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Title
Lighting Up Cancer Dynamics

Permalink

Journal

ISSN

Authors
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Publication Date

DOI

5. Alexander, B.M. *et al.* (2018) Hazards of hazard ratios — deviations from model assumptions in immunotherapy. *N. Engl. J. Med.* 378, 1158–1159

6. Huang, A.C. *et al.* (2017) T-cell invigoration to tumour burden ratio associated with anti-PD-1 response. *Nature* 545, 60–65

7. Anagnostou, V. *et al.* (2017) Evolution of neoantigen landscape during immune checkpoint blockade in non-small cell lung cancer. *Cancer Discov.* 7, 264–276

8. Mansfield, A.S. *et al.* (2016) Temporal and spatial discordance of programmed cell death-ligand 1 expression and lymphocyte tumor infiltration between paired primary lesions and brain metastases in lung cancer. *Ann. Oncol.* 27, 1953–1958

9. Chen, D.S. and Mellman, I. (2013) Oncology meets immunology: the cancer-immunity cycle. *Immunity* 39, 1–10

Spotlight

Lighting Up Cancer Dynamics

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Live-cell microscopy has revealed that signaling pathways carry elaborate time-varying activities. Yet, the connection between these dynamics and cellular disease has remained elusive. Recent work leverages cellular optogenetics to analyze the Ras-to-Erk transfer function in cancer cells. These analyses reveal how changes to the filtering properties of a pathway lead to the misperception of extracellular events. Overall, these studies suggest that mutations do not simply hyperactivate pathways but rather can also change their transmission properties in more subtle ways.

For a cell to function properly, it must faithfully relay information about itself and its environment through its signaling pathways. Recent advances in live-cell biosensors have revealed that these pathways exhibit intricately patterned, time-varying activity. This has led many to hypothesize that the dynamics of a signaling pathway, rather than its instantaneous activity, bear the information that cells use to make decisions. Indeed, *p53*, the most frequently mutated gene in cancer, was shown to transmit cell-state information via its nuclear translocation dynamics, with oscillations resulting in cell cycle arrest and continuous nuclear localization resulting in senescence (Figure 1A) [1].

Thus, it follows that altering signaling dynamics changes cell fates. In the case of p53, oscillatory dynamics can be drugged into sustained signals, leading cells that would otherwise undergo temporary cell −cycle arrest to senesce [1]. While corrupting p53-mediated transmission using drugs can lead to the misperception of the initial stimuli, it is unclear whether signal misperception is a useful framework with which to describe oncogenic mutations.

Recently, evidence has emerged that the Ras-Erk pathway also exhibits elaborate pulsatile dynamics. Through a tour de force of multiplexed fluorescent reporter engineering, a link has been established between extracellular stimuli, Erk signaling dynamics, gene expression, and proliferation [2,3]. Specifically, it has been demonstrated that growth factor stimulation increases the frequency of Erk activity pulses, which positively correlated with an increased propensity of cells to enter mitosis (Figure 1A) [4].

This work established a connection between homeostatic Erk dynamics and cell fate choices, but only recently, with the advent of cellular optogenetic tools, have investigators gained the ability to precisely and reversibly toggle the activity of signaling pathways. Such tools enable scientists to mimic and systematically modify pathway activity *in vivo*, thereby allowing them to understand what 'features' of time-varying signals are being 'read out' by cells [5,6]. Finally, investigators are poised to ask whether signaling dynamics lead to disease.

Recent work published in *Science* takes a monumental first step towards understanding the relationship between signaling dynamics and cancer [7]. Previous work by corresponding authors Toettcher and Lim established OptoSOS, which utilizes the light-gated heterodimerization of the Phy and Pif proteins from *Arabidopsis thaliana* to activate the Ras-Erk pathway. Upon red light stimulation, Pif, fused to the Ras-activator SOS, binds to Phy, recruiting SOS to the membrane, where it activates Ras. Infrared light then turns the pathway off by dissociating Pif from Phy (Figure 1B) [3]. By coupling OptoSOS to downstream reporters of pathway activity, this tool can yield a picture of the input–output relationship, or transfer function, of the Ras-Erk module.

