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Novel *SDHA*-knockout cell line enables sensitive functional characterization of *SDHA* VUS

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Abstract

Genetic inactivation of *SDHA* can lead to succinate dehydrogenase (SDH)-deficiency and is implicated in a variety of human diseases. In particular, germline loss-of-function (LOF) mutations in *SDHA* increase the lifetime risk for developing a number of SDH-deficient cancers in heterozygous carriers. Currently, there are no highly effective medical therapies for unresectable or metastatic SDH-deficient cancer, but most are curable if resected at an early stage; therefore, early detection is critical. However, it has been difficult to determine which variants disrupt enzyme activity and actually confer cancer risk; over 700 *SDHA* missense variants have been reported in ClinVar, yet 97% are considered variants of uncertain significance (VUS) due to insufficient functional evidence. Carriers of *SDHA* VUS cannot be recommended enhanced clinical surveillance or genetic counseling, leaving those at risk unlikely to benefit from early detection. It is clear we are in great need of a model that enables the functional characterization of *SDHA* VUS, allowing us to identify which patients are truly at risk and should receive further screening.

To this end, we used CRISPR-Cas9 technology to create a stable *SDHA*-knockout in the HAP1 cell line. Biochemical analysis revealed complete loss of SDH-activity, allowing us to express different *SDHA* VUS via lentiviral transduction and interrogate their functional consequences with high sensitivity. As expected, known benign variants displayed SDH-activity similar to that of wild-type SDHA, whereas known tumor-associated variants failed to restore activity to a detectable level. Due to the ability to discriminate tumor-associated variants from benign variants, we functionally profiled over 20 *SDHA* VUS, many of which failed to restore any SDH-activity. The functional data obtained from this model can be used as strong evidence for clinical reclassification of *SDHA* VUS, leading to the discovery of novel pathogenic *SDHA* variants and aid in identifying those at-risk for developing SDH-deficient cancers.