Drugging Drug Resistance

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Resistance to anticancer drugs is widely observed in vitro and in cancer patients, but its prevalence is too high to be solely explained by the acquisition of mutations. Sharma et al. (2010) now report that dynamic chromatin modifications may be an independent route to drug resistance in cancer cells that can be reversed by epigenetic drugs.

Drug resistance is the nightmare of every cancer patient receiving cytotoxic or targeted therapy for disseminated cancer. Well-established mechanisms underpin the different routes to drug resistance in tumor cells, including drug export, augmented drug metabolism, and secondary mutations in the drug target or in parallel pathways (Redmond et al., 2008). Cancer-initiating cells have also been proposed as potential culprits because of their capacity to escape from the effects of drug treatment by becoming quiescent (Frank et al., 2010; Peacock and Watkins, 2008). Heterogeneity, in general, enables organisms to adapt to changing environmental conditions, constituting the basis of Darwinian selection. Genetic variability is the hardwired version of heterogeneity. However, heterogeneity also occurs within genetically identical populations. In bacteria, up to 1% of the population might show drug resistance that is caused not by genetic mechanisms (such as the acquisition of plasmids encoding antibiotic resistance genes) but by mechanisms that reduce proliferation, a characteristic associated with a dormant, nondividing state. Bacteria exhibiting this characteristic are called "persisters" (Lewis, 2007) and resemble cancer-initiating cells (Frank et al., 2010). But what determines this resistance state and how can it be overcome? In a provocative paper in this issue of *Cell*, Settleman and colleagues (Sharma et al., 2010) not only provide intriguing insights into how such resistance mechanisms might operate but also suggest how they might be tackled and abolished.

These investigators exposed the PC9 non-small cell lung cancer cell line, carrying an activating mutation in the epidermal growth factor receptor (EGFR), to the drug erlotinib that abrogates EGFR signaling. Although this treatment (at a dose 50 times the IC_{50}) killed nearly all PC9 parental cells, a small fraction of mostly nondividing cells (the drug-tolerant persisters) survived the treatment (Figure 1). Roughly 20% of these cells reinitiated growth in the presence of erlotinib, becoming so-called drug-tolerant expanded persisters. This acquired resistance was not caused by genetic alterations known to confer resistance to erlotinib, including secondary mutations (such as T790M) in EGFR or ERBB3 (HER3) activation through amplification of the receptor tyrosine kinase MET (Engelman et al., 2007). Rather, the acquired drug resistance was due to a different chromatin configuration in the drug-tolerant persisters present in the PC9 parental population. The drug-tolerant persisters, comprising ~0.3% of the parental tumor cell population, all expressed CD133 and were enriched for CD24-positive cells, markers preferentially expressed by stem cells. Interestingly, the drug-tolerant expanded persisters, which arose from the quiescent drug-tolerant persister population by resuming proliferation in the presence of erlotinib, exhibited the marker heterogeneity of the parental PC9 cell line. This indicated that the "stem cell phenotype" of drug-tolerant persisters was only important for their survival during initial drug exposure. Cells surviving erlotinib exposure also showed cross-resistance to the chemotherapeutic cisplatin. Notably, expanded single-cell clones from the parental PC9 cell line gave rise to drug-tolerant persisters and expanded persisters at frequencies similar to the uncloned population, supporting a mechanism of nonmutational heterogeneity. In line with this notion, continued culture of the drug-tolerant expanded persisters in the absence of erlotinib gave rise to drug-sensitive cells, although it required many passages to "unlock" the drug-tolerant state. A similar behavior was also observed for other tumor cell lines, indicating that many, if not all, tumor cells share this mechanism. This regained drug sensitivity has a corollary in clinical practice and is described as the "re-treatment response," the reacquisition of a drug response after a "drug holiday."

To gain insight into the underlying mechanism of PC9 tumor cell drug resistance, Settleman and coworkers analyzed gene expression profiles of the PC9 parental cell line and of drug-tolerant persisters and expanded persisters. They noted a highly nonrandom distribution of differentially expressed genes along chromosomes, pointing to global chromatin alterations in the drug-tolerant expanded persister population. One gene showing significant elevated expression in both drug-tolerant persisters and expanded persisters was *KDM5A* encoding a histone demethylase. This could explain the observed reduction in H3K4 methylation in these drug-resistant cell populations. KDM5A appears to be critical for acquiring the resistance phenotype as knockdown of this histone demethylase reduced the generation of drug-tolerant persisters and expanded persisters from the PC9 parental cell line. Msc1, the yeast ortholog of KDM5A, is known to reduce H3K14 acetylation (Ahmed et al., 2004), and acetylation of H3K14 appeared to be decreased in drug-tolerant persist-

ers and expanded persisters, probably through the association of KDM5A with histone deacetylases (HDACs) (Klose et al., 2007). So, the authors next tested the effects of HDAC inhibitors on the parental PC9 tumor cells as well as the drug-tolerant (expanded) persisters. Surprisingly, HDAC inhibitors caused the rapid death of drug-tolerant persisters and expanded persisters but not the PC9 parental cell line (Figure 1). Cell death was associated with an altered DNA-damage response, as indicated by phosphorylation of the histone variant H2AX (γH2AX) in the drug-tolerant persister and expanded persister cells but not in parental cells treated with an HDAC inhibitor. The mechanistic basis for the DNA-damage response of the persister and expanded persister cells after HDAC inhibitor treatment is unclear, and the killing of these cells compared with the killing of PC9 parental cells by erlotinib seems to occur through a different mechanism.

