Cell

Network Medicine Strikes a Blow against Breast Cancer

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DOI 10.1016/j.cell.2012.04.014

Drug development for complex diseases is shifting from targeting individual proteins or genes to systems-based attacks targeting dynamic network states. Lee et al. now reveal how the progressive rewiring of a signaling network over time following EGF receptor inhibition leaves triple-negative breast tumors vulnerable to a second, later hit with DNA-damaging drugs, demonstrating that time- and order-dependent drug combinations can be more efficacious in killing cancer cells.

Aberrant signaling drives the pathological behavior of cells during tumor development. However, signaling networks are highly complex, involving a large ensemble of dynamic interactions that flux in space and time. Thus, to understand how inappropriate cell decisions arise and determine the best strategy to modulate them requires a global view of cell signaling network dynamics, underpinned by both quantitative measurements and computational modeling (Janes et al., 2005; Linding et al., 2007). In particular, it is vital to identify network states or attractors in cancer cells and to utilize these to prevent disease progression. Such understanding holds great promise for the development of therapeutics that target systems and networks ("network drugs") (Erler and Linding, 2009; Pawson and Linding, 2008). However, direct translational impacts of systems biology studies have so far been scarce (Huang et al., 2007; Schoeberl et al., 2009).

In this issue of *Cell*, Lee et al. (2012) from Mike Yaffe's team at MIT harness the immense power of systems biology to identify therapeutic strategies against cancer. Instead of shying away from the biological complexity, Lee and colleagues take the bull by the horns, monitoring the dynamic network response of breast cancer cells to a set of different clinically relevant therapeutic agents, including DNA-damaging reagents and kinase inhibitors. The team integrates multiple types of quantitative data using advanced computational network modeling and find that the most effective strategy for killing aggressive triple-negative breast cancer (TNBC) cells in vitro and in vivo is a time- and order-dependent combination of drugs (Figure 1), specifically, treatment with the EGF receptor kinase inhibitor erlotinib followed by doxorubicin. This finding has two profound consequences. First, it demonstrates that drugs such as kinase inhibitors can push cells toward a new cellular state, rendering them vulnerable to other drugs such as DNA-damaging agents. Second, it shows that drugs target dynamic molecular networks and highlights the importance of studying the dynamics of the system to be targeted, including time dependency and the order of treatments.

The context-dependent nature of signaling networks driving cell decisions was convincingly illustrated in a seminal paper from Lauffenburger and Yaffe in 2005, which showed that the outcome of JNK kinase activity on cell fate depends on the state of the cellular signaling network prior to its activation (Janes et al., 2005). To predict cell behavior, it is thus a requirement to asses temporal- and/or state-based network dynamics in response to perturbations such as those induced by anticancer drugs. Yaffe's paper in the current issue of *Cell* takes this to the next level, showing that highly effective tumor targeting strategies can be developed with an a priori knowledge of the multivariate nature of the network being targeted. Importantly, the work clearly demonstrates that network activity, and not expression levels of EGFR, is a marker for response to treatment. Understanding how a network will respond to a particular drug is therefore essential to find the best strategy to target it. This has deep implications for the deployment of biomarkers, and translating such network-based biomarkers into the clinic will clearly be a challenge (Erler and Linding, 2009). Though our understanding of adaptation, compensation, and network rewiring is currently not sufficient to allow ab initio predictions of the cellular response in any cell type, it is now clear that attractor states in networks of cancer cells (for example, through sustained EGFR inhibition) can open up for second-phase attacks using DNA-damaging drugs to drive apoptosis.

Why have such studies been, until recently, lacking in the drug development field? Put simply, there are two strictly required aspects that have been missing. First, and perhaps most importantly, there has been a lack of systems approaches to understanding and targeting disease, with most studies focusing on individual targets and the identification of specific mutations, without investigating the impact of these on signaling and other molecular networks. Second, only in the last decade has it become technologically feasible to monitor thousands of

