CHROMATIN THERAPEUTICS

Cancer Drug Discovery Faces the FACT

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In this issue of *Science Translational Medicine*, Gasparian *et al*. use a clever cell-based screen to identify a family of DNA-binding small molecules—curaxins—that inhibit tumor cell growth and division. The curaxins' mechanism of action pinpoints a new chromatin-remodeling factor as a therapeutic target for cancer.

Cell-based phenotypic screens in oncology drug discovery have rarely resulted in the identification of potent compounds with novel mechanisms of action. In this issue of *Science Translational Medicine*, Gasparian *et al*. (*1*) show that small molecules called curaxins inhibit tumor cell growth and division by binding to the minor groove of DNA and interfering with the facilitator of chromatin transcription (FACT) complex. Contrary to expectations, the potent antitumor activity of curaxins may not depend on the induction of apoptosis or autophagy and is not antagonized by antiapoptotic mechanisms.

Compounds that insert themselves into DNA almost unequivocally induce DNA damage, which spurs mutagenesis, stimulates DNA repair cascades, and induces adaptive cell-survival mechanisms, including upregulation of the stress-activated transcription factor nuclear factor κB (NF-κB). Curaxins were identified on the basis of their ability to simultaneously activate the tumor suppressor protein p53 and inhibit NF-κB signaling pathways in intact tumor cells, a property that was previously demonstrated for the old antimalaria drug quinacrine but with no mechanistic explanation (*2*). Through a series of elegant studies, Gasparian *et al*. (*1*) now demonstrate that curaxins intercalate into DNA by placing their side chains into the minor groove, which is followed by rapid depletion of the FACT complex from the nucleoplasm (Fig. 1).

FACT is a heterodimer of two proteins: SPT16—a chaperone protein that repositions histones on DNA after it is transcribed—and SSRP1—a high-mobility group (HMG) domain–containing protein that recognizes DNA bound by intercalating agents (*3*). FACT was discovered as a result of its ability to interact with DNA modified by the anticancer drug cisplatin (*4*) and to facilitate transcription by disassembling nucleosomes, the histone-containing DNA packaging units of chromatin (*5*). A structure composed of nuclear DNA and organizing proteins that condense to form chromosomes, chromatin regulates various aspects of the packaging of DNA and its accessibility to gene regulatory factors.

Gasparian *et al*. postulated that curaxins bind to DNA and cause the trapping of FACT in chromatin followed by phosphorylation of p53 at Ser^{392} by the CK2 protein kinase, which is known to interact with FACT. Phosphorylation inhibits the degradation of p53 and activates its transcriptional regulatory—and thus its cell growth–inhibitory—functions. The FACT deficiency caused by its binding to curaxin-modified DNA inhibited

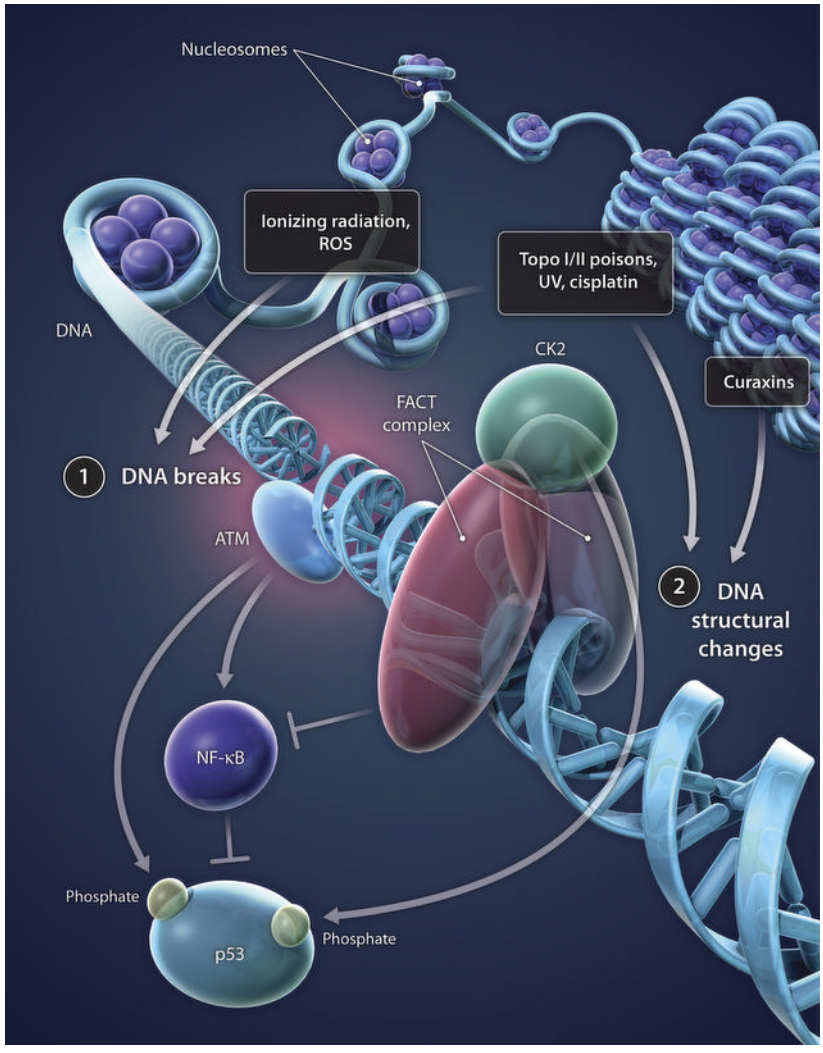

Fig. 1. Curaxins' actions. (1) Curaxins bind to the DNA minor groove and induce a conformational change in the DNA (but not DNA breaks or mutations) and recruitment of the FACT complex. Binding of FACT results in cell growth inhibition, which involves the induction of DNA damage and repair mechanisms but not apoptosis or autophagy. NK-κB is inhibited, and p53 is activated by CK2 phophorylation. (2) The impact of ionizing radiation and other DNA damaging agents is shown for comparison. DNA breaks and mutations are introduced; NF-κB is activated, and p53 either is activated via the ataxia telangiectasia mutated (ATM) protein kinase or is inhibited via NF-κB. [modified from Gasparian *et al*. (*1*)]. Topo I/II, topisomerases type I and II; UV, ultraviolet radiation; ROS, reactive oxygen species.

