The International Journal of Biochemistry & Cell Biology 55 (2014) 264–268

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Electric field regulated signaling pathways

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ARTICLE INFO

Article history:
Received 7 July 2014
Received in revised form
10 September 2014
Accepted 12 September 2014
Available online 22 September 2014

Keywords:
Electric field
PI3K/Akt
PTEN
EGFR
AChR and NMDAR
MAPK
Electrotaxis

ABSTRACT

Physiological electric field (EF) is a potent guidance cue for many physiological development and pathological conditions. The EF induced cellular responses such as migration and proliferation, are considered to be regulated by multiple signaling pathways in a coordinated way. Unlike the signaling transduction regulating the cellular responses toward chemical gradients, the signaling network involved in electric stimulation shows a unique manner, combining the regulation of ion channels, membrane receptors and associated intracellular signaling pathways. This review shall discuss the cellular responses in EF, and summarize the primary signaling network activated during the EF-induced cellular response.

1. Introduction

Electric field (EF) stimulation is among many potential guidance cues regulating signaling pathways essential for multiple cellular events in both physiological and pathological conditions, including embryonic development, tissue regeneration, wound healing, *etc.* It has been well documented that EF could improve wound healing, restore neuromuscular function, treat neurological and psychiatric disorders and maintain the tissue health status (Zhao et al., 2010; Jahanshahi et al., 2014; Creed et al., 2012; Nuccitelli, 2003a,b). Mechanistic investigation has revealed that EF stimulation can affect the ion channel and receptor functions on cell membrane, which constantly monitors the cellular response to the microenvironment. Consequently, EF induces the transduction of intracellular signaling molecules downstream the cascades to drive the cell hyperpolarization/depolarization, migration, proliferation, differentiation and other activities (Babona-Pilipos et al., 2011; Sato et al., 2009). The accumulation of such signaling molecules triggers the cellular response at tissue level, which is reflected as improving wound healing and tissue regeneration, exercising paralyzed muscles, relaxing the neural stress, *etc.* (Jahanshahi et al., 2014; Creasey et al., 2004). Also with the development of stem cell transplantation research, EF stimulation is found beneficial for supporting the survival of grafted stem cells, driving their migration and improving their differentiation to function at the lesion point (Meng et al., 2011). These advances have urged the investigation into the therapeutic potential of EF treatment in wound healing, neurodegenerative diseases of central nervous system (CNS), and stem cell replacement therapy, and the associated signaling pathways and molecular mechanisms underlying these events (Fig. 1).

The mechanism underpinning the cellular/molecular responses toward EF treatments, however, is still not clear. Generally, EF stimulation is considered to polarize the signaling molecules at the targeted cell membrane which induces an asymmetric activation of the signaling molecules and downstream cytoskeleton, thereby leads to the directed cell migration in EF, namely electrotaxis or galvanotaxis (McCaig et al., 2005; Pullar et al., 2006; Li et al., 2008; Mycielska and Djamgoz, 2004). Previous studies on the regulation of electrotaxis have demonstrated several important candidate signaling molecules, including Na/K-ATPase (NaKA),

Abbreviations: AchR, acethylcholine receptor; AP-1, activator protein 1; cAMP, cyclic adenosine monophosphate; cdc42, cell division control protein 42 homolog; cGMP, cyclic guanosine monophosphate; CNS, central nervous system; EF, electric filed; EGFR, epidermal growth factor receptor; ERKs, extracellular signal-regulated kinases; GbpC, cGMP binding protein; MAPK, mitogen-activated protein kinase; NADPH, nicotinamide adenine dinucleotide phosphate; NaKA, Na^+/K^+-ATPase; NHE, Na^+/H^+ exchanger; NMDAR, N-methyl-D-aspartate receptor; NPCs, neural progenitor cells; pHi, intracellular pH; PI3K, phosphoinositide 3-kinase; PIP2, phosphatidylinositol of 4,5-trisphosphate; PIP3, phosphatidylinositol of 3,4,5-trisphosphate; PKC, protein kinase C; PTEN, phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase.