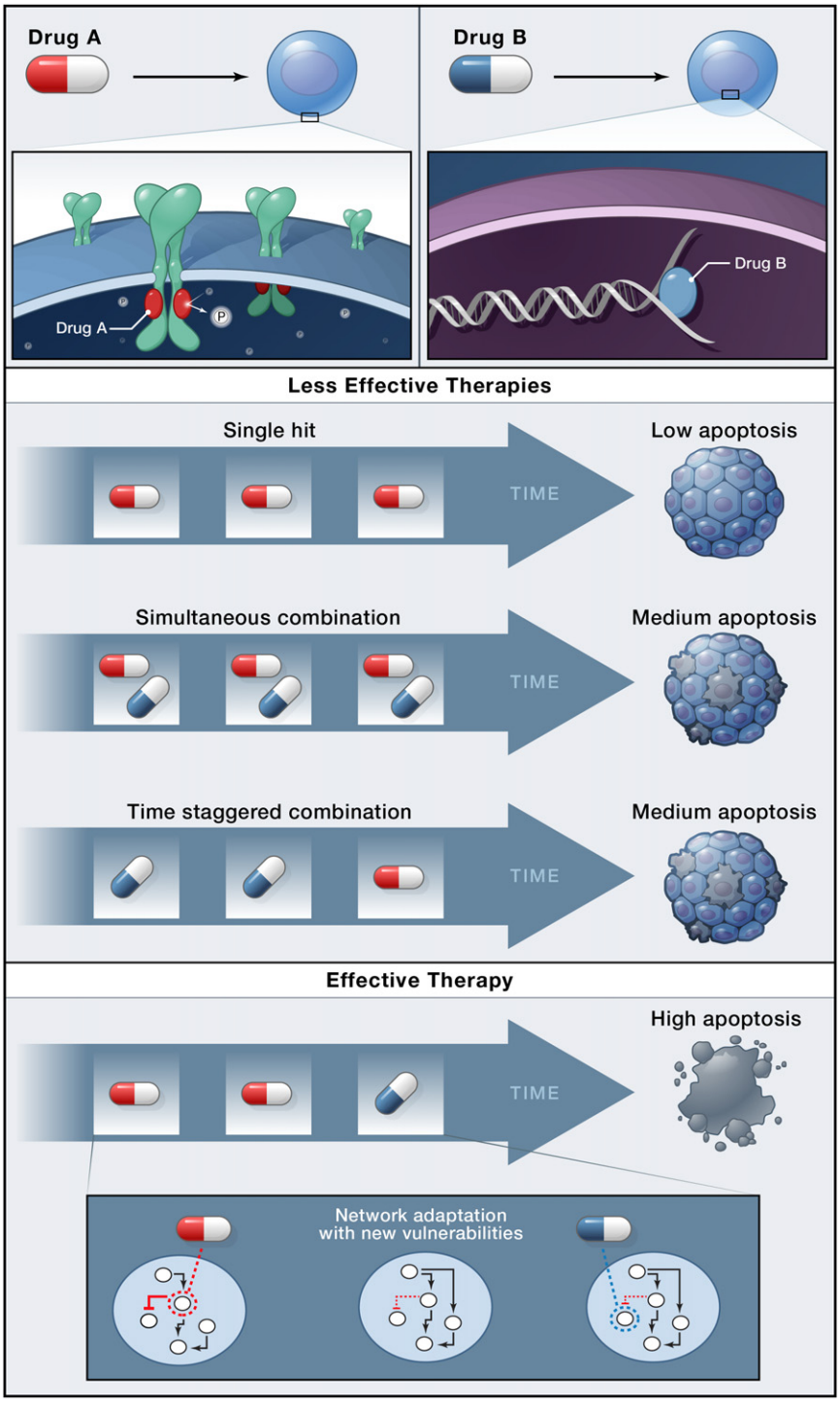

Figure 1. Targeting Systems Dynamics in Cancer

Lee et al. used a combinatorial, time-, and order-dependent therapy to effectively induce apoptosis in triple-negative breast cancer cells. The most effective therapy, which consisted of a combination of erlotinib (drug A) 4 hr before administration of doxorubicin (drug B), showed significantly more potent efficacy than administrating erlotinib alone (top), erlotinib and doxorubicin at the same time (middle), or erlotinib 4 hr after doxorubicin (bottom). The authors show that this time and order dependency is achieved by erlotinib rewiring the signaling network and pushing the cell toward a state of higher sensitivity to doxorubicin.

molecular signals embedded in dynamic signaling networks and to integrate them computationally in order to gain biological insight into drug mechanisms and efficacy.

The study by Lee et al. highlights the need to take systematic biochemical approaches to understand and target disease effectively and to computationally integrate molecular and phenotypic data to model the system under study. Future studies should address what underlies the signaling network heterogeneity in the cell lines that did not respond to the order- and time-dependent apoptotic effect of the combinatorial therapy. In addition, the authors' current approach relies on affinity reagents and prior knowledge of the organization of so-called "pathways." Signaling networks are far too dynamic and intertwined to be well described by the classical static "pathway" framework (Jørgensen and Linding, 2010). Thus, this approach will bias the performance of the model due to limited sampling of network states. An alternative approach would be to perform an ab initio study in which all network elements were sampled and monitored using proteome-wide, mass spectrometry integrated with multidimensional, quantitative phenotyping to derive unbiased predictive network models (Jørgensen and Linding, 2010). We predict that such strategies integrating multiple types of quantitative molecular and phenotypic data to study network dynamics will dramatically impact our understanding of cells and disease.

