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Research Article

IDH1^{R132H} Mutation Increases U87 Glioma Cell Sensitivity to Radiation Therapy in Hypoxia

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Objective. IDH1 codon 132 mutation (mostly Arg132His) is frequently found in gliomas and is associated with longer survival. However, it is still unclear whether IDH1 mutation renders the cell more vulnerable to current treatment, radio- and chemotherapy. Materials and Methods. We transduced U87 with wild type IDH1 or IDH1^{R132H} expressing lentivirus and analyzed the radiosensitivity (dose ranging 0 to 10 Gy) under normoxia (20% O\textsubscript{2}) and moderate hypoxia (1% O\textsubscript{2}). Results. We observed that IDH1^{R132H} U87 cells grow faster in hypoxia and were more sensitive to radiotherapy (in terms of cell mortality and colony formation assay) compared to nontransduced U87 and IDH1\textsuperscript{wt} cells. This effect was not observed in normoxia. Conclusion. These data suggest that IDH1^{R132H} mutation increases radiosensitivity in mild hypoxic conditions.

1. Introduction

The IDH1 gene encoding the cytoplasmic NADP+ dependent isocitrate dehydrogenase—and more rarely IDH2, encoding the mitochondrial isozyme—are frequently mutated in gliomas, especially low grade gliomas and secondary glioblastomas [1]. IDH1/IDH2 mutation is associated with better clinical outcome, whatever the grade, but it is still not clear whether it is merely a prognostic marker or a predictor of the response to radiotherapy or chemotherapy [2–6]. Recent data IDH1/IDH2 mutation results in a new enzyme function catalyzing the NADPH-dependent reduction of alpha-ketoglutarate to D-2-hydroxyglutarate (D-2HG) [7]. IDH1/IDH2 mutations result in D-2HG accumulation and lowering NADPH levels. On one hand D-2HG inhibits various alpha-ketoglutarate dependant reactions, including histone and DNA demethylation, and is likely to promote—rather than inhibit—HIF1α degradation [8–11]. On the other hand, low NADPH levels might sensitize tumors to oxidative stress, potentiating response to radiotherapy, and may account for the prolonged survival of patients harboring the mutations.

Since the majority of gliomas are poorly responsive to current treatment regimens, the ability to enhance cell
radio-chemosensitivity would be of clinical benefit. In this study, we characterized the impact of IDH1 mutation on U87 glioma cell growth and radiosensitivity.

2. Methods and Materials

2.1. Cell Culture and Hypoxia Treatment. The human glioblastoma cell line U87 MG (HTB14) was obtained from the American Type Culture Collection (ATCC, Rockville, MD) and maintained in Dulbecco’s modified Eagle’s medium (DMEM), supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Normoxic cells (21% O₂) were grown in a humidified-air atmosphere incubator containing 95% air/5% CO₂ at 37°C. Hypoxia experiments were performed in a controlled atmosphere chamber (INVIVO2 1000, Ruskinn, Awel, France) set at 1% O₂, 94% N₂, and 5% CO₂ at 37°C.

2.2. Production of Recombinant Expression Lentiviruses. A recombinant pLenti7.3/V5-TOPO expression vector (Invitrogen’s ViraPower™ HiPerform™ Lentiviral Expression Systems; catalog number K5320-00) containing the human IDH1 wild type and IDH1R132H cDNA was generated. The expression clones and the ViraPower Packaging Mix were cotransfected into the 293FT Cell line to produce lentiviral stocks, which were used to transduce the mammalian U87 cell line. U87-IDH1wt and U87-IDH1R132H stable cell lines were acquired using EmGFP selection by flow cytometry. The constructs was verified by DNA sequencing and RT-qPCR analysis.

