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PERSPECTIVE

Sleuthful Pharmacology

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How many times have you found yourself in the position of not knowing how your favorite novel compound really works in a cell or organism? Your hypothesis, so carefully constructed, just does not seem to align with the data. You dream wearily of the day when a useful, if not universal, approach for determining the real mechanism of action becomes available. The current article, authored by T. Efferth and coworkers from nine international laboratories (Efferth et al., 2003), provides a clever illustration of one such approach. Armed with a semisynthetic derivative of artemisinin, the active principle of the *Artemisia annua* (sweet wormwood), the authors exploit a valuable public asset developed by the Developmental Therapeutics Program of the U.S. National Cancer Institute to decipher the mechanism of action of artesunate (Fig. 1), an antimalarial agent with previously described anticancer activity (Efferth et al., 2001). Artesunate is not a new agent; it is first-line therapy for *Plasmodium falciparum* and *Plasmodium vivax* malaria in some areas of Asia and also displays antischistosomal properties. More than 200 peer-reviewed articles on artesunate have been published in the last seven years, a testimony to its importance as a therapeutic agent. Nonetheless, only three of those references refer to its potential use as an anticancer agent.

The work of Efferth et al. (2003) illustrates how a hypothesis-generating (derisively termed "ignorance-based" by some) approach rather than a hypothesis-driven approach can yield useful insights into the possible mechanisms of action of a new anticancer compound. The National Cancer Institute has tested tens of thousands of compounds against their 60-tumor cell panel for growth inhibition and has provided additional information about the molecular phenotype of each cell line. Information from this substantial undertaking has been placed in the public domain on a readily accessible website (http://dtp.nci.nih.gov) that can be mined with the National Cancer Institute's COMPARE program. As Efferth et al. (2003) showed, this informatics tool can generate testable candidate molecular targets for a compound that does not have any obvious relationship, at least based on this algorithm, to clinically used anticancer drugs. Rapidly growing tumors were more sensitive to artesunate than slowly growing tumors, but this is seen with many existing anticancer agents with the exception of the drugs that cause DNA adducts, such as carboplatin, dacarbazine, and isosfamide.

With artesunate, the authors selected 465 genes whose expression levels were obtained by microarray hybridization and are available in the National Cancer Institute's data base. They used hierarchical cluster analysis and found 60 genes whose expression correlated with sensitivity or resistance to artesunate. Three genes were studied in greater detail because of the high correlation of their cDNA levels with a cytotoxic response to artesunate, their mathematically determined low false positive discovery rate, and the availability of appropriate cell systems to test their importance. All three selected gene products were validated as genes involved in the artesunate cytotoxic response using gene transfer methodology. Transfection of cells with the cDNA for epidermal growth factor receptor, the target of the recently approved anticancer agent gefitinib (Iressa), and γ-glutamylcysteine synthetase altered sensitivity to artesunate. The authors also used a tetracycline repressor expression vector system, first developed by Blomberg and Hoffmann (1999), to confirm a role for the CDC25A gene in the cytotoxic mechanism of artesunate. This latter observation is particularly interesting because, like the epidermal growth factor receptor, the Cdc25A protein has been shown to be over-expressed in a number of human tumors, including breast cancer (Cangi et al., 2000), and has been implicated in several aspects of the malignant phenotype (Fig. 2). Thus, Cdc25A controls cell cycle checkpoints that regulate progression through G_1/S, S, and mitosis due to its ability to dephosphorylate and, thus, activate cyclin-dependent kinases. Consequently, elevated levels of functional Cdc25A are thought to allow cells to replicate and duplicate damaged DNA and, thus, to encourage genetic instability. Cdc25A also has been shown to block apoptosis signal-regulating kinase-1 (ASK-1) (Zou et al., 2001) and to affect epidermal growth factor receptor (Wang et al., 2002), raf-1 (Xia et al., 1999), and steroid

ABBREVIATIONS: ASK-1, apoptosis signal-regulating kinase-1.

receptors (Ma et al., 2001). The interaction between Cdc25A and ASK-1 or steroid receptors did not seem to require a Cdc25A protein with phosphatase activity. Thus, an agent that preferentially affects cells that over-express Cdc25A is of considerable pharmacological interest (Lyon et al., 2002). Efferth et al. (2003) also show that the growth-inhibitory activity of artesunate was not influenced by the most common cellular multidrug resistance mechanisms or by the p53 or p21 status of cells.

