

6 GENERAL DISCUSSION

The NCI-60 cell lines are the most extensively characterized set of cancer cell lines in existence. The various data amassed on the molecular pharmacology of these cells has been of tremendous value to cancer research. However, cancer cell lines are limited with respect to representation of the histopathologic diversity of any given cancer type and may have acquired further genetic events *in vitro* during the cell culture process. Despite this limitation, cell lines remain mainstays in drug development programs, because unlike primary tumors, they are available in abundance, are generally genetically homogeneous and are experimentally tractable.

In terms of molecularly targeted drug screens, the NCI-60 cell line set may need to be reevaluated. There are several limitations to the use of the NCI-60. First, there are only nine tissue types represented in the panel. Second, there are a limited number of cell lines represented within each subtype. Our mutational analysis of 24 cancer genes in the NCI-60, the largest for any set of publicly available cell lines, reveals that a majority of the lines are not fully representative of the tissue types they represent. Ultimately, deriving a cell line panel to represent fully the genetic diversity of primary tumors may be impossible. However, the more we learn about cancer genetics the better we can assess the validity of results from molecularly targeted drug screens.

Our analysis of the relationship between somatic mutations in frequently altered cancer genes and drug activity reveals that we have more to learn about the molecular genetics of drug response. We find that differential drug response, in most cases, will not be due to a single genetic lesion and possibly not even a combination of cancer gene mutations. We did, however, identify a statistically significant association between the presence of V600E BRAF mutation in melanomas and increased anti-proliferative activity of phenothiazine compounds. We have subsequently demonstrated that the *in vitro* anti-proliferative activity of phenothiazines is greatest in codon 600 BRAF mutant melanomas compared with non-codon 600 BRAF mutant, RAS mutant and RAS/RAF wild type melanomas.

Phenothiazine compounds are FDA approved for use in the treatment of psychiatric illnesses due to their antagonist activity at dopamine (D2) receptors. Our work, and the work of others, clearly shows that phenothiazine compounds have pleiotropic effects other than their effects of D2 receptors. Among the many proposed mechanisms of phenothiazine action, its calmodulin antagonist activity is well documented. The anti-neoplastic and anti-proliferative activities of phenothiazines are yet unknown. Based on our work, it would be interesting to evaluate the hypothesis that the increased activity of phenothiazines in V600E BRAF mutant melanoma is due to inhibition of the RAF-MEK-ERK pathway. A first set of experiments to address this hypothesis would be to evaluate the total protein levels of MEK and ERK and total levels of phosphorylated states of MEK and ERK in V600E BRAF mutant, RAS mutant, and RAS/RAF wild type melanoma lines after

treatment with phenothiazines. Enhancing our knowledge of the phenothiazine anti-proliferative mechanism of action may prove useful for future anti-oncogenic BRAF drug development efforts.

Because phenothiazines are already FDA approved drugs they have been deemed safe to administer. Therefore, it would be possible to conduct a phase II trial with a standard dose of phenothiazine alone or in combination with a cytotoxic agent, dacarbazine, for the treatment of advanced melanoma. Given the precedent with sorafenib, it is quite likely that the *in vitro* selectivity of response in V600E BRAF mutant melanomas would not translate into a clinical predictor of response to phenothiazines. However, given the lack of any curative agents for melanoma, it may be worth pursuing the use of phenothiazines for the treatment of melanoma.