neurons.[1] The absence of an effective blood–nerve barrier in sensory neurons and nerves of the peripheral nervous system (PNS) places these cells at increased risk compared to the central nervous system, which is well protected by the blood–

Abbreviations: 3-D, three-dimensional; AFM, atomic force microscopy; CIPN, chemotherapy-induced peripheral neuropathy; DRG, dorsal root ganglion; NB, neurobasal; PNS, peripheral nervous system.

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None of the authors declare a conflict of interest and commercial interest related to this work.

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brain barrier. An estimated 40-50% of cancer patients who undergo chemotherapy often experience sensory and motor symptoms, a condition known as chemotherapy-induced peripheral neuropathy (CIPN).[2-4] Although CIPN is one of the most common reasons that cancer patients stop their life-saving treatment early, the mechanism of CIPN has not been fully elucidated yet. There are currently no preventative or effective treatments for CIPN.

Vincristine is widely used for treating many types of cancer, such as lymphoma, sarcoma and leukemia.[5] Over 60% of vincristine-treated patients experience sensation disturbance and muscle weakness, which affect physical ability and daily life.[4] Early symptoms include numbness, tingling, and weakness of distal muscle, followed by motor dysfunction, which in some cases can be irreversible.[4-7] The anti-cancer action of vincristine is due to its binding to β-tubulin (major component of cytoskeleton, such as microtubules and axons), which disrupts mitotic spindle formation in actively dividing cells, thereby resulting in depolymerization and inhibition of cell division.[8,9] Most of the reported studies focused on the cytoskeleton of cancer cells or non-neuronal cells;[8,10] however, little is known about the direct effect of vincristine on peripheral sensory neurons (i.e., dorsal root ganglion, DRG), particularly the axonal regeneration and cytoskeletal organization of DRG neurons.

Microtubules and actin filaments are two major components of cytoskeleton that generate the extension and traction force[11] that maintains cell integrity and allows the cell to undergo normal biological processes, such as neurite extension, mitosis and cell migration. In most cases, disruption of regular biological processes is attributed to changes in the cell integrity resulting from the rearrangement of cytoskeletal components. Because the cytoskeletal rearrangement plays a critical role in influencing the forces exerted on cells and the mechanical behavior of entire cellular structures, it is of great importance to investigate the cell mechanical properties to have a better understanding of the underlying mechanism that drives the axonal regeneration. In recent years, a number of techniques, including optical[12] and magnetic tweezers,[13] microneedle pulling,[14] traction force microscopy[15] and nanoindentation based on atomic force microscopy (AFM),[16] have been developed to measure cell mechanical properties such as cell stiffness. In addition, AFM is the most promising technique to allow the real-time monitoring and study of live cells at molecular level because of its capabilities in providing high spatial resolution images, detailed topographical information and precise quantitative elasticity measurement.[17,18]

Due to the significant advantages of using AFM for biological studies, AFM has been used extensively to study a wide range of biological structures, such as growth cone, synapse, cell junction and microtubule distribution, through high-resolution scanning.[17,19,20] Recent reports have been found on the use of AFM to study the growth cone of fixed DRG neuron[19] and to determine the mechanical properties of non-neuronal cells under different physiological conditions such as apoptosis.[21] It has been shown that the mechanical properties of cells after fixation were altered significantly.[22] Live cell AFM imaging studies are more likely to detect minor molecular changes in cytoskeletal elements and reflect the true physiological condition of cells with drug treatments when compared with fixed cell studies.

Animal studies of CIPN are widely used to study possible mechanisms of vincristine in inducing peripheral neuropathy. Intraperitoneal injection of vincristine induces loss of epidermal sensory fibers in the hind paws of mice, which results in pain hypersensitivity, and axonal degeneration of peripheral nerves, which results in motor function impairments.[23] Circulating chemotherapy agent access injured nerves and adversely affect nerve repair.[24,25] However, the mechanisms of CIPN remain largely unclear. Here, we present a quantitative approach to investigate the mechanisms of CIPN based on AFM. To the best of our knowledge, we provide the first report to reveal a direct linkage between axonal degeneration and cell mechanical properties in live adult DRG neurons under CIPN conditions using AFM. Our *in vitro* system presented here not only demonstrates the site of action of vincristine in DRG neurons but also serves as a cell-based screening tool for future chemotherapy drug discovery.

Materials and methods

AFM instrumentation, imaging and surface roughness analysis

A BioScope Catalyst atomic force microscope (Bruker Nano, Santa Barbara, CA) was used for live cell imaging and DRG culture analysis. A conically shaped silicon nitride AFM probe with a spring constant of 0.7 N/m (SCANASYST-FLUID, Brukernano, Santa Barbara, CA) was used. The movement of AFM probe was controlled by a piezo-electric AFM scanner with a maximum *x-y* range of 100 μm × 100 μm and *z* range of 16 μm. To achieve high-resolution imaging and prevent any damage of cell samples during the scanning process, a new scanning operation mode called the peak force tapping mode was used. For all imaging experiments, the applied force was set to 500 pN and the scan frequency was set to 0.3 Hz. AFM images were acquired with a pixel resolution of 256 × 256. Detailed procedures of AFM imaging and analysis are described in Supplemental Materials.

Cell surface roughness of DRG neurons was calculated based on AFM height images. A region of 1 μm × 1 μm was selected from the height image, and two surface roughness parameters, namely the average surface roughness (R_a) and root-mean-squared roughness (R_q), were calculated from equations (1) and (2), respectively.

$$R_a = \frac{1}{n} \sum_{i=1}^{n} | y_i | \tag{1}$$

$$R_q = \sqrt{\frac{1}{n} \sum_{i=1}^{n} y_i^2} \tag{2}$$

i is the sample order and y_i is the difference between the ith value and the average value of n samples.

AFM-based single-cell indentation

AFM was also utilized as a nano-indenter to determine the mechanical properties of DRG neurons. When the AFM probe was brought into contact with cell surface, cell membrane was deformed and deflection of AFM cantilever was recorded. The deflection-displacement curve was recorded, and the applied