

MUC1 mucin stabilizes and activates hypoxia-inducible factor 1 alpha to regulate metabolism in pancreatic cancer

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Aberrant glucose metabolism is one of the hallmarks of cancer that facilitates cancer cell survival and proliferation. Here, we demonstrate that MUC1, a large, type I transmembrane protein that is overexpressed in several carcinomas including pancreatic adenocarcinoma, modulates cancer cell metabolism to facilitate growth properties of cancer cells. MUC1 occupies the promoter elements of multiple genes directly involved in glucose metabolism and regulates their expression. Furthermore, MUC1 expression enhances glycolytic activity in pancreatic cancer cells. We also demonstrate that MUC1 expression enhances in vivo glucose uptake and expression of genes involved in glucose uptake and metabolism in orthotopic implantation models of pancreatic cancer. The MUC1 cytoplasmic tail is known to activate multiple signaling pathways through its interactions with several transcription factors/coregulators at the promoter elements of various genes. Our results indicate that MUC1 acts as a modulator of the hypoxic response in pancreatic cancer cells by regulating the expression/stability and activity of hypoxia-inducible factor-1 α (HIF-1 α). MUC1 physically interacts with HIF-1 α and p300 and stabilizes the former at the protein level. By using a ChIP assay, we demonstrate that MUC1 facilitates recruitment of HIF-1 α and p300 on glycolytic gene promoters in a hypoxia-dependent manner. Also, by metabolomic studies, we demonstrate that MUC1 regulates multiple metabolite intermediates in the glucose and amino acid metabolic pathways. Thus, our studies indicate that MUC1 acts as a master regulator of the metabolic program and facilitates metabolic alterations in the hypoxic environments that help tumor cells survive and proliferate under such conditions.

cancer metabolism | glutamine accumulation | pentose phosphate pathway | 2-ketoglutarate

MUC1, a type I transmembrane protein, plays a significant role in the progression of cancer, particularly pancreatic adenocarcinoma (1–4). Although expressed in the normal pancreas, its expression is elevated in pancreatic adenocarcinoma and its expression pattern changes from a strictly apical localization on normal polarized epithelial cells to a broad distribution across the cell surface membrane of nonpolarized tumor cells (2). This results in aberrant signaling that enhances tumor progression and metastasis. MUC1 protein is expressed in >90% of pancreatic tumors (5), and MUC1 expression in tumors and its serum levels are associated with a poor prognosis and recurrence in patients with resected tumors (6). Much of the oncogenic role of MUC1 can be attributed to the participation of the small, cytoplasmic tail of MUC1 (MUC1.CT) in signal transduction and transcriptional events (2). ChIP-chip analyses have demonstrated that MUC1 occupies a plethora of promoter elements in which MUC1 modulates the recruitment and activity of

transcription factors, thus regulating transcription of the corresponding genes (7).

Several studies have established a role for MUC1 in tumor growth, invasion and metastasis in pancreatic cancer (4, 8, 9). MUC1 expression significantly correlates with tumor cell growth and invasiveness in vitro and tumor growth and metastasis in the orthotopic injection models of pancreatic cancer (3, 4). MUC1-overexpressing cells (S2-013.MUC1) produced larger tumors than the mock-transfected S2-013 cells (S2-013.Neo), indicating that MUC1 overexpression facilitates tumor growth (3). Pancreatic cancer cells metastasize to liver, lung, lymph nodes, and other tissues in patients with pancreatic cancer. MUC1 overexpression in pancreatic cancer cells significantly enhances the number of metastases in lymph nodes and lungs in orthotopic implantation models (3). Furthermore, mice lacking MUC1 have a profound defect in tumor growth and metastasis (1). These mice also have defects in MAPK activity and oncogenic signaling.

Pancreatic tumors exhibit significant levels of desmoplasia that results in hypoxic microenvironments (10, 11). Hypoxia is known to stabilize the activity of hypoxia-inducible factors (HIFs) that reprogram cancer cell metabolism to facilitate cancer cell growth in harsh environments (12–14). HIFs can also be stabilized by several oncogenes, which act through hypoxia-independent mechanisms. HIF-1 α activates transcription of several genes regulating glucose uptake, glycolysis, and flux through the tricarboxylic acid cycle. Thus, increased protein levels of HIF-1 α result in increased glucose metabolism through the glycolytic pathway, but reduced entry of glucose into the TCA cycle (12). Such metabolic alterations provide the tumor cells with increased biomass production, thus enhancing proliferation.