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http://dx.doi.org/10.1016/j.biocel.2014.09.014
1357-2725/

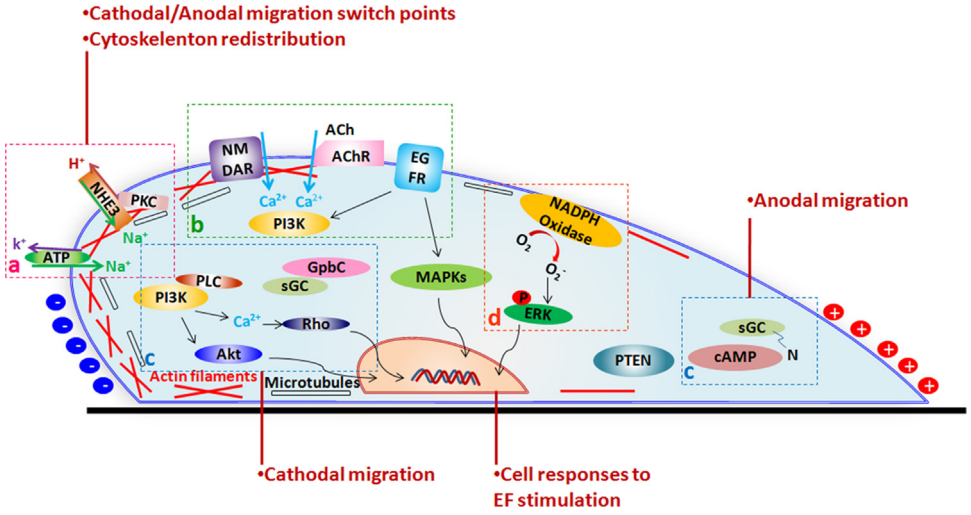

Fig. 1. Signaling network in EF stimulated cells. (a) The ion channels NaKA and NHE3 are considered as a switch point of the cathodal/anodal migration. When the cell is exposed to EF, NaKA and NHE3 are reported to accumulate at the cathodal/anodal edge of the migration cell, depending on different cell types. This activation induces Na^+ and Ca^{2+} influxes, which lead to the formation of the ion gradient along/against the EF direction; ultimately this leads to cell depolarization and cytoskelenton redistribution (*i.e.* actin, tubulin, myosin, *etc.*) through intracellular signaling. NHE3 could also induce cytoskelent on redistribution through complex with PKC and tubulin when treated with EF. (b) Cell membrane receptors are also reported to contribute towards the ion influxes and signaling activation under EF stimulation. AChR and NMDAR are both evidenced to be activated at the cathodal pole of the EF and play certain role in inducing the Ca^{2+} influxes and cell depolarization; while EGFR could be activated by the EF in a ligand-independent manner, to trigger the downstream MAPKs and PI3K activations. The activation of the membrane receptors will finally contribute to the cytoskeleton redistribution and cellular responses through Ca^{2+} influxes and downstream effectors. (c) PI3K/Akt signaling pathway is widely explored and plays an important role in electrotaxis and other cellular responses. Post the Ca^{2+} influxes and cell depolarization, PI3K and downstream effectors are found to accumulate and activate at the leading edge of the electrotaxing cell; in return, PI3K is found to play an important role in the EF-induced cathodal migration. The activated PI3K at the leading edge could initiate other cellular responses through downstream effector Akt, or combining with PLC to activate Rho through Ca^{2+}. PTEN has been reported to down regulate at the cathodal side and show more activation at the anodal side. Apart from PI3K, the catalytic domain of sGC with GbpC are also involved in cell migration to the cathode pole; while the N-domain of sGC and cAMP-activated pathways may contribute to mediate the anodal migration. (d) Other molecules are also explored to participate in the signaling transduction during EF stimulation, *i.e.* NADPH oxidase could be activated by EF to generate superoxide, which triggers the up regulation of phosphorylation of ERK, and thereby influences the cell response to EF.

