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## TFAP2E-DKK4 and Chemoresistance in Colorectal Cancer

**TO THE EDITOR:** Ebert et al. (Jan. 5 issue)<sup>1</sup> report that hypermethylation of the gene encoding transcription factor AP-2 epsilon (TFAP2E) reduces its expression and is associated with chemoresistance in patients with colorectal cancer and in colorectal-cancer cell lines in vitro.

The test has dramatic clinical value: the response rate increased from less than 15% among hypomethylated tumors to more than 80% among hypermethylated tumors. Methylation of this gene had a huge biologic and clinical effect.

However, we have several questions. First, the methylation status differs between primary tumors and metastases in approximately 30% of tumors: were these tumors considered to be hypermethylated or not?

Second, the design of in vitro tests is not consistent with clinical data: patients were treated with drug combinations or chemoradiation, and cells were exposed to single drugs.

The antiproliferative activity reported was limited, even if drug concentrations for irinotecan (20  $\mu\text{M}$ ), oxaliplatin (60  $\mu\text{M}$ ), and fluorouracil (382  $\mu\text{M}$ ) were very high and not comparable to clinical data.<sup>2,3</sup> Concerning the evaluation of methylation in vitro, drug combinations might confound the effects,<sup>4</sup> and it is unclear whether azacytidine or aza-2-deoxycytidine was used.

Finally, in vitro data show increased resistance only to fluorouracil, whereas clinical data suggest that alterations of *TFAP2E-DKK4* could represent a more global chemotherapy-resistance marker.

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No potential conflict of interest relevant to this letter was reported.

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**THE AUTHORS REPLY:** We are fully aware of the shortcomings and limitations of our study. Since most patients with colorectal cancer underwent combination therapy, we were not able to address the role of *TFAP2E* in fluorouracil monotherapy. Thus, based on our findings, we concluded that *TFAP2E* may present a more global resistance marker. For the in vitro studies, different agents, including azacytidine, were used; the role of drug combinations in the predictive value of *TFAP2E* was not addressed in our study. We agree that the methylation status differs in a subgroup of primary and metastatic lesions; however, no response data on the patient cohorts that were used to assess methylation in primary and metastatic lesions were available. We believe that our observations need to be prospectively evaluated in a clinical trial. So far, our study is a retrospective analysis with evidence from in vitro experiments that may indicate a role of *TFAP2E* in predicting treatment response.

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Since publication of their article, the authors report no further potential conflict of interest.