2.3. Cell Proliferation Assay in Normoxia and in Hypoxia. To evaluate the impact of IDH1 mutation on cell growth in normoxia and in hypoxia by trypsin blue dye exclusion method, U87, U87-IDH1wt, and U87-IDH1R132H cells (4000/well) plated in 24-well plates (6 plates in total) were incubated at 37°C for six hours in normoxia to adhere; then 3 plates were removed at 37°C in the controlled atmosphere chamber overnight. At 1, 3, and 7 days after exposure to normoxia and hypoxia, the cells were trypsinized, and the number of viable cells
2.4. Comparative Cell Viability Assay after Irradiation, in Normoxia and in Hypoxia. To evaluate the effect of IDH1R132H in the response to radiotherapy, U87 cells, IDH1wt-U87, and IDH1R132H-U87 cells were plated (4.103 per well) in 24-well plates. Six hours later at 37°C in normoxia, plates were either kept in normoxia or incubated in the controlled atmosphere chamber 1% O2 overnight. The next day, cells were irradiated with doses ranging from 0 to 10 Gy in order to determine the most discriminating dose. Cells were fixed in paraformaldehyde (PFA) 4%, then stained with Hoechst 33342 (10 μg/mL in PBS, Sigma-Aldrich, France) and photographed in a blinded fashion under fluorescence (4 wells per condition; 4 photographs per well) at 24 h, 48 h, and 120 h, respectively. Cells were counted with ImageJ software (Rasband, WS, ImageJ, US NIH).

2.5. Colony-Formation Assay in Normoxia and in Hypoxia. U87, IDH1wt-U87, and IDH1R132H-U87 cells were plated in 6-well containing 0.3% base agar layer. Six hours later, cells were either incubated in the hypoxic or normoxic chamber overnight. The next day, the cells were treated by radiotherapy at the Radiotherapy Department of the Centre de Lutte Contre le Cancer (CLCC) François Baclesse (Caen, France) using an X-ray generator with doses ranging 0–8 Gy (Therac 15-Saturne with a dose rate of 2 Gy/min) and then incubated again for colony formation. One month later, the colonies were fixed in 20% ethanol and stained with 0.05% crystal violet. Colonies that contained more than 50 cells were counted. Survival was calculated as the average number of colonies counted divided by the number of cells plated multiplied by plating efficiency (PE), where PE is the fraction of colonies counted divided by cells plated without radiation. The clonogenic survival data were generated using JMP software. The experiment was performed five times in triplicate each.

2.6. Statistical Analysis. Results obtained in vitro were expressed as mean ± SEM. Image analysis was performed with in-house macros under the ImageJ Software (Rasband, WS, ImageJ, US NIH). All statistical analyses were determined using post hoc tests after significant ANOVA. Values of \( P < 0.05 \) were considered statistically significant.

3. Results

3.1. Transduced Cells Express High Quantities of IDH1wt and IDH1R132H. The presence of IDH1R132H transduced gene was confirmed by DNA sequencing. Real time PCR showed a high expression of gene IDH1wt and IDH1R132H in transduced U87 cells compared to nontransduced cells (Figure 1).
3.2. IDH1R132H Expressing U87 Glioma Cells Grow Faster in Hypoxia. We determined whether IDH1R132H expression directly influences cell growth in normoxia and in hypoxia. The viable cell number per well was determined by counting with trypan blue at 1, 3, and 7 days after incubation in normoxia and in hypoxia. Proliferation rate of U87-IDH1R132H cells was significantly higher in normoxia than in hypoxia for all the three cell lines. In normoxia, U87, U87-IDH1wt, and U87-IDH1R132H cells grew at the same rate, whereas U87-IDH1R132H grew faster than U87 and U87-IDH1wt in hypoxia (Figure 2).

3.3. Effect of Transduced IDH1R132H on Cell Viability upon Exposure to Doses Ranging 0 to 10 Gy in Normoxia and in Hypoxia. To evaluate the role of IDH1R132H in the response to radiotherapy, U87, U87-IDH1wt, and U87-IDH1R132H were exposed to different doses (range: 0–10 Gy): in normoxia the three cell lines showed the same radiosensitivity profile, whereas in hypoxia, the viability of U87-IDH1R132H cells was significantly lower after 5 days compared to control cells and IDH1wt cells (Figure 3) (13% versus 23% and 22% for a dose of 10 Gy, P < 0.001, respectively). This result suggests that IDH1R132H makes the cells more radiosensitive in hypoxic, but not in normoxic conditions.

