Details about detection of *IDH1/2* mutations by CADMA PCR

Chapter Supplement – Isocitrate Dehydrogenase Mutations are Better Prognostic Marker than O^6-methylguanine-DNA Methyltransferase Promoter Methylation in Glioblastomas – a Retrospective, Single-centre Molecular Genetics Study of Gliomas

CADMA detection combines allele specific PCR with intended mismatch and competition for template between primers [1]. All qPCR reactions were designed and carried out according to the MIQE guidelines [2]. *IDH1* R132H and R132C mutation specific, overlapping wild-type and reverse primers were designed elementary and for each mutation selectively to able to distinguish the mutated and wild-type allele after melting analysis. *IDH2* R172K primers were designed using uMELT software [3]. Ten microliters of PCR reaction mixture contained – 1xHotStarTaq Plus Master Mix kit (Qiagen, Hilden, Germany), 1xEVAgreen (Biotinum, Hayward, USA), mutation specific primer (0.4 nmol), overlapping wild-type primer (0.1 nmol), wild-type primer (0.4 nmol) (Generi-Biotech, Hradec Kralove, Czech Republic) (Tab. 1) and 4 ng of DNA. PCR protocol consisted of 95 ℃ 15 sec, 35× (95 ℃ 10 sec, 60 ℃ 20 sec, 72 ℃), 95 ℃ 10 sec, 60 ℃ 45 sec, melt to 95 ℃ with a temperature increase of 0.06 ℃/sec. PCR and HRM analysis were performed on the LightCycler 480 System (Roche Diagnostics Corporation, Basel, Switzerland). *IDH1* R132H, *IDH1* wild-type, *IDH2* R172K and *IDH2* wild-type were purchased from Horizon Diagnostics (Horizon Diagnostics, Cambridge, UK) and *IDH1* R132C patient DNA was a gift from CGB laboratory (CGB laboratory, AGEL company, Czech Republic). DNA of standards were amplified by REPLI-g Single Cell Qiagen kit (Qiagen, Hilden, Germany) according to manufacturer's instructions concentrations of DNA standards were measured by PCR-based calibration curve method and mutation DNA fraction was assessed by massive parallel sequencing on MiSeq (Illumina). Amplicons were prepared with using of *IDH1* forward (ACCAAATGGCACCATACGA), *IDH1* reverse (GCAAAATCACATTATTGCC AAC), *IDH2* forward (GCTGCAGTGGG ACCACTATT) and *IDH2* reverse (TGTGG CCTTGTACTGCAGAG) primers (Generi-Biotech, Hradec Kralove, Czech Republic) were purified, tagmented and indexed using Nextera XT DNA

The authors declare they have no potential conflicts of interest concerning drugs, products, or services used in the study.

Autoři deklarují, že v souvislosti s předmětem studie nemají žádné komerční zájmy.

The Editorial Board declares that the manuscript met the ICMJE recommendation for biomedical papers.

Redakční rada potvrzuje, že rukopis práce splnil ICMJE kritéria pro publikace zasílané do biomedicínských časopisů.

doc. Mgr. Jiri Drabek, Ph.D.
Institute of Molecular
and Translational Medicine
Faculty of Medicine and Dentistry,
Palacky University and University
Hospital in Olomouc
Hnevotinska 5
779 00 Olomouc
Czech Republic
e-mail: jiri.drabek@upol.cz

Submitted/Obdrženo: 3. 5. 2017
Accepted/Přijato: 23. 7. 2017

doi: 10.14735/amko20171

Tab. 1. Primers used in CADMA PCR.

Gene	Mutation	Mutation specific primer	Overlapping wild-type primer	Wild-type primer
IDH1	R132H	gatgggtaaaacctatAatcatagAtcA	tgagtggatgggtaaaacctatcatcat	cttacttgatccccataagcatga
IDH1	R132C	ggatgggtaaaacctatAatcataAgtT	tgagtggatgggtaaaacctatcatcat	cttacttgatccccataagcatga
IDH2	R172K	gctggacGaagcccatcaAcattgAcaA	ctggctggaccaagcccatcaccat	cctacctggtcgccatgggcgt

*mismatched positions of mutation specific primers are marked in capital letters
IDH1 – isocitratedehydrogenase 1, *IDH2* – isocitratedehydrogenase 2

