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Advances in Brain Cancer: Creating Monoallelic Single Point Mutation in IDH1 by Single Base Editing

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Abstract

Mutations in the Isocitrate Dehydrogenase 1 (IDH1) gene occur in 70% of grade II and grade III gliomas, 10% of acute myeloid leukemia, as well as cholangiocarcinomas, melanomas, and chondrosarcomas. Numerous mechanisms have been proposed to illustrate the biological function of mutant IDH1. Most functional studies of mutant IDH1 have been conducted in exogenous overexpression systems with the IDH1 wild type background. This mini-review comments on recent publication by Wei et al, in which a highly efficient “single base editing” approach was employed to generate monoallelic IDH1 R132H mutation without the induction of a double strand break in the IDH1 gene.

Keywords

Glioma; Heterozygous IDH1 R132H Mutation; Single Base Editing; Yes-Associated Protein (YAP)

3. Introduction

Gliomas are the most prevalent type of tumors of the central nervous systems, accounting for up to 30% of all primary lesions and nearly 80% of all malignant forms [1,2]. Given their anatomical localization and locally infiltrative nature, these tumors are associated with high morbidity and mortality. Despite radical surgical resection coupled with chemo- and radiotherapy, these tumors often recur, leading to a dismal overall prognosis. With an approximate incident rate of 6.6 per 100,000 individuals annually in the USA, these malignancies result in a majority of deaths from primary brain tumors. Historically, gliomas have been classified based on their histological features and graded by their degree of

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4. Compliance with Ethical Standards

4.2. **Conflict of Interest:** The authors declare no conflict of interest.

4.3. **Ethical approval:** This article does not contain any studies with human participants or animals performed by any of the authors.

anaplasia according to WHO criteria, serving as a “gold standard” for decades. However, in the case of low grade gliomas and particularly diffusely infiltrative gliomas, these methods are subject to intra observer variability. Thus, with the advent of molecular profiling, these tumors have now been further interrogated to identify diagnostically relevant alterations, including genomic, transcriptomic, and epigenetic variants, complementing the histological-based classification system [3–5].

3.1. IDH1 Mutation in Glioma

The recent identification of frequent mutations in the metabolic gene Isocitrate Dehydrogenase (IDH) 1 and 2 suggests the existence of different molecular subclasses of diffusely infiltrative gliomas with distinct biological and clinical attributes, prompting the WHO to propose revised classification guidelines [6]. Originally discovered in 2008 [7], it is now appreciated that 70–80% of grade II/III and 20% grade IV gliomas harbor mutations in IDH1; and that these alterations frequently coexist with TP53, ATRX mutations, and co-deletions of chromosome 1p and 19q [8,9]. Prior studies have identified mutations in IDH1 as one of the earliest events in gliomagenesis, possibly playing a significant role during tumor initiation and subsequent transformation [9–11]. The majority of IDH1 mutants contain heterozygous single amino acid missense mutation in its active site at arginine 132, altering its enzymatic activity that results in the neomorphic production of the oncometabolite 2-Hydroxyglutarate (2-HG) using α-ketoglutarate (α-KG) [12]. This aberrant production of 2-HG in turn inhibits α-KG-dependent dioxygenases, including histone demethylases and DNA demethylase Ten-Eleven Translocation 2 (TET 2) [13–15]. Consequently, IDH mutation is associated with global changes in DNA and histone methylation patterns as indicated by widespread hypermethylation of CpG islands [16]. Clinically, mutations in IDH1 prolong survival of glioma patients [8]. Given the pronounced frequency of IDH1 mutation in gliomas coupled with its impact on the biology and clinical progression of the disease, it is vital to further delineate the role of monoallelic IDH1 point mutations in gliomas.

3.2. Current Models for Mutant IDH1

While previous studies have investigated the biological function of mutant IDH1 in the context of tumorigenesis and tumor progression, these studies are often limited by the paucity of appropriate endogenous mutant IDH1 systems [17,18]. For instance, most prior studies have relied on the use of overexpression systems, which do not necessarily recapitulate the naturally occurring heterozygous IDH1 mutational status in this cancer [17]. Moreover, the underlying wild type IDH1 background in these exogenously overexpressing IDH1 mutant clonal cells may obscure the true biological and clinical impact of IDH1 mutation in this cancer. Although techniques to establish primary cultures carrying monoallelic IDH1 mutants from human tumor samples has been improved, it remains difficult to generate isogenic cellular models to study the function of mutant IDH1, especially during tumorigenesis [19]. Likewise, while orthotopic xenograft models are available, their utility is often limited [20]. Thus, it is important to establish clinically relevant cellular models that recapitulate the parental disease to methodically characterize the role of IDH1 mutation in this cancer. Such clinically representative *in vitro* disease

models will enable systematic delineation of the molecular network driven by mutated IDH1, a prerequisite for effective therapeutic design.