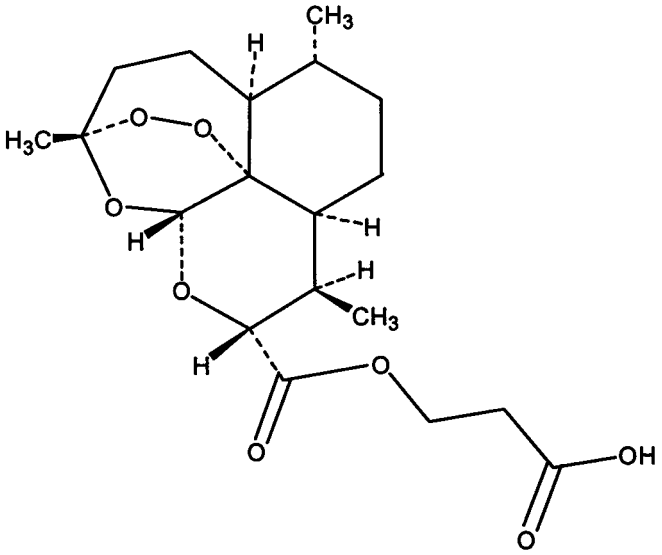

Fig. 1. Chemical structure of artesunate.

As with any manuscript, the current contribution is not without potential issues. For example, the fundamental tool for selecting the candidate genes was cDNA microarray data. No persuasive evidence was provided that glutamate-cysteine ligase regulatory subunit, epidermal growth factor receptor, and CDC25A protein expression was elevated in concert with the cDNA levels. Moreover, if one accepts that the Cdc25A acts as an oncogene because it promotes genetic instability, then it is difficult to deduce theoretically how the very transient Cdc25A over-expression occurring after tetracycline withdrawal could render a cell more sensitive to artesunate. Perhaps artesunate was acting to disrupt Cdc25A interactions with ASK-1, raf-1, or epidermal growth factor (Fig. 2). The current article provides no mechanistic information on how the candidate gene products alter sensitivity to artesunate. Furthermore, the failure to see any effect of Cdc25A expression on doxorubicin-induced growth inhibition is not fully in agreement with a recent report (Xiao et al., 2003), which was published after the manuscript by Efferth et al. was submitted. Xiao et al. (2003) demonstrated that