Here, we present data implicating MUC1 as a facilitator of glucose uptake and glycolysis in pancreatic cancer cells. In addition, we demonstrate that MUC1 physically occupies promoter regions and regulates expression of multiple metabolic genes. Furthermore, we show that MUC1 enhances HIF-1 α stabilization and activity in pancreatic cancer cells. Our metabolomic

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analysis demonstrates that MUC1 acts as a master regulator of the metabolic program in pancreatic cancer.

Results

MUC1 Physically Occupies Promoters of Key Metabolic Enzymes and Regulates Their Activity. Activation of MUC1. CT signaling in response to various growth factors leads to differential gene expression (2). To further investigate the mechanism by which MUC1. CT activation triggers differential gene expression, we performed ChIP-chip promoter analysis. We used CT2 mAb, which recognizes MUC1. CT, to pull down the sonicated DNA elements that were occupied by MUC1 in S2-013.MUC1F cells, and the DNA elements were then identified by performing a promoter microarray analysis, as described previously (7). Results from these studies showed that the promoters of several metabolic genes are physically occupied by MUC1 (Fig. S1 A and C). We confirmed *ENO1* and *PGM2* promoter occupancy by MUC1 in independent ChIP studies (Fig. S1 B and D).

MUC1 Regulates Expression of Glycolytic Genes. To determine if MUC1 regulates the expression of glycolytic genes, we performed real-time quantitative PCR (qPCR) analysis of RNA extracted from control (S2-013.Neo), MUC1-overexpressing (S2-013.MUC1), vector control [FG.shScr (scrambled control)], and MUC1-knockdown (FG.shMUC1) cells cultured under normoxic or hypoxic conditions (at 1% oxygen), which enhance glycolytic gene expression (15). The relative mRNA expression levels for HK2, PFKFB2, *ENO1*, *PGK1*, *PGM2*, and *LDHA* are shown in Fig. S24. These results indicate that MUC1 has an additive role with hypoxia on some of the glycolytic gene promoters (e.g., *LDHA*), whereas, on others (e.g., *PGK1*, *PGM2*), there is synergism between MUC1 and hypoxia.

MUC1 Regulates Glucose Uptake and Lactate Production in Pancreatic Cancer Cells. As MUC1 occupies promoters of multiple genes involved in glycolysis, we then determined the effect of MUC1 expression on glucose uptake and lactate production. To achieve this, we performed glucose uptake assays in S2-013.MUC1 or S2-013.Neo cells by using [³H]2-deoxyglucose (DG). Cells treated with labeled and excess unlabeled 2-DG were used as controls to set a baseline for nonspecific tritium uptake. After normalization for total cell count, we observed a twofold increase in glucose uptake by S2-013.MUC1 in comparison with S2-013.Neo cells (Fig. S2B). Similarly, we observed a more than twofold reduction in glucose uptake upon knockdown of MUC1 expression in FG.shMUC1 cells in comparison with FG.shScr (Fig. S2C). These results demonstrate that MUC1 expression facilitates glucose uptake in pancreatic adenocarcinoma cells. Levels of lactate, an end product of aerobic glycolysis, were also similarly modulated by MUC1 (Fig. S2 B and C).

MUC1 Regulates in Vivo Glucose Uptake in an HIF-1 α -Dependent Manner. Next, we sought to determine if the differences in the rate of glucose uptake were not simply an artifact of cell culture. To achieve this, we implanted the S2-013.MUC1 or S2-013.Neo cells orthotopically into the pancreas of athymic female nude mice. Three weeks later, the mice were injected with IR800 dye-coupled 2-DG (IRDye 800CW 2DG; LI-COR Biosciences). The mice were imaged 24 h after injection. We observed significantly higher glucose uptake by tumors generated from S2-013.MUC1 cells than the tumors generated from S2-013.Neo cells (Fig. 1A). The glucose uptake values were quantified by normalizing to the tumor size (Fig. S3) determined by euthanizing the animals after glucose measurements (Fig. 1B). Similar results were obtained with orthotopically implanted Capan1.MUC1 and Capan1.neo cells (Fig. 1B). However, MUC1-mediated increase in glucose uptake was significantly diminished by HIF-1 α knockdown (Fig.