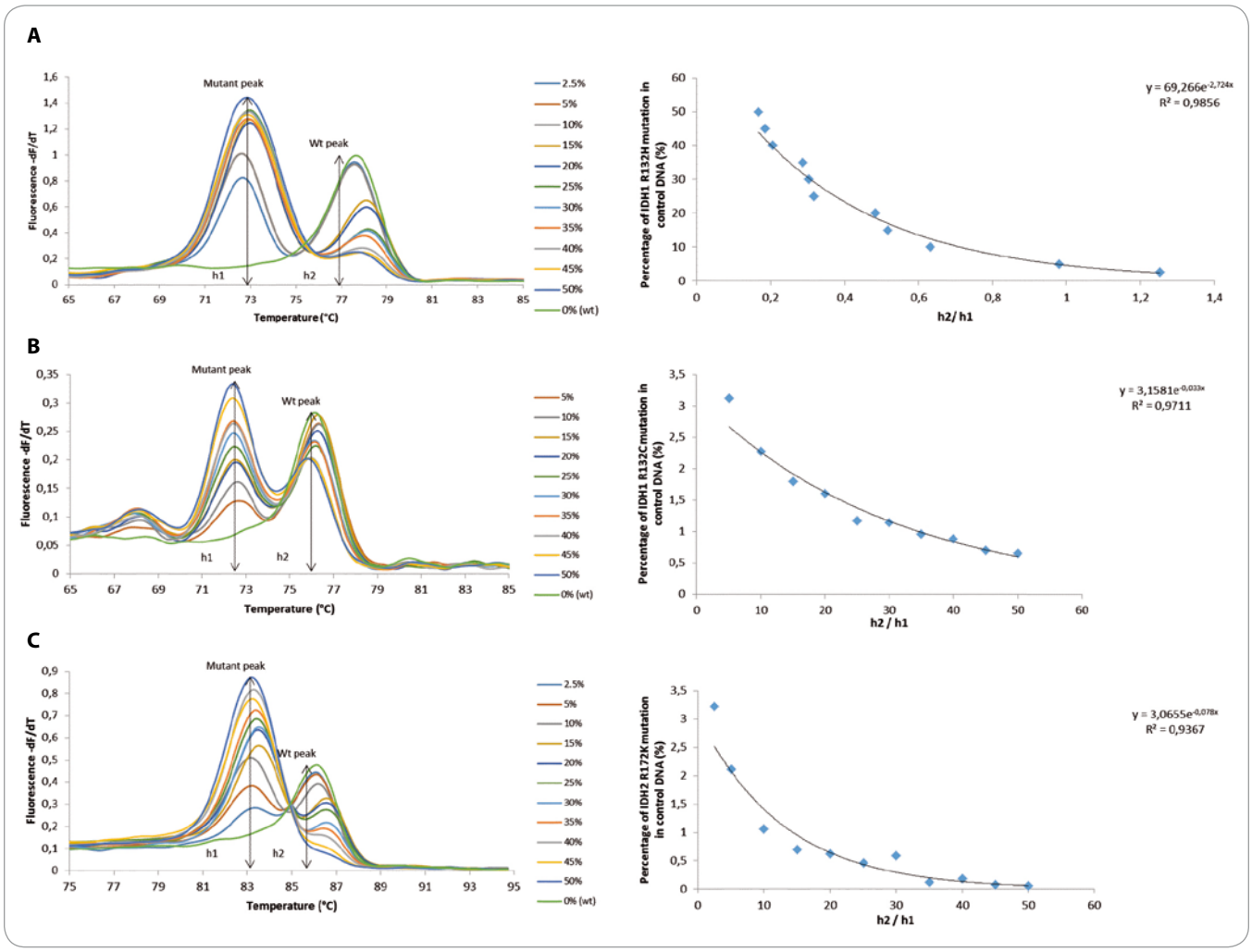

Fig. 1. An example of CADMA PCR results for dilution series and regression analysis used for quantification.
A – dilution series for IDH1 R132H mutation, B – dilution series for IDH1 R132C mutation, C – dilution series for IDH2 R172K mutation

Sample Preparation kit (Illumina, San Diego, USA) and then sequenced using MiSeq Reagent kit v2 300 cycles with coverage exceeding 10,000 in every sample tested. The output data were preprocessed using MiSeq Reporter 2.3 (Illumina) and then variants calls were processed using homemade MS Excel VBA script [4]. DNA from each mutated standards was serially diluted into wild-type reference standards from Horizon Diagnostics to the following fractions of mutated alleles in a wild-type background (0, 2.5, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50%) and used for quantification of *IDH* mutations. The LightCycler 480 System software was used for analysis. CADMA PCR results of dilution series of *IDH1* R132H, *IDH1* R132C and *IDH2* R172K are shown in Fig. 1. The presence of wild-type peak (higher Tm) with or without the mutant peak (lower Tm) was used for qualification of *IDH1* R132H, R132C and *IDH2* R172K in the samples. The ratio of mutant and wild-type peaks height in relation to percentage of mutated allele was used for quantification of *IDH1* R132H, R132C and *IDH2* R172K in the samples in each analysis (Fig. 1). All CADMA PCR analysis was performed retrospectively between years 2013–2014 from stock DNA samples and three times repeated for mutated samples.

References

1. Kristensen LS, Andersen GB, Hager H et al. Competitive amplification of differentially melting amplicons (CADMA) enables sensitive and direct detection of all mutation types by high-resolution melting analysis. Hum Mutat 2012; 33(1): 264–271. doi: 10.1002/humu.21598.
2. Bustin SA, Benes V, Garson JA et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. ClinChem 2009; 55(4): 611–622. doi: 10.1373/clinchem.2008.112797.
3. Dwight ZL, Palais R, Kent J et al. Heterozygote PCR product melting curve prediction. Hum Mutat 2014; 35(3): 278–282. doi: 10.1002/humu.22494.
4. Stranska J, Jancik S, Slavkovsky R et al. Whole genome amplification induced bias in the detection of KRAS-mutated cell populations during colorectal carcinoma tissue testing. Electrophoresis 2015; 36(6): 937–940.