Correction: Phosphoglycerate mutase 1 regulates dNTP pool and promotes homologous recombination repair in cancer cells

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After publication, the authors realized that an antibody and siRNAs used in the paper were incorrect. Specifically, the antibody reported as Cdh1 in Fig. 5 E was instead an antibody against CDH1 (Cadherin). In addition, the three siRNAs in Fig. 5 E reported as siCdh1#1, siCdh1#2, and siCdh1#3 were also inadvertently synthesized against CDH1 (Cadherin). Upon discovering the error, the authors generated three correct siRNAs against Cdh1, obtained an antibody against Cdh1, and repeated the experiment in Fig. 5 E. The incorrect resources were not used in any other part of the paper and the conclusions are not affected by this correction.

The authors also identified errors in the reported sequences of shPGAM1 #2, shPGAM1 #3, and siNC. The data resources generated were accurate. The error was only in the reported sequence in the manuscript.

Fig. 5 has been corrected and the following text corrections, in bold, have been made in the HTML and PDF versions of the article. The errors remain only in the print version.
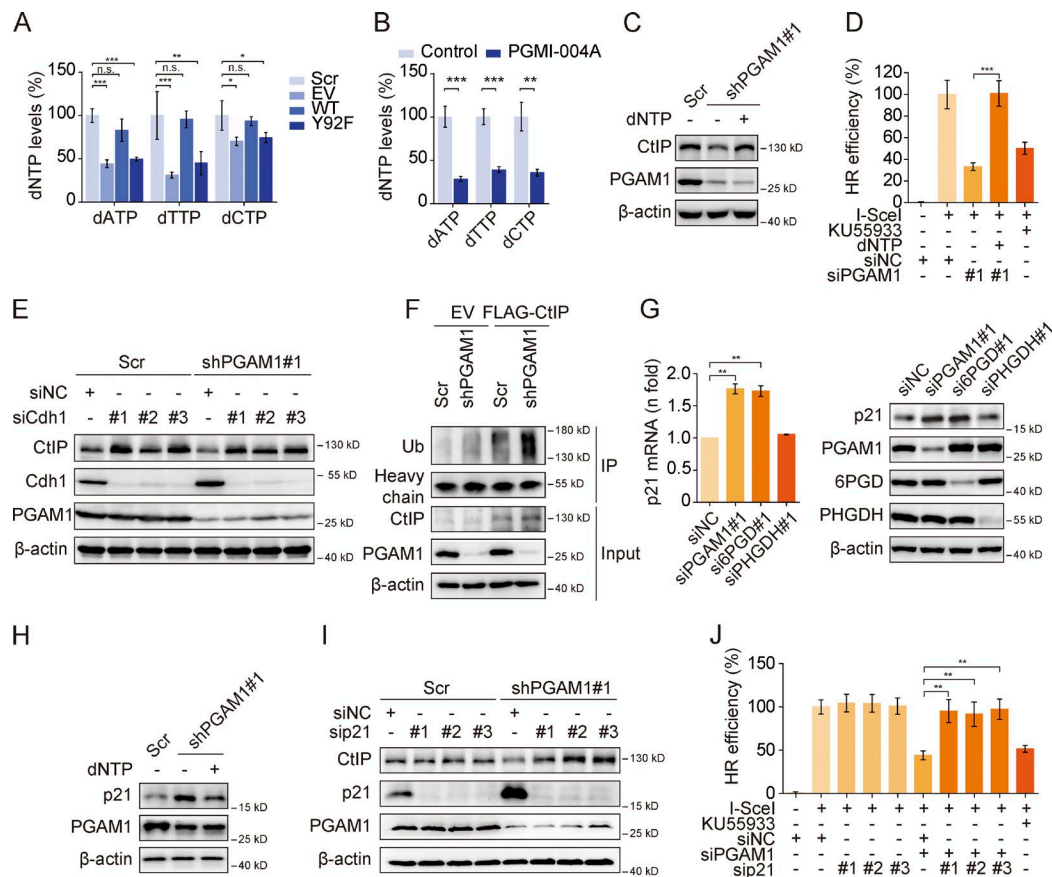

Figure 5.   **Decreased CtIP stability results from deficient dNTP synthesis.** (A and B) dNTP level change. Intracellular individual dNTP levels were measured using LC-MS/MS analysis. (A) HeLa shPGAM1#1 cells reconstituted with empty vector (EV), WT, or mutant PGAM1; Scr, scramble cells. (B) HeLa cells treated with PGMI-004A (20 µM) for 24 h. (C) CtIP protein level change. HeLa shPGAM1#1 cells were treated with 100 µM dNTPs for 24 h before being subjected to immunoblotting. (D) HR repair assay. DR-U2OS cells were transfected with indicated siRNAs for 24 h followed by I-SceI transfection. dNTP (100 µM) or KU55933 (10 µM) was added at the time of I-SceI transfection. GFP-positive cells were analyzed by FACS analysis 48 h later. (E) CtIP protein level change. **Cells transfected with indicated siRNAs for 72 h were harvested for immunoblotting analysis.** (F) CtIP ubiquitylation assay. HeLa shPGAM1#1 or scramble (Scr) cells were transfected with FLAG-CtIP for 48 h, and MG132 (10 µM) was added 6 h before harvest. Cell lysates were subjected to immunoprecipitation using FLAG M2 beads followed by blotting with anti-ubiquitin antibody. (G and H) p21 level change. p21 mRNA or protein levels were examined using real-time PCR or immunoblotting analysis. (G) HeLa cells transfected with indicated siRNAs for 48 h. (H) HeLa shPGAM1#1 cells treated with dNTP (100 µM) for 24 h. (I) CtIP protein level change. Cells were transfected with indicated siRNAs for 48 h before being subjected to immunoblotting analysis. (J) HR repair assay. DR-U2OS cells were transfected with indicated siRNAs for 24 h. HR repair was assessed as in D. siNC, negative control siRNA. Error bars represent mean ± SD of triplicates. *, P < 0.05; **, P < 0.01; ***, P < 0.001; n.s., not significant.

