CORRESPONDENCE

Re: Synergistic Anticancer Effects of Ganciclovir/Thymidine Kinase and 5-Fluorocytosine/Cytosine Deaminase Gene Therapies

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Aghi et al. (1) recently reported that combining cytosine deaminase (CD) and herpes simplex virus type-1 thymidine kinase (HSV-TK) prodrug-activating gene therapies resulted in synergistic anticancer effects. The 9L/CD-TK cells used were generated by transfecting parental 9L rat gliosarcoma cells with three retroviral vectors (pTLKRL-1, pBABE-Puro, and pCD2) carrying the desired CD and HSV-TK genes. We feel it inappropriate to conclude the aforementioned synergistic effects by simply comparing the cytotoxic effects of the prodrug combinations between 9L and 9L/CD-TK cells. Some rational experiments, such as comparing the cytotoxic effects of the prodrug combinations in 9L/CD-TK and 9L cells with three control plasmids, should be included to ensure that the observed anticancer effects were indeed enhanced by the combined CD/HSV-TK treatment and not by other unexpected effects from plasmids or antibiotic stress during the process of clone selection. The authors speculated that these synergistic effects are due to enhancement of ganciclovir phosphorylation by 5-fluorouracil. To support their hypothesis, two direct approaches can be considered. First, the effect of 5-fluorouracil on the phosphorylation of ganciclovir in 9L/TK cells can be tested to clarify the cross-talk of the combined gene therapies. Second, the bystander effect can be analyzed by examining the toxicity of the prodrug combinations in control cells exposed to the conditioned medium from 9L/CD-TK cells treated with 5-fluorocytosine. Finally, the traditional combination effect analyses [the Loewe isobologram (2) and the Chou–Talalay multiple drug-effect method (3)] used by Aghi et al. may not be reliable enough to analyze quantitatively the interactions between two prodrug-activating systems. There are some genetic factors such as transgene expression that should be carefully put into consideration to design a proper cause–effect analysis for the combined gene therapies.

We disagree with the contention of Liao et al. that the use of parental 9L cells as a control for the combined prodrug therapy experiments is not appropriate. In light of experiments reported in previously published literature as well as the experiments that we describe at the end of the results section of page 328, the suggested 5-fluorouracil (5-FU) experiment on enhancement of GCV phosphorylation in 9L/TK and/or 9L/CD-TK appears superfluous, although it may provide additional confirmation of the veracity of the proposed mechanism. Prichard et al. [(1) and (28) of the article] and Harmenberg et al. [(2) and (29) of the article) showed that inhibitors of thymidylate synthetase (such as 5-FU) potentiated the antiviral effect of acyclovir by decreasing intracellular pools of thymidine. Additional work by Tattersall and Harrap [(3) and (32) of the article], Ives et al. [(4) and (33) of the article], and Chen et al. [(5) and (30) of the article) also shows that this process occurs by relief of the feedback inhibition of mammalian TK by deoxythymidine-5’-triphosphate and that thymidine is the primary inhibitor of GCV phosphorylation. On page 328, we mention that supplementation of thymidine completely abolished the increased phosphorylation observed by treatment of 9L/CD-TK cells with 5-FC. We are convinced that the latter experiment alone in conjunction with published literature supports the proposed mechanism of synergy. Nevertheless, we did perform the suggested experiment and, as expected, found that 5-FU alone did enhance the phosphorylation of GCV in 9L/CD-TK cells (Fig. 1).

3) The suggested bystander experiment would not be helpful because phosphorylated GCV (the active metabolite) does not act through conditioned medium (6).

4) The two analyses have been extensively employed in numerous publications, cited in the article. Furthermore, the finding of synergy by the employed analyses is supported/confirmed by the ganciclovir phosphorylation experiments of Fig. 7. We disagree that tradi-
tional combination analyses cannot be employed, because measurements were performed on a traditional pharmacologic variable (prodrug dose that produces an antiproliferative effect) in the context of a stably transfected (i.e., stably expressing) clonal cell line.

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References


Notes

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