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Linking Cancer Metabolism to DNA Repair and Accelerated Senescence

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Abstract

Conventional wisdom ascribes metabolic reprogramming in cancer to meeting increased demands for intermediates to support rapid proliferation. Prior models have proposed benefits toward cell survival, immortality and stress resistance while the recent discovery of oncometabolites has shifted attention to chromatin targets affecting gene expression. To explore further effects of cancer metabolism and epigenetic deregulation, DNA repair kinetics were examined in cells treated with metabolic intermediates, oncometabolites and/or metabolic inhibitors by tracking resolution of double strand breaks (DSBs) in irradiated MCF7 breast cancer cells. Disrupting cancer metabolism revealed roles for both glycolysis and glutaminolysis in promoting DSB repair and preventing accelerated senescence after irradiation. Targeting pathways common to glycolysis and glutaminolysis uncovered opposing effects of the hexosamine biosynthetic pathway (HBP) and tricarboxylic acid (TCA) cycle. Treating cells with the HBP metabolite N-acetylglucosamine (GlcNAc) or augmenting protein O-GlcNAcylation with small molecules or RNAi targeting O-GlcNAcase enhanced DSB repair, while targeting O-GlcNAc transferase reversed GlcNAc's effects. Opposing the HBP, TCA metabolites including α -ketoglutarate blocked DSB resolution. Strikingly, DNA repair could be restored by the oncometabolite 2-hydroxyglutarate (2-HG). Targeting downstream effectors of histone methylation and demethylation implicated the PRC1/2 polycomb complexes as the ultimate targets for metabolic regulation, reflecting known roles for

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Polycomb group proteins in non-homologous end-joining (NHEJ) DSB repair. Our findings that epigenetic effects of cancer metabolic reprogramming may promote DNA repair provide a molecular mechanism by which deregulation of metabolism may not only support cell growth but also maintain cell immortality, drive therapeutic resistance and promote genomic instability.

Keywords

Warburg effect; glutaminolysis; oncometabolite; DNA repair

INTRODUCTION

Otto Warburg was first to describe a diminished Pasteur effect in tumors, which actively take up and ferment glucose even in the presence of oxygen (1). Prior models ascribed deregulated glucose fermentation in cancer cells primarily to compensation for mitochondrial defects or adaptation to tumor hypoxia (2), but the focus has shifted to roles for metabolic products beyond ATP. Indeed, aerobic glycolysis promotes accumulation of intermediates that can serve as precursors for the proteins, lipids, and nucleic acids needed to support rapid cancer cell growth (3, 4). In turn, glutamine “addiction” in cancer, first observed by Eagle as an elevated requirement for cells in culture (5), has similarly been ascribed to answering increased demand for building blocks for cell proliferation (6–8). Beyond biosynthesis, recent attention has focused on potential regulatory functions for metabolic intermediates produced by glycolysis and/or glutaminolysis, via their roles as co-factors and inhibitors of chromatin-modifying enzymes (9–11, 12). Relevant chromatin-modifying enzyme/coenzyme pairs include histone acetyltransferase (HAT) and acetyl-CoA, poly(ADP-ribose) polymerase (PARP) and NAD⁺, histone lysine methyltransferases (HMT) and S-adenosyl methionine, Jumonji-domain containing histone lysine demethylases (JmjC HDM) and α -ketoglutarate (α -KG), O-linked N-acetylglucosamine (O-GlcNAc) transferase (OGT) and GlcNAc and, of course, Ser/Thr and Tyr protein kinases and ATP. These considerations have raised the hypothesis that via its epigenetic effects, cancer metabolic reprogramming may influence gene expression to drive oncogenesis and maintain cancer cell identity. For example, glycolytic metabolism in cancer cells impacts global chromatin structure by modulating histone acetylation (13), potentially altering transcription but also impinging on DNA repair. Indeed, along with their well-studied roles in epigenetic regulation of transcription, HATs, PARPs, and HMTs are also key regulators of DNA damage response (DDR) (14), suggesting a mechanism by which cancer metabolism might directly influence genomic instability and resistance to genotoxic stress.

In particular, specific patterns of histone modification are associated with ionizing radiation induced foci (IRIF), the multi-kilobase chromatin domains that form rapidly at sites of chromosomal double strand breaks (DSBs) and mark eroded telomeres (15–18). While DSBs are difficult to visualize in intact nuclei, IRIF are easily detected and can serve as a proxy for DNA damage (19). To probe the interaction of cancer metabolism and DNA repair, we used small molecule inhibitors, cell-permeable metabolic intermediates and RNA interference to perturb metabolic pathways in MCF7 breast adenocarcinoma cells. We observed that inhibition of glycolysis before irradiation allowed IRIF to form but blocked

their timely resolution. Detecting residual DNA breaks by comet assays confirmed a defect in DSB repair. In the face of persistent damage, rather than undergoing apoptosis, many cells entered accelerated senescence. Additional chemical probes pointed to two pathways downstream of glycolysis, the hexosamine biosynthetic pathway (HBP) and tricarboxylic acid (TCA) cycle, mediating opposing effects on IRIF persistence, DSB repair and cell senescence. Finally, we were able to implicate Polycomb Repressive Complex (PRC) 1 and 2 as the ultimate targets of cancer metabolic reprogramming in DSB repair. Taken together, these findings reveal critical connections between cancer cell metabolism, DSB repair and senescence with implications for genomic instability, carcinogenesis and therapeutic resistance.

METHODS

Cell Lines and Tissue Culture

The MCF7 Tet-On Advanced cell line was obtained from Clontech. The generation and characterization of the MCF7^{GFP-IBD} cell line has been described (24) and was used with further authentication by IDEXX BioResearch within the last 6 months. Panc 02^{GFP-IBD}, U-87 MG^{GFP-IBD}, and hTERT-HME1^{GFP-IBD} cell lines were developed similarly from parent cell lines purchased from American Type Culture Collection (ATCC). Briefly, GFP-IBD cloned into the pLVX-Tight-Puro vector was transfected along with pLVX-Tet-On Advanced vector (Clontech) into each cell line. Following G418 and puromycin selection, cells were induced with 1 µg/mL doxycycline and sorted to establish the IRIF reporter cell lines. The Panc 02^{GFP-IBD} and U-87 MG^{GFP-IBD} cell lines were maintained in RPMI (Invitrogen), supplemented with 10% Tet system approved FBS (Clontech). The hTERT-HME1^{GFP-IBD} cell line was maintained in MEM media supplemented with MEGM SingleQuot (Lonza). For studies requiring glucose and glutamine limitation, media was prepared using DMEM base, D-glucose (Sigma), and L-glutamine solutions (Gemini Bioproducts) at appropriate concentrations with 10% FBS.