phosphorylated Na^+/H^+ exchanger isoform 3 (NHE3), Ca^{2+} channel, and N-methyl-D-aspartate (NMDA) receptors (Shanley et al., 2006; Ozkucur et al., 2011; Li et al., 2008; Jeong et al., 2009). EF stimulation can also trigger the activation of EF sensitive receptors on the cell membrane and initiate the signaling transduction to downstream effectors, which ultimately determines the cell fate and responses towards stress, survival, migration, proliferation or differentiation. Membrane receptors such as Epidermal growth factor receptor (EGFR) and Acetylcholine receptor (AchR) are reported to act as such EF sensitive receptors (Wu et al., 2013; Zhang and Peng, 2011; Zhao et al., 2002a), whose activation by EF stimulation will initiate the intracellular pathways such as phosphatidylinositol-3-OH kinase (PI3K)/Akt, MAPK/ERKs, integrin, and Rho (Fukata et al., 2003; Etienne-Manneville and Hall, 2001; Li et al., 2012). This review shall elucidate the mechanism of the cellular response toward EF treatment, and discuss the signaling regulation from the membrane polarization to the activation and regulation of intracellular signaling pathways.

2. Cellular response to EF stimulation

Ever since German physiologist Emil Du Bois-Reymond (1818–1896) first measured the electric current at the human skin wound over 150 year ago, the modern electrophysiology started to develop (Bois-Reymond, 1843, 1860). In the last few decades, the research field has moved on from the endogenous EF improved wound healing to the artificial EF stimulation therapy for nerve excitation, muscle contraction, wound healing and other fields (Zhao, 2009). To explore the mechanism of the EF stimulation, several tissue and cell models are established to investigate the cellular and molecular regulation. The migration behavior of electrotaxis can be cell type and experimental condition dependent, for example single cells tend to show better/faster electronic response compared to monolayer/cell sheet at 2D and cell clusters within ex vivo 3D tissue. Cell clusters at 2D and 3D tend to respond as well coordinated collective migration in an EF-polarity dependent manner (Zhao et al., 2006). A recent study however showed U-turn effect of the Madin-Darby canine kidney (MDCK) cells within the cell sheet boundary, in the absence of EF polarity change and overall movement of the cell sheet/leading cells (Cohen et al., 2014). Cellular response to EF stimulation is considered to start from the trailing or leading edge polarization of the cells along the EF direction (Jahanshahi et al., 2014). Although the leading edge depolarization associated with the ion flow has been theoretically determined by several studies (Mycielska and Djamgoz, 2004; Borys, 2012; Djamgoz et al., 2001), Ozkucur et al. (2011) have provided a contrasting evidence of a depolarization at the trailing edge of Calvaria cells. Either on the leading or trailing edge, EF-triggered polarization of the cells is considered to ultimately attract the membrane lipids and EF sensitive receptors to asymmetrically redistribute towards the leading edge of the electrotaxing cells (Zhao et al., 2002b). This unique mechanism of electrotaxis works differently from chemotaxis, which activates the randomly distributed signaling molecules only on one pole of the cell facing upstream of the chemoattractant gradient in a biased manner. The difference between chemotaxis and electrotaxis is also confirmed by the result that cytokine receptor (C–X–C chemokine receptor type 4, CXCR4) mediated chemotaxis of neural stem cells in brain, whereas CXCR4 did not affect the electrotaxis of the same cells (Feng et al., 2012).

Previous studies demonstrate that anodal or cathodal electrotaxis is cell type dependent. The majority of the cell types investigated so far, recruit towards the cathode of the EF (Feng et al., 2012; Lin et al., 2008; Meng et al., 2011; Nuccitelli and Erickson, 1983; Stump and Robinson, 1983; Zhao et al., 2006). A small proportion of cell types however do respond to anodal migration in EF