Babij and colleagues report that STK33 is dispensable for KRASmut-dependent cancer cells (1). A finding in contrast to previous investigations (2–4). We believe the data do not support this conclusion and wish to bring selected methodologic issues to the authors’ attention. Because of space constraints, we have focused on the RNA interference (RNAi) experiments presented.

Transient transfection of siRNAs was used to target STK33 and KRAS in leukemia cell lines, but transfection efficiency was not reported. Leukemic cells are difficult to transfect, typically resulting in a large proportion of cells without the respective siRNA whose outgrowth may obscure the effects of gene knockdown, especially when inefficient siRNAs are used.

The siRNAs used by Babij and colleagues had inconsistent, context-dependent effects. For example, STK33_7Q, a weak siRNA, also suppressed KRAS and inhibited the growth of some KRASmut cell lines but not others. Furthermore, while all siRNAs targeting KRAS decreased KRAS mRNA levels, their phenotypic effects in KRASmut cell lines were highly variable. Surprisingly, these siRNAs had a strong antiproliferative effect in MDA-MB-231, even though this cell line was KRAS-independent in the screens by Babij and colleagues.

There is nominal overlap of two KRASmut and three KRASwt cell lines between the siRNA screens conducted by Babij and colleagues and our previous study that used a lentiviral short hairpin RNA (shRNA) library (2). Strikingly, the only two KRASmut lines included in both studies (PANC-1 and MDA-MB-231) were KRAS-independent in the screens by Babij and colleagues, whereas we observed substantial growth inhibition of PANC-1 and MDA-MB-231 following KRAS knockdown in multiple experimental systems. Given the apparent lack of KRAS essentiality in PANC-1, it is perplexing that these cells were chosen to evaluate the impact of kinase-deficient STK33.

To select "hits" from our shRNA screens, we applied relaxed criteria to guard against overlooking genes that, when suppressed, are not "strong killers" but nevertheless segregate with KRASmut dependence, such as STK33 (2). This strategy was influenced by our experience that the effects of KRAS and STK33 knockdown on adherent cells are weaker in standard tissue culture than in soft agar or immunocompromised mice. Because of the limited sensitivity of cellular assays "on plastic," we confirmed our findings in colony formation and xenotransplantation experiments, a strategy not used by Babij and colleagues. We concur that it is critical to guard against off-target effects in RNAi studies. This can be achieved by in vitro and in vivo validation of candidate genes.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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STK33 Kinase Is Not Essential in KRAS-Dependent Cells–Letter

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