IRIF Imaging

MCF7^{GFP-IBD} cells were seeded at 2.5×10^4 per well in 24-well plates on cover glass (Fisher Scientific) in 4.5 g/L glucose media. GFP-IBD expression was induced with 1 µg/mL doxycycline (Sigma) for 48 hours. For glucose- and glutamine-limiting conditions, media was exchanged one day after plating. Cells were incubated with small molecule inhibitors or cell-permeable metabolites (Supplementary Table 1) for 1 hour prior to 6 Gy irradiation by a GammaCell (MDS Nordion) ⁶⁰Co source unless otherwise noted and IRIF persistence was evaluated at 24 hours. Control, nonirradiated cells treated with each inhibitor or metabolite were examined at 24 hours to confirm lack of toxicity and no increase in IRIF formation. In turn, cells treated with inhibitors or metabolites were examined at 2 hours after irradiation to detect any suppression of IRIF formation.

For imaging, cells were fixed with 2% paraformaldehyde in PBS for 5 minutes, followed by two washes with PBS. Slides were mounted with ProLong Gold (Invitrogen) after staining with 5 µg/mL Hoechst 33342 (Sigma) or mounted with SlowFade Gold anti-fade reagent with DAPI (Invitrogen). Images were captured on a Zeiss Axiovert 40 CFL microscope with

a 40X Plan-Neofluar objective and AxioCam digital camera controlled by AxioVision 4.8 software and pseudo-colored in Adobe Photoshop or ImageJ (<http://imagej.nih.gov/ij/>). Numbers of foci per nucleus were determined using ImageJ, and means $\pm$ SEM were plotted. Statistical significance of IRIF phenotypes was determined by two-tailed, unpaired *t*-test with Welch's correction using GraphPad Prism 6 software. $P \leq 0.05$ are considered to be significant, with *** denoting $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$. $P > 0.05$ is not significant (n.s.).

RNAi Gene Silencing Experiments

Sets of 3 validated gene-specific Trilencer-27 siRNA duplexes targeting expression of OGT and OGA (MGEA5) and the Trilencer-27 Universal scrambled negative control siRNA duplex were obtained from OriGene Technologies.

The siRNA sequences used in this study were:

OGT(a) - ACUACUCAGAUCAACAAUAAGGCTG;

OGT(b) - CCUACUCUAAUAUGGGAAACACUCT;

OGT(c) - GGCACAUCGAGAAUAUCAGGCAGGA;

MGEA5(a) - CCUCUAGAAUGGUAACAAUUCAGCC;

MGEA5(b) - GCACGAGAAUAUGAGAUAGAGUUCA;

MGEA5(c) - CGAGCAAUAGUAGUGUUGUCAGTG.

For siRNA analysis, MCF7^{GFP-IBD} cells were seeded in 6-well plates to achieve 60–80% confluence after 24 hours. Transfections of the individual duplex siRNAs, mixtures of 3 gene-specific duplex siRNAs or the scrambled control were performed using FuGENE HD (Promega) according to the manufacturer's instruction. After 24 hours, transfected cells were seeded in 24-well plates with coverglasses in high or low glucose media with 1 μ g/mL doxycycline with or without PUGNAc. After overnight incubation, cells were irradiated with 6 Gy. After 24 hours, cells were fixed and GFP-IBD foci were imaged. OGT protein expression and O-GlcNAc protein modification were analyzed by Western blot 48 hours after siRNA transfection. O-GlcNAc transferase antibody from Thermo Fisher Scientific was used for Western blot analysis of OGT protein expression and anti-O-linked N-acetylglucosamine antibody from Abcam for detection of O-GlcNAc protein modification.

SA- β -Gal Senescence Assay

MCF7^{GFP-IBD} cells were seeded at 3×10^4 per 35 mm Fluorodish (World Precision Instruments) in 4.5 g/L glucose media. After 1 day, cells were treated with small molecule agents for 1 hour prior to irradiation at 6 Gy. To monitor effects of glucose or glutamine limitation on senescence induction, growth media was changed to the appropriate media 1 day after seeding and cells cultured overnight before further treatment. The SA- β -Gal assay was performed as described (66), fixing cells 5 days after irradiation. Images were captured on a Zeiss Axiovert 200M microscope with 20X Plan-Neofluar objective and AxioCam digital camera controlled by OpenLab software. Images were corrected for white balance using an ImageJ macro (http://digital.bsd.uchicago.edu/%55Cimagej_macros.html).

To estimate the level of SA- β -Gal staining for each condition, SA- β -Gal-positive and SA- β -Gal-negative cells were counted in multiple fields, percent SA- β -Gal-positive determined, and these values were averaged. Percent positive staining is indicated in each SA- β -Gal image as mean $\pm$ SEM.

Western Blotting

MCF7^{GFP-IBD} cells were seeded at 1×10^6 on 10 cm dishes. 2-DG, 2-FDG, and mannose were added to the media 1 hour before irradiation. Cells were harvested the next day, and lysed in lysis buffer (50 mmol/L Tris-HCl pH 7.5, 150 mmol/L NaCl, 1% NP-40, 1 mmol/L EGTA, 0.05% SDS) with HALT protease and phosphatase inhibitor cocktail (Thermo Scientific). 10 μ g of total cell lysates were loaded per lane on NuPage Bis-Tris precast gels (Invitrogen), transferred to a 0.45 μ meter nitrocellulose membrane (Bio-Rad), and probed with an anti-BiP antibody (C50B12, Cell Signaling). For the detection of poly(ADP-ribose) (PAR) chains, cells were treated with 50 mmol/L 2-DG and/or 10 μ mol/L PARP inhibitor veliparib (ChemieTek) 1 hour before the induction of DNA damage using 1 mmol/L H₂O₂ for 10 minutes. 20 μ g of total cell lysate was loaded per lane, and analyzed by Western blotting using an anti-PAR antibody (10H, GeneTex).