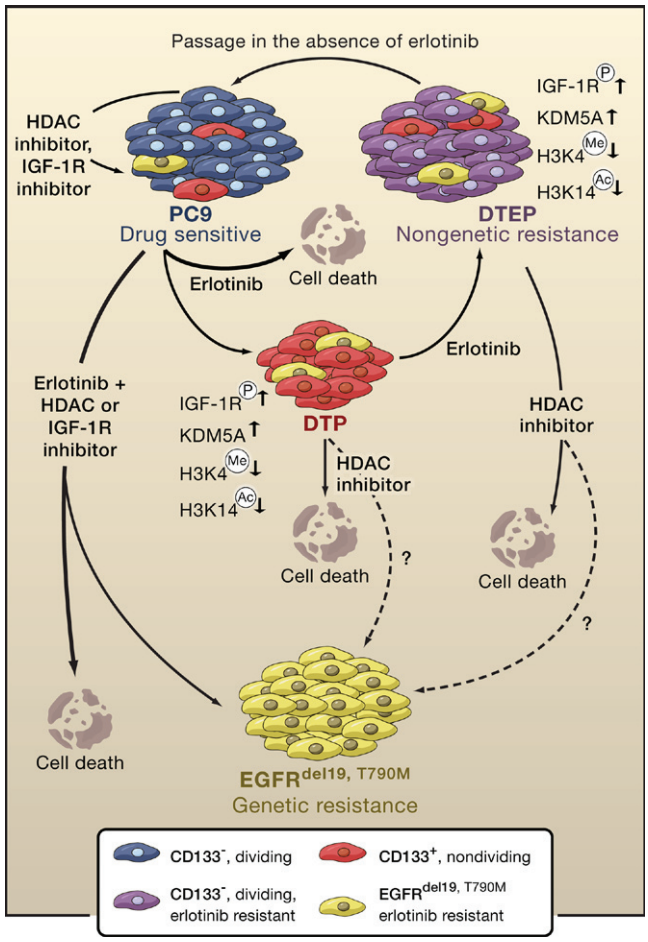

Figure 1. Drug Resistance in Lung Tumor Cells

A population of non-small cell lung tumor cells (PC9 parental cancer cell line), carrying the activating mutation $EGFR^{del19}$, consists of a heterogeneous mix of drug-sensitive cells (blue), nongenetic, drug-tolerant persisters (DTP, red), and possibly spurious tumor cells carrying the secondary T790M mutation in the epidermal growth factor receptor ($EGFR^{T790M}$ mutants; yellow). Upon treatment with the targeted drug erlotinib, the majority of the tumor population is eradicated, but drug-tolerant persisters (red) survive and expand to generate drug-tolerant expanded persisters (DTEP, purple). DTP and DTEP are characterized by increased phosphorylation of the IGF-1 receptor (IGF-1R), increased expression of the histone demethylase KDM5A, reduced H3K4 methylation, and reduced H3K14 acetylation. Drug resistance of DTEP tumor cells is reversible as passaging these cells in the absence of erlotinib results in the emergence of a drug-sensitive heterogeneous tumor cell population. Treatment with chromatin-modifying drugs such as HDAC inhibitors eliminates DTPs and DTEPs but does not kill the vast majority of the PC9 parental tumor cell population. Concomitant treatment with erlotinib and HDAC inhibitors or IGF-1 receptor inhibitors results in effective ablation of drug-tolerant persisters thereby abrogating nongenetic drug resistance. Tumor cells surviving this treatment (yellow) have acquired genetic resistance due to secondary mutations (e.g., the T790M mutation) in EGFR.

The investigators set out to identify other compounds in addition to HDAC inhibitors that could prevent the development of drug resistance. They pinpointed a selective inhibitor of the IGF-1 receptor, AEW541. IGF-1 receptor signaling is required for the induction of drug tolerance, and its inhibition leads to a substantial reduction in KDM5A expression, indicating that IGF-1 receptor signaling likely results in chromatin modifications mediated by KDM5A. Although synergy between cytotoxic drugs and chromatin-modifying drugs has been reported (Camphausen and Tofilon, 2007), this is the first study to connect inhibition of HDACs with "reversible drug resistance." Although future work will undoubtedly reveal the genes that confer drug resistance in response to KDM5A-mediated chromatin modifications, the work described by Sharma et al. elucidates an important pathway that operates in the emergence of drug-tolerant persisters and expanded persisters.