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the transcriptional activity of NF-κB, an effect that appeared to result from direct interference with NF-κB's transcriptional or posttranscriptional activity, as curaxins did not affect the stress-activated translocation of NF-κB to the nucleus.

ROADS LESS TRAVELED

Drug discovery screens predominantly use purified biochemical assay systems that most often measure the ability of added compounds to affect enzymatic activities, protein-protein interactions, or protein–nucleic acid interactions. The "hits" identified in such screens are then subjected to potency determination [median inhibitory concentration (IC_{50}) or inhibition constant (K_i)], and for those deemed to be of potential interest, this step is followed by iterative cycles of chemical synthesis aimed at generating suitable drug candidates with improved potency and specificity, as well as suitable pharmaceutical characteristics.

A much tougher yet unbiased and, therefore, potentially more rewarding and groundbreaking strategy focuses instead on intact cell–based phenotypic screens. In their quest for the identification of previously unknown inhibitors of cell proliferation, Gasparian *et al.* (*1*) set out to identify compounds that were able to activate p53 without directly inducing DNA damage. They were also interested in simultaneously suppressing NF-κB activation, a known mechanism for potent inhibition of cell death (*6*).

A common issue with cell-based screens aimed at inducing p53 function is that they have often yielded compounds that activate p53 indirectly through induction of DNA damage. By using a cell line that was unable to activate p53's transcriptional regulatory ability upon DNA damage, the authors identified a class of carbazole-based compounds called curaxins that had the special ability to both activate p53 and repress the activation of transcription by NF-κB, as measured by reporter gene assays. Gasparian *et al.* then went on to demonstrate that these compounds shared a consistent potency trend for the ability to activate p53 and to repress NF-κB activation, thus modulating in the desirable directions two major cancer treatment targets (*7*). The authors also showed that pharmacologically optimized curaxins were able to inhibit xenograft tumor growth in mice, both in fully formed xenograft tumors derived from conventional human tumor cell lines and in primary human tumor tissue–derived xenografts, without apparent induction of bone marrow toxicities or weight loss.

Through another less-than-usual approach, the authors then sought to identify the cellular target (or targets) of the curaxins. They found that p53 targeting occurred through its phosphorylation at Ser^{392} and that this phosphorylation is mediated through p53 interaction with FACT. Intriguingly, they showed that curaxins induced a massive delocalization of FACT from the nucleoplasm to chromatin, where it remained tightly bound. This chromatin-trapping of FACT is likely mediated by the ability of curaxins to bind DNA and induce changes in its conformation. FACT-trapping through tight association with chromatin was observed both in vitro as well as in vivo by using tumor samples derived from animals treated with curaxins. Lastly, to mimic curaxin-induced FACT depletion, the authors treated tumor cells in culture with short hairpin RNAs that mediated knockdown of the genes that encode the FACT subunits; they found that depletion of free FACT by a second method caused death of the tumor cells by a mechanism that involved inhibition of NF-κB transcription.

These results introduce FACT as a novel target for anticancer therapy. The broad antitumor activity of curaxins, their previously unidentified mechanism of action—which apparently does not rely on the induction of DNA damage—and their ability to affect multiple pathways fully justify a continued effort in evaluating them as anticancer agents that could possibly hit the clinic. It will be important to assess the true potential of curaxins as therapeutics by performing additional mechanistic, validation, and experimental therapeutic testing in the preclinical setting.

ROADS NOT YET TRAVELED

Several questions arise from this new work: What drives the compounds' antitumor activity? How could we predict tumor subpopulations that might best respond to treatment? What are the in vivo molecular targets of curaxins? Is their antitumor activity mediated completely via the compounds' ability to bind to the DNA minor groove? In that case, it would be important to determine the therapeutic window of these molecules in vivo because other DNA minor-groove binders have proven to have very limited tolerability in the clinic. Is inhibition of NF-κB activation an essential component of curaxins' observed antiproliferative activity? What is the relevance of curaxin-mediated activation of p53 Ser^{392} phosphorylation? The authors show that p53 activation might not contribute substantially to the compounds' antitumor capacity, as curaxins are also active in cells that carry a mutated version of p53 that cannot be phosphorylated or activate transcription. Also, the antitumor activity of curaxins is not inhibited by overexpression of BH3-domain (Bcl-2) antiapoptotic proteins, suggesting that p53-induced apoptosis does not contribute to curaxins' antiproliferative capacity.

Most important, there should be an aggressive plan to select an optimal clinical candidate, either among those curaxins already identified or by applying medicinal chemistry as needed, and then to extensively explore the candidate's potential as a therapeutic agent in animal models of specific cancers for which the underlying genetics and clinicopathological context are well characterized. Such experiments should always be performed with standard of care as a comparator. Rigorous implementation of such efforts could make effective cancer therapies a matter of FACT.

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