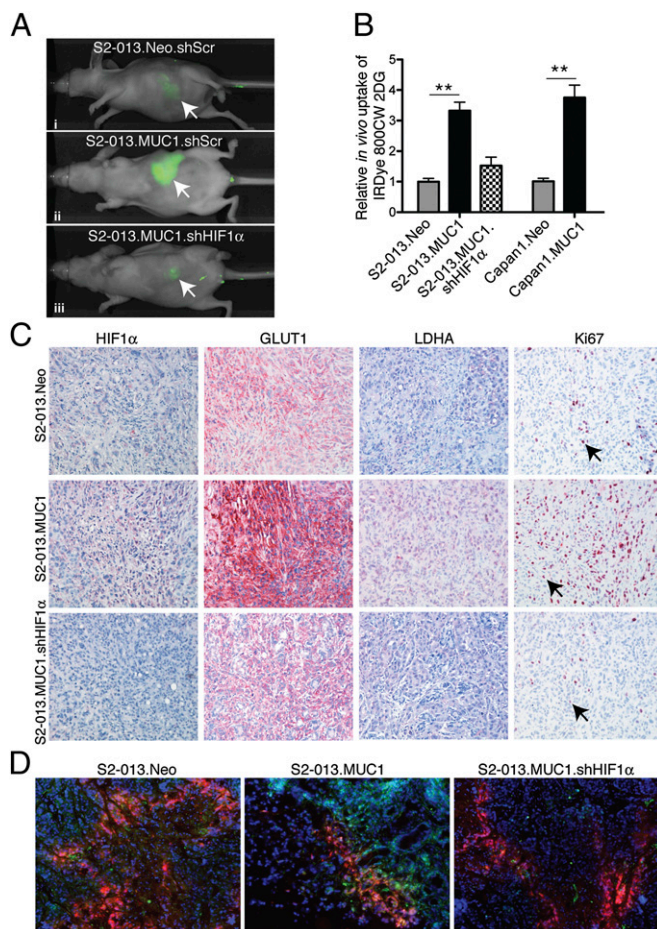


Fig. 1. MUC1 regulates in vivo glucose uptake and expression of glycolytic genes. (A) Glucose uptake by tumors formed by orthotopically implanted S2-013.Neo.shScr (scrambled control), S2-013.MUC1.shScr (scrambled control), or S2-013.MUC1.shHIF-1 α (HIF-1 α knockdown). Orthotopic xenograft tumors in athymic nudes were imaged with IRDye 800CW 2DG. Arrowheads indicate the tumors. (B) Quantification of in vivo glucose uptake by tumors (normalized to tumor size). (C) Immunohistochemical staining of formalin-fixed paraffin-embedded tumor sections reveals increased expression of HIF-1 α , GLUT1, and LDHA and increased nuclear Ki67 staining in S2-013.MUC1 cells in comparison with S2-013.Neo cells. Arrowheads point to nuclear Ki67 staining. (D) Immunofluorescence staining for hypoxia (EF5, red), HIF-1 α (green), and nuclei (DAPI) in tumor sections from S2-013.Neo, S2-013.MUC1, and S2-013.MUC1.shHIF-1 α -implanted mice (* P < 0.05 and ** P < 0.01).

1B). Also, we determined the expression of genes involved in aerobic glycolysis in the tumor sections from orthotopic implantation models with S2-013.Neo and S2-013.MUC1 cells (Fig. 1C). The expression of HIF-1 α , GLUT1, and LDHA was significantly more enhanced in tumors from S2-013.MUC1 cells in comparison with that of S2-013.Neo cells. Also, S2-013.MUC1 cells exhibited higher staining levels of Ki67, a marker of cell proliferation, compared with the S2-013.Neo cells (Fig. 1C). The increases in the expression of glycolytic genes were abrogated by HIF-1 α knockdown (Fig. 1C). Reciprocally, MUC1 knockdown in orthotopically implanted cells expressing naturally high levels of MUC1 (Capan2.shMUC1 vs. Capan2.shScr) demonstrated decreased glucose uptake (Fig. S4). Immunofluorescent EF5 staining indicated that, although the hypoxia levels were similar in the different tumor groups, MUC1 expressing tumor sections demonstrated enhanced HIF-1 α levels (Fig. 1D). These results

confirm that MUC1 overexpression enhances glucose uptake in pancreatic cancer cells.