3.4. Effect of Transduced IDH1R132H on Cell Mortality over Time following 8 Gy Irradiation in Normoxia and in Hypoxia. We quantified then cell death at 24 h, 48 h, and 120 h after 8 Gy irradiation. There was no substantial cell death after 24 h. The effect appeared at 48 h in both normoxia and in hypoxia (data not shown) and was maximal after 5 days. Cell death was significantly higher for IDH1R132H transduced cells in hypoxia but not in normoxia (Figure 4).

3.5. Radiosensitivity of U87- IDH1R132H in Hypoxia Is Confirmed by Colony-Formation Assay. A colony-formation assay was used to confirm the effect of IDH1R132H on the response to radiotherapy. Cells were treated with graded doses of radiation (0, 2, 4, 6, and 8 Gy). Colony-forming efficiency was determined 1 month later and surviving fractions were calculated. In normoxia, U87, U87-IDH1wt, and U87-IDH1R132H had the same colony-formation capacity after radiotherapy. In hypoxia, the colony number of U87-IDH1R132H after radiotherapy was significantly lower than U87 and U87-IDH1wt (Figure 5). Thus, U87-IDH1R132H significantly sensitized U87 glioma cells to radiation.

4. Discussion

We observed here that IDH1 mutated U87 grew faster in moderate hypoxic conditions (1% O2) than in normoxia (21% O2). This contrast with data obtained in normoxia, IDH1R132H overexpression in established glioma cell lines in vitro, resulted in a marked decrease in proliferation and mice injected with IDH1R132H-U87 cells had prolonged survival compared to mice injected with IDH1wt-U87 cells [12].

We found then that IDH1R132H-U87 were more sensitive to radiotherapy in hypoxic condition. Indeed a high rate of cell proliferation is per se a sensitive factor of the radiation therapy response. But on the other hand, IDH1/IDH2 mutated cells may be more sensitive to oxidative stress. The role of isocitrate dehydrogenase in cellular defense against oxidative stress has been suggested [13]. Indeed, IDH1/IDH2 serves as a major source of cytosolic and mitochondrial NADPH production necessary to regenerate reduced glutathione (GSH) by glutathione reductase and for the activity of NADPH-dependent thiorredoxin system, both are important in the protection of cells from oxidative damage [14, 15]. Thus, the
decrease of NADPH in IDH1/IDH2 mutated cells might result in an increase of ROS that can damage DNA. Partially in line with our results, U87 cells transduced with IDH1<sup>R132H</sup> or IDH2<sup>R172K</sup> demonstrated increased sensitivity to radiation but the effect observed in normoxia and hypoxic conditions was not investigated [16].

Despite hypoxia being considered as a factor of radioresistance, we observed here a radiosensitizing effect of IDH1<sup>R132H</sup> in glioblastoma cell line in hypoxic but not in normoxic condition. Until recently, IDH1/2 mutations were believed to result in the stabilization of HIF1α [10, 17]. Interestingly Koivunen et al. [11] showed that D-2HG (but not L-2HG) instead of being an inhibitor of EGLN (HIF prolyl 4-hydroxylases) activity acts as a partial agonist of EGLN and promotes the degradation of HIF1α. Because HIF protects cells from irradiation therapy under hypoxic condition, we may hypothesize that IDH mutation, by inducing an inappropriate degradation of HIF, could make the mutated cell more vulnerable to RT.

In conclusion, this study suggests a radiosensitizing effect of IDH1<sup>R132H</sup> in glioblastoma cell lines U87 grown under mild hypoxic conditions, which are close to in vivo conditions. We need to confirm this finding on clinical setting: the lp19q codeletion is a known marker of chemosensitivity. Whether the IDH1/2 mutation is a marker of radiosensitivity should be determined. The ongoing EORTC trial on low grade gliomas, which randomizes radiotherapy versus chemotherapy in low grade gliomas at progression and includes also a prospective observational cohort, will be pivotal to answer this question.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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