Detection of DNA Damage

Neutral comet assays were performed according to the manufacturer's protocol (CometAssay, Trevigen). Briefly, MCF7^{GFP-IBD} cells were seeded at 2×10^5 per well on 6-well plates, treated as indicated for IRIF imaging and harvested at 24 hours by trypsinization, washed once with PBS, and re-suspended at 1×10^5 cells/mL. Cells were then mixed with Comet LM agarose at 1:10, and 5000 cells were spotted per area on CometSlides. After incubation in Lysis Solution for 1 hour at 4° C, electrophoresis was performed as recommended. Slides were fixed with 70% methanol for 30 minutes at room temperature, dried, and stained with SYBR green for imaging using a Zeiss Axiovert 40 CFL with 10X Plan-NeoFluar objective, and an Axiocam digital camera controlled by AxioVision 4.8 software. Images were analyzed using an Image J comet assay macro (<http://www.med.unc.edu/microscopy/resources/imagej-plugins-and-macros/comet-assay>), and pseudo-colored in Adobe Photoshop.

Synthesis of Cell Permeable (R)-2-HG and (S)-2-HG

Ester-protected analogs of (R)-2-HG and (S)-2-HG were synthesized as previously described (42). (R) or (S)-5-oxotetrahydrofuran-2-carboxylic acid (650 mg, 5.0 mmol/L) was dissolved in acetonitrile (15 mL), followed by addition of i-Pr₂NEt (1.05 mL, 6.0 mmol/L, 1.2 equiv) and 3-(trifluoromethyl)benzyl bromide (0.92 mL, 6.0 mmol/L, 1.2 equiv). The mixture was refluxed for 15 minutes, allowed to cool to RT and then stirred overnight. The solvent was removed under vacuum and the resulting white residue was re-dissolved in ethyl acetate (50 mL). The organic layer was washed with 10% HCl (50 mL), 10% sat NaHCO₃ (50 mL) and brine (50 mL), and dried with Na₂SO₄. Rotary evaporation yielded a yellow oil which was purified using flash chromatography, eluting with 1:1 hexanes:ethyl acetate to give the pure compound as an oil [(R)-2-HG: 1.26g, 89%; or (S)-2-HG: 1.08g, 75%] which solidified to a white solid after co-evaporation with ether.

^1H NMR (500 MHz, CDCl_3) δ 7.65 – 7.56 (m, 2H), 7.56 – 7.51 (m, 1H), 7.48 (t, $J = 7.9\text{ Hz}$, 1H), 5.24 (s, 2H), 5.00 – 4.94 (m, 1H), 2.61 – 2.47 (m, 3H), 2.33 – 2.23 (m, 1H). ^{13}C NMR (126 MHz, CDCl_3) δ 175.83, 169.59, 135.77, 131.67, 129.35, 125.59, 125.56, 125.53, 125.50, 125.06, 125.03, 125.00, 124.97, 75.56, 66.58, 26.65, 25.75.

RESULTS

Targeting glycolysis blocks IRIF resolution, slows DSB repair and accelerates senescence

The constitutively nuclear-localized DNA damage and repair signaling adapter protein 53BP1 is recruited to IRIF within minutes after DNA damage and then disperses as DSBs are repaired (20). We have exploited GFP fused to the 53BP1 minimal IRIF binding domain (IBD), which consists of a nuclear localization domain, dimerization domain, paired Tudor domains and an ubiquitination-dependent recruitment (UDR) motif (21–23), as a live-cell reporter for DNA double strand break formation and repair in MCF7 human breast cancer cells (MCF7^{GFP-IBD}) (24). Upon irradiation of MCF7^{GFP-IBD} cells with 6 Gy, GFP-IBD relocalizes to form several dozen IRIF. In response to the DNA damage signal, cells arrest proliferation, primarily in G1. A phase of rapid repair ensues during the first 2 hours and most remaining IRIF resolve over 48 hours. While most surviving cells return to proliferation, a fraction fail to complete repair and remain arrested. Over several days, these cells eventually adopt features characteristic of senescent cells including a flat morphology, altered ploidy and expression of senescence-associated β -galactosidase (SA- β -Gal). When MCF7^{GFP-IBD} cells are treated with higher radiation doses and/or radiation sensitizers such as the PARP inhibitor veliparib, IRIF persistence and the proportion of senescent cells each increase (24, 25). Consistent with the known role of PARP in non-homologous endjoining (NHEJ) DSB repair (26), treating MCF7^{GFP-IBD} cells with 10 $\mu\text{mol/L}$ veliparib for 1 hour prior to 6 Gy irradiation significantly increased both IRIF persistence ($P = 0.001$) (Supplementary Fig. S1A–B) and DNA fragmentation measured by neutral comet assays ($P = 0.001$) at 24 hours (Supplementary Fig. S1C–D).

In searching for other agents that might similarly impair the DNA damage response, we observed that treating MCF7^{GFP-IBD} cells with the novel glucose transporter (GLUT1) inhibitors Compounds 11 and 12 (27) (see Supplementary Table 1 for structures and references for all small-molecule probes) prior to irradiation induced both significant IRIF persistence ($P = 0.001$) and increased senescence at 5 days (Fig. 1A and Supplementary Fig. S2A). Suggesting a role for glucose uptake in DNA repair, limiting glucose transport with the conventional inhibitors 2-deoxy-D-glucose (2-DG) and phloretin as well as simply lowering media glucose from 4.5 g/L to 1 g/L or removing glucose altogether each recapitulated this effect (Fig. 1A–C and Supplementary Fig. S2A–B). In turn, conventional small-molecule probes of glycolysis, 3-bromopyruvate (3-BP) to inhibit hexokinase, 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3-PO) to target phosphofructokinase-2 (PFK-2), alizarin red S to block phosphoglycerate mutase 1 (PGAM1) or oxamate to inhibit lactate dehydrogenase (LDH) (Fig. 1D), each promoted IRIF persistence and senescence ($P = 0.001$, Fig. 1E and Supplementary Fig. S2C), establishing a requirement for glucose metabolism in IRIF resolution. Consistent with effects observed with MCF7^{GFP-IBD} cells, the glucose uptake inhibitor phloretin and hexokinase inhibitor 3-BP similarly promoted

IRIF persistence in other cell lines. When either the mouse pancreatic cell line Panc 02^{GFP-IBD} or human glioma cell line U-87 MG^{GFP-IBD} were irradiated with 6 Gy in 4.5 g/L glucose media, both resolved IRIF by 24 hours. While treatment with phloretin or 3-BP prior to irradiation did not induce IRIF on its own, blocking glycolysis delayed IRIF resolution at 24 hours after irradiation in both cell lines (Supplementary Fig. S2E and S2F).

