Molecular pathology selected abstracts

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Evaluation of mismatch repair deficiency for eliciting tumor immunogenicity

December 2023—Immune checkpoint blockade therapy has dramatically altered treatment options for a variety of cancers. A high tumor mutation burden (TMB) is considered one of the strongest predictors of immune checkpoint blockade response. DNA mismatch repair deficiency (MMRd) is associated with a high TMB, and many tumors associated with MMRd have shown excellent response to immunotherapy. However, most MMRd tumors do not show durable response to treatment with immune checkpoint blockade (ICB). Intratumor heterogeneity may further mediate response to ICB therapy. Many of the mutations present in a tumor may be subclonal. The resulting subclonal neoantigens may not produce sufficient T-cell responses and targeting them may have minimal therapeutic impact. To investigate this hypothesis, the authors used an autochthonous mouse model of MMRd lung and colon cancer and whole exome sequencing of tumors to confirm the mutation of MMR genes and evaluate TMB. They estimated cancer cell fractions and performed whole exome sequencing on single-cell clones to assess the clonal composition of mutations. The tumors showed significant intratumor heterogeneity with a high burden of subclonal mutations. They did not show increased baseline immunogenicity or response to ICB. Most mutations were present in less than a quarter of cells, indicating that TMB was predominantly subclonal. The authors showed that T-cell depletion had no significant overall effect on TMB or tumor neoantigen burden (TNB). However, clonal, but not subclonal, TMB and TNB increased significantly. The authors also isolated clonal cell lines and transplanted them into other mice. They found that the tumors were highly immunogenic when retransplanted at clonal, but not subclonal, levels. These findings suggest that immunosurveillance may facilitate intratumor heterogeneity and shape clonal architecture, with the immune response targeting a subset of dominant neoantigens and failing to delete other neoantigens. The authors also reanalyzed sequencing data from two clinical trials of anti-PD-1 treatment in advanced MMRd colorectal cancer and gastric cancer. Clonal, but not subclonal, TNB was significantly associated with objective response and longer progression-free survival. A high intratumor heterogeneity index (subclonal to clonal neoantigen ratio) was significantly associated with nonresponse and shorter progression-free survival. Although total TNB was also associated with objective response, that may be because the tumors in these studies tended to have more clonal rather than subclonal neoantigens. The study supports a link between intratumor heterogeneity and the lack of durable response to ICB in some MMRd cancers and raises important questions about the interplay between tumor neoantigens and the immune system. Larger prospective clinical studies would be needed to better understand the role of intratumor heterogeneity and its potential utility as a biomarker of ICB response in MMRd cancers.

Westcott PMK, Muyas F, Hauck H, et al. Mismatch repair deficiency is not sufficient to elicit tumor immunogenicity. *Nat Genet*. 2023;55(10):1686–1695.

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Accuracy of a machine-learning model for predicting the effects of proteome-wide missense variants

Genetic sequencing has shed light on the pathophysiology, diagnosis, and treatment of many inherited and acquired human diseases. However, a significant amount of naturally occurring genetic variation—that is, germline

polymorphisms—is present in the human population. How a genetic variant impacts protein function is not always clear. Only an estimated two percent of missense variants have been clinically classified as pathogenic or benign. Consequently, the vast majority remain variants of uncertain significance. Machine learning may be useful in classifying these variants as it leverages patterns in biological data to predict the pathogenicity of unannotated variants. The authors (employees of Google DeepMind) developed AlphaMissense, an adaptation of DeepMind's AlphaFold artificial intelligence system for predicting protein structure from protein sequence, to improve predictions of the pathogenicity of missense variants. Like AlphaFold, AlphaMissense was trained to perform single-chain structure prediction and protein language modeling using a reference sequence, training variant samples, and multiple sequence alignments. The authors fine-tuned the model using population-frequency data. Unlike many prediction models, AlphaMissense was not trained directly on clinical databases, thereby avoiding inherited biases from human curators and prior in silico models. The authors tested the model against multiple benchmarks, including ClinVar variants, the Deciphering Developmental Disorders cohort, and a selection of clinically actionable genes from the American College of Medical Genetics. Using the area under the receiver operator curve as a metric of performance, AlphaMissense outperformed other in silico models, ranking consistently high across curated clinical benchmarks. The authors also used AlphaMissense to generate predictions for missense variants across the human proteome, producing 71 million missense variant predictions. Calibrated prediction scores were used to classify variants as likely pathogenic, likely benign, and ambiguous. The proportion of ClinVar variants classified with 90 percent precision was increased to 92.9 percent using AlphaMissense from 67.1 percent using the well-performing unsupervised evolutionary model of variant effect (EVE). The authors have provided the research community with a database of AlphaMissense predictions for all possible single amino acid substitutions in the human proteome. Data on the performance of AlphaMissense and the potential applications of the database of predictions are promising. Researchers could use predictions to prioritize missense variants for further investigation. While AlphaMissense may prove to be a useful tool for classifying missense variants, in silico predictions are only one of many lines of evidence used when clinically classifying variants according to American College of Medical Genetics/Association for Molecular Pathology guidelines.

Cheng J, Novati G, Pan J, et al. Accurate proteome-wide missense variant effect prediction with AlphaMissense. *Science*. 2023. doi:10.1126/science.adg7492

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