To investigate the transfer function of the Ras-Erk module across a panel of lung cancer cell lines, Bugaj *et al.* delivered an identical input pulse to each cell line using OptoSOS and assessed the phosphorylation state dynamics of Erk. With this optoprofiling technique, they discovered that H1395 lung cancer cells distort pathway inputs as they are transmitted down the MAPK phosphorylation cascade. Specifically, after a fixed-duration pulse of Ras activity, Erk is phosphorylated for longer in H1395 cells than in normal cells. Reasoning that a single mutation was responsible for this delay, the authors performed optogenetic epistasis experiments by observing how inputs to each subsequent node are transmitted to Erk activity. The authors utilized a combination of small molecules and optogenetic tools to control subsequent nodes in the pathway, Raf and Mek. Intriguingly, an optogenetic B-Raf restored the wild-type off-kinetics of phosphorylated Erk in H1395 cells, implying that B-Raf is the node at which signaling dynamics are distorted. Based on the mutational
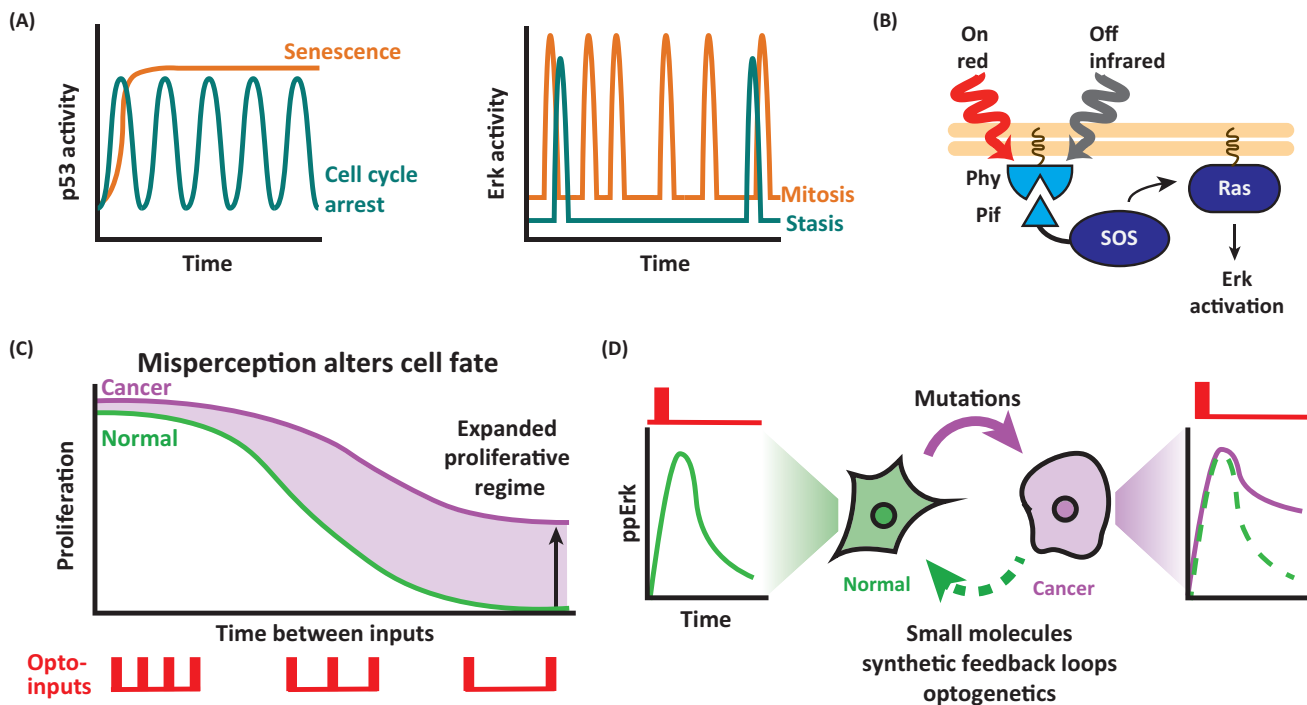
Trends in Cancer

Figure 1. Signaling Dynamics: From Homeostasis to Cancer (and Back?). (A) Canonical examples of signaling dynamics determining cell fate. (left) Oscillations in p53 result in transient cell cycle arrest, while sustained activity leads to senescence. (right) Erk activity in cells without growth factors are in stasis, while stimulation-induced pulsing correlates with mitosis. (B) Schematic of the OptoSOS system. Red light activates Ras by recruiting Pif-SOS to the membrane. Infrared dissociates Pif from Phy. (C) Bugaj et al. [7] showed that, as the time between pulses increases, normal cells have decreased proliferation, while the cells with slow Erk off-kinetics still proliferate. (D) Mutations can cause changes in the transmission of upstream inputs. Future treatments, such as small molecules, engineered feedback loops, or optogenetics, could be designed to push the dynamics back into a normal regimen.

landscape of H1395 cells, the authors identified a mutation in B-Raf (G469A) known to increase its dimerization affinity. To demonstrate that this G469A mutation is sufficient to explain the sluggish Erk phosphorylation kinetics, the authors expressed the mutated B-Raf in an untransformed lung cell line (Beas2B). This was indeed sufficient to recapitulate the sluggish dynamics, showing, for the first time, that a single oncogenic mutation can alter the Ras-Erk transfer function.

What are the phenotypic consequences of sluggish signal transmission and how might it contribute to cancer proliferation? To answer these questions, the authors built a custom 96-well plate light-stimulation device to screen for the effects of altered signal transmission across a range of input dynamics [8]. Remarkably, they found that misperception of extracellular signals expanded the range of growth-promoting inputs. Specifically, both wild-type cells and cells treated with a drug to mimic the slow Erk off-kinetics of H1395 cancer cells exhibited similar proliferation rates in the presence of high-frequency inputs. However, as the time between pulses of Ras activity increased, cells with slow off-kinetics maintained a high proliferation rate, while proliferation in normal cells precipitously dropped (Figure 1C). Thus, the altered Ras-Erk transfer function may increase fitness by expanding the proliferative regime.

