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A novel approach to study the effects of mutations on melanoma phenotype

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Melanoma is one of the most highly mutated malignancies, with a frequency of mutations ranging from 0.1–100/Mb. Although the major cancer drivers have been identified, the effects of a single mutation or distinct combinations of mutations on melanoma development and progression are not well understood. A study in *Science* sequentially introduced mutations commonly associated with melanoma into human melanocytes to produce a series of genetically distinct cell models. In-depth phenotypic characterization of these melanoma models in vitro and in vivo after xenograft injection in mice revealed the effects of different mutations on tumor and microenvironment.

The investigators used a stepwise CRISPR/Cas9-based gene editing approach to introduce mutations into the retinoblastoma tumor suppressor (RB) pathway (*CDKN2A, "C"*), then the mitogen-activated protein kinase (MAPK) pathway (*BRAF, "B"*), and then the telomerase regulation pathway (*TERT, "T"*), resulting in the creation of C, CB and CBT cell models respectively. In the immortalized CBT cells, the team further introduced mutations in *PTEN* ("*P*"), *TP53* ("*3*"), or *APC* ("*A*") to create the CBTP, CBT3 and CBTA respectively; and then the CBTP3 and CBTPA models carrying 5 mutations each.

Single-cell RNA sequencing (scRNA-seq) analysis of the different cell models revealed progressive changes in gene expression as melanocytes acquired mutations. "These results demonstrate that melanoma-associated mutation combinations activate and repress specific expression programs that are shared across genotypes and, in some cases, help explain the overall cellular phenotypes," write the investigators in their report.

Next the researchers injected the immortalized cell models into the dermis of immunodeficient mice to characterize the disease phenotypes caused by the mutations in vivo. CBT melanocytes were malignant and formed slow-growing tumors in the mice, while quadruple and quintuple-mutant melanoma models grew faster and resembled patient melanomas. A fifth mutation notably led to aggressive melanocytic disease. ScRNA-seq analysis of the *in vivo* tumors showed that tumor genotypes also shaped the composition and expression state of infiltrating stromal and immune cells, including neutrophils.

Altogether these new models of melanoma established a causal relationships between mutations and tumor phenotypes. "The same editing approach can be applied to generate human models of uveal, acral, and mucosal melanomas, diseases for which precise cellular models are still lacking and targeted therapies are still not available," conclude the investigators.

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