In some cells, glycolysis is critical for maintaining ATP levels, raising the concern that glycolysis inhibitors might delay DNA repair via ATP depletion. However, that MCF7 cells display active respiration and avid utilization of alternate fuels (28) suggests they can compensate for decreased glycolytic flux. Alternatively, glucose fermentation might augment NAD⁺ pools, supporting PARP activity to promote NHEJ repair. However, neither media glucose limitation nor treatment with 2-DG blocked PARylation upon H₂O₂ treatment (Supplementary Fig. S3A–D). To explain his observation that elevating glycolysis blocked senescence (29), Kondoh invoked the Crabtree effect, where increased fermentation suppresses respiration. He proposed that the resulting decrease in mitochondrial reactive oxygen species (ROS) would protect cells from DNA damage. To test this hypothesis, cells were irradiated in 1 g/L glucose in the presence of antioxidants N-acetyl-L-cysteine (NAC), butylated hydroxyanisole (BHA), or EUK134. The antioxidants failed to restore IRIF kinetics (Supplementary Fig. S4A–B), suggesting a direct role for glucose metabolism in DNA repair.

Glucose limitation results in IRIF persistence independent of UPR activation

As a potential confounding factor linking glucose limitation to IRIF persistence, we considered the potential impact of inhibition of secretory pathway N-glycosylation and resulting activation of the unfolded protein response (UPR) (30). Much like 1 g/L glucose media, both the N-glycosylation inhibitor tunicamycin (TM) and SERCA inhibitor thapsigargin (THA) induced IRIF persistence (Fig. 2A–B). Arguing against a primary role for UPR in IRIF kinetics, 1 g/L glucose media failed to induce upregulation of the UPR marker GRP78/BiP (31) (Fig. 2C). To dissect this further, we examined suppression of UPR by mannose in cells treated with 2-DG versus the negative control 2-FDG (32) (Fig. 2D). Even though it blocked GRP78/BiP induction in 2-DG-treated cells (Fig. 2E), mannose failed to restore IRIF resolution (Fig. 2F–G), unlinking the UPR and DSB repair pathways.

Targeting the hexosamine biosynthetic pathway (HBP) with chemical probes or RNA interference similarly modulates IRIF kinetics

In glucose-depleted cells, the core glycosylation subunit N-acetylglucosamine (GlcNAc) can enter the hexosamine biosynthetic pathway (HBP) to restore levels of UDP-GlcNAc (Fig. 3A) but fails to repopulate glycolysis, the pentose phosphate pathway, or TCA cycle (33). Beyond secretory pathway glycosylation, UDP-GlcNAc serves as a co-factor for nucleocytoplasmic O-GlcNAc transferase (OGT) in O-linked protein GlcNAcylation (34, 35) (Fig. 3A). Suggesting a role for the HBP pathway in DNA repair, the addition of GlcNAc to 1 g/L glucose media restored IRIF resolution and shortened comet tails in irradiated MCF7^{GFP-IBD} cells (Fig. 3B–C). Confirming a role for the HBP and OGT in DNA repair, inhibiting the HBP rate-limiting enzyme glutamine fructose-6-phosphate amidotransferase (GFAT) with azaserine or directly blocking OGT with either alloxan,

BADGP or ST060226 each significantly induced IRIF persistence in 4.5 g/L glucose ($P = 0.001$, Fig. 3D–E). In turn, blocking the deglycosylating enzyme O-GlcNAcase (OGA) with PUGNAc restored IRIF resolution in 1 g/L glucose ($P = 0.01$, Fig. 3D–E, Supplementary Figure S5B). Consistent with the IRIF kinetics, neutral comet assays demonstrated that GlcNAc and PUGNAc restored DSB repair in 1 g/L glucose ($P = 0.001$) while alloxan had an opposing effect ($P = 0.01$, Fig. 3F–G).

To confirm links between the HBP pathway and DNA repair in a non-transformed cell line, we used hTERT-HME1^{GFP-IBD}, a human telomerase reverse transcriptase immortalized mammary epithelial cell line expressing the IRIF reporter. Like MCF7^{GFP-IBD}, hTERT-HME1^{GFP-IBD} displayed greater IRIF persistence at 24 hours after irradiation when treated with glucose uptake inhibitor Compound 11 or OGT inhibitor ST060266 and faster IRIF resolution when treated with GlcNAc or the OGA inhibitor PUGNAc (Supplementary Figure S5C).

As a complementary strategy to analysis with chemical probes, we examined targeting O-GlcNAcylation via RNA interference to knock down expression of OGT or OGA in MCF7^{GFP-IBD} cells. When cells were treated with 27-mer duplex siRNAs targeting OGT to impair nucleocytoplasmic O-GlcNAcylation prior to irradiation, IRIF persistence at 24 hours was increased in both 4.5 g/L and 1 g/L glucose media, recapitulating the effects of OGT inhibitors alloxan, BADGP and ST060226 ($P = 0.01$, Fig. 3H–I and Supplementary Fig. S5D, S5E, S5F and S5G). In turn, duplex siRNA targeting OGA enhanced IRIF resolution in 1 g/L glucose media, much like the OGA inhibitor PUGNAc. Further confirming the analogy between the chemical probes and siRNA knockdown, the OGA inhibitor PUGNAc failed to restore IRIF resolution in cells treated with siRNA to silence OGT.

TCA cycle activation results in IRIF persistence

Given the requirement for glutamine in the conversion of fructose-6-P to glucosamine-6-P to enter the HBP (Fig. 3A), we expected that glutamine metabolism might also impinge on IRIF kinetics. Indeed, restricting media glutamine enhanced IRIF persistence in 4.5 g/L glucose media, but paradoxically restored IRIF resolution in 1 g/L glucose ($P = 0.001$, Fig. 4A and Supplementary Fig. S6A). Glutamine also enters the TCA cycle via conversion to glutamate and α -ketoglutarate (α -KG), joining the glycolytic product pyruvate in formation of acetyl-CoA (Fig. 4B). Down-regulation and/or mutation of TCA cycle enzymes are observed in cancer (36) and activation of the TCA cycle may promote senescence (37). Toward establishing links between the TCA cycle and DSB repair, we augmented TCA cycle activity via the addition of cell permeable pyruvate or inhibition of pyruvate dehydrogenase kinase (PDK) with dichloroacetate (DCA). Both perturbations resulted in IRIF persistence ($P = 0.01$ and $P = 0.001$ respectively, Fig. 4C and Supplementary Fig. S6B). Similarly, the cell-permeable TCA intermediates oxaloacetate and malate each induced IRIF persistence ($P = 0.001$, Fig. 4C and Supplementary Fig. S6B). Dimethyl 2-oxoglutarate (DM- α -KG), a cell-permeable analog of α -KG, induced IRIF persistence in both 4.5 g/L and 1 g/L glucose media ($P = 0.001$, Fig. 4D and Supplementary Fig. S6A). In turn, blocking glutamine-dependent anaplerosis with the glutaminase inhibitor Compound 968 or glutamate dehydrogenase inhibitor EGCG restored normal IRIF kinetics in 1 g/L

glucose media ($P = 0.001$, Fig. 4E and Supplementary Fig. S6A). Comet assays confirmed that IRIF persistence after treatment with DM- α -KG and Compound 968 reflected inhibition of DSB repair ($P = 0.001$, Figs. 4F–G).

