Abstract

Isocitrate dehydrogenase (IDH) is a metabolic enzyme that converts isocitrate to α-ketoglutarate (α-KG). Mutations in this enzyme have been linked to human cancers such as glioma and acute myeloid leukemia (AML). While there are three isoforms of IDH, mutations that lead to cancer have only been identified in IDH1 and IDH2, which result in simultaneous loss of normal catalytic activity, the production of α-KG, and the gain of a new function—the production of 2-hydroxyglutarate (D-2HG). D-2HG is structurally similar to α-KG and acts as an α-KG analogue, competitively inhibiting multiple α-KG-dependent enzymes, including pyruvate dehydrogenase (PDH) and DNA repair mechanisms (TP53), causing widespread changes in histone and DNA methylation and potentially promoting tumorigenesis. A number of studies from the mutant IDH initiators indicate that IDH is a valid target for a new class of cancer therapeutics.

The most prominent IDH1 mutation takes place at residue R132H and plays a role in the development of gliomas, while the majority of IDH2 mutations take place at residue R140Q, which is linked to AML. Given the presence of these mutations, we sought to use CRISPR/Cas9 gene-editing technology to create two in vitro disease models that either harbour either the IDH1 or IDH2 mutations. An IDH1R132H mutation was introduced in the malignant glioblastoma U-87 MG (ATCC® HTB-14™) cell line, and an IDH2R140Q mutation was introduced in the TF-1 (ATCC® CRL-2000™) erythroleukemia cell line that was derived from an AML patient. To validate that the isogenic IDH mutations confer gain-of-function mutations, an IDH1R132H G-to-A mutation was introduced in parental U-87 MG cells and potential IDH1R132H clones were separated on agarose gels. As expected, Sanger sequencing data for parental U-87 MG cells and IDH1R132H knock-in cells showed the successful knock-in of IDH1R132H p.R132H (c.400G>A).

Materials and Methods

II. Identification of IDH1R132H and IDH2R140Q Alleles in the Genome

Figure 1. Enzymatic activities of wild-type (WT) and mutated IDH enzymes. WT IDH enzymes catalyze the production of α-KG and produce NADH, whereas the mutated IDH enzymes catalyze the production of α-KG and α-KG-dependent enzymes, resulting in the accumulation of D-2HG.

Figure 2. Gene-editing knock-in strategy for the generation of the IDH1R132H allele in U-87 MG cells. (A) IDH1R132H allele in TF-1 cells (B). U-87 MG and TF-1 parental cells were transfected with respective gRNA and donor, allowing for Cas9 expressing plasmid. Transfected cells were then subjected to transient antibiotic selection and the surviving pooled cells were used for single cell sorting. The expanded single cell clones were then subjected to genotyping to identify clones harbouring the desired knock-in mutations.

Figure 4. Confirmation of the IDH1R132H knock-in allele in U-87 MG cells and the IDH2R140Q knock-in allele in TF-1 cells. (A-D) Diagram of IDH1 gFwd and IDH1 gRev primers used in this study. (B) PCR products from the IDH1 parental U-87 MG cells, IDH1R132H U-87 MG cells, parental TF-1 cells, and IDH2R140Q TF-1 cells were separated on agarose gels. (C) DNA amplification Sanger sequencing data for parental U-87 MG cells and IDH1R132H U-87 MG cells showed the successful knock-in of IDH1R132H p.R132H (c.400G>A) in the genomic DNA of U-87 MG cells. (D) Sanger sequencing data for parental TF-1 cells and IDH2R140Q TF-1 cells showed the successful knock-in of IDH2R140Q p.R140Q (c.419G>A).

Figure 5. Morphology of IDH1R132H mutant U-87 MG cells and IDH2R140Q mutant TF-1 cells. (A) IDH1R132H U-87 MG cells displayed morphological similarity to parental U-87 MG cells. (B) Suspension culture of parental TF-1 cells and IDH2R140Q TF-1 cells in complete culture media. (C) After removing the suspension cells, a larger population of IDH2R140Q TF-1 cells attached to the tissue culture plate and exhibited spindle-like morphology (unmodified neomorphic-like cells) when compared to parental TF-1 cells.