MUC1 Regulates Expression/Stability and Activity of HIFs. As our data indicated an additive effect of hypoxia on MUC1-mediated transcriptional activation of glycolytic genes, we next examined if MUC1 modulated the stability of hypoxia-inducible transcription factors at protein level. By performing Western blot analysis, we evaluated if culturing S2-013.MUC1 cells under hypoxia-mimicking conditions (CoCl₂, which stabilizes HIFs) differentially altered the expression of HIF-1 α and HIF-2 α in comparison with the control S2-013.Neo. Our CoCl₂ dose-course (Fig. S5A) and time-course (Fig. S5B) experiments suggest that MUC1 expression enhances expression/stabilization of HIF-1 α and HIF-2 α in pancreatic cancer cells. However, the increased expression of HIF-1 α under conditions of MUC1 overexpression is not caused by changes in mRNA levels of HIF-1 α or the prolyl hydroxylase domain-containing proteins (PHDs)/von Hippel–Lindau tumor suppressor protein (VHL) that regulate HIF-1 α turnover (Fig. S6). S2-013.MUC1 cells also demonstrated increased expression of HK2, GLUT1, and PDK1 at protein level, in contrast to the S2-013.Neo cells (Fig. S5C). We performed lentiviral shRNA-mediated knockdown of HIF-1 α in S2-013.Neo and S2-013.MUC1 cells. HIF-1 α knockdown, but not scrambled control, diminished hypoxia mimetic-induced expression of the glycolytic gene HK2 (Fig. S5D). Furthermore, by performing promoter-reporter assays by using hypoxia response element (HRE)-containing human *enolase (ENO) 1* promoter-Luciferase reporter constructs, we observed that S2-013.MUC1 cells exhibited higher HRE-promoter activity under treatment with DMOG, which inhibits PHDs and stabilizes HIF-1 α (Fig. S5E) (16). Strikingly, even when transfected with constitutively active HIF-1 α , which has been rendered resistant to proteasomal degradation via deletion of part of the oxygen-dependent domain of HIF-1 α and mutation of specific prolyl residues (17), S2-013.MUC1 cells exhibited higher activity than S2-013.Neo cells (Fig. S5E). Thus, we can infer that MUC1 enhances HIF-1 α activity, independent of its effect on HIF-1 α expression levels. MUC1 expression also enhanced hypoxia-induced glucose uptake in S2-013 and Capan1 pancreatic cancer cell lines (Fig. S5F–G). Knockdown of HIF-1 α in S2-013.MUC1 cells reverted MUC1 overexpression-induced increase in glucose uptake (Fig. S5F). Reciprocally, the expression levels of MUC1 were also induced by hypoxic conditions in

a HIF-1 α -dependent manner (Fig. S5H and I). Furthermore, knockdown of MUC1 in Capan2 pancreatic cancer cell line diminished hypoxia-induced increase in the expression levels of HIF-1 α , HK2, and GLUT1 (Fig. S7A and B). Also, MUC1 knockdown in Capan2 diminished glucose uptake and lactate production (Fig. S7C and D). As MUC1 and HIF-1 α facilitate expression of some of the same glycolytic genes, we tested if the synergist activity is a result of their physical interaction. Proximity ligation assays (PLAs) demonstrate significant MUC1 and HIF-1 α interaction in S2-013.MUC1 and FG cells under HIF-1 α stabilizing conditions (Fig. 2A and B). Also, we could coimmunoprecipitate HIF-1 α with MUC1 by using an mAb against MUC1.CT, from S2-013.MUC1 cells treated with CoCl₂ or solvent control (Fig. 2C).

