Science Signaling
AAAS

` Combinatorial Nanotherapeutics: Rewiring, Then Killing, Cancer Cells

Ronnie H. Fang and Liangfang Zhang (13 May 2014)
Science Signaling **7** (325), pe13. [DOI: 10.1126/scisignal.2005386]

Science Signaling (ISSN 1937-9145) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue, NW, Washington, DC 20005.

DRUG DEVELOPMENT

Combinatorial Nanotherapeutics: Rewiring, Then Killing, Cancer Cells

Ronnie H. Fang and Liangfang Zhang*

Tumors are inherently resilient and often develop resistance against cancer therapies, leading to poor patient outcomes. With the sophisticated analytical and computational tools now available, much has been revealed about how molecularly targeted drugs affect the biochemical networks of cancer cells, enabling the directed design of treatment regimens that can better thwart resistance. In this issue of *Science Signaling*, Morton *et al*. demonstrate a nanoparticle system capable of sequentially delivering two drugs: The first inhibits an oncogenic pathway to sensitize the cells to DNA damage-induced apoptosis; the second is a genotoxic drug that takes advantage of the vulnerable state of the cancer cells to kill them with enhanced efficiency.

Single-drug therapies have long been lacking when used for the treatment of various diseases, including many forms of cancer (*1*). Because of the heterogeneity that exists within tumors, selective pressure weeds out the most susceptible clones, leaving room for resistant ones to grow and ultimately making the drug ineffective. Traditional combinatorial chemotherapies were designed to decrease the likelihood of resistance by simultaneously using different drugs with varying modes of action, thereby providing a multipronged attack in hopes of generating a more durable response. However, without intimate knowledge of the inner workings of tumors on a personalized basis, such dual-drug treatments for circumventing resistance are also susceptible to eventual failure (*2*). This is made evident by the fact that, even when driven by the same oncogene, the ways in which different cancers develop resistance can vary greatly (*3*, *4*). Elucidation of the exact mechanisms for discrete subsets of cancer is an ongoing process that requires careful analysis of the network of interconnected pathways that contribute to the pathogenic phenotype. Current progress is encouraging, because the advanced genomic and proteomic tool sets now available to cancer researchers have accelerated the rate of discovery.

As scientists begin to identify the molecular mechanisms that contribute to a tumor's survival, more informed and personalized treatment regimens can be developed. This increased insight has led to the directed design of combinatorial therapies that better exploit the weaknesses of cancer cells (*5*, *6*). For example, two targeted drugs can be used in conjunction such that one complements the other by preemptively cutting off alternative oncogenic signaling pathways. Additionally, targeted therapies can be used to resensitize cancer cells to chemotherapeutics that have lost effectiveness. Not only is the identity of the individual drugs important, but the order in which they are administered can also play a crucial role. In 2012, Lee *et al*. reported that subsets of epidermal growth factor receptor (EGFR)–driven cancer cells were more effectively killed by administering an EGFR inhibitor and a genotoxic drug in a time-staggered fashion (*7*). Taking a systems-based approach to analyzing cellular signaling networks, they showed that EGFR inhibition led to the dynamic rewiring of the cancer cells' signaling pathways, recapitulating the action of certain apoptotic pathways. During this rewiring, the cells once again became susceptible to death triggered by DNA damage. This work highlights the need for combinatorial treatments that are specific to individual cancers, and it also opens up new avenues for exploration into the temporal aspect of dual-drug administration.

A great challenge in the field of drug discovery is translating the findings acquired in a basic research setting from the bench to bedside. Although potential treatment modalities can be easily tested and verified in vitro, maintaining their promising efficacy with animal models and eventually with patients is a much harder task. To this end, nanoparticle technology offers many advantages for drug delivery that can aid in the translation process (*8*, *9*). Nanoscale carriers can be loaded with a large amount of drug molecules, either hydrophobic or hydrophilic, such that successful delivery of the payload from a single particle can kill cancer cells (*10*). Nanoparticles can be localized to tumor vasculature through both passive and active targeting mechanisms, thereby improving the specificity of the loaded drug and decreasing systemic side effects. Further, drug release can be precisely controlled such that it happens slowly over time or is environmentally triggered, which enables fine control over exactly how the drug is delivered upon reaching the target site.