Interestingly, when PC9 parental cells were treated with both erlotinib and AEW541, a small number of drug-tolerant expanded persister cells carrying the EGFR T790M mutation eventually emerged. Thus, under this drug regimen, genetic mutations became the preponderant mechanism of drug resistance. The fact that the EGFR T790M mutation is often found in cancer patients treated with erlotinib (Engelman and Jänne, 2008) indicates that in vivo "reversible" drug resistance is not a substitute for mutational mechanisms of drug resistance. However, the mutational stress imposed on cells exhibiting "reversible" drug resistance could facilitate the generation of hard-wired drug resistance mutations.

The ramifications of the work of Settleman and colleagues could be substantial. The prevention of "reversible" drug resistance by inhibitors of HDACs or IGF-1 receptor signaling is intriguing, as it might provide a more effective way to treat tumors. The authors report that they have already initiated a clinical trial in which patients with non-small cell lung cancer are treated with a combination of erlotinib and a chromatin-modifying agent. However, rare tumor cells with EGFR mutations (or MET amplification) causing erlotinib resistance might still survive and cause cancer relapse. To eliminate such pre-existing erlotinib-resistant tumor cells, treatment with cisplatin and HDAC (or IGF-1 receptor) inhibitors, followed by erlotinib and HDAC (or IGF-1 receptor) inhibitors, could be considered. This would kill rare genetically resistant tumor cells harboring any erlotinib-resistant mutations that were already present in the tumor prior to treatment. Time will tell whether these exciting observations that appear common in vitro also apply to real tumors in real people.

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Exposing p120 Catenin's Most Intimate Affair

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p120-catenin regulates cell-cell adhesion by controlling cell surface retention of cadherin. In this issue, Ishiyama et al. (2010) present the first crystal structure of p120 in complex with cadherin, revealing molecular details of the functional interface and providing sophisticated new tools for dissecting p120's role in cell-cell adhesion.

The p120 catenin (p120) protein celebrates its 21st birthday in the spring of 2010. Described initially as a substrate of Src (Reynolds et al., 1989), p120 has emerged as a master regulator of cadherin retention and stability at the cell surface (Davis et al., 2003; Xiao et al., 2003). The classical cadherin family of receptors (which has 26 members in humans) is widely considered to be the most important of the cell-cell adhesion proteins in eukaryotes. Cadherins essentially compete at the cell surface for interaction with a limited pool of p120; if p120 is unavailable, unbound cadherins are removed from the cell surface for destruction or recycling (for review, see Reynolds and Roczniak-Ferguson, 2004). This stabilization effect by p120 clearly stems from its direct interaction with the cytoplasmic region of cadherin, but how this interaction is modulated to control cell adhesion is unclear. In this issue of *Cell*, Ishiyama et al. (2010) present the crystal structure of p120 in complex with a fragment of cadherin; this structure provides new insight into how p120 might influence the stability and function of cadherin in cell-cell adhesion complexes.

Cadherins are homophillic cell-cell adhesion receptors with essential roles in development, tissue morphogenesis, and cancer (Takeichi, 1995). Epithelial cadherin (E-cadherin) is the major cell-cell adhesion molecule in most epithelial tissues and is widely regarded as a master organizer of the epithelial phenotype. In most types of carcinoma, the downregulation of E-cadherin is closely linked to the emergence of metastasis and poor prognosis for patients.

β-catenin and p120 are key regulators of E-cadherin. Both proteins are armadillo repeat domain proteins (Reynolds et al., 1992), which bind directly to the cytoplasmic domain of cadherin (Reynolds et al., 1994) (Figure 1): β-catenin interacts with the catenin binding domain at the C-terminal end, and p120 catenin interacts with the juxtamembrane domain, which comprises ~40 amino acids at the N-terminal end. It is believed that the binding of p120 to the juxtamembrane domain of cadherin blocks factors such as the ubiquitin ligase Hakai and components of the endocytic machinery, which tag and target cadherin for destruction and internalization. β-catenin also interacts with α-catenin, and together they modulate interactions with the underlying actin cytoskeleton (Figure 1).

With the p120/E-cadherin crystal structure presented by Ishiyama et al. (2010), all core components of the cadherin complex are now available at high resolution (2.4 to 2.8 Å), and it is possible to assemble in silico a complete cadherin complex from the molecularly defined components (Figure 1, left panel). The resulting model nicely illustrates how the short polypeptide tail of the cadherin cytoplasmic domain, surprisingly, can anchor p120 and β-catenin simultaneously, even though each of these proteins is more than ten times the size of the cadherin fragment. The otherwise unstructured tail threads across and through the two catenins as if they were giant beads on a string, which would be a perfect analogy if not for the exquisitely choreographed