Methylation of the DPYD Promoter and Dihydropyrimidine Dehydrogenase Deficiency

To the Editor: In their important article, Ezzeldin et al. (1) report a positive association between dihydropyrimidine dehydrogenase (DPD) deficiency and the DPYD gene (DPYD) promoter hypermethylation in peripheral blood mononuclear cells of studied individuals. The authors focused on methylation in the individuals with the DPD deficiency, but did not elaborate on the impact of hypermethylation of the DPYD promoter region on the DPD activity across individuals. We have recently completed a study of methylation of the DPYD promoter in both colon tumor and nonmalignant tissues from a cohort of colorectal cancer patients, all of which were wild-type for known DPYD variants. We used pyrosequencing to evaluate the DPYD promoter methylation at positions −119 to −86, which contains six CpG loci. Our result showed that 10 out of 48 (21%) tumor DNA samples were methylated, and 4 of 48 (8%) normal DNA samples were methylated at all six CpG loci in the region of the DPYD promoter mentioned above. DPYD promoter methylation was a dichotomous event, with little variation between the six loci. In the same cohort of patients, the overall RNA expression was nearly 3-fold lower in tumors than normal tissues in our previous study (2). DPYD RNA expression was not different between the DPYD methylated and unmethylated samples for both tumor and normal tissues. The majority of tumors with low DPYD RNA expression had no DPYD promoter methylation. This suggests that the effect of DPYD promoter methylation on DPD activity could be tissue-specific. There has been evidence (3) that hypermethylation of gene promoter may be correlated with an increased, rather than a decreased level of expression in some of the genes, and methylation at different regions of the gene sequence may have variable effects on gene expression. Especially, methylation within the transcribed sequences may not suppress gene expression in most of the cases (4). A specific pattern of DNA methylation within the promoter region has a distinctive influence on gene expression and is tissue-specific (3). In fact, Ezzeldin et al. highlight that in addition to the methylation mechanism in the DPD deficiency, a variety of polymorphisms in DPYD play an important role in the 5-fluorouracil-induced toxicity (5). These important findings now need to be evaluated in patient cohorts to determine the clinical utility of DPYD methylation analysis.

In response: We appreciate the comments in the letter to the editor from Yu and McLeod. The authors are correct to point out that dihydropyrimidin dehydrogenase (DPD) activity varies according to tissue. Of note, the National Center for Biotechnology Information Expressed Sequence Tags online published expression levels of DPD in normal tissue vary greatly according to type of tissue, with ~3.5-fold higher DPD expression in blood compared to that of normal colon tissue.

In our study (1), we examined a 209-bp region (nucleotides +44 to −165 from tsp). This region includes two regulatory elements, which harbor potential transcription factor binding sites (e.g., Sp1), and has been shown to be associated with the regulation of DPD expression (2). In addition, 27 CpG sites were detected in this region, 11 of which lie within the two regulatory elements (1). Methylation and expression analysis of this region showed its significant effect on DPD expression in colorectal cancer cell lines and patients samples (1, 2).

In their letter to the editor, Yu and McLeod examined a small fragment (~86 to −119) which lies 5’ upstream of the two regulatory elements. Of interest, this fragment encompassed only six CpG sites, in a region lacking the presence of known regulatory elements with no evidence for direct association with the regulation of DPD expression (2). It is therefore not unexpected that the associated change, within this small fragment, would have no effect on DPD expression.

Lastly, we would note that the genotype-phenotype approach used in our study had the advantage of not only measuring the enzyme activity in peripheral blood mononuclear cells but also the phenotypic characterization of systemic DPD enzyme activity using the 13C-uracil breath test. We agree with the authors that future studies are needed to emphasize the role of methylation in predicting response to fluoropyrimidine treatments, specifically in patients with wild-type DPYD.

References
Methylation of the DPYD Promoter and Dihydropyrimidine Dehydrogenase Deficiency

Jinsheng Yu and Howard L. McLeod