Antibodies

The following antibodies were used in this study: anti-PGAM1 (NBP1-49532; Novus Biologicals), anti-CtIP (sc-271339; Santa Cruz Biotechnology, Inc.), anti-Mre11 (ab214; Abcam), anti–H2AX-pS139 (9718; Cell Signaling Technology), anti–β-actin (60008-1-Ig; Proteintech), anti–Lamin B1 (12987-1-AP; Proteintech), anti-GAPDH (60004-1-Ig; Proteintech), anti-RPA32 (ab2175; Abcam), anti–RPA32-pS4S8 (NBP1-23017; Novus Biologicals), anti-BrdU (5292; Cell Signaling Technology), **anti-Cdh1 (ab3242; Abcam)**, anti-p21 (2947; Cell Signaling Technology), anti-RAD51 (sc-8349; Santa Cruz Biotechnology, Inc.), anti-PGD (sc-398977; Santa Cruz Biotechnology, Inc.), anti-PHGDH (ab57030; Abcam), anti-IgG (2729; Cell Signaling Technology), anti-Histone H3 (4620; Cell Signaling Technology), anti-p53 (9282; Cell Signaling Technology), anti-p73 (ab202474; Abcam), and anti–Cleaved Caspase-3 (9661; Cell Signaling Technology).

Plasmid, shRNA, and siRNA transfection

PGAM1 stably depleted cells were generated using pLKO.1 lentiviral system (Addgene). The target sequences of shRNAs were as follows: Scramble (Scr), 5′-CAAATCACAGAATCGTCGTAT-3′; shPGAM1#1, 5′-CCATCCTTTCTACAGCAACAT-3′; **shPGAM1#2, 5′-CCTGTGAGAGTCTGAAGGATA-3′; and shPGAM1#3, 5′-CGCCTCAATGAGCGGCACTAT-3′**.

Coding sequences of FLAG-PGAM1 and indicated mutants were cloned to pCDNA3.1 vector. Nonsense point mutations to the underlined nucleotides 5′-CCA<u>C</u>CC<u>A</u>TT<u>T</u>TTACAGCAACAT-3′ in the corresponding coding sequence of PGAM1 in the pCDNA3.1 plasmid confer resistance to shRNA#1 silencing. WT or mutant PGAM1 reconstituted cells were stable lines generated by Lipofectamine 2000 (Invitrogen) transfection followed by G418 selection. The lines used in this study were selected monoclones with expression levels comparable to those of endogenous PGAM1.

For siRNA transfection, cells were plated at 30–60% confluence in OPTI-MEM serum-free medium and transfected with a specific siRNA duplex using Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer's instructions. siRNAs were ordered as reverse-phase HPLC–purified duplexes from Sigma-Aldrich and **Shanghai GenePharma Co., Ltd**. The sequences were as follows: **negative control siRNA (siNC), 5′-UUCUCCGAACGUGUCACGUTT-3′**; siPGAM1#1, 5′-CGACUGGUAUUCCCAUUGUTT-3′; siPGAM1#2, 5′-GUCCUGUCCAAGUGUAUCUTT-3′; si6PGD#1, 5′-GGCCAGAACUUAAUUCUGATT-3′; si6PGD#2, 5′-CUGGUGACAUCAUCAUUGATT-3′; si6PGD#3, 5′-GCUGCAUCAUUAGAAGUGUTT-3′; siPHGDH#1, 5′-CUUAGCAAAGAGGAGCUGAUA-3′; siPHGDH#2, 5′-CAGACUUCACUGGUGUCAGAU-3′; sip21#1, 5′-GAUGGAACUUCGACUUUGUTT-3′; sip21#2, 5′-CCUCUGGCAUUAGAAUUAUTT-3′; sip21#3, 5′-CAGGCGGUUAUGAAAUUCATT-3′; **siCdh1#1, 5′-GGAUUAACGAGAAUGAGAATT-3′; siCdh1#2, 5′-AAUGAGAAGUCUCCCAGUCAGTT-3′; siCdh1#3, 5′-GCAACGAUGUGUCUCCCUATT-3′**; sip73#1, 5′-CCAUGCCUGUUUACAAGAATT-3′; sip73#2, 5′-CCAUCCUGUACAACUUCAUTT-3′; sip73#3, 5′-GUGGAAGGCAAUAAUCUCUTT-3′; sip53#1, 5′-GUACCACCAUCCACUACAATT-3′; and sip53#2, 5′-GUAAUCUACUGGGACGGAATT-3′.