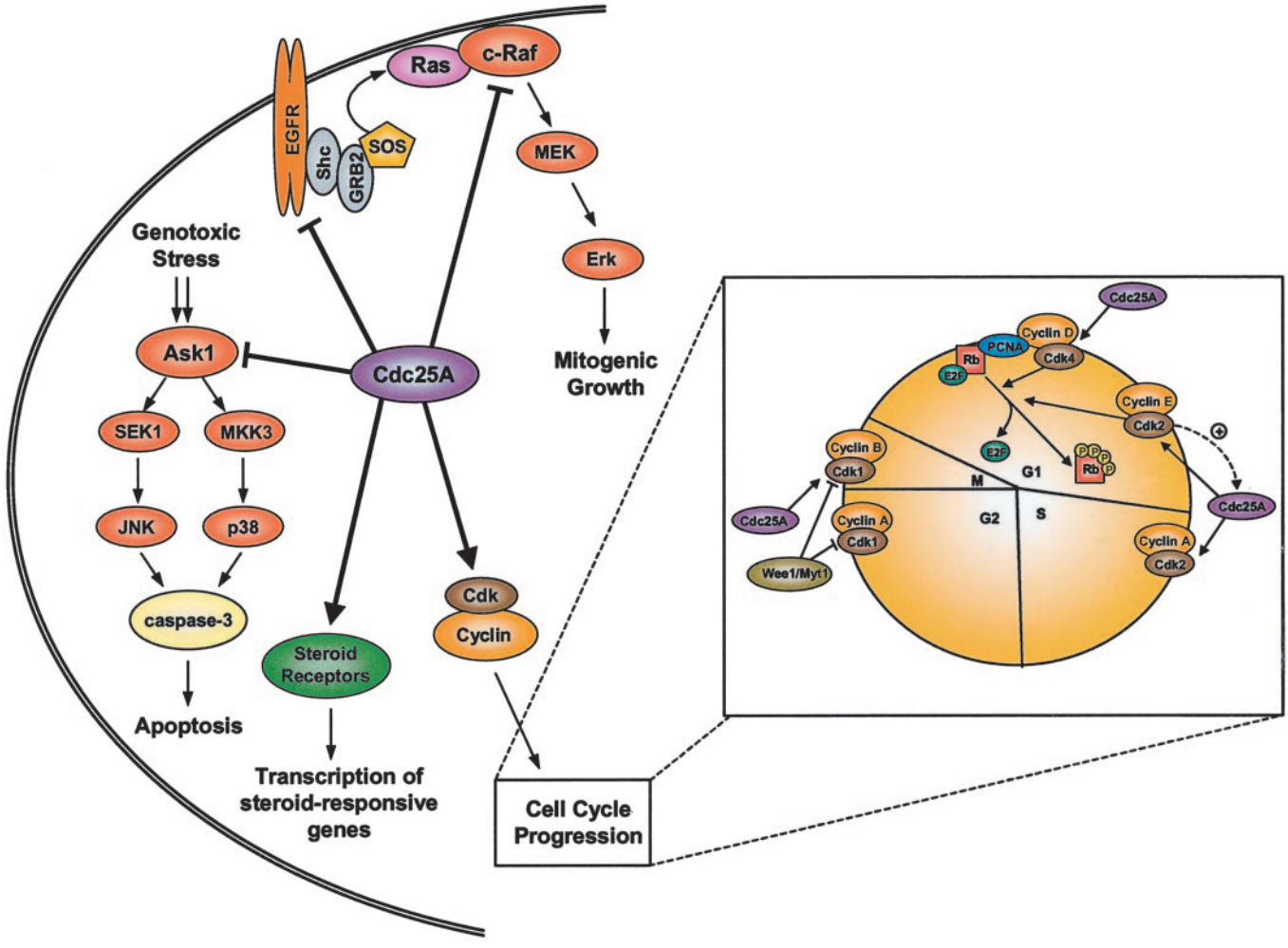

Fig. 2. Potential intracellular actions of Cdc25A. Evidence for potential positive (arrows) and negative (T-bar) effects of Cdc25A are indicated. The role of Cdc25A on cell cycle progression is indicated in the right inset.

high expression of Cdc25A caused resistance to doxorubicin, whereas low-level expression did not alter doxorubicin sensitivity. Although the difference in results of the two groups might reflect the use of different species, knowledge about the Cdc25A protein expression levels in the Efferth study would be useful comparative information.

Like many good articles, the hypothesis-generating study of Efferth et al. provides as many new questions as it answers. What was the mechanism of action by which γ-glutamylcysteine synthetase, epidermal growth factor receptor, and Cdc25A phosphatase affected cell sensitivity to artesunate? What about the other 57 genes? How does one determine the hierarchical importance of the 60 identified genes, really, in the ultimate antiproliferative activity of artesunate? These and other questions may arise in the mind of the readers of this article, perhaps stimulating them to answer these questions or even emulate the sophisticated approach taken by the authors of this contribution.

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