MUC1 Facilitates Hypoxia-Dependent Recruitment of HIF-1 α and p300 to Glycolytic Gene Promoters. Next, we sought to ask if MUC1 expression enhances occupancy of glycolytic gene promoters by HIF-1 α . To achieve this, S2-013.Neo and S2-013.MUC1 cells were cultured under normoxic or hypoxic conditions (1% oxygen) for 4 h and subjected to ChIP assays with HIF-1 α Ab. qPCR assays with the immunoprecipitated chromatin indicated increased HIF-1 α occupancy on *ENO1* and *PGM2* glycolytic gene promoter regions containing functional HREs; $-109/+44$ (i.e., flanking sequence and 44 nt inside the transcription start site) for *ENO1* and $-63/+52$ for *PGM2* (Fig. 3A) (16). No enrichment was observed for the distal regions. Furthermore, MUC1 coimmunoprecipitated with p300, a HIF-1 α coactivator, in a hypoxia-dependent manner (Fig. 3B). ChIP assays revealed p300 occupancy at *ENO1* and *PGM2* HRE regions, which was significantly enhanced by hypoxic conditions. The p300 occupancy was further enhanced by MUC1 overexpression in a hypoxia-dependent manner (Fig. 3C). The levels of histone H3 acetylated at lysine-9 (H3K9-ac) at *ENO1* and *PGM2* HREs was also significantly enhanced by MUC1 overexpression in a hypoxia-dependent manner (Fig. 3D). MUC1 overexpression had no effect on total cellular levels of H3K9-Ac or histone 3 (Fig. 3E).

MUC1 as Global Metabolic Regulator. To identify the effect of MUC1 on global metabolite levels in pancreatic adenocarcinoma cells, we performed NMR-based differential metabolomic studies. Principle component analysis of one-dimensional ¹H NMR spectra obtained from unlabeled S2-013.Neo and S2-013.MUC1

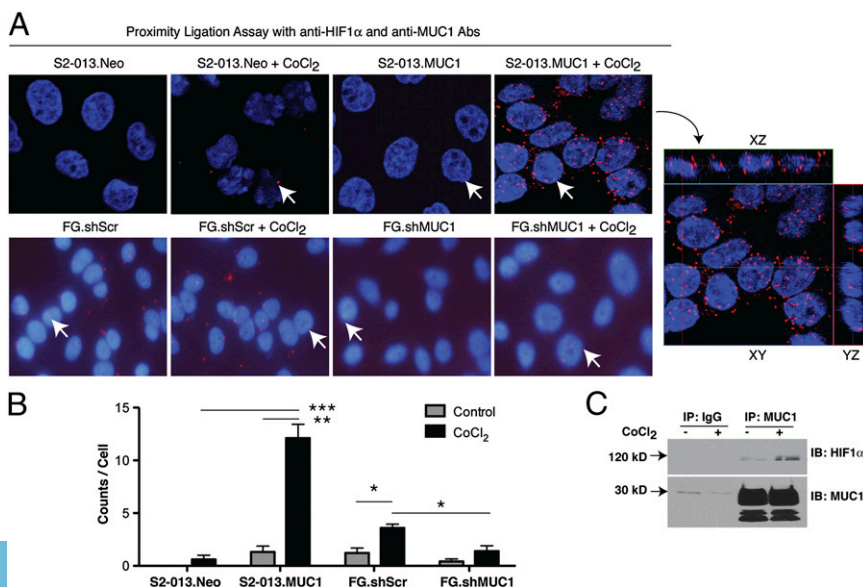


Fig. 2. Interaction of MUC1.CT with HIF-1 α . (A) PLA with Abs against MUC1.CT (CT2) and HIF-1 α demonstrate increased interaction of MUC1 with HIF-1 α under conditions of MUC1 expression and 6 h CoCl₂ treatment. Arrowheads point to the interaction spots. *Right*: 3D localization of interaction spots in S2-013.MUC1 cells under CoCl₂ treatment. (B) Quantification of interaction spots per cell in S2-013.Neo, S2-013.MUC1, FG.shScr, and FG.shMUC1 cells under conditions of 6 h CoCl₂ treatment or control. (C) Coimmunoprecipitation of HIF-1 α along with MUC1 from Nonidet P-40 extracts of 6 h CoCl₂ treated or control S2-013.MUC1 cells. Immunoprecipitations with isotype IgG were performed as a control (* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$).

