Characterization of cancer-associated IDH mutations in GeneArt Engineered Cell Models

Introduction

The enzyme isocitrate dehydrogenase (IDH) metabolizes isocitrate to α-ketoglutarate (α-KG). Mutations in two genes, *IDH1* and *IDH2*, are associated with a number of different cancers, including glioma, prostate, colon, and acute myeloid leukemia (AML).

Mutations in the *IDH1* gene result in an enzyme that converts α-KG to 2-hydroxyglutarate (2-HG) [1]. The function of 2-HG has not yet been fully elucidated, but it has been hypothesized that it could contribute to alteration of metabolic pathways within cancer cells [2]. The most common adaptation is a switch to consuming glucose at an elevated rate, even in the presence of oxygen (the Warburg effect) [3]. This elevated glucose consumption requires an increased rate of glucose uptake and, as such, an increased expression of the key glucose transporter GLUT1 has been demonstrated in cancer cells [4].

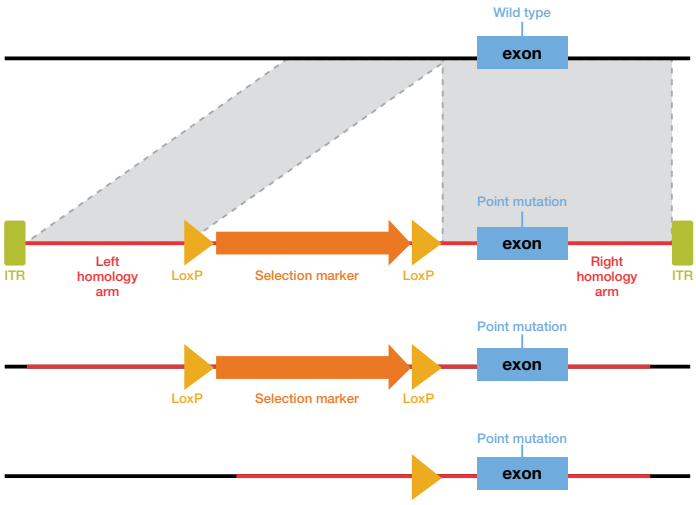

Figure 1. Targeting strategy for the insertion of a point mutation.

In this application note, we describe the use of a panel of Invitrogen™ GeneArt™ Engineered Cell Models with *IDH1* and *IDH2* mutations in the HCT116 colon cancer cell line to investigate how introduction of IDH mutations could affect the generation of 2-HG and the regulation of GLUT1.

Gene engineering using recombinant adeno-associated virus (rAAV) vectors can be used to generate cell lines with defined genetic alterations (Figure 1). The impact of the mutational changes can be assessed by comparing the genetically altered cell lines with unmutated parental control cell lines. This provides a valuable tool for validating therapeutic targets.

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Results and discussion

Genotyping of the IDH1-mutant cell lines

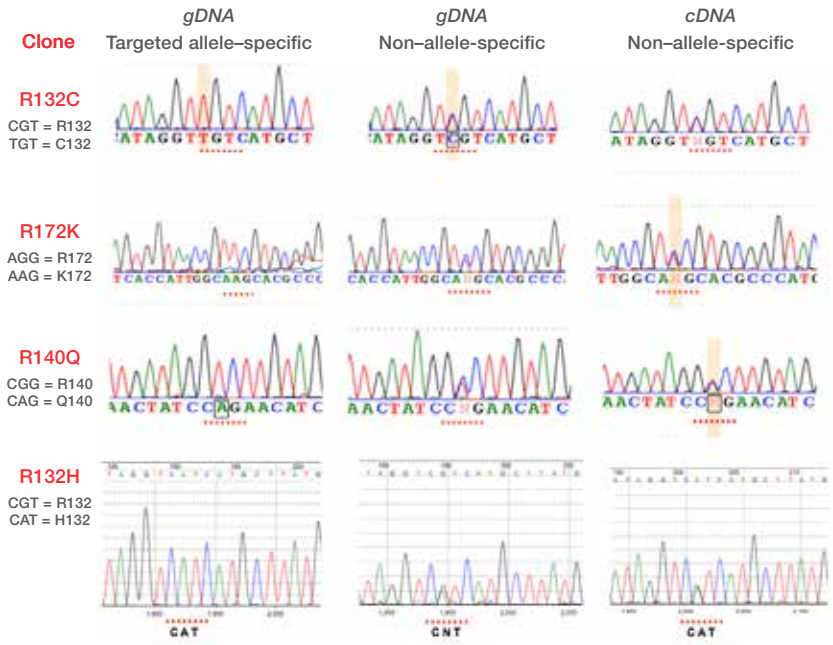
The IDH gene mutations were confirmed by Sanger sequencing of PCR products amplified from the engineered mutant cell lines. In all cases, sequencing that targeted the specific mutations confirmed the presence of the engineered mutation, and non–allele-specific sequencing confirmed each mutant allele and its wild type counterpart to be present in a 1:1 ratio as expected for a heterozygous locus in a diploid (2n) genome. Sequencing of an RT-PCR (cDNA analysis) product also confirmed that the engineered mutations were expressed as RNA transcripts (Figure 2).