The age of "network medicine" has clearly begun. In addition to supporting the original definition of network medicine (Pawson and Linding, 2008) as a pharmacology that defines both the network connectivity and dynamics as components of drug targets, the paper is arguably one of the first examples of systems biology really making a difference in translational research and beyond. As disease researchers, we must consider network states, and this and other studies serve as a model for a new generation of cancer biologists. The work by Lee et al. is groundbreaking in its demonstration that the principles of order and time are essential to the development of effective therapies against complex diseases. We are convinced that approaches such as those

used by Lee et al., in combination with large-scale profiling of individual patients (Chen et al., 2012), will drive the discovery of powerful effective drug combinations and new therapeutic strategies. Once we learn how cells become rewired and reach new network states, it will be much more feasible to force tumor cells out of these pathological states in order to kill or ''normalize'' them through drug-induced dynamic rewiring of signaling networks. It remains to be seen whether these approaches will be effective in clinical trials. But for now, the future looks very bright for network medicine.

ACKNOWLEDGMENTS

Thanks to Pau Creixell (C-SIG, DTU) for essential input and help with the manuscript and creation of the figure. R.L. is supported by a Sapere Aude Starting Grant from The Danish Council for Independent Research, a Lundbeck Foundation Group Leader Fellowship, and a Career Development Award from Human Frontier Science Program. J.T.E. is supported by a Hallas Møller Stipend from the Novo Nordisk Foundation. See http://www.networkbio.org, http://www.lindinglab.org, and http://www.erlerlab.org for more information on cancer-related network biology.

REFERENCES

Chen, R., Mias, G.I., Li-Pook-Than, J., Jiang, L., Lam, H.Y., Chen, R., Miriami, E., Karczewski, K.J., Hariharan, M., Dewey, F.E., et al. (2012). Cell *148*, 1293–1307.

Erler, J., and Linding, R. (2009). J. Pathol. *220*, 290–296.

Huang, P.H., Mukasa, A., Bonavia, R., Flynn, R.A., Brewer, Z.E., Cavenee, W.K., Furnari, F.B., and White, F.M. (2007). Proc. Natl. Acad. Sci. USA *104*, 12867–12872.

Janes, K.A., Albeck, J.G., Gaudet, S., Sorger, P.K., Lauffenburger, D.A., and Yaffe, M.B. (2005). Science *310*, 1646–1653.

Jørgensen, C., and Linding, R. (2010). Curr. Opin. Genet. Dev. *20*, 15–22.

Lee, M.J., Ye, A.S., Gardino, A.K., Heijink, A.M., Sorger, P.K., MacBeath, G., and Yaffe, M.B. (2012). Cell *149*, this issue, 780–794.

Linding, R., Jensen, L.J., Ostheimer, G.J., van Vugt, M.A., Jørgensen, C., Miron, I.M., Diella, F., Colwill, K., Taylor, L., Elder, K., et al. (2007). Cell *129*, 1415–1426.

Pawson, T., and Linding, R. (2008). FEBS Lett. *582*, 1266–1270.

Schoeberl, B., Pace, E.A., Fitzgerald, J.B., Harms, B.D., Xu, L., Nie, L., Linggi, B., Kalra, A., Paragas, V., Bukhalid, R., et al. (2009). Sci. Signal. *2*, ra31.

RNP Export by Nuclear Envelope Budding

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DOI 10.1016/j.cell.2012.04.018

Nuclear export of mRNAs is thought to occur exclusively through nuclear pore complexes. In this issue of *Cell*, Speese et al. identify an alternate pathway for mRNA export in muscle cells where ribonucleoprotein complexes involved in forming neuromuscular junctions transit the nuclear envelope by fusing with and budding through the nuclear membrane.

The canonical model of macromolecular trafficking between the nucleus and the cytoplasm stipulates that all communication between these compartments occurs exclusively via the nuclear pore complexes (NPCs), which perforate the double membrane of the nuclear envelope (NE). Import and export through the NPCs relies on specific signal sequences and associated transport factors. Regulated transport through the NPCs maintains proper gene expression by ensuring that only correctly formed mRNAs access the translation machinery in the cytoplasm. In this issue of *Cell*, Speese et al. (2012) add a new wrinkle to the process and show that large RNP granules formed in *Drosophila* muscle cells during synaptogenesis are exported by budding through the NE instead of passing through the NPCs. (Figure 1).

Newly transcribed mRNAs assemble into large ribonucleoprotein complexes prior to export from the nucleus. These particles vary in size and some exceed the NPC diameter (Grünwald et al., 2011). One of the largest known RNPs, the Balbiani ring particle, overcomes this difficulty by undergoing extensive remodeling to conform to the NPC diameter (Daneholt, 2001). In addition, certain viruses export large particles by using a mechanism that completely bypasses the NPCs. Herpes viruses assemble large capsids in the nucleus that bind to the inner nuclear membrane, dissolve the lamina, a protein network that supports the nucleus, and bud into the perinuclear space between the inner and outer nuclear membranes (INM and ONM). From there the capsid buds fuse with the ONM to exit to the cytoplasm (Johnson and Baines, 2011). Now, Speese and colleagues suggest that the virus may have co-opted a mechanism already used for RNP transport.

Signaling at neuromuscular junctions (NMJs) during *Drosophila* development involves the internalization and cleavage of the wnt receptor DFrizzled2. Processing of DFrizzled2 to DFz2C is required for proper synapse formation, but how