Review

Exploiting epigenetic vulnerabilities for cancer therapeutics

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Epigenetic deregulation is a hallmark of cancer, and there has been increasing interest in therapeutics that target chromatin-modifying enzymes and other epigenetic regulators. The rationale for applying epigenetic drugs to treat cancer is twofold. First, epigenetic changes are reversible, and drugs could therefore be used to restore the normal (healthy) epigenetic landscape. However, it is unclear whether drugs can faithfully restore the precancerous epigenetic state. Second, chromatin regulators are often mutated in cancer, making them attractive drug targets. However, in most instances it is unknown whether cancer cells are addicted to these mutated chromatin proteins, or whether their mutation merely results in epigenetic instability conducive to the selection of secondary aberrations. An alternative incentive for targeting chromatin regulators is the exploitation of cancer-specific vulnerabilities, including synthetic lethality, caused by epigenetic deregulation. We review evidence for the hypothesis that mechanisms other than oncogene addiction are a basis for the application of epigenetic drugs, and propose future research directions.

Anticancer therapy and epigenetics

The success of an anticancer drug critically depends on its ability to kill cancer cells without causing severe side effects in healthy tissues. Hence, most therapeutics in the clinic today exploit inherent differences between cancer cells and normal cells such as defective DNA repair pathways and checkpoints, and the presence of oncogenic kinases. Another important feature that discriminates tumor from normal cells involves epigenetic deregulation. In normal cells, gene expression is tightly controlled by epigenetic mechanisms that regulate chromatin accessibility for the transcriptional machinery through post-translational modifications of histones (e.g., methylation, acetylation, and phosphorylation) and DNA (methylation and hydroxymethylation). Modified chromatin in turn recruits 'reader' proteins to carry out further regulatory functions. However, many cancers display global changes in histone modifications, aberrant methylation patterns, and mutations in or altered expression levels of key chromatin-modifying proteins, and some tumors even harbor mutations in histone variants. Here we discuss how drugs that inhibit epigenetic modifiers can specifically target cancer cells by exploiting this deregulated chromatin state, including the targeting of synthetic lethal interactions (also known as non-oncogene addiction or induced essentiality; Box 1). We only briefly touch on epigenetics and cancer (Box 2) and refer readers to recent reviews on these topics [1–8].

Network rewiring in cancer generates new synthetic lethal targets

During tumorigenesis, normal cells acquire a set of hallmark characteristics that allow survival and proliferation even in the presence of counteracting signals [9–11]. This dramatic transition is reflected by extensive rewiring of cellular signaling networks, and is driven by mutations and the deregulated expression of oncogenes and tumor suppressor genes [12,13]. Thus, the cellular networks of cancer cells are distinct from those of normal cells, and most of the mechanisms of action of anticancer drugs can be explained by understanding the rewiring involved. Such network changes can lead to a critical reliance on an oncogenic driver event (oncogene addiction, Box 1 and Figure 1A–C), which underlies most of the targeted agents that inhibit overactive oncogenes such as BCR-ABL1 (breakpoint cluster region c–abl oncogene 1 fusion protein), BRAF (v-raf murine sarcoma viral oncogene homolog B), EGFR (epidermal growth factor receptor), and HER2 (ERBB2; v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2) inhibitors.

Network rewiring can also result in strict dependence on the activity of gene products that are not essential in normal cells. Thus, inactivation of such a *de novo* essential gene through mutation would lead to cell death or a decrease in fitness. This phenomenon has traditionally been studied in model organisms such as the budding yeast *Saccharomyces cerevisiae* and the fruit fly *Drosophila melanogaster*, and has been termed synthetic lethality or synthetic sickness (Box 1) [14–17]. The concept of synthetic lethality (in a broader sense also termed non-oncogene addiction or induced essentiality) has been proposed as a therapeutic avenue to specifically target cancer cells and leave normal cells largely unaffected: introduction of a perturbation (i.e., a drug) would represent a 'single mutant' and hence would be non-toxic to normal cells, but the transformed cancer cells would be 'double mutant' and thus synthetically lethal/sick [6,11,18–21]. Theoretically, exploitation of such synthetic interactions for therapeutic intervention by definition has less toxic side effects on

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Box 1. Genetic interactions, synthetic lethality, and oncogene addiction

Background and nomenclature

In complex cellular systems, genes do not act independently from each other but function in a concerted manner, constituting a genetic interaction network. The topology of genetic interaction networks has evolved to confer robustness such that an organism can cope with various types of internal and external perturbation while maintaining the capacity to evolve over generations. This has led to the identification of various mechanisms, and corresponding terms, that underlie but also undermine this robustness.