Histone demethylases mediate effects of α -KG on IRIF persistence

By virtue of their catalytic requirement for molecular oxygen and α -KG as cofactors, dioxygenases such as the hypoxia-inducible factor (HIF)-1 α prolyl hydroxylases, TET DNA demethylases, and JmjC-domain containing (JMJD) histone lysine demethylases are considered key epigenetic enzymes linking metabolism to gene expression (10, 38, 39)(Fig. 5A). Extending this paradigm to metabolic control of DNA damage response, a 1 hour pretreatment with the dioxygenase inhibitor IOX1 prior to irradiation in 1 g/L glucose media restored IRIF resolution ($P = 0.001$, Figs. 5B and Supplementary Fig. S7A). Similarly, the oncometabolite and α -KG antagonist 2-hydroxyglutarate (2-HG) (40, 41) also restored IRIF resolution ($P = 0.001$, Figs. 5B and Supplementary Fig. S7A). In turn, IOX1 and 2-HG partly blocked induction of senescence after irradiation in 1 g/L glucose (Fig. 5C).

Toward determining the presumptive enzyme target of α -KG, we found that the S enantiomer of 2-HG that can inhibit HIF prolyl hydroxylases and the R enantiomer that cannot conferred similar effects on IRIF resolution (41, 42) ($P = 0.001$, Fig. 5B and Supplementary Fig. S7A), suggesting a minor role if any for HIF-1 α . While TET-dependent DNA demethylation has been linked to excision repair, a role for TET proteins in DSB repair remains to be established. By contrast, activation of JMJD proteins to demethylate histones H3 at K4, K9, K36, K79 and/or H4 at K20 could antagonize well-known protein-protein interactions critical to IRIF function and DSB repair (17). Indeed, treating cells with either PBIT to inhibit H3 K4 demethylase Jarid1A/B or with GSKJ4 to block H3 K27 demethylase JMJD3 each restored IRIF resolution after irradiation in 1 g/L glucose ($P = 0.01$ and $P = 0.001$ respectively, Fig. 5B and Supplementary Fig. S7A), decreased senescence (Fig. 5C), and accelerated DNA repair as measured by comet assay ($P = 0.001$, Supplementary Fig. S7B). In turn, R-2-HG, IOX1 and GSKJ4 each blocked the effect of DM- α -KG, restoring IRIF resolution and DNA repair ($P = 0.001$, Fig. 5D–E and Supplementary Fig. S7B).

Toward determining the order of function between O-GlcNAcylation and histone demethylase activity in IRIF resolution, we examined relevant combinations of cell-permeable metabolites and specific inhibitors. Both GlcNAc and PUGNAc restored IRIF resolution and DNA repair in cells treated with DM- α -KG, suggesting that O-GlcNAcylation might promote methylation or protect against demethylation ($P = 0.001$, Fig. 5D–E and Supplementary Fig. S7C–D). In a reciprocal experiment, the demethylase inhibitors IOX1 and R-2-HG overcame the effects on IRIF resolution of OGT inhibitors alloxan and ST060266 ($P = 0.001$, Fig. 5F and Supplementary Fig. S7E). A conservative interpretation places α -KG-dependent lysine demethylation downstream of O-GlcNAcylation in DNA repair and IRIF resolution.

Inhibition of histone methyltransferase G9a or Polycomb repressive complexes 1 or 2 blocks DNA repair downstream from O-GlcNAcylation and demethylation

Confirming a role for protein methylation in DSB repair, blocking the H3K9 methyltransferase G9a with BRD4770 or the H3K27 methyltransferase EZH2 with GSK126 each promoted IRIF persistence and delayed repair in both 4.5 and 1 g/L glucose ($P = 0.001$, Fig. 6A–D). We placed O-GlcNAcylation upstream of histone methylation, insofar as neither GlcNAc nor PUGNAc could overcome inhibition of IRIF resolution after treatment with methyltransferase G9a inhibitor BRD4770 (Supplementary Fig. S7F–G).

We were particularly struck by the IRIF persistence observed upon treatment with the H3K27 methyltransferase inhibitor GSK126. Its target, the SET domain protein EZH2, serves as a catalytic subunit for the Polycomb Repressive Complex 2 (PRC2). PRC2-dependent H3K27 trimethylation marks genes for epigenetic silencing, partly via recruitment and activation of the PRC1 histone H2A K119 ubiquitylation complex (43). Notably, beyond its key role in Polycomb-mediated gene repression, the PRC1 ubiquitin ligase catalytic subunit BMI-1 rapidly relocates to IRIF where it regulates DSB repair via ubiquitylation of H2A and H2AX (44, 45). In line with the results of Ismail *et al.* (46), treating cells with the BMI-1 inhibitor PRT4165 markedly delayed IRIF resolution ($P = 0.001$, Fig. 6E–F). Importantly, IRIF persistence induced by PRT4165 was not reversed by increasing GlcNAcylation with PUGNAc or blocking demethylation with IOX1 ($P = 0.001$, Fig. 6E–F). These results are consistent with Polycomb-group (PcG) proteins and specifically BMI-1/PRC1 as the ultimate target of cancer metabolism mediating epigenetic control of DSB repair, IRIF dynamics and senescence (Fig. 7).

