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Mechanical cytoprotection: A review of cytoskeleton-protection approaches for cells

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ABSTRACT

We review a class of cutting-edge approaches for cytoprotection of cells exposed to assaults such as sustained deformations, chemotherapy, radiotherapy, or ischemia. These approaches will enhance cell survival by mechanically protecting the structure and dynamics of the actin cytoskeleton (CSK). Cortical actin provides structural support to the plasma membrane (PM), protecting its integrity. Consequently, assaults can fragment the actin cortex leading to local, mechanical failure of the PM and poration of the cell. This disrupts normal trafficking of biomolecules across the PM, leading to loss of homeostasis and eventually, to cell death and tissue necrosis. Two different approaches to cytoskeletal protection are covered in this review paper. The first is to supply energy-related molecules to maintain and enhance the energy-consuming dynamics of the actin CSK. The second is to stabilize newly formed actin CSK directly, for example through cross-linking or reinforcement at PM anchoring sites. Research in this area is clearly still in its infancy. Very few studies have gone beyond characterizing the effects of induced damage to the actin CSK (and subsequent PM collapse). Recent work, focusing instead on sustaining the actin under non-physiological or pathophysiological conditions, has shown great promise. Such cytoskeletal-protection may find medical applications in preventing or minimizing tissue damage when tissues are unhealthy or at risk, or in enhancing cell performance under stress. Here, we condense the relevant cell biology and biomechanics background, assess candidate cytoskeletal protective agents, and review published works that have shown potential for medical benefit in experimental model systems.

1. Introduction

In this paper, we review cutting-edge approaches to enhance cell survival under various assaults that damage cell integrity, such as sustained large deformations, chemotherapy, irradiation and ischemia. These approaches will enhance cell survival by targeted treatments to mechanically cytoprotect the cell cytoskeleton (CSK) dynamics, reconstitution ability, and stability. Cytoprotection is a general therapeutic concept referring to protecting cells against any harmful physiological or non-physiological effects or agents. One commonly used example is gastric cytoprotection which is the stimulation of gastric cells (by pharmacological therapy) to produce more mucosa, for protecting these cells against gastric acid which may cause gastric ulcers (Takeuchi, 2014). Another frequently encountered example is cytoprotection of the vascular endothelium which is exposed to potentially harmful substances such as cytokines that could lead to endothelial dysfunction preceding arthrosclerosis. Cytoprotective drugs aimed at conditioning the endothelium are developed to minimize vascular injuries and atherogenesis e.g. in patients with systemic inflammatory disease (Peshavariya et al., 2014).

The present paper highlights a particular aspect of cytoprotection that has a pivotal biomechanical aspect – namely, *mechanical cytoprotection*. Specifically, we focus on the mechanical function of the cortical actin network which structurally supports the plasma membrane (PM) of the cells; most of the approaches reviewed here still require additional experimental evidence, yet have shown promise in maintaining cell integrity and viability. Two different mechanical cytoskeletal protective approaches are covered here: (1) enhancement of CSK remodeling dynamics by supplementing cell energy and (2) stabilization of newly formed actin e.g. through cross-linking or reinforcement at its anchoring sites to the PM. The combined treatments facilitate CSK fluidization followed by its rapid reconstitution, to restore cell homeostasis. There are currently massive research gaps to fill prior to applying mechanical cytoprotection of cells in clinical practice, specifically when tissues are subjected to non-physiological or

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pathophysiological conditions. However, published seed work in the field already points to the medical benefits in treating cells with energy molecules, actin-stabilizing molecules, or a combination of the two. Here, we explain the role of the CSK and its interactions with the PM in normal and abnormal conditions, and then assess candidate biomolecules for which there is sufficient published evidence to indicate cytoskeletal mechano-protective efficacy, with potential medical applications.

Living cells are active, non-equilibrium systems that continuously adapt their internal environment and external connections in response to changes in their environment. The cells constantly exchange energy and matter with the surrounding extracellular matrix (ECM) and neighboring cells, and remodel their CSK to facilitate the dynamic responses. The CSK, situated in the crowded cell cytoplasm, is continuously regulated to maintain cell shape and morphology and support normal cell functions (Alberts et al., 2008; Cooper and Hausman, 2007). The CSK dynamics, in particular, are critical for cell function. The CSK responds to various perturbations by breakdown and remodeling. Its dynamic-biomechanical remodeling and structural fluctuations have been studied extensively (Brangwynne et al., 2008, 2007; Burov et al., 2011; Bursac et al., 2005; Caspi et al., 2000; Gal and Weihs, 2010; Wilhelm, 2008), and have been shown to become enhanced for example in cancerous cells (Gal and Weihs, 2012; Goldstein et al., 2013). Concurrently, the PM, a fragile thin (5-nm) structure that continuously fluctuates (Girshovitz and Shaked, 2015; Reed et al., 2008), relies on the CSK for stability (Pelling et al., 2007; Raupach et al., 2007). The CSK anchors at the PM to reinforce it and responds to forces that are externally applied through the PM or the cell environment (Arcizet et al., 2010; Mizrahi et al., 2012). The combined dynamics of the CSK and the PM facilitate cell processes such as morphology changes, force application and migration (Discher et al., 2005; Ehrlicher et al., 2015; Fabry et al., 2001; Trepat et al., 2009). For example, in cancer, cells are not only internally more dynamic (Gal and Weihs, 2012; Goldstein et al., 2013), but they are also externally softer and can apply greater or varying forces to their environment (Cross et al., 2007; Dvir et al., 2015; Guck et al., 2005; Kristal-Muscal et al., 2013; Mierke et al., 2008). Importantly, responses of single cells can affect the neighboring cells and the surrounding ECM (Ben-Or Frank et al., 2015; Shoham et al., 2015). This induces signals that can elicit biochemical and biomechanical responses throughout the tissue volume, especially during tissue development (Teo et al., 2015).