Nanoparticles are also particularly well suited for the simultaneous delivery of more than one therapeutic (*11*). A hurdle specific to combinatorial therapies is the dosing of different drugs, each with varying degrees of solubility, such that they are all present at the tumor site in therapeutically relevant doses. For free-drug formulations, this is often determined simply by the maximum amount that a patient can tolerate, which can be suboptimal. To address this, nanoparticles can be engineered to co-encapsulate multiple drugs, regardless of hydrophobicity, with precise ratiometric control. Targeted delivery to tumors by means of the same vehicle makes it possible to unify the pharmacokinetics of different drugs. This effective drug co-delivery enables dosing strategies that are driven by and more closely mimic in vitro findings, thereby increasing the ultimate likelihood of success.

In this issue of *Science Signaling*, Morton *et al*. build upon the previous study (*7*) and demonstrate the use of a nanoparticle platform for implementing the combinatorial approach to rewiring cancer cells to sensitize the cells to genotoxic damage agents (*12*). They developed a liposomal system that encapsulates erlotinib (an EGFR inhibitor) into the hydrophobic bilayer and doxorubicin (a genotoxic drug) into the hydrophilic center (Fig. 1). The compartmentalization of the two drugs resulted in differential release, such that the erlotinib was released much more quickly than the doxorubicin. This recreated the time-staggering effect necessary for maximal efficacy. Indeed, only the dual drug-loaded formulation effectively killed

Department of NanoEngineering and Moores Cancer Center, University of California, San Diego, La Jolla, CA 92093, USA.

***Corresponding author. E-mail: zhang@ucsd.edu**

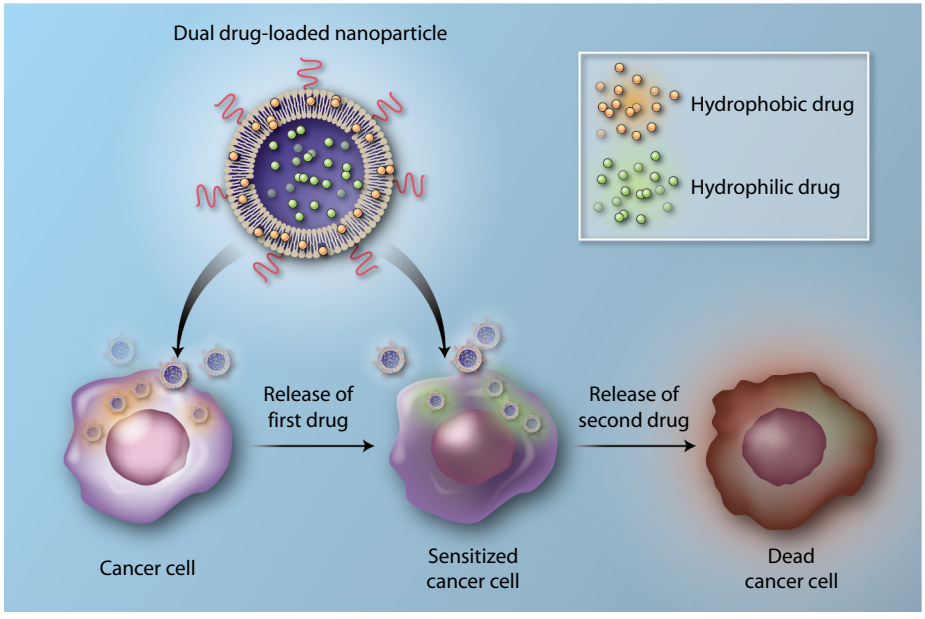

Fig. 1. Time-staggered release of drugs from a liposomal nanoparticle rewires cancer cell signaling networks and promotes more efficient cell killing. The drug incorporated in the hydrophobic lipid bilayer compartment is released first, rewiring the cancer cell into a state more susceptible to the cytotoxic effects of the second drug. The cancer cell can then be efficiently killed by subsequent release of a second drug loaded into the hydrophilic aqueous compartment of the liposome.

the cancer cells, whereas a doxorubicin-only formulation induced substantial DNA damage, underscoring the need for initial exposure to erlotinib to sensitize the cells. Using a folate receptor–targeting strategy, they delivered the dual drug-loaded nanoparticles to tumor xenografts grown in immunodeficient mice, achieving regression of tumor growth over time. The authors also showed that the platform could be generalized for the time-staggered release of several other drug combinations, thus opening up a wide range of possibilities for further applications.

