Editorial

Transgenic mouse models of *Idh*-mutated neural stem cells: an appropriate model for low grade glioma?

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Introduction

The large majority of diffuse gliomas, classified by World Health Organization (WHO) guidelines as grade II and III, and the grade IV gliomas that have progressed from these lower grade cancers (the so-called secondary glioblastomas) carry heterozygous hotspot mutations in *IDH1* or *IDH2*, the genes encoding the metabolic enzymes isocitrate dehydrogenase 1 (IDH1, cytosolic/peroxisomal) or IDH2 (the mitochondrial variant) (1,2). Besides glioma, the occurrence of these mutations is limited to a small number of cancer types, including acute myeloid leukemia (AML), enchondromas, chondrosarcoma and hepatic cholangiocarcinoma (3).

Since the discovery of *IDH1/2* hotspot mutations in gliomas in 2008 (4) a lot of information has become available on the biology of *IDH*-mutated cancer cells and it is commonly accepted now that these mutations drive carcinogenesis. The molecular events that underly gliomagenesis are however complex and not well understood. There is an interesting ambiguity with these mutations: although they cause glioma, *IDH1* mutations (especially when combined with loss of chromosome arms 1p and 19q in oligodendrogliomas) also confer the best prognosis to glioma patients (5).

Isocitrate dehydrogenase (IDH)

IDH1 converts isocitrate to alpha-ketoglutarate (αKG) in cytosol and peroxisomes, simultaneously reducing $NADP^+$ to NADPH. Especially in brain this reaction is important because NADPH facilitates the synthesis of fatty acids and cholesterol, comprising 50% of the brains' dry weight. In parallel, NADPH ensures redox homeostasis by generating reduced glutathione and protecting against oxidative stress. In IDH wild-type glioblastoma, IDH1 is estimated to be responsible for the production of 65% of total NADPH (6). Next to NADPH, αKG is also an important metabolite with many functions. It is a substrate in the mitochondrial tricarboxylic acid cycle and under hypoxic conditions can be converted back to isocitrate in a reverse IDH reaction (7), isocitrate being a substrate for sequential citrate, acetyl-CoA and fatty acid synthesis. Another important function of αKG is its role as cofactor for enzymes that regulate epigenetic events, such as DNA demethylation [the Ten-Eleven-Translocation (TET) family of 5-methylcytosine hydroxylases] (8) and histone demethylation (the Jumonji-C domain containing family of proteins) (9,10).

IDH mutations

Rare loss-of-function-only mutations in IDH1 have been described (11), but the large majority of mutations occur at a hotspot involving arginine 132 (R132) and result in loss of normal and gain of novel function. The mutant protein subunits have lost affinity for isocitrate, but instead use αKG as substrate and convert it to D-2-hydroxyglutarate (D-2-HG). Although these metabolites differ only in one group (a ketone in αKG, a hydroxyl in D-2-HG), this conversion has huge consequences. D-2-HG dehydrogenase (D-2-HGDH, the enzyme that converts D-2-HG back to αKG)

is scarce, allowing D-2-HG to accumulate to millimolar concentrations. At these concentrations D-2-HG competes with αKG as cofactor for TET enzymes, resulting in the glioma CpG-island methylator phenotype (G-CIMP). Similarly, histone hypermethylation occurs (12). Together this results in epigenetic alterations and transcriptome profiles that favour dedifferentiation.

This reaction of αKG to D-2-HG oxidizes NADPH to $NADP^+$. In light of the important roles of αKG and NADPH in metabolism, and their excessive consumption in *IDH*-mutated cancers, it is no surprise that *IDH*-mutant gliomas suffer from metabolic stress, and it has been postulated that this is one of the reasons why patients with *IDH*-mutated gliomas have the better prognosis and respond better to radiotherapy (2,13). Metabolic stress may however also explain why reliable research models of IDH mutated gliomas are scarce: whereas there is a high number of patient-derived *IDHwt* glioma cell lines and xenograft models available worldwide, common experience is that it is very difficult to generate such models from *IDH*-mutated gliomas (14). Only few cancer models with the endogenous mutation have been reported, mostly orthotopic xenograft models (15-17).

Models of IDH-mutated glioma

In the absence of available alternatives, experimental models are often used in which mutant IDH is overexpressed in cell lines with a wild-type IDH background. It is conceivable that these models are not appropriate for the following reasons:

(I) IDH mutations are without exception heterozygous, and the stoichiometry of IDH wild-type and mutant subunits is possibly important for a balanced production of αKG (by the wild-type subunits) as substrate for D-2-HG production (by the mutant-subunits).

(II) IDH-mutated gliomas have adapted their metabolism to accommodate growth (18). One of these adaptations involves epigenetic silencing of the gene encoding branched chain amino acid aminotransferase (*BCAT1*), that converts αKG to glutamate (19) resulting in low glutamate levels in $IDH1^{R132H}$ models (20). It was previously postulated that this may relate to increased consumption of glutamate, a ubiquitous neurotransmitter in the brain, to generate αKG (21).

