Chemistry & Biology, Vol. 10, 583–589, July, 2003, ©2003 Elsevier Science Ltd. All rights reserved. DOI 10.1016/S1074-5521(03)00152-2

Previews

New Insights into Drug Resistance in Cancer

New findings by Kavallaris et al. characterize mechanisms of resistance to epothilones and paclitaxel in cell lines. One significant discovery is a novel tubulin point mutation positioned outside the paclitaxel binding site, which confers a high degree of drug resistance.

Several antimitotic drugs that disrupt microtubule dynamics have been introduced to the arsenal of the chemotherapeutics in the fight against cancer over the last two decades. Prime examples of this class of anticancer therapeutics are the *Vinca* alkaloids (vinblastine and vincristine), which were introduced to treat hematopoietic malignancies, and the taxanes (paclitaxel and docetaxel), which are arguably the most effective and clinically successful agents from the last decade. Taxanes interfere with microtubule dynamics by accelerating the polymerization of tubulin monomers and stabilizing microtubules, whereas *Vinca* alkaloids, colchicinoids, and combretastatins inhibit tubulin polymerization.

The clinical success of the taxanes has stimulated the search for new molecules with a similar mode of action and has resulted in the discovery of natural products including epothilones A and B from myxobacterium, discodermolide from marine sponge, and eleutherobins/sarcodictyns from soft coral. Interestingly, it appears that these structurally unrelated molecules from diverse origins share a common pharmacophore with paclitaxel [1]. Such a hypothetical pharmacophore was used to model the drug binding site in wild-type and mutant tubulins [2]. Although taxanes represent a practical improvement in the clinical management of solid tumors, these drugs have not solved one of the most important clinical problems, drug resistance. Also, patients resistant to other chemotherapeutic agents but who initially responded to taxanes often relapse, unfortunately, and do not respond to these drugs anymore. Several mechanisms of drug resistance have been reported for paclitaxel. Since taxanes are substrates for the P-glycoprotein efflux pump (P-gp), resistant cells may overexpress P-gp, thereby hampering intracellular drug retention [3]. An alternative means by which taxane resistance occurs is by point mutation at the target β-tubulin within or near the paclitaxel binding site [4]. A third relevant mechanism underlying drug resistance is the expression of β-tubulin isotypes that are less sensitive to paclitaxel inhibition [5]. In fact, β-tubulin is encoded by a large multigene family, with the most significant differences at the carboxyl terminus region. Cleveland and Sullivan used this region to devise a classification system to distinguish tubulin isotypes in vertebrates [6]. In humans, six possible β-tubulin isotypes have been identified and characterized for tissue expression. Class I and IVb are constitutively expressed in all tissues, while class III, Iva, and II are typical of brain tissues and are expressed only at low levels in other tissues. Class VI possesses the lowest degree of homology to the others and is specifically expressed in the hematopoietic compartment. In functional terms, the presence of the class III inhibits the assembly of β-tubulin subunits promoted by paclitaxel, and the class III-depleted subunits show enhanced sensitivity to paclitaxel [7].

