Imaging and Mathematical Modeling of Molecular Activities in Living Cells

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1. Introduction

In multicellular organisms, cell proliferation is a highly regulated process that maintains tissue architectures and organ size [1]. The inhibition of cell proliferation observed in cells with high cell density is referred to as contact inhibition of proliferation, or simply contact inhibition, and a defect of this process has been closely associated with uncontrolled cell proliferation, leading to the cancer [2].

One of the key molecules that embody the regulation of cell proliferation is the ERK MAP kinase. The ERK MAP kinase is a serine/threonine kinase that serves as the output of the Ras-Raf-MEK-ERK intracellular signal transduction pathway [3]. Upon Ras activation, which could be triggered by a number of growth factors or differentiation factors, Raf is recruited to Ras at the plasma membrane. The Ras-Raf complex activates MEK, which in turn phosphorylates tyrosine and threonine residues in the activation loop of ERK in the cytoplasm. The activated ERK catalyzes the phosphorylation of many downstream proteins, thereby regulating a large variety of cellular processes, including cell proliferation, differentiation, and tumorigenesis [4].

Differences in the kinetics of signaling molecules, including protein kinases, have been suggested to dictate appropriate outcomes in diverse biological systems [5]. For example, sustained ERK activation for at least several hours is required for the induction of genes, such as cyclin D, and consequent entry into S phase [6]. On the other hand, in PC12 pheochromocytoma cells, sustained ERK activation inhibits cell growth and induces neuronal differentiation [7]. However, it has not been demonstrated how dynamic behavior of ERK activity is processed and decoded to alter cellular function in single cell level.

2. Results

Here, we show that stochastic ERK activity pulses regulate cell proliferation rates in a cell density-dependent manner [8]. A fluorescence resonance energy transfer (FRET) biosensor [9] revealed that stochastic ERK activity pulses fired spontaneously or propagated from adjacent cells. Frequency, but not amplitude, of ERK activity pulses exhibited a bell-shaped response to the cell density and correlated with cell proliferation rates. Consistently, synthetic ERK activity pulses generated by a light-switchable activation system accelerated cell proliferation. Furthermore, a mathematical model based on coupling oscillator model clarified that 80% and 20% of ERK activity pulses are generated by the noise and cell-to-cell propagation, respectively. Finally, gene expression analysis with RNA sequencing

in cells subjected to the synthetic ERK activity pulses suggested the involvement of serum responsive factor (SRF) transcription factors in the gene expression driven by the ERK activity pulses.

3. Conclusion

We have demonstrated that the cell density-dependent control of proliferation in mammalian cells is associated with the frequency of the ERK activity pulses, which consist of pulses from both spontaneous firing and cell-to-cell propagation. An understanding of the quantitative relationship between ERK signaling and cell proliferation would provide a useful framework to predict the clinical efficacy of drugs targeting the Ras-ERK pathway to impede the proliferation of cancer cells.

References

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