Expression of recombinant $IDH1^{R132H}$ in cell lines without exception results in production of *D*-2-HG, but how long it takes to have full penetrance of D-2-HG effects in the epigenome (i.e., G-CIMP) is difficult to estimate. This raises questions on the physiological relevance of overexpressing mutant IDH1 in cells that are not adapted to conditions of αKG- and NADPH shortage. Therefore, there is an ongoing search for cancer models that faithfully recapitulate IDH-mutant low grade gliomas.

The prevailing view of glioma development is that these cancers originate from neural stem cells (NSC) in the subventricular zone, giving rise to growth-deregulated glioma stem-like cells that sustain tumour growth. To reflect a situation of IDH1 mutations occurring in NSCs, Bardella and colleagues created a transgenic mouse model that builds upon this concept (22). They crossed mice expressing Cre-recombinase under control of the Nestin promoter (active in NSCs from day 10.5) with mice carrying a floxed $IDH1^{wt}$ minigene, followed by an $IDH1^{R132H}$ gene. Cre-mediated recombination in progeny mice results in selective expression of $IDH1^{R132H}$ in NSCs. A similar approach has been used before and resulted in perinatal lethality due to haemorrhages, presumably because D-2-HG interferes with maturation of vessel wall collagens (23).

In line with this previous report, Bardella *et al*. also observed perinatal lethality as a result of brain haemorrhages (22). To study the effects of IDH1 mutations on NSC behaviour in adult animals, authors elegantly introduced tamoxifen-based control on Cre expression in NSC, by crossing the mice carrying the floxed $IDH1^{R132H}$ construct with mice carrying a tamoxifen-inducible nestin promoter-Cre construct. When offspring animals were fed tamoxifen at age of 5-8 weeks, Cre-mediated $IDH1^{R132H}$ knock-in in NSCs was tolerated, allowing investigation of the early effects of mutant IDH1 expression in these stem cells. This shows that the developmental stage at which a somatic IDH mutation is acquired is an important factor in these models, possibly because of D-2-HG toxicity in the developing brain. Offspring mice with induced $IDH1^{R132H}$ expression in their NSCs during adulthood ultimately died because of ventricle dilatation and hydrocephalus.

As expected, D-2-HG levels were significantly elevated in brains of offspring mice after tamoxifen treatment. The $IDH1^{R132H}$ knock-in NSCs in the subventricular zone displayed high migratory behaviour and increased proliferation as compared to wild type IDH counterparts, suggesting increased neurogenesis. Based on the expression of neuronal and glial lineage markers NeuN, Dcx,

GFAP and Olig2, authors concluded that in the NSC compartment, differentiation capacity was not markedly affected. This is an interesting finding because consensus has been till now that genome hypermethylation, which was also demonstrated in tamoxifen-treated animals, is responsible for an aberrant differentiation state which may contribute to tumorigenesis. To what extent the hypermethylation in the model was genome-wide, or was confined to CpG islands, is however an open question.

The authors find a number of similarities between IDH1^{R132H} NSCs and *IDH*- mutated glioma (CpG island hypermethylator phenotype, expression of *Wnt*- and *MYC* target genes and features of the proneural glioma phenotype(24), increased infiltration in the brain parenchyma and increased proliferation of IDH1^{R132H} NSCs). Whereas Sasaki *et al*. (23) suggested that D-2-HG stabilizes hypoxia-inducible factor and induce a pseudohypoxic response, the study of Bardella did not show such an effect. Of note, in clinical low grade (IDH-mutant) gliomas, HIF responses and neo-angiogenesis are generally absent, supporting the notion that D-2HG stimulates, rather that inhibits, HIF prolyl hydroxylases, the enzymes mediating proteasomal HIF degradation (25).

Based on the above, and on the finding of subventricular nodules of proliferating IDH1^{R132H}-expressing NSCs, authors postulate that this transgenic mouse model recapitulates features of gliomagenesis. However, obviously additional events in NSCs are needed to faithfully recapitulate glioma: the majority of IDH1-mutated low grade astrocytomas contain additional mutations in *TP53*, whereas clinical oligodendrogliomas often contain deletion of chromosome arms *1p* and *19q*, events that cannot be recapitulated in mouse. It would be interesting to investigate the effects of additional inclusion of Cre-activatable *p53* mutations, although one may expect that the high number of affected NSC cells would quickly result in death of the animals. In this respect, local and controlled injection of lentiviruses, expressing Cre under control of GFAP or nestin promoters could yield useful information. Such models would represent great tools to test novel treatments for *IDH1*-mutated gliomas, and might be of special interest for testing of immunotherapy approaches since these involve immunocompetent animals, in contrast to patient derived xenografts that need to be grown in immunodeficient mice.

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