Screening for the biomarker 2-HG in IDH-mutant lines

The IDH-mutant cell lines were characterized by LC-MS for production of 2-HG. The IDH mutant cell lines all exhibited higher levels of intracellular 2-HG than the parental line, in accordance with published data [1]. Interestingly, while IDH2 R140Q cells still produced more 2-HG than parental cells, they produced markedly less 2-HG than the other IDH-mutant lines (Figure 3).

Characterization of IDH1 R132H

Further characterization of the effects of introducing an IDH mutation was then carried out using the IDH1 R132H cell line. First, the level of mutant IDH1 expressed was assessed by western blot using an antibody specific to the IDH1 R132H mutation. Additionally, the expression of the key glucose transporter GLUT1 was investigated. Increased expression of GLUT1 was observed in cells containing the R132H mutation, suggesting that the introduction of an IDH1 mutation results in altered cellular metabolism (Figure 4).



1:1 ratio of mutant:wild type peaks at the gDNA level, as would be expected for a 2n locus, indicating true clonality.

Expression analysis. The ratio of mutant:wild type is at a ratio of 1:1 as expected for a 2n locus.

Figure 2. Genomic validation of targeted cell lines.

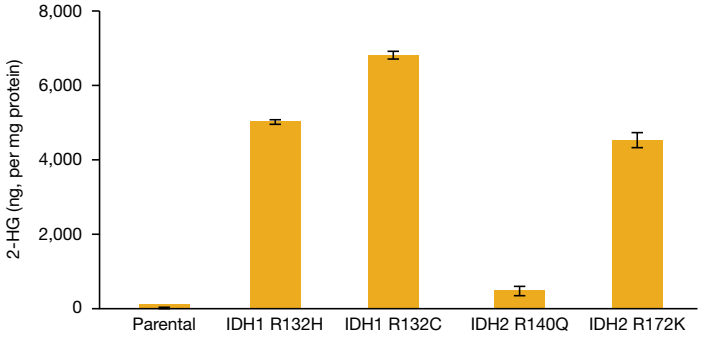


Figure 3. Intracellular levels of 2-HG measured in HCT116 isogenic cells by LC-MS.

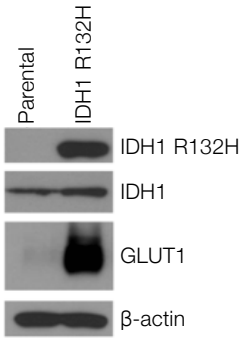


Figure 4. HCT116 cells analyzed by western blot. An antibody specific to IDH1 R132H was used to confirm expression of the mutant IDH1 protein. Expression of GLUT1 was also investigated.

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Conclusion

By using an isogenic cell line system, it has been possible to demonstrate that IDH mutations confer a gain-of-function phenotype, resulting in increased 2-HG production. Further characterization of the IDH1 R132H cell line additionally revealed that introduction of the mutation led to increased expression of the key glucose transporter GLUT1.

References

1. Dang L, White DW, Gross S et al. (2009) Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature* 462(7274):739–744.
2. Borodovsky A, Seltzer MJ, Riggins GJ et al. (2012) Altered cancer cell metabolism in gliomas with mutant IDH1 or IDH2. *Curr Opin Oncol* 24(1):83–89.
3. Cairns RA, Harris IS, Mak TW et al. (2011) Regulation of cancer cell metabolism. *Nat Rev Cancer* 11(2):85–95.
4. Ganapathy V, Thangaraju M, Prasad PD (2009) Nutrient transporters in cancer: relevance to Warburg hypothesis and beyond. *Pharmacol Ther* 121(1):29–40.

Ordering information

Cell line	Gene	Genotype	Cell ID No.
HCT116	*IDH1*	IDH1 (R132H/+)	HD 104-013
HCT116	*IDH1*	IDH1 (R132C/+)	HD 104-021
HCT116	*IDH2*	IDH2 (R140Q/+)	HD 104-020
HCT116	*IDH2*	IDH2 (R172K/+)	HD 104-020

Related products

Product	Quantity	Cat. No.
Gibco™ RPMI 1640 Medium	500 mL	11875-093*
Gibco™ Fetal Bovine Serum	500 mL	10270106*
Gibco™ DMEM/F-12 Medium	500 mL	11330-032*
Gibco™ Horse Serum, heat inactivated	500 mL	26050088*
AlamarBlue™ Cell Viability Reagent	100 mL	DAL1100

*For *In Vitro* Use Only.