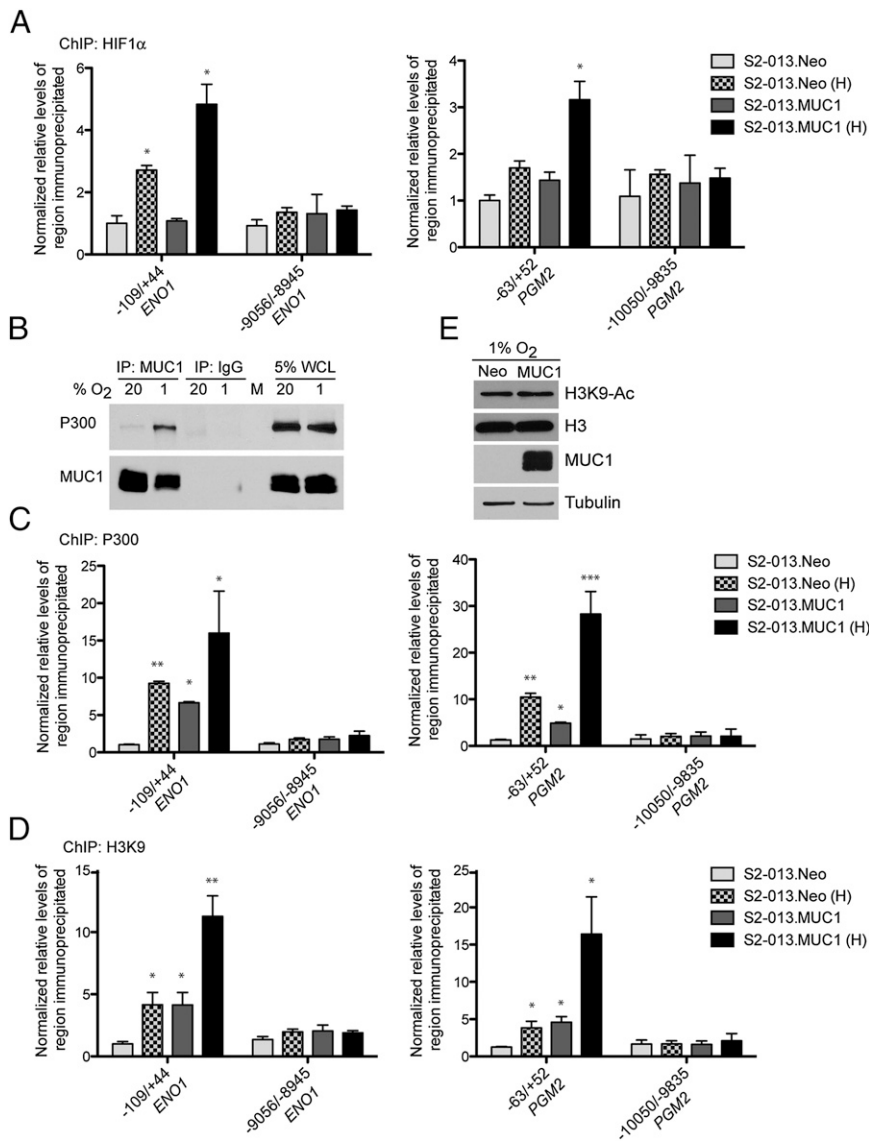


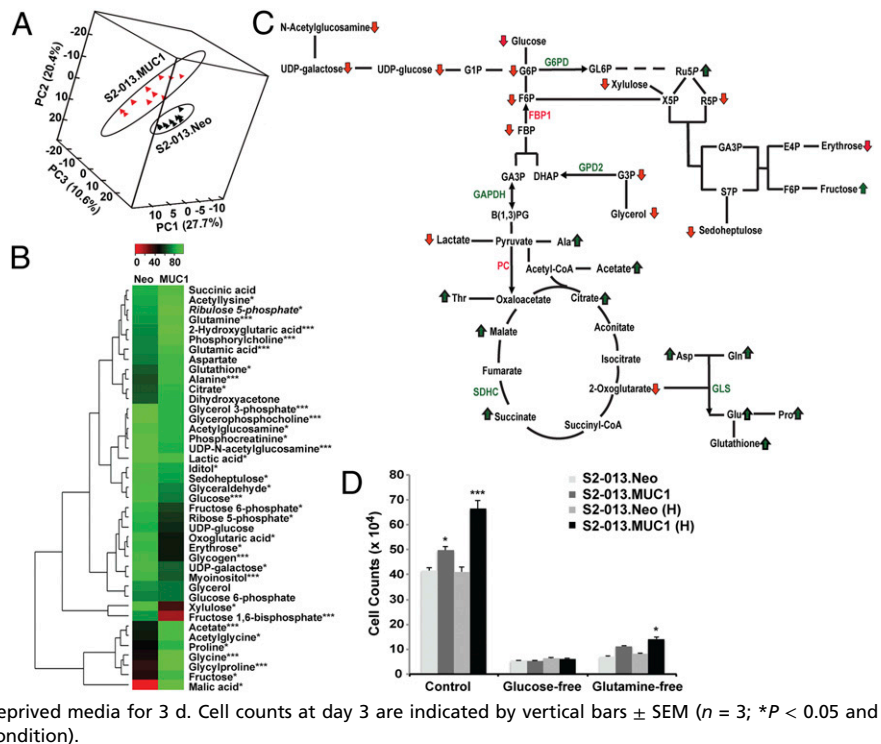
Fig. 3. MUC1 facilitates HIF-1 α recruitment to glycolytic gene promoters. (A) Occupancy of *ENO1* and *PGM2* promoters by HIF-1 α was confirmed by ChIP using anti-HIF-1 α Ab or IgG control, followed by qPCR analysis. Enrichment of proximal and distal *ENO1* and *PGM2* promoter regions from S2-013.Neo and S2-013.MUC1 cells under normoxic or hypoxic conditions (1% O₂, 6 h) was compared with that from S2-013.Neo under normoxic conditions. (B) Coimmunoprecipitation of p300 along with MUC1 from Nonidet P-40 extracts of 6 h hypoxia-treated or control S2-013.MUC1 cells. M, marker lane; WCL, whole-cell lysate. (C) Occupancy of *ENO1* and *PGM2* promoters by p300 was confirmed by ChIP using anti-p300 Ab or IgG control, followed by qPCR analysis. Enrichment of proximal and distal *ENO1* and *PGM2* promoter regions from S2-013.Neo and S2-013.MUC1 cells under normoxic or hypoxic conditions was compared with that from S2-013.Neo under normoxic conditions. (D) ChIP assays were performed with IgG or anti-acetylated H3K9 (H3K9-Ac) antibody. (E) Immunoblotting to determine the total cellular levels of acetylated lysine 9 residue in histone 3 (H3K9-Ac) and the protein expression levels of histone 3 (H3) and MUC1 under hypoxic conditions in S2-013.Neo and S2-013.MUC1 cells. Tubulin was used as a loading control. (Normalized relative values are presented as vertical bars \pm SEM; * P < 0.05, ** P < 0.01, and *** P < 0.001).