DISCUSSION

Among the many hallmarks of cancer (47), altered metabolism has returned to the forefront as a potential therapeutic target. From Warburg's initial description of aerobic glycolysis to the present, the defining feature of cancer metabolic reprogramming has remained the deregulated uptake and utilization of glucose beyond cellular needs for ATP production. This apparent inefficiency is commonly ascribed to the elevated demands for biosynthetic intermediates required for rapid cell proliferation (3, 6, 7, 48) and/or as a means of resistance to cell death (49). Supporting the former model, a moderate correlation has been observed across multiple studies between ^{18}F -fluorodeoxyglucose (FDG) uptake as measured by PET in human tumors and fraction of Ki-67 positive nuclei or other measures of cell proliferation in biopsies. However, recent discoveries that the oncogenicity of mutations of TCA cycle enzymes IDH1 and IDH2 is mediated by excess production of the oncometabolite 2-HG, leading to inhibition of histone and/or DNA demethylases (10, 50, 51), have lent support to an alternative model that implicates metabolic reprogramming in deregulation of gene expression. Our work extends this model beyond epigenetic regulation of transcription to effects of chromatin modification on DNA repair. We found that disrupting glycolysis and/or glutaminolysis impaired the response to ionizing radiation, thereby linking cancer metabolism to enhanced DNA double strand break repair. The increased tolerance to genotoxic stress induced by metabolic reprogramming may promote

genomic instability and cell immortality in cancer cells by evading DNA-damage induced senescence, independent of cell proliferation *per se*.

We note that our primary strategy was to exploit small molecule probes rather than genetic tools to perturb cell metabolism. Beyond offering finer control of dose and timing compared to RNAi (52), chemical probes are particularly suited to the analysis of metabolic networks, where intermediates and metabolites are often considered nodes connected by metabolic enzymes as edges. While RNAi, CRISPR or other nucleic acid-targeted perturbations are powerful tools for analysis of specific gene products and linear pathways, they are poorly matched to analysis of cell metabolism, given the complex web of reactions that affect many intermediates. By contrast, a single chemical probe may target multiple isoforms or whole classes of binding sites. In turn, probes are readily used in combination and temporal order, allowing straightforward interrogation of order-of-function, functional redundancy and other pathway relationships (53, 54). Of translational significance, many of the small molecules used here have been evaluated for therapeutic use (55).

Among the most dramatic results was the block to DNA repair induced by TCA cycle intermediates including the cell permeable analog of α -KG. In turn, the TCA cycle enzyme-derived oncometabolite 2-HG blocked all these effects. Providing a strong argument that α -KG and its competitive inhibitor 2-HG mediate their effects via epigenetic targets, 1) α -KG serves as a co-factor for multiple dioxygenases including the Jumonji domain-containing (JMJD) histone lysine demethylases while 2-HG is a dioxygenase and JMJD inhibitor, 2) α -KG effects were recapitulated by G9a histone lysine methyltransferase inhibitor BRD4770 and EZH2 methyltransferase inhibitor GSK126, and 3) α -KG effects were blocked by Jarid1A/B histone lysine demethylase inhibitor PBIT and JMJD3 demethylase inhibitor GSKJ4. These results appear to provide a link between mitochondrial metabolism and genomic instability that may be independent of oxidative phosphorylation and reactive oxygen species.

Among our results with the greatest potential for translation, we implicated the hexosamine biosynthetic pathway (HBP) in regulation of DNA repair and senescence. We found that activating OGT by treating cells with GlcNAc, a readily available nutraceutical used to relieve inflammation, or targeting OGA with its inhibitor PUGNAc or by knockdown with siRNA each promote DSB repair. Blocking the pathway with small-molecule OGT inhibitors or by OGT knockdown had the opposite effect. Of potentially relevant OGT targets, surveys of the O-GlcNAc-ome have revealed modification of histones, DNA damage repair proteins, histone methyltransferases and PcG proteins (56–58). Specifically, OGT mediates O-GlcNAcylation of EZH2 on Ser75 in MCF7 cells, enhancing protein stability and maintaining H3 K27 trimethylation (59). Depletion of EZH2 is sufficient to delay DSB repair (60). However, given the multiple targets of O-GlcNAcylation in epigenetic regulation, its precise roles in DSB repair and IRIF kinetics remain to be elucidated.

Multiple lines of evidence point to cancer metabolism accelerating DSB repair via activation of non-homologous end-joining (NHEJ). First, our use of GFP-IBD as a reporter may have skewed our study toward tracking end-joining, given the known role of 53BP1 in promoting

NHEJ over homologous recombination (HR) (20, 61). In turn, NHEJ inhibitors promote IRIF persistence and senescence after irradiation (62), while candidate HR inhibitors fail to promote either response (our unpublished results). Providing a potential mechanistic link, several PcG proteins have been linked to NHEJ by detection of physical interactions and/or functional studies. One implication is that cancer metabolic reprogramming may speed resolution of DSBs by rapid NHEJ repair before they can initiate slow, but accurate, HR repair. Cancer cells may gain an advantage not only from more efficient DSB repair that supports proliferation in the face of genotoxic stress, but also from the increased genomic instability due to error-prone repair.

The Warburg effect has been widely recognized as a driver of cancer cell growth, tumorigenicity and therapeutic resistance. Our chemical genetics approach revealed that cancer metabolism also promotes DNA repair and thereby blocks accelerated senescence, inactivating a recognized tumor suppressor mechanism that serves as a critical barrier to carcinogenesis (63–65). These studies identified roles for both glycolysis and glutaminolysis via their dual contributions to the TCA cycle and HBP as key determinants of cellular responses to DNA damage. Our data also suggest a reexamination of the mechanisms of carcinogenesis by oncometabolites to include mechanisms beyond deregulation of gene expression. Indeed, 2-HG alone appears sufficient to recapitulate the benefits of metabolic reprogramming in promoting DNA repair and blocking cell senescence. Similarly, that treating cells with GlcNAc mirrors the effects of the oncometabolite 2-HG supports a reevaluation of O-GlcNAcylation as a mediator and target in cancer and aging.

Taken together, our results suggest that along with an established role in supporting cell proliferation, increased glycolysis and glutaminolysis may also support cancer cell survival in the face of genotoxic stress. By promoting DNA repair by error-prone non-homologous end-joining, metabolic reprogramming may serve a previously unrecognized role in tumor progression to increase genomic instability and maintain cellular immortality.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Implications

By defining a pathway from deregulated metabolism to enhanced DNA damage response in cancer, these data provide a rationale for targeting downstream epigenetic effects of metabolic reprogramming to block cancer cell immortality and overcome resistance to genotoxic stress.

Figure 1.

