Chemical Inhibitors: The Challenge of Finding the Right Target

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A paper in this issue of *Chemistry & Biology* shows that the diaryl oxazole compound UA62784 that targets pancreatic cancer cells interacts with tubulin near the colchicine binding site (Tcherniuk et al., 2011). These findings differ from previous observations, highlighting the challenges of identifying the biological target for chemical inhibitors.

Cancer is linked to abnormal cell proliferation and differentiation and this loss of regulation induces escape from senescence and death. The main cytotoxic agents are classified into four categories: (1) DNA alkylating agents, (2) inhibitors of topoisomerases I and II, (3) antimetabolite agents, and (4) microtubule targeting agents (MTAs). The latter are also called antimitotics and are known to interact with tubulin at four main binding sites: the taxane/epothilone, laulimalide, colchicine, and vinca alkaloid sites (Figure 1). Binding to taxane and laulimalide sites stabilizes microtubules, whereas binding to the others induces tubulin depolymerization. These microtubule-targeting agents perturb not only mitosis but also cellular trafficking during interphase. MTAs induce microtubule polymerization or depolymerization at high concentrations, while low doses kinetically stabilize microtubules without changing their polymer mass (Jordan and Wilson, 2004). The success of antimitotics is overshadowed by the occurrence of resistance owing to a number of different factors. For example, resistance can occur due to the overexpression of the P-glycoprotein, an ABC transporter involved in the efflux of antineoplastic drugs from cancer cells (Sandor et al., 1998), or tubulin mutations at the drug binding site (Mozzetti et al., 2008). Other cytoskeletal proteins (γ-actin and actin-regulating proteins), as well as variations in tubulin isotypes or microtubule-associated proteins expression, can also regulate the pool of microtubules and disturb the activity of MTAs (Kavallaris, 2010). In addition, the success of MTAs, which is usually attributed to the induction of mitotic arrest, is counterbalanced by deleterious side effects owing to essential microtubule functions beyond mitosis. This led to the development of new compounds that inhibit cellular targets with exclusive mitotic functions. These proteins include Aurora kinases, kinesin spindle protein (KSP), polo-like kinases 1 (PLK1), and the centromeric protein E (Cenp-E), which are only active during mitosis where they are responsible for controlling different steps in the assembly and function of the mitotic spindle (Jackson et al., 2007). Aurora kinases control the spindle checkpoint and cytokinesis, whereas KSP is responsible for establishing mitotic spindle bipolarity. PLK1 regulates centrosome maturation and formation of the mitotic spindle and Cenp-E is involved in chromosome congression. Agents that disrupt mitosis have, however, shown only limited clinical input to date (Komlodi-Pasztor et al., 2011), so the screening for new compounds is still in progress.

Two recent studies (Henderson et al., 2009 and Tcherniuk et al., this issue) reported the characterization of a new diaryl oxazole compound, UA62784, which selectively targets pancreatic cancer cells. This compound showed remarkable activity in both studies, providing new hope for the treatment of this extremely aggressive neoplasia. However, the proposed mechanism of action differs in the two studies. Henderson et al. attributed UA62784 effects to a direct inhibition of Cenp-E ATPase activity, whereas Tcherniuk et al. now show that UA62784 promotes microtubule depolymerization by binding to microtubules near the colchicine binding site. In brief, Henderson et al. observed that UA62784 had no effect on tubulin polymerization, but found that UA62784 inhibits Cenp-E ATPase activity, without affecting Cenp-E ability to bind microtubules or BubR1 at the kinetochore, which are critical for Cenp-E function. Interestingly, it should be noted that a related study showed that only a couple of the UA62784 derivatives, produced as a part of an extensive SAR exploration, inhibited Cenp-E while the majority had no inhibitory effect on Cenp-E or other kinesins tested (Shaw et al., 2009), leading the authors to conclude that UA62784 derivatives did not reproduce UA62784 Cenp-E inhibitory ability. The same study showed that UA62784 derivatives inhibited a subset of kinases, suggesting a possible alternative mechanism of antitumor action. This example of potentially incorrect conclusion, i.e., UA62784 inhibits Cenp-E activity, is not unprecedented since other cases can be found in the literature. For example, anthracyclines were classified as DNA-interacting agents but later were found to damage cellular components including cancer cells membranes, via multiple cellular targets (Komlodi-Pasztor et al., 2011).