cell lysates indicated that they cluster into two separate groups and that they can be metabolically differentiated (Fig. 4A). We then labeled S2-013.MUC1 and S2-013.Neo cells with ¹³C₆-glucose and performed 2D ¹H-¹³C heteronuclear single quantum coherence (HSQC) NMR experiment to identify the ¹³C-labeled metabolites in the cell extracts. Pathway information for each of the metabolites was obtained from Cytoscape and Metscape. The metabolites were linked with the nearest possible metabolite in the pathway. These studies identified MUC1-regulated metabolites involved in multiple metabolic pathways in pancreatic cancer (Fig. 4B). Overall, metabolites involved in glucose metabolism, amino acid metabolism, and TCA cycle were found to be altered by overexpression of MUC1, suggesting their role in MUC1-mediated induction of cell growth and proliferation in pancreatic adenocarcinoma (Fig. 4C). Flux through the pentose phosphate pathway, which contributes to nucleotide synthesis, is enhanced by MUC1 overexpression. Also, we observed increased levels of several amino acids, including glutamine, in MUC1-overexpressing S2-013 cells. When subjected to glutamine-deprived conditions, S2-013.MUC1 cells demonstrated increased cell growth, in a hypoxia-dependent manner, in comparison with the controls (Fig. 4D). However, MUC1 expression did not provide any growth advantage under glucose-deprived conditions.