Targeting glycolysis blocks IRIF resolution and accelerates senescence. **A**, Glucose uptake inhibitors do not induce DNA damage in nonirradiated cells (NIR) but promote IRIF persistence and accelerated senescence after irradiation with 6 Gy (IR) in 4.5 g/L glucose. Representative cell images, with GFP-IBD fluorescence examined 24 hours after irradiation (IRIF, green; foci numbers per nucleus are shown (mean ± SEM) and senescence-associated β-galactosidase activity detected 5 days after irradiation by X-Gal (SA-β-Gal, blue). Degree of senescence phenotype was assessed by % SA-β-Gal (+) cells and indicated as mean ± SEM. **B**, Lowering media glucose to 1 g/L promotes IRIF persistence and senescence after irradiation. Representative images, with IRIF per nucleus (mean ± SEM) indicated. **C**, Plots of IRIF per nucleus in individual cells after 6 Gy in 4.5 g/L and 1 g/L glucose (NIR, ●; IR, ●), red bar indicates mean ± SEM. ***, $P = 0.001$, unpaired t-test. **D**, Glycolysis pathway and chemical probes. **E**, Small molecule inhibitors of glycolysis induce IRIF persistence and accelerated senescence in 4.5 g/L glucose. See Supplementary Table 1 for concentrations of all probes used. Shown are representative images of cell nuclei with IRIF, with means ± SEM indicated, and SA-β-Gal assay cell images. Scale bars, 10 μm (IRIF); 50 μm (SA-β-Gal).

Figure 2.

Inhibition of O-GlcNAcylation results in IRIF persistence independent of UPR activation.

A, Induction of unfolded protein response (UPR) causes IRIF persistence. MCF7^{GFP-IBD} cells were grown in 1 g/L or 4.5 g/L glucose media, treated with TM or THA 1 hour before 6 Gy irradiation, and observed after 24 hours. IRIF (green) per nucleus (blue) are shown for irradiated cells (mean ± SEM). Scale bar, 10 μm. **B**, Plots of IRIF per nucleus from **A**. Red bar, mean ± SEM. ***, $P = 0.001$, unpaired t-test. **C**, Glucose limitation does not induce UPR. MCF7^{GFP-IBD} cells were treated as in **A** and cell lysates were analyzed for GRP78/BiP induction by Western blot. Tub, tubulin. **D**, N-linked glycosylation and inhibitors. G, GlcNAc; M, mannose; P, phosphate. **E**, Mannose blocked GRP78/BiP induction in 2-DG-treated cells. MCF7^{GFP-IBD} cells were grown in 4.5 g/L glucose media, and treated with 2-DG, 2-FDG, and mannose at 1 hour before 6 Gy irradiation. Cells were lysed after 24 hours and analyzed for GRP78/BiP induction by Western blot. Tub, tubulin. **F**, IRIF persistence induced by 2-DG is not restored by mannose supplementation. MCF7^{GFP-IBD} cells were treated as in **E**, and examined after 24 hours. Representative cell images (IRIF, green; nucleus, blue) are shown with foci numbers per nucleus (mean ± SEM). Scale bar, 10 μm. **G**, IRIF per nucleus in **F** was plotted and analyzed for significance (mean ± SEM).

Figure 3.

Inhibition of O-GlcNAcylation results in persistent DNA damage after irradiation. **A**, Hexosamine biosynthesis pathway (HBP) metabolites are shown in black, enzymes in blue, and inhibitors in red. G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; GlcN-6P, glucosamine-6-phosphate; GlcNAc, N-acetylglucosamine; GlcNAc-6P, N-acetylglucosamine-6-phosphate; UDP-GlcNAc, uridine diphosphate N-acetylglucosamine; GFAT, glutamine fructose-6-phosphate amidotransferase; NAGK, N-acetylglucosamine kinase; OGT, O-linked GlcNAc transferase; OGA, O-GlcNAcase. **B**, N-acetylglucosamine (GlcNAc) restores IRIF resolution in 1 g/L glucose. Representative images, with IRIF per nucleus (mean ± SEM) indicated. **C**, IRIF persistence and neutral comet assay after 3 Gy in 1 g/L glucose, ± GlcNAc, display similar kinetics, suggesting O-GlcNAcylation promotes DSB repair. Paired plots of IRIF per nucleus (●) and percent tail DNA (○) after 3 Gy in 1 g/L glucose, red bar, mean ± SEM. ***, $P < 0.001$, n.s., not significant, unpaired t-test relative to NIR control for foci and comets. **D**, Small molecule probes link O-GlcNAcylation to IRIF persistence. MCF7^{GFP-IBD} cells were grown in 1 g/L or 4.5 g/L glucose media for 24 hours, and treated with inhibitors or activators of O-GlcNAcylation for overnight, irradiated, and harvested for imaging after 24 hours. Representative images of irradiated cells, with IRIF per nucleus (mean ± SEM) indicated. Nonirradiated controls are shown on Figure S5A. **E**, Plots of IRIF per nucleus from **D**. Red bar indicates mean ± SEM. ***, $P < 0.001$, **, $P < 0.01$, unpaired t-test relative to DMSO control of each glucose condition (4.5 g/L, ●; and 1 g/L, ○). **F**, Modulators of O-GlcNAcylation affect DNA repair. MCF7^{GFP-IBD} cells were grown in 1 g/L glucose media and treated with GlcNAc, alloxan, or PUGNac for overnight, irradiated (3 Gy), harvested at 24 hours, and examined by neutral comet assay.

Representative comet images are shown. Percent tail DNA (mean $\pm$ SEM) indicated. **G**, Percent tail DNA was plotted and analyzed for significance (mean $\pm$ SEM). ***, $P = 0.001$, **, $P = 0.01$, unpaired t-test relative to 3 Gy control. Scale bars, 10 μ m (IRIF); 20 μ m (comets). **H**, RNAi-mediated silencing of OGT or OGA in MCF7^{GFP-IBD} cells affects IRIF persistence and confirms results obtained with chemical probes. Representative images, with IRIF per nucleus (mean $\pm$ SEM) indicated. **I**, Plot of IRIF per nucleus after indicated treatment. Red bar indicates mean $\pm$ SEM (4.5 g/L, ●; IR, ●). ***, $P = 0.001$, unpaired t-test relative to scrambled control RNA for each glucose concentration.

Figure 4.