- **Buffering:** Robust genetic interaction networks buffer genetic variation, as illustrated by the observation that under standard laboratory culture conditions, the great majority of yeast single mutants are viable.
- **Synthetic lethality and synthetic sickness:** On occasion, single mutants become highly sensitive to specific second mutations, resulting in cell death or a decrease in fitness. These phenomena have been termed synthetic lethality and synthetic sickness, respectively (from the Greek *syn-tithenai* for to put together). Uncovering synthetic interactions is not trivial because they are rare and not always directly conserved from model organisms.
- **Non-oncogene addiction and induced essentiality:** Synthetic lethality has been revisited as a promising strategy for specific targeting of cancer, because cancer cells harbor numerous genetic aberrations that distinguish them from normal cells. To discern these applications from the precise genetic meaning, they have also been dubbed non-oncogene addiction or induced essentiality.
- **Oncogene addiction:** Distinct from synthetic lethality, oncogene addiction describes the rewired state of the signaling network of a cancer cell that has become critically dependent on the expression of a driver oncogene by shutting off parallel signaling pathways. A typical example of oncogene addiction with clinical relevance is the dependence of chronic myeloid leukemia (CML) cells on the BCR-ABL fusion gene, inhibition of which causes cell death.

Box 2. Epigenetics and cancer

What evidence links chromatin and cancer?

The earliest hints of a connection between epigenetics and cancer came from DNA methylation studies more than three decades ago, but were merely correlative in nature. With the advent of high-throughput sequencing technologies and the analysis of large sample numbers in recent years, it is now evident that epigenetic changes play a widespread role in the establishment of cancer via various mechanisms. For instance, many tumor suppressor genes are epigenetically inactivated by hypermethylation of their promoters. A number of mouse models have shown that epigenetic regulators are required for tumorigenesis, and most importantly, mutated chromatin-modifying enzymes have frequently been found in various types of cancer.

What consequences arise from a deregulated epigenome?

It has been proposed that aberrant epigenetic mechanisms allow for oncogenic reprogramming reminiscent of the differentiation process. In another model presented by Timp and Feinberg [46], it is hypothesized that deregulation of the epigenome occurs in the very early stages of tumorigenesis and causes flattening of the epigenetic landscape, leading to a stochastic departure from the normal epigenetic signature. This in turn allows for establishment of heterogeneity, acquisition of cancer hallmarks, and alteration of mutation rates.

Changes in DNA methylation also affect the cell mutation rate, for instance, through higher rates of spontaneous deamination at methylated compared to unmethylated cytosines. Moreover, in the absence of DNMTs, cells suffer microsatellite instability, destabilization of repeats, and alterations in telomere length (and lengthening, ALT) and recombination. Not only DNA methylation but also histone methylation, especially H3K9me3, and other indicators of heterochromatin have been correlated with elevated mutation rates. Furthermore, it has been shown that overexpression of EZH2 contributes to genomic instability.

normal tissue, thereby increasing the therapeutic index. Importantly, synthetic lethality would allow targeting of not only gain-of-function oncogenic events but also of loss-of-function mutations in tumor suppressor genes, as well as oncogenes that are not directly druggable.

Epigenetic deregulation as a cancer vulnerability

Interestingly, several studies have highlighted that chromatin modifiers play a special role in buffering genetic interactions. One such study involved a screen for genetic interactions in the worm *Caenorhabditis elegans* [22]. By systematically screening 65 000 pairs of genes using RNA interference, the authors found that chromatin modifiers were strongly enriched among the gene pairs displaying synthetic lethality. This included components of several conserved chromatin modifier complexes such as the histone acetyl transferase NuA4–Tip60 complex, the transcriptional co-activator Mediator complex, and the Mi2–NuRD (nucleosome remodeling deacetylase) complex. Conceptually, it appears logical that the fine tuning of transcriptional processes is well positioned to buffer perturbations in other regulatory layers and is reminiscent of the role of the protein folding chaperone Hsp90 in buffering genetic variation [23]. Whereas heat shock proteins adjust protein folding and stability, epigenetic buffering directly targets transcription and may thus compensate for deleterious passenger mutations via transcriptional activation of the second allele, repression of the mutant allele, or regulation of alternative pathways.

Enrichment of chromatin modifiers was also observed in a large screen in *S. cerevisiae* [24]. A genome-scale genetic interaction screen consisting of 5.4 million pairwise interactions showed that genes involved in chromatin function were among the most highly connected genes in the network. Furthermore, chromatin modifiers are often found bridging different cellular functions, further supporting a buffering function in the cell.

Although similar high-throughput studies in human (cancer) cells are lacking, experiments using histone deacetylase (HDAC) inhibitors suggest a conserved function. There are reports that HDAC inhibitors frequently synergize with other compounds such as DNA methyltransferase (DNMT) inhibitors, proteasome inhibitors, or inhibitors of pro-survival proteins, resulting in greater anticancer effects than their additive individual effects [25]. Moreover, they seem to be enriched in drug combination screens aimed at killing cancer cells (S.K., unpublished observation). Together, these studies suggest that chromatin modifiers are important in maintaining homeostasis on genetic perturbations and that this function is conserved. Below we discuss some of the implications of this notion in the context of specific epigenetic drugs.

Do approved HDAC and DNMT inhibitors work via synthetic lethal mechanisms?

Chromatin emerged as a drug target long before epigenetics was considered a druggable area of biology or even established as a scientific field. For instance, DNA-damaging and -intercalating agents cause not only a