

Mutant IDH1 differently affects redox-state and metabolism in glial cells of normal and tumor origin

Biedermann, Julia¹; Preussler, Matthias¹; Conde, Marina²; Peitzsch, Mirko³; Richter, Susan³; Wiedemuth, Ralf²; Abou-El-Ardat, Khalil¹; Krüger, Alexander^{1,4,5,6}; Meinhardt, Matthias⁷; Schackert Gabriele^{2,5,6}; Leenders, William P.⁸; Herold-Mende, Christel⁹; Niclou, Simone P.¹¹; Bjerkvig, Rolf^{11,12}; Eisenhofer, Graeme^{3,10}; Temme, Achim^{2,5,6}; Seifert, Michael^{5,13}; Kunz-Schughart, Leoni A.^{4,5}; Schröck, Evelin^{1,5,6}; Klink, Barbara^{1,5,6,14*}

¹ Institute for Clinical Genetics, Faculty of Medicine Carl Gustav Carus, Technische Universität Dresden, Fetscherstraße 74, 01307 Dresden, Germany, Evelin.Schrock@tu-dresden.de

² Department of Neurosurgery, University Hospital Carl Gustav Carus, Technische Universität Dresden, 01307 Dresden, Germany, Achim.Temme@ukdd.de, Gabriele.Schackert@ukdd.de

³ Institute of Clinical Chemistry and Laboratory Medicine, University Hospital Carl Gustav Carus, Technische Universität Dresden, 01307 Dresden, Germany, Susan.Richter@ukdd.de

⁴ OncoRay - National Center for Radiation Research in Oncology, Faculty of Medicine and University Hospital Carl Gustav Carus, Technische Universität Dresden and Helmholtz-Zentrum Dresden-Rossendorf, 01307 Dresden, Germany, Leoni.Kunz-Schughart@oncoray.de

⁵ National Center for Tumor Diseases (NCT), partner site Dresden, Germany

⁶ German Cancer Consortium (DKTK), Dresden; German Cancer Research Center (DKFZ), Heidelberg, Germany

⁷ Institute for Pathology, University Hospital Carl Gustav Carus, Technische Universität Dresden, 01307 Dresden, Germany, Matthias.Meinhardt@ukdd.de

⁸ Department of Biochemistry, Radboud University Medical Center, Nijmegen, The Netherlands, William.Leenders@radboudumc.nl

⁹ Experimental Neurosurgery, Department of Neurosurgery, University Hospital Heidelberg, Heidelberg, Germany, h.mende@med.uni-heidelberg.de

¹⁰ Department of Medicine III, University Hospital Carl Gustav Carus, Technische Universität Dresden, 01307 Dresden, Germany, Graeme.Eisenhofer@ukdd.de

¹¹ Department of Oncology, NorLux Neuro-Oncology Laboratory, Luxembourg Institute of Health (LIH), Luxembourg, Luxembourg, simone.niclou@lih.lu

¹² Department of Biomedicine, University of Bergen, Bergen, Norway, Rolf.Bjerkvig@uib.no

¹³ Institute for Medical Informatics and Biometry, Faculty of Medicine Carl Gustav Carus, Technische Universität Dresden, Germany, michael.seifert@tu-dresden.de

¹⁴ National Center of Genetics (NCG), Laboratoire national de santé (LNS), Dudelange, Luxembourg, barbara.klink@Ins.etat.lu

*Corresponding author. E-mail: Barbara.klink@Ins.etat.lu; Phone: +352 28100 418; Fax: - 441

Supplementary Tables**Table S1. qPCR Primes for cDNA**

primer name	sequence	product length
cNAMPT_Exon1_forward	GGCAGAAGCCGAGTTCAA	82 bp
cNAMPT_Exon2_reverse	GCTTGTGTTGGGTGGATATTG	
cNAPRT1_Exon11_forward	ACTGGTGCCAATGCCAAT	107 bp
cNAPRT1_Exon10_reverse	GGCTGGAGTCAGTCCTCATC	
cNmrk1_Exon8_forward	ATCAGCCTCCAGACTCTCCG	125 bp
cNmrk1_Exon9_reverse	CTCTTCAGATTTTGTTCATCCA	
cQPRT_Exon2_forward	GAGGGCTGGTGATGGTGA	115 bp
cQPRT_Exon4_reverse	CTGCATTCCACTTCCACCTT	
cARF1_Exon2_forward	GACCACGATCCTCTACAAGC	111 bp
cARF1_Exon3_reverse	TCCCACACAGTGAAGCTGATG	
cGAPDH_Exon6_forward	ATGTTTCGTCATGGGTGTGAA	89 bp
cGAPDH_Exon7_reverse	GGTGCTAAGCAGTTGGTGGT	

Table S2. Antibodies (Ab) applied for western blotting (WB).

Primary Ab	Clone	Host	Species reactivity*	Distributor	dilution/working
IDH1 R132H	H09	Ms	H	Dianova	1:500
IDH1	2H9	Ms	H	Abcam	1:2000
QPRT	5D11	Ms	H	Sigma Aldrich	2 µg/ml
NAPRT1	n.d.	Ms	H	Sigma Aldrich	2 µg/ml
HAAO	n.d.	Ms	H	Abnova	2 µg/ml
NAMPT	P4D5AT	Ms	H	Enzo Life Science	1:2000
GAPDH	n.d.	Ms	H, B, Gt Ms, Rb, Rt Sw, F	Meridian Life Science	1:20.000
Secondary Ab					
Mouse IgG	n.d.	Gt	Ms	Santa Cruz Biotechnology	1:2000

* species reactivity according to distributor

n.d. - not defined, B – bovine, F-fish, Gt - goat, H- human, Ms - mouse, Rb - rabbit, Rt-Rat, Sw - swine

The primary antibodies were incubated at 4°C over night. The secondary antibody was incubated for 1 hour at room temperature.