Tcherniuk et al.'s study now adds another layer of insight into the UA62784 mechanism of action. The authors use biophysical binding studies coupled with in vivo imaging fluorescence data, to provide evidence that UA62784 interacts with tubulin with nanomolar affinity. Binding studies were performed in solution with microtubules that had been polymerized from purified tubulin and provide valuable information regarding the kinetics of microtubule dynamics and the interaction of UA62784 with microtubules. Based on

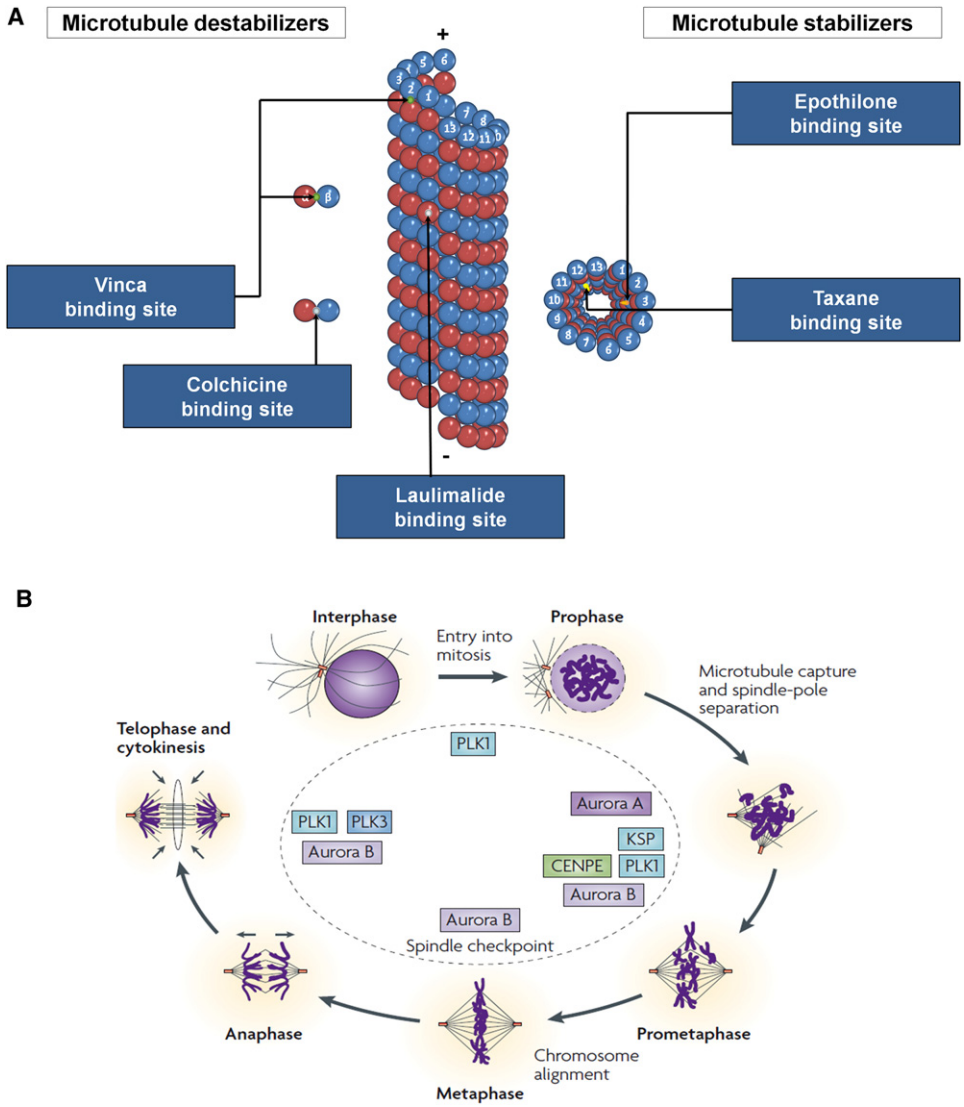

Figure 1. Targets of Chemotherapy for Cell Cycle Perturbation

(A) Tubulin binding sites of antimicrotubule agents. Taxanes preferentially bind to the tubulin subunit of polymeric microtubule at the lumen of the microtubule pore. Epothilones bind the same site as taxanes, but exploit the tubulin-binding pocket in a unique and independent manner. Laulimalide binds at a site that is distinct from the paclitaxel/epothilone site. Colchicine and related molecules that bind at the same site induce microtubule depolymerization by inhibiting lateral contacts between microtubule protofilaments, but they have a better affinity for free tubulin heterodimers. Vinca alkaloids preferential bind free tubulin, but also bind microtubule ends.