TCA cycle activation results in IRIF persistence. A, A balance of glucose and glutamine determines IRIF persistence. Representative images of irradiated cells, with IRIF per nucleus (mean $\pm$ SEM) indicated. **B,** TCA cycle metabolites are shown in black, enzymes in blue, and inhibitors in red. GLS, glutaminase; GLDH, glutamate dehydrogenase; DCA, dichloroacetate; EGCG, epigallocatechin gallate. **C,** Promoting TCA cycle flux induces IRIF persistence. MCF7^{GFP-IBD} cells were grown in 4.5 g/L glucose media for 24 hours, and treated with activators of TCA cycle 1 hour prior to 6 Gy irradiation, and fixed after incubation for 24 hours. Representative images of cells with IRIF per nucleus (mean $\pm$ SEM) indicated. **D,** Providing exogenous α -KG (DM- α -KG) promotes IRIF persistence regardless of the glucose levels. MCF7^{GFP-IBD} cells were grown in 1 g/L or 4.5 g/L glucose media for 24 hours, and treated with α -KG 1 hour prior to 6 Gy irradiation, and fixed after incubation for 24 hours. Representative images of irradiated cells, with IRIF per nucleus (mean $\pm$ SEM) indicated. **E,** Inhibiting glutamine metabolism results in IRIF resolution. MCF7^{GFP-IBD} cells were grown in 1 g/L glucose media for 24 hours, treated with glutaminase inhibitors 1 hour prior to 6 Gy irradiation, and fixed after incubation for 24 hours. IRIF per nucleus (mean $\pm$ SEM) indicated. **F,** Glutamine metabolite α -KG delay DNA repair. MCF7^{GFP-IBD} cells were grown in 1 g/L glucose media and treated with DM- α -KG or glutaminase inhibitor Compound 968, irradiated (3 Gy), harvested at 24 hours, and examined by neutral comet assay. Representative comet images are shown. Percent tail DNA (mean $\pm$ SEM) indicated. **G,** Percent tail DNA was plotted and analyzed for significance (mean $\pm$ SEM). ***, $P = 0.001$, unpaired t-test relative to 3 Gy control. Scale bars, 10 μ m (IRIF); 20 μ m (comets).

Figure 5.

Histone demethylases mediate effects of α -KG on IRIF persistence. **A**, Pathway for hydroxylation by α -KG-dependent dioxygenases and specific inhibitors (red). **B**, α -KG-dependent dioxygenase inhibitors promote IRIF resolution in 1 g/L glucose. MCF7^{GFP-IBD} cells were grown in 1 g/L or 4.5 g/L glucose media for 24 hours as indicated, and treated with α -KG-dependent dioxygenase inhibitors 1 hour prior to 6 Gy irradiation, and fixed after incubation for 2 or 24 hours. Representative images, with IRIF per nucleus (mean $\pm$ SEM) indicated. **C**, The inhibitors IOX1, PBIT, and GSKJ4 as well as the oncometabolite R-2-HG prevent accelerated senescence. SA- β -Gal activity was detected 5 days after irradiation by X-Gal. Degree of senescence phenotype was assessed by % SA- β -Gal (+) cells and indicated with mean $\pm$ SEM. **D**, O-GlcNAcylation, oncometabolite R-2-HG and JMJD3 histone demethylase inhibition block α -KG effects on IRIF persistence. MCF7^{GFP-IBD} cells were grown in 1 g/L glucose media for 24 hours, treated as indicated, and fixed after incubation for 24 hours. Representative images of irradiated cells, with IRIF per nucleus (mean $\pm$ SEM) indicated. **E**, Neutral comet assays after 3 Gy in 1 g/L glucose reveal IOX1 and R-2-HG promote DSB repair and block α -KG effects (mean $\pm$ SEM). ***, $P < 0.001$, unpaired t-test relative to 3 Gy + DM- α -KG. **F**, Inhibition of α -KG-dependent dioxygenases restores IRIF resolution after inhibition of O-GlcNAcylation. MCF7^{GFP-IBD} cells were grown in 1 g/L or glucose media for 24 hours, and treated overnight with O-GlcNAcylation inhibitors with or without α -KG-dependent dioxygenase inhibitors, irradiated, and fixed after 24 hours. Representative images of irradiated cells, with IRIF per nucleus (mean $\pm$ SEM) indicated. Scale bars, 10 μ m (IRIF); 50 μ m (SA- β -Gal).

Figure 6. Inhibition of histone methyltransferases G9a and Polcomb Repressive Complexes (PRC2/1) block DNA repair regardless of O-GlcNAcylation and histone demethylase activity. **A**, Roles for histone methyltransferase G9a and PRC1 and ubiquitin ligase PRC2 in chromatin modification and their specific inhibitors (red) are shown. **B**, Blocking the H3K9 methyltransferase G9a with BRD4770 or the H3K27 methyltransferase EZH2 with GSK126 each promoted IRIF persistence both in 4.5 g/L and in 1 g/L glucose media (mean ± SEM). **C**, Number of foci per nucleus from **B** were plotted and analyzed for significance (4.5 g/L, ●; 1 g/L, ●; mean ± SEM). ***, $P = 0.001$, unpaired t-test relative to IR control (6 Gy) of each glucose condition. **D**, Inhibitors of histone methyltransferases G9a and EZH2 block DSB repair. Neutral comet assays after 3 Gy in 1 g/L glucose. Percent tail DNA was plotted and analyzed for significance (mean ± SEM). ***, $P = 0.001$, unpaired t-test relative to 3 Gy control. **E**, Small-molecule inhibition of PRC1 (BMI1/RNF2) E3 ubiquitin ligase induces IRIF persistence, independent of α -KG-dependent dioxygenase activity or O-GlcNAcylation (mean ± SEM). **F**, Number of IRIF per nucleus from (**E**) was plotted and analyzed for significance. ***, $P = 0.001$, n.s., not significant, unpaired t-test relative to IR control. Scale bars, 10 μ m.

Figure 7.

Model linking metabolic reprogramming to cancer cell immortality via modulation of DNA repair. These studies with chemical probes and RNA interference have implicated increased glucose and glutamine metabolism in mediated increased DNA double strand break repair, thereby supporting continued proliferation and resisting accelerated senescence. Prior work has established a key role for chromatin in regulation of DNA damage repair, mediated by phosphorylation of histone H2AX at sites of damage, leading to recruitment of 53BP1 and other repair and signaling factors, inducing a checkpoint signal that promotes accelerated senescence. Our data implicate cancer metabolic reprogramming in accelerating DNA repair, leading to H2AX dephosphorylation, release of 53BP1 and rapid return to proliferation. We found a role for the hexosamine biosynthetic pathway (HBP) metabolite N-acetyl-glucosamine and O-GlcNAcylation (O-GlcNAc) in promoting histone methylation by EZH2 and/or G9A upstream of the E3 ubiquitin ligase activity of the PRC1 polycomb group complex, which has a well-established role in non-homologous end-joining repair. This activity is normally opposed by the TCA cycle product α-ketoglutarate (α-KG), which can promote histone demethylation by JMJD demethylases. Downregulation of the TCA cycle or overproduction of the oncometabolite 2-hydroxyglutarate (2-HG) blocks demethylation to accelerate DNA repair and resist senescence.