

Commentary

Gene profiling and the cyclin-dependent kinase inhibitor flavopiridol: What's in a name?

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Flavopiridol represents one in an ever-expanding group of compounds currently called molecularly targeted agents. Such compounds, in contrast to conventional cytotoxic drugs, which exert a more general disruptive effect on key cellular structures or processes (e.g., DNA replication, formation of the mitotic spindle, etc.), target pathways directly implicated in neoplastic transformation. The prototype of this type of compound is imatinib mesylate, or Gleevec, an inhibitor of the Bcr/Abl kinase that has enjoyed considerable success in the treatment of chronic myelogenous leukemia and related disorders (1). Flavopiridol is a semi-synthetic rohitukine alkaloid that functions as a broad inhibitor of cyclin-dependent kinases (CDKs), enzymes critically involved in cell cycle progression and the disordered proliferation of tumor cells (2). In addition to its cell cycle inhibitory effects, preclinical studies have shown that flavopiridol is a highly potent inducer of apoptosis in malignant cells, particularly those of hematopoietic origin (3). Flavopiridol has also been shown to potentiate the lethal effects of both conventional cytotoxic as well as more novel agents in epithelial and leukemic cells (4, 5). On the basis of its very promising activity in preclinical studies, flavopiridol was the first CDK inhibitor to enter clinical trials in humans (6). Currently, flavopiridol continues to undergo extensive clinical evaluation, either as a single agent or in combination with other cytotoxic drugs. Although flavopiridol has demonstrated some, albeit limited, activity in early trials (7), to date it has not fulfilled the promise predicted for it based on encouraging preclinical results. However, evidence is now emerging that the activity of flavopiridol in humans may have been limited by pharmacokinetic factors, including suboptimal administration schedules as well as binding of the drug to

plasma proteins. Consequently, attention is now focusing on newer hybrid schedules (e.g., bolus in conjunction with a prolonged infusion) to circumvent these problems. In the intervening period, interest in CDK inhibitors has persisted, and several other agents of this class, including UCN-01 (8), CYC202 (9), and BMS 387032 (10) have now entered clinical trials.

Efforts to elucidate the mechanism of action of CDK inhibitors have provided the following insights: such agents may have multiple targets in addition to CDKs, and it is unclear whether CDK inhibition is primarily responsible for lethality. For example, UCN-01 was originally developed as a selective PKC inhibitor (11) but was subsequently found to act as a CDK inhibitor (12). Since then, UCN-01 has been shown to exert multiple other actions that may be as or possibly more directly related to lethality, including inhibition of Chk1 (13) and Akt/PDK1 (14). Flavopiridol, in particular, has been found to exert an exceptionally broad spectrum of actions in addition to CDK inhibition, all of which might plausibly contribute to cytotoxicity. These include anti-angiogenic activity (15), transcriptional repression via inhibition of the positive transcription elongation factor-B (PTEF-b), CDK9/cyclin T complex (16), down-regulation of anti-apoptotic proteins (e.g., XIAP, Mcl-1, Bcl-xL; refs. 17, 18), repression of p21^{CIP1} (19), DNA duplex formation (20), inhibition of survivin phosphorylation (21), down-regulation of cyclin D₁ (22), and most recently, inhibition of IKK and NF- κ B (23). It is important to note that the pleiotropic mode of actions of such agents is not restricted to CDK inhibitors; in fact, the putatively specific Bcr/Abl kinase inhibitor imatinib mesylate is known to inhibit other tyrosine kinases, including c-Kit, a finding that has significant clinical implications, for example, in gastrointestinal stromal tumors (24).

It is clear that in the case of novel agents, such as flavopiridol, a better understanding of which pathways are being targeted will be critical to the development of optimal therapeutic strategies. However, until recently, identification of such targets has been a largely empirical endeavor. With the development of gene profiling strategies, this situation seems likely to change. For example, in a study reported in this issue by Lü et al. (25), a DNA array approach was employed to characterize changes in gene expression in four epithelial tumor types (prostate and gliomas) exposed to cytotoxic concentrations of flavopiridol. A number of findings, including some that were quite unexpected, emerged from this study. Lü et al. identified a diverse group of 220 genes that were either significantly up- or down-regulated following exposure of

Mol Cancer Ther 2004;3(7):873–5

Grant support: Awards CA63753, CA 93738, and CA 100866 from the National Cancer Institute, and awards 6045-03 from the Leukemia and Lymphoma Society of America and DAMD17-03-0262 from the Department of Defense.

