Reviewer's report

Title: Quantitative Correlation between Promoter Methylation and Messenger RNA Levels of the Reduced Folate Carrier

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Reviewer: Jeffrey A Moscow

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This paper reports that the cell line M805 has evidence of promoter methylation of the RFC gene, and that this cell line, along with a previously reported cell line with RFC promoter methylation (MD-231), have lower RFC RNA levels than 6 cell lines that do not demonstrate RFC promoter methylation. Reversal of M805 methylation with azacytidine increased RFC RNA levels but only resulted in a marginal increase in MTX sensitivity; this is an important observation, but one in which the data was not shown, and not further explored. The conclusion is that promoter methylation 'is possibly a mechanism involved in the fine regulation of RFC transcription.'

An alternative interpretation of the data is that RFC promoter methylation status is not related to RFC gene expression or MTX sensitivity in 6 of the 8 cell lines examined. Data that would help establish the context of the reported observations include MTX sensitivity of all the cell lines, effect of azacytidine on all of the cell lines in regard to MTX sensitivity and uptake (to compare RFC methylated and unmethylated cell lines and to determine the effect of other genes involved in MTX sensitivity). Also, it would be of interest whether the recently described low-pH folate transporter, which is also an important regulator of MTX uptake, is expressed in these cell lines, and whether it is also regulated by methylation.

Since most cell lines do not show RFC methylation, and since demethylation of M805 did not result in increased MTX sensitivity, the relevance of the incrementally new findings reported in the paper is not established. If clinical samples showed promoter methylation, this would help establish some relevance of the observation. In this context, if demethylation does not change MTX sensitivity experimentally, this would be a relevant observation in that it would discourage clinical trials of demethylating agents in combination with methotrexate.