This proof-of-concept work will undoubtedly inspire the advancement of novel nanoparticle-based systems that are specifically tailored toward the internal state of tumor cells. It is a perfect example of how basic science and engineering go hand in hand. By leveraging our ever-growing knowledge of cellular biology, it is possible to engineer personalized treatments that are rationally designed for maximal efficacy. Also highlighted is the incredible versatility of nanoparticle technology, because different platforms can be adapted to meet the complex requirements for overcoming drug resistance. Overall, the progress toward more personalized cancer therapies is encouraging, and there is hope that further development of new nanotherapeutic platforms will help to bring forth better clinical outcomes.

References

1. M. M. Gottesman, Mechanisms of cancer drug resistance. *Annu. Rev. Med.* **53**, 615–627 (2002).
2. C. R. Chong, P. A. Jänne, The quest to overcome resistance to EGFR-targeted therapies in cancer. *Nat. Med.* **19**, 1389–1400 (2013).
3. D. A. Nathanson, B. Gini, J. Mottahedeh, K. Visnyei, T. Koga, G. Gomez, A. Eskin, K. Hwang, J. Wang, K. Masui, A. Paucar, H. J. Yang, M. Ohashi, S. J. Zhu, J. Wykosky, R. Reed, S. F. Nelson, T. F. Cloughesy, C. D. James, P. N. Rao, H. I. Kornblum, J. R. Heath, W. K. Cavenee, F. B. Furnari, P. S. Mischel, Targeted therapy resistance mediated by dynamic regulation of extrachromosomal mutant EGFR DNA. *Science* **343**, 72–76 (2014).
4. K. Yonesaka, K. Zejnullahu, I. Okamoto, T. Satoh, F. Cappuzzo, J. Souglakos, D. Ercan, A. Rogers, M. Roncalli, M. Takeda, Y. Fujisaka, J. Philips, T. Shimizu, O. Maenishi, Y. G. Cho, J. S. Sun, A. Destro, K. Taira, K. Takeda, T. Okabe, J. Swanson, H. Itoh, M. Takada, E. Lifshits, K. Okuno, J. A. Engelman, R. A. Shivdasani, K. Nishio, M. Fukuoka, M. Varella-Garcia, K. Nakagawa, P. A. Jänne, Activation of ERBB2 signaling causes resistance to the EGFR-directed therapeutic antibody cetuximab. *Sci. Transl. Med.* **3**, 99ra86 (2011).
5. B. Y. Zhao, J. R. Pritchard, D. A. Lauffenburger, M. T. Hemann, Addressing genetic tumor heterogeneity through computationally predictive combination therapy. *Cancer Discov.* **4**, 166–174 (2014).
6. B. Al-Lazikani, U. Banerji, P. Workman, Combinatorial drug therapy for cancer in the post-genomic era. *Nat. Biotechnol.* **30**, 679–692 (2012).
7. M. J. Lee, A. S. Ye, A. K. Gardino, A. M. Heijink, P. K. Sorger, G. MacBeath, M. B. Yaffe, Sequential application of anticancer drugs enhances cell death by rewiring apoptotic signaling networks. *Cell* **149**, 780–794 (2012).
8. M. E. Davis, Z. G. Chen, D. M. Shin, Nanoparticle therapeutics: An emerging treatment modality for cancer. *Nat. Rev. Drug Discov.* **7**, 771–782 (2008).
9. D. Peer, J. M. Karp, S. Hong, O. C. Farokhzad, R. Margalit, R. Langer, Nanocarriers as an emerging platform for cancer therapy. *Nat. Nanotechnol.* **2**, 751–760 (2007).
10. D. J. Irvine, Drug delivery: One nanoparticle, one kill. *Nat. Mater.* **10**, 342–343 (2011).
11. C.-M. J. Hu, L. F. Zhang, Nanoparticle-based combination therapy toward overcoming drug resistance in cancer. *Biochem. Pharmacol.* **83**, 1104–1111 (2012).
12. S. W. Morton, M. J. Lee, Z. J. Deng, E. C. Dreaden, E. Siouve, K. E. Shopsowitz, N. J. Shah, M. B. Yaffe, P. T. Hammond, A nanoparticle-based combination chemotherapy delivery system for enhanced tumor killing by dynamic rewiring of signaling pathways. *Sci. Signal.* **7**, ra44 (2014).

10.1126/scisignal.2005386

Citation: R. H. Fang, L. Zhang, Combinatorial nanotherapeutics: Rewiring, then killing, cancer cells. *Sci. Signal.* **7**, pe13 (2014).