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all four cell lines to flavopiridol. Intriguingly, only a minority of genes (e.g., 11%) were specifically related to the cell cycle; other broad categories of involved genes included those related to transcriptional regulation, signaling, cell structure, protein synthesis regulation, and cellular metabolism, among others. As one might anticipate, perturbations in gene expression were both drug dose- and exposure interval-dependent. Interestingly, and rather unexpectedly, no apoptosis regulatory genes appeared in the screen; moreover, while flavopiridol reduced clonogenic survival of each of the cell lines, apoptosis was not detected, at least at the interval at which it was monitored. The authors conclude that molecularly targeted agents, such as flavopiridol, may, when administered at cytotoxic concentrations, induce a characteristic "transcriptosome," that is, a constellation of genetic changes that is associated with or leads to a form of reproductive cell death. A corollary of this concept is that, in addition to their predicted effects (e.g., inhibition of CDKs), agents such as flavopiridol may exert their lethal actions primarily or in part by modulating the expression of diverse genes, including some which have no obvious relationship to cell cycle events.

The results of this study, intriguing as they are, must be viewed with several caveats. First, as noted by the authors, the functional significance of the observed changes in gene expression remains to be determined. For example, many or most of the changes could simply represent secondary responses to stresses resulting from CDK inhibition and/or other actions of flavopiridol. The functional contribution of such changes to the cell death process will have to be defined using genetic approaches, for example, by strategies involving ectopic expression or down-regulation of the gene in question. Temporal considerations will also have to be taken into account. For example, effects of an agent on gene expression cannot be understood by examining perturbations at a single time point; in many cases, the duration of a signal, or the temporal pattern of its expression, has been found to be critical in determining the biological effects of such events (26). Sorting out these issues will obviously be a complex task, given the large number of genes displaying altered expression following flavopiridol administration. Another potentially confounding factor stems from the fact that perturbations in gene expression do not operate in a vacuum, but may exhibit cooperativity. Consequently, stimulation (or inactivation) of a pathway, in isolation, will not mimic the actions of flavopiridol simply because such actions may be linked to various other signaling or cell cycle-related events. According to this hypothetical model, induction (or repression) of a gene may not be lethal by itself, but will become so only in concert with CDK inhibition and/or other changes. In support of this notion, recent studies have shown that CDK inhibition is particularly lethal to leukemic cells when combined with down-regulation/inactivation of the PI3K/Akt (27) or NF- κ B pathways (28). Thus, as far as CDK inhibitor-mediated lethality is concerned, context may be everything.

Two of the more surprising findings of this study were that in the epithelial tumor cell systems examined, apoptosis did not seem to represent the mode of flavopiridol-induced

cell death, and that changes in expression of apoptotic regulatory genes were not prominent in the gene profiles of treated cells. One question remaining to be answered is that if these cells did not undergo an apoptotic form of cell death in response to flavopiridol, how exactly did they die? Answers to this question may reflect the use of clonogenic assays to monitor lethality. Such assays can be influenced by diverse processes which either induce cell death or inhibit self-renewal capacity. Possibilities include, in addition to the delayed induction of apoptosis, alternative forms of reproductive cell death, including necrosis, mitotic catastrophe, differentiation, senescence, and paraptosis, among others. It is also noteworthy that several anti-apoptotic genes known to be down-regulated in response to flavopiridol in malignant hematopoietic cells, including XIAP, Bcl-x_L, and Mcl-1 (17, 18), were noticeably absent from the present screen. Such findings raise the possibility that the failure of flavopiridol to down-regulate such genes in epithelial cells may account for, at least in part, the non-apoptotic response of these cells to flavopiridol administration.

What, then, is the significance of the present findings? The answer to this question could lie in the possibility that, as in the case of more conventional cytotoxic drugs, the lethal actions of more novel, "molecularly targeted" agents, such as flavopiridol may ultimately depend on changes in the expression of genes only tangentially related to their perceived primary mode of action, for example, in this case, disruption of the cell cycle. The challenge now is to determine which of the large number of such candidate genes play functional roles, either alone or in concert with others, in drug lethality. It should be recalled that slightly over a decade ago, Gudkov et al. (29) used related techniques to identify genetic suppressor elements (GSEs), such as kinesin, which conferred resistance to the topoisomerase inhibitor etoposide. The jury is still out about whether perturbations in such genes play a physiologic role in drug responsiveness. In any case, with the advent of DNA array technology, it seems likely that novel agents like flavopiridol will be found to exert exceptionally pleiotropic and in many cases, unexpected effects on gene expression and function. Moreover, the scope of such actions will undoubtedly expand further with the use of even newer technologies, including proteomics and phospho-proteomics. The central point is that while it is tempting to assign a name (e.g., CDK inhibitor) to a drug such as flavopiridol, and to try to understand its mode of action in this context, many if not most of what we consider to be "targeted" agents may prove to act through completely unanticipated mechanisms. Hopefully, the use of gene profiling and related strategies will result in an improved understanding of the mechanisms underlying the lethal mode of action of these agents, leading in turn to optimization of their therapeutic potential.

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