2. The cell cytoskeleton

The cell houses organelles with various metabolic, functional, and dynamic-mechanical roles. The largest organelle is the stiff and elastic nucleus, which contains the genetic material of the cell. Cells are self-enclosed systems bounded by the inelastic PM (Gralka and Kroy, 2015) that separates the outside microenvironment from the intracellular region, and facilitates cell-cell and cell-matrix communication, nutrients and waste transport. The PM is connected to the nucleus through the CSK that spans the cytoplasm. The dynamic cytoskeleton and especially the molecular motors acting on it provide the cell with its remodeling capabilities and allow active transport within the cell.

The cell CSK is composed of different types of three-dimensional (3D) bio-polymer protein networks: microtubules, intermediate filaments (IFs), and actin filaments, networks, or stress fibers. Each element has different roles due to its dynamic, structural remodeling abilities. The actin and microtubules are more dynamic, facilitating faster cell responses while the IFs mostly provide structural support. The CSK has three key roles in: (a) spatially organizing the cell to allow efficient intracellular transport; (b) physically (biochemically) connecting the cells, through their PM, with their external microenvironment, i.e. the ECM and neighboring cells; (c) generating and sustaining mechanical forces between cells and the ECM or neighboring cells, where for example coordinated forces enable the cell to change shape or move (Fletcher and Mullins, 2010).

Actin filaments are simply constructed to facilitate dynamics and adaptability. Actin is a simple biopolymer composed of repeated units of globular actin (G-actin) monomers that spontaneously polymerize into long actin filaments (F-actin); actin is typically a-few-microns long in cells. The resulting single microfilaments are flexible and highly dynamic, and may form more mechanically stable bundles or stress fibers. Concurrently, actin filaments can be cross-linked by various myosin molecular-motors, forming a dynamically regulated network. The resulting actomyosin network enables local transport along filaments, and more importantly, facilitates cell motility and force application through localized contractions of the network. For transmitting such localized forces extracellularly, trans-membranal integrins (that connect to the actin network) are then clustered to form dynamic, yet structurally stable cell-matrix connections, which are called focal adhesions. The actin filaments typically organize into networks and localize to the cortical or submembranal region (Pollard and Cooper, 2009). The cortical actin network mechanically supports the PM against external deformations, and can form connections with the external cell environment (through transmembranal integrins). The actin can be reorganized in the cell rapidly, to produce forces and change cell morphology to facilitate processes such as cell motility and orientation with a stress field (Gourlay and Ayscough, 2005).

In contrast to actin, microtubules span the cytosol, ranging from the microtubule centrosome (their "hub") at the nucleus and to the PM. Microtubules are essential in cell motility, morphogenesis, division, organelle transport, and organization. Microtubules are grouped into stable (detyrosinated) and dynamic (tyrosinated) populations (Kreis, 1987), respectively, with lifetimes of 18 h and 5 min. Microtubule biopolymers are composed of α/β tubulin heterodimers that polymerize into long strands, typically $\sim$10 μm long in mammalian cells; microtubules are 25 nm in diameter, hollow and consists of 13 linear protofilaments. Heterodimer orientation gives microtubules a directional polarity, where the minus side is located near the cell nucleus at the centrosome, and the plus side is peripheral at the PM. This directionality is used to determine direction-of-motion of molecular motors, where families of dynein and kinesin, move to- and from-the nucleus, respectively (Valiron et al., 2001).

The least dynamic element of the CSK is the structurally complex IFs; the naming relates to their diameter being between that of actin and microtubules. IFs are short ($\sim$50 nm long) hollow biopolymers composed of 32 strands of intertwined and bundled protein chains. The structural complexity of the IFs makes them less dynamic and therefore less involved in cell motility and migration. It is assumed that their main role is mechanical, in absorbing mechanical stress and serving as a binding factor for all the CSK elements (Herrmann et al., 2007; Nagle, 1994).

The normal function of a cell requires constant generation of forces and precise regulation of internal and external stiffnesses on short time scales (Discher et al., 2005). One of the most important roles of the CSK is in maintaining the cell's morphology and stiffness as well as sustaining PM stability and integrity under applied forces and deformations. Specifically, the actin CSK typically accumulates at the cell periphery, provides structural support to the thin PM and facilitates formation of lamellipodia and filopodia. Thus, for example, in cancer cells, changes in CSK organization result in reduced external and internal stiffness (Cross et al., 2007; Gal and Weihs, 2012; Goldstein et al., 2013; Guck et al.,

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