Letters

COMMENT & RESPONSE

In Reply Maruvka et al analyzed the associations of *MUC16* mutation with tumor mutation load (TML) and survival outcome in the Cancer Genome Atlas gastric cohort by using the Curveball algorithm[1] to randomize the mutation matrix without altering the row and column totals. In our view, their analysis underestimated the variability of genetic mutation in the cohort. Their interpretations of the conclusions of our study[2] may have resulted from some misunderstanding.

We agree that most frequently mutated genes are associated with high TML. A higher TML in the tumor genome is largely reflected by frequent mutation in genes. However, the association of *MUC16* mutation with TML does not indicate that *MUC16* mutation is the driver of high TML. We did not make this claim in our article.

Maruvka et al used the Curveball algorithm to permutate the mutation matrix by fixing the total number of mutations in each patient and each gene. The Curveball algorithm, as described by Strona and colleagues,[1] was developed to randomize presence-absence matrices in numerical ecology by fixing row and column totals. Herein we demonstrate that the variability of the mutation matrix is likely to be underestimated when both margin totals are fixed. Let A and B denote 2 binary mutation matrices with identical dimension ordered by TML. The dissimilarity d of A and B was measured as 1 − sum(A and B) / sum($A|B$). For the binary mutation matrix of the Cancer Genome Atlas data set, we performed random permutation with the Curveball algorithm 1000 times and calculated the dissimilarity d between each permutated matrix and the original binary mutation matrix; the final dissimilarity vector was denoted as $d_{Curveball}$. In addition, we randomly sampled with replacement 2 matrices from the original binary mutation and calculated their dissimilarity. This procedure was also repeated 1000 times; we denoted the dissimilarity vector as $d_{sampling}$. The average value of $d_{Curveball}$ was significantly smaller than $d_{sampling}$ (t test, 0.83 vs 0.88; $P < .001$), which suggests that randomizing the binary mutation matrix with the row and column totals fixed cannot fully capture the variability of the original mutation matrix. Furthermore, the TML of a tumor also varies over the course of disease development and progression. Thus, application of the Curveball algorithm to examine the associations of gene mutation with TML and clinical phenotypes is rather conservative.

In our study,[1] the association of *MUC16* mutations with TML and improved survival in patients with gastric cancer was addressed for confounding factors in multivariate models including DNA mismatch repair signatures that can be used as surrogate variables for high TML. These associations were validated in the independent Asian cohort. The *CDH23* and *ANK1* genes mentioned by Maruvka et al were not associated with prognosis in the multivariate Cox analysis of the Asian cohort (hazard ratio [HR], 2.15; 95% CI, 0.55-8.30; $P = .27$ and HR, 2.15; 95% CI, 0.59-7.76; $P = .24$, respectively).

Different algorithms often give rise to different results. The association of *MUC16* mutations with high TML and improved survival obtained from traditional multivariate logistic and Cox models in our study should not be negated when the results were not significant based on the Curveball algorithm, especially when the application of this algorithm in such a circumstance still needs to be discussed. We would like to note that 2 recent studies reported the association of *MUC16* mutations with favorable prognosis in gastric cancer[3] and endometrial cancer by enhancing cytotoxic T-lymphocyte infiltration.[4]

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