The article by Kavallaris and collaborators published in this issue [8] raises several very important points concerning drug resistance to antimitotic anticancer agents that inhibit microtubule dynamics. In this study, the authors generated a panel of cells with collateral drug resistance to paclitaxel and sensitivity to *Vinca* alkaloids through stepwise exposure to increased concentrations of desoxyepothilone B (dEpoB). Since epothilones are not a substrate for P-gp, it was expected that the drug-resistant cell lines would not overexpress P-gp. Moreover, when drug-resistant cell lines are generated through chronic and stepwise increase in exposure to a drug, it is highly unlikely that only a single mechanism of drug resistance is operating, i.e., several mechanisms should coexist and be contributing to the drug-resistant phenotype. In fact, in this article the authors observed that multiple drug-resistance mechanisms were operating in the same cells. Drug-resistant cells displayed increased expression at the protein level of the most dynamic class III β-tubulin isotype. In a recent paper, Nicoletti and coworkers quantified the relative ratio of the tubulin isotypes in a panel of 17 human cancer cells [9], wherein a statistically significant increase in the class III β-tubulin is correlated to an inherent drug-resistant phenotype toward paclitaxel and vincristine. This finding indicates that it is unlikely that changes in the relative amounts of β-tubulin isotypes could explain the collateral sensitivity to *Vinca* alkaloids exhibited by dEpoB-resistant cells. Besides the overexpression of the class III β-tubulin, Kavallaris et al. also noticed increased expression of microtubule associated proteins (MAPs). Indeed, the increased level of MAP4 could compensate for the reduced microtubule stability induced by the acquired β-tubulin mutation or by the increased levels of the class III β-tubulin. In addition, a couple of important point mutations in the class I β-tubulin were found in the dEpoB-resistant cells. A heterozygous point mutation at nucleotide 691, resulting in an alanine to threonine substitution at amino acid 231, was observed in all dEpoB-resistant cells. The second heterozygous point mutation at nucleotide 874, substituting a glutamine to glutamic acid at amino acid 292, was found only in the most stringent conditions of drug exposure. These two mutations are actually translated into the corresponding mutated proteins, as demonstrated by peptide mass fingerprinting using MALDI-TOF and ESI-TOF tandem mass analyses. Both mutations are novel and different from those reported previously involving amino acid residues 270, 274, 282, and 364 [2]. A231T mutation resides on the helix 7 of β-tubulin, which is located within the binding

pocket of a paclitaxel molecule [10], whereas Q292E mutation is located in the helix H9 near the M loop. The mutation at 292 is particularly interesting because it is located apparently outside of the paclitaxel binding site. The most likely explanation is that, at 292, the presence of an extra-positive ionic charge due to glutamine (instead of glutamic acid) induces a distortion in the M loop, thereby hampering the entry of paclitaxel (and epothilones) into the pocket of the binding site. As a matter of fact, this mutation is linked to an extremely high degree of drug resistance (around 400-fold) and greatly reduced binding of [^{3}H]paclitaxel as compared to the drug-sensitive parental cells.

Consequently, the work of Kavallaris and her collaborators provides novel insights into drug-target interactions in microtubules. It is now essential to understand which of the reported drug-resistance mechanisms actually occurs in clinical settings so that we can focus our efforts on the generation of new and efficacious molecules as well as therapeutic strategies to overcome drug resistance.

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Chemistry & Biology, Vol. 10, July, 2003, ©2003 Elsevier Science Ltd. All rights reserved. DOI 10.1016/S1074-5521(03)00153-4

RNA as a Transcriptional Activator

Two recent reports demonstrate that in vivo selection can isolate novel RNAs that activate transcription when tethered to a gene promoter. This highlights the structural plasticity that allows RNA to fulfill many functions normally carried out by proteins.

Eukaryotic transcription factors are modular proteins that are generally comprised of a sequence-specific DNA binding domain (DBD) tethered to a transcriptional activation (AD) or repression domain (RD). Extensive structural diversity can be found in the various motifs utilized for both DNA binding and activation/repression. DBDs are very well characterized, and numerous high-resolution structures are now available for zinc finger, helix-turn-helix, leucine zipper, winged helix, and other types of DBDs [1]. The molecular details underlying base-sequence specificity have been unraveled for many of these DBDs. While activation and repression domains are also well characterized at the level of amino acid sequence composition and character, structural studies lag far behind the DBDs, and the precise mechanisms leading to gene activation or repression in many cases have not yet been elucidated. Activation domains are thought to be responsible for the recruitment of coactivators, components of the basal transcription machinery, or enzymes for chromatin modification (chromatin remodeling factors and histone-modifying enzymes) [2]. Common targets may be shared between different transcriptional activators, but many activation domains share little sequence homology to one another.

The chemical biology community has long sought to devise methods to control gene expression by targeting specific DNA sequences with novel peptides, oligonucleotides, or small molecules [3–5]. Natural or synthetic DBDs have been tethered to peptides derived from potent viral activators [6–9], and fully synthetic peptides have been fused to natural DBDs for gene activation [10, 11]. In another approach, peptide libraries