3.3. An Efficient Approach to Create Heterozygous IDH1 R132H Mutation

To this end, we recently demonstrated the use of “Single base editing” method to generate isogenic cellular models carrying monoallelic IDH1 mutants [21]. Using a recently reported CRISPR-Cas9 technology which functions without the induction of a double strand break in IDH1 [22], we precisely introduced heterozygous IDH1 R132H point mutation in human astroglial cells with a successful rate of 20%. Compared with other nuclease and homology directed repair-based knock-in methods used to date [23–25], our work provides an efficient and easy approach to generate monoallelic IDH1 R132H mutation, and can be valuable to others in the field searching for models of endogenous heterozygous IDH1 mutation.

The monoallelic IDH1 mutants in our model displayed global alterations in DNA methylation and gene expression pattern coupled with dramatic changes in cellular behavior including decreased cell proliferation. Notably, we uncovered a previously unknown link between expression of YAP, an effector of the pro-growth Hippo pathway, and IDH1 mutation status (Figure 1). Specifically, our work revealed a Hippo-independent, 2-HG-dependent regulation of YAP expression in these monoallelic IDH1 mutant astroglial clones. The Hippo-YAP pathway has emerged as a critical network driving tumor growth and progression [26–28]. Thus, it is of interest to identify potent regulators of YAP and their role in cancer development. Our study suggests that YAP is responsive to changes in metabolic state, highlighting the intimate relationship between proto-oncogenes and cellular metabolism. While further mechanistic investigation is warranted to precisely elucidate the biological implication of YAP inhibition by IDH1 mutation, this study lays the groundwork in establishing a novel connection between oncometabolite production and activity of pro-growth signaling network in early disease development. Overall, this versatile and efficient “Single base gene editing” technique will permit thorough interrogation of the biological function of heterozygous IDH1 mutants in the context of glioma development and progression, and serve as a valuable model to test effective therapies for the management and treatment of gliomas.

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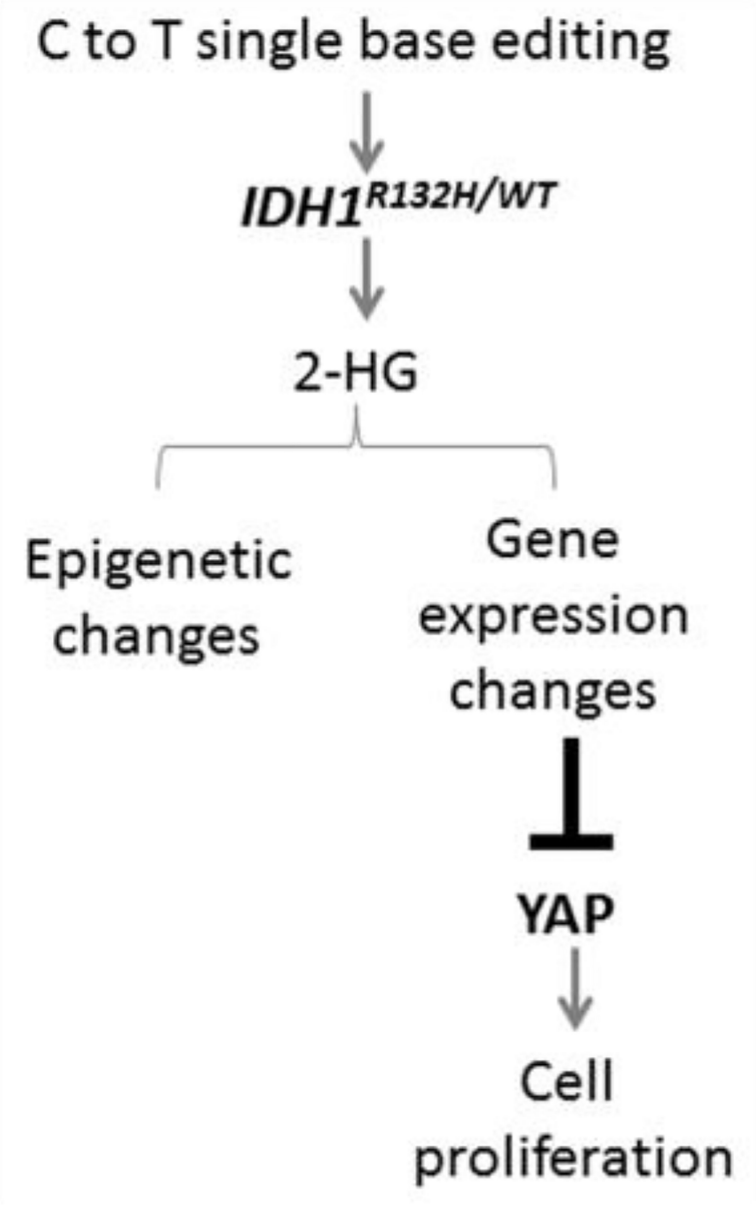

Figure 1:
Schematic of the mechanistic details and functional effects of mutant IDH1-YAP signaling.