(B) Progression of mitosis through the canonical morphological stages. Specific druggable protein targets that function during mitosis are highlighted. Kinesin spindle protein (KSP) is required to establish mitotic spindle bipolarity by driving centrosome separation. Centromeric protein E is required for accurate chromosome congression at metaphase. Aurora A is crucial for centrosome maturation and separation during early prophase. Aurora B is involved in chromosomal alignment on the metaphase plate, bipolar kinetochore-microtubule attachments, spindle checkpoint, and cytokinesis. During mitosis, Polo-like kinase 1 (PLK1) is involved in centrosome maturation and formation of the mitotic spindle. PLK1 is also required for exit from mitosis and the separation of sister chromatids during anaphase. PLK1 might also have a role in cytokinesis through the phosphorylation of the kinesin-like motor protein MKLP1. Reprinted from Jackson et al. (2007) Nat. Rev. Cancer **7**, 107–117, with permission from Macmillan Publishers Ltd., copyright 2007.

state-of-the-art imaging in living cells and biophysical analysis, Tcherniuk et al. concluded that UA62784 could provide a better treatment strategy at lower doses due to UA62784 higher binding affinity for tubulin, when compared with other well-known antimicrotubule agents, such as vinblastine.

From the discussion above, it is clear that probing the exact spatial relationships between proteins and drugs requires the use of investigation tools that can be applied at molecular level, such as optical tweezers (Calligaris et al., 2010). Alternatively, molecular mass spectrometry (MS) imaging has recently emerged as a promising tool to address the challenge of drug-target colocalization. This sensitive technique allows rapid and quantitative profiling of drugs in tissues without any prior knowledge and avoids the use of antibodies. Mass spectrometry imaging is therefore more powerful in this regard than classical immunochemistry;

however, its spatial resolution and sensitivity are limited. Numerous matrix-assisted laser desorption/ionization (MALDI) MS imaging studies have reported biomarker identification for prognosis of lung and brain cancers, and also for elucidating disease mechanisms (McDonnell et al., 2010). In cytoskeleton research, MALDI imaging has been used to locate the interaction of unlabeled small molecules with microtubules.

In conclusion, studies that examine effects of small molecules and aim to identify their specific cellular targets need to include specific cellular readouts at the molecular level together with a complementary set of in vivo and in vitro techniques for their unambiguous validation, thus focusing basic and clinical research on the right targets and avoiding erroneous conclusions and unfounded claims.

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Activity-Based Profiling of 2-Oxoglutarate Oxygenases

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2-Oxoglutarate oxygenases (2-OGs) are a large enzyme family involved in numerous processes in health and disease. Rotili et al. (2011) describe in this issue of *Chemistry & Biology* an activity-based protein profiling-based strategy with which the activity of individual members of the 2-OG family can be addressed in the context of complex biological systems.

Activity-based protein profiling (ABPP) has emerged as a powerful strategy in chemical biology research involving enzymes (Evans and Cravatt, 2006). In ABPP research, activity-based probes (ABPs) are designed to recognize, and subsequently bind covalently to, an enzyme or enzyme family, preferably in complex biological systems. An ABP is normally assembled from three individual functional parts: a recognition element (generally but not necessarily derived from the substrate of the enzyme at hand), a reactive group (for instance, electrophilic trap, photoreactive group), and an affinity tag. ABPP studies on enzymes are complementary to classical enzyme inhibition studies, in which an isolated enzyme reacts with a fluorogenic substrate, either in the presence or absence of a competitive inhibitor. Inhibition constants can be accurately measured in this fashion, which provides valuable information in case the inhibitor at hand is considered as a lead for drug development.

The kinetics studies of covalent, irreversible inhibitors are considerably more complicated. For this reason, and perhaps more importantly also for the widespread belief in medicinal chemistry that such compounds would make poor drug candidates, covalent inhibitors have been neglected for some time. This situation changed drastically a decade ago with a number of seminal studies. Biotinylated broad-spectrum inhibitors of serine hydrolases (Liu et al., 1999) and cysteine proteases (Greenbaum et al., 2000) proved highly useful in the profiling of these hydrolytic enzymes in cell extracts and living cells. At the same time, biotinylation of the natural product epoxomicin led to the identification of proteasomes as the target of this toxic, but potentially therapeutic, agent (Meng et al., 1999). These groundbreaking studies have opened the field of ABPP, and several attractive aspects of the strategy have been addressed in the following years. As said, transforming a natural product into an ABP may reveal its biomolecular target. Enzyme activities may be unearthed, or the presence (or absence) of