This study suggests the intriguing possibility that oncogenic mutations in signaling pathways do not simply cause hyperactivation of a pathway, but instead lead to more subtle defects by altering aspects of the dynamics of a pathway, such as its gain, frequency, duty cycle, or relative phase. Even more provocatively, this work implies the possibility of a new cancer treatment modality that targets dynamics. For cancers harboring the BRaf G469A mutation, we hypothesize that tumor growth arrest could be achieved by any therapeutic that returns the Ras-Erk transfer function back to normal. We not only imagine that this could be accomplished with traditional small molecules that reduce a specific kinase phosphorylation rate, but also foresee a

role for engineered synthetic feedback loops (Figure 1D), which have already been implemented to modify the Ras-Erk module in yeast [9]. In addition, this work exemplifies the utility of cellular optogenetics for ascertaining a systems-level understanding of cancer. Not only can optogenetics serve as a diagnostic technique, but also may even aid in the treatment of cancer through the use of light-activated drugs. Overall, this work ushers in a shift in how we think of cancer: mutations may not simply hyperactivate a pathway, but rather can cause network-level defects that change how information is transmitted and interpreted.

Resources

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References

1. Purvis, J.E. et al. (2012) p53 dynamics control cell fate. Science 336, 1440–1444
2. Aoki, K. et al. (2013) Stochastic ERK activation induced by noise and cell-to-cell propagation regulates cell density-dependent proliferation. Mol. Cell 52, 529–540
3. Toettcher, J.E. et al. (2013) Using optogenetics to interrogate the dynamic control of signal transmission by the Ras/Erk module. Cell 155, 1422–1434
4. Albeck, J.G. et al. (2013) Frequency-modulated pulses of ERK activity transmit quantitative proliferation signals. Mol. Cell 49, 249–261
5. Hannanta-Anan, P. and Chow, B.Y. (2016) Optogenetic control of calcium oscillation waveform defines NFAT as an integrator of calcium load. Cell Syst. 2, 283–288
6. Wilson, M.Z. et al. (2017) Tracing information flow from Erk to target gene induction reveals mechanisms of dynamic and combinatorial control. Mol. Cell 67, 757–769
7. Bugaj, L.J. et al. Cancer mutations and targeted drugs can disrupt dynamic signal encoding by Ras/Erk pathway. Science (in press)
8. Bugaj, L.J. and Lim, W.A. University of California. Apparatus, systems and methods for programmable tissue culture illumination. US Patent No. US20180016538A1
9. Mitchell, A. et al. (2015) Oscillatory stress stimulation uncovers an Achilles' heel of the yeast MAPK signaling network. Science 350, 1379–1383

Forum

Mutation Signatures Depend on Epigenomic Contexts

Neil Coleman[1] and Subhajyoti De[1,*]

Mutation signatures – the patterns of acquired genetic changes in somatic genomes – provide critical insights into DNA repair defects and exposure to mutagenic processes during development, aging, and cancer progression. Efforts to decipher the etiology of the emerging computationally predicted mutation signatures in cancer genomes are currently underway. Since chromatin and epigenomic contexts influence DNA damage and repair pathway choices, taking both epigenomic and sequence contexts of the mutations into consideration is likely to benefit interpretation of mutation signatures.

DNA damage, replication errors, and defects in DNA repair during development, aging, and cancer progression result in accumulation of thousands of genomic alterations, including point mutations and structural variations (e.g., deletions, translocations), in somatic genomes. While a majority of mutations is passenger, and is inconsequential in disease contexts, the associated patterns of genetic changes, known as mutation signatures, provide insights into underlying DNA damage and repair mechanisms, which can be used to infer past exposure to mutagens, DNA repair defects, and normal physiological processes during development and aging. This has important implications for understanding disease etiology, minimizing hazardous environmental exposure, and also for predicting efficacy of therapies [1]. For instance, mutation signatures related to smoking, a higher neoantigen burden, and DNA repair pathway mutations are associated with sensitivity to anti-PD-1 therapy in lung cancer [2].

Mutational landscapes of cancer genomes are usually montage of genetic changes resulting from multiple different mutagenic processes such that it is not trivial to identify and separate different mutation signatures. In a direct approach, one may characterize mutation patterns in cells exposed to known mutagens to determine corresponding signatures and then estimate the contribution of such signatures in cancer genomes. For instance, Boot et al. [3] characterized the mutation signature of cisplatin and showed that liver and esophageal cancer patients who previously received platinum treatment had high burden of mutation signature of cisplatin-mediated DNA damage in their tumors. However, not all sources of DNA damage and mutations in tumor genomes are known a priori, and in such cases, indirect, computational blind source separation techniques (e.g., non-negative matrix factorization) can infer mutation signatures and estimate their relative burden in tumor genomes in a data-driven manner. Using such an approach and analyzing sequence contexts of mutations from thousands of samples from all major cancer types, Stratton and colleagues [4,5] elegantly identified a number of point mutations and genomic rearrangement signatures (Figure 1A), with a majority of these signatures corresponding to known mutagenic processes. For instance,