Discussion

In the present study, we report the finding that MUC1 regulates metabolism in pancreatic cancer cells. First, we demonstrated that MUC1 physically occupies the promoter elements of multiple genes involved in glycolysis and glucose metabolism in pancreatic cancer cells (Fig. S1). To confirm if physical occupancy correlates with transcriptional activation of the genes, we performed qPCR analyses. The qPCR analyses indicated that MUC1 facilitates expression of multiple key glycolytic genes at mRNA level and that the effect is further pronounced under the hypoxic conditions, indicating a role of HIF factors. Assaying MUC1-overexpressing S2-013 cells or MUC1-knockdown FG cells, along with control cells, indicated that MUC1 expression enhanced glucose uptake and lactate production, in contrast to control cells. Furthermore, MUC1 expression correlated with increased glucose uptake in vivo in the orthotopic xenograft implantation model of pancreatic cancer. Also, MUC1 expression correlated with increased expression of glucose metabolic enzymes such as GLUT1 and LDHA. These results indicate that MUC1 expression transcriptionally activates glycolytic genes and thus facilitates increased glucose uptake and consumption in pancreatic cancer cells. These findings correlate well with the

Fig. 4. MUC1 regulates metabolite levels in pancreatic cancer cells. (A) Three-dimensional principal component analysis scores plot comparing S2-013.Neo cells (black) with S2-013.MUC1 cells (red). (B) Heat map generated from the normalized-mean peak intensities for each metabolite identified from the triplicate set of 2D ^1H - ^{13}C HSQC NMR experiments. The normalized-mean intensities are plotted on a color-scale from 0% (red) to 100% (green). Dendrogram depicts hierarchical clustering of relative metabolite concentration changes between the S2-013.MUC1 and S2-013.Neo cells ($*P < 0.05$ and $***P < 0.001$; metabolites in italics have lower levels of confidence in NMR peak assignment). (C) Metabolic pathway depicting the metabolites identified in the S2-013.MUC1 and S2-013.Neo cell cultures by the 2D ^1H - ^{13}C HSQC NMR experiments. Three replicates of S2-013.MUC1 and S2-013.Neo cell cultures were used for metabolite identification. Dark green arrows correspond to metabolites with an increased concentration in S2-013.MUC1 cells relative to S2-013.Neo cells. Arrows colored red correspond to metabolites with a decreased concentration in S2-013.MUC1 cells relative to S2-013.Neo cells. Metabolic genes up- or down-regulated by MUC1 expression are indicated by green and red, respectively. Most glycolytic genes are not depicted for clarity. (D) Cells (5×10^4) were seeded and cultured under normoxic or hypoxic conditions (1% O_2) in regular or glucose/glutamine-deprived media for 3 d. Cell counts at day 3 are indicated by vertical bars \pm SEM ($n = 3$; $*P < 0.05$ and $***P < 0.001$ vs. counts of S2-013.Neo cells for that condition).



previous studies that suggest a role of MUC1 in modulating growth and invasive properties in multiple cancer types (3, 4). Increased tumor cell metabolism has been identified as a hallmark of cancer and a requirement for rapid tumor cell growth.

Our data demonstrate that MUC1 interacts with HIF-1 α and regulates the stability/activity of the latter. HIF-1 α stability is regulated by prolyl hydroxylation. Under well-oxygenated conditions, HIF-1 α is hydroxylated on Pro-402 and/or Pro-564 by PHD2, which uses oxygen and 2-oxoglutarate, also known as α -ketoglutarate, as substrates in a reaction that generates CO_2 and succinate as byproducts (12). Prolyl hydroxylation of HIF-1 α triggers its interaction with the von Hippel-Lindau tumor suppressor protein, which recruits an E3-ubiquitin ligase that targets HIF-1 α for proteasomal degradation. Under hypoxic conditions, the prolyl hydroxylation is inhibited by substrate (i.e., O_2) deprivation and/or the mitochondrial generation of reactive oxygen species, which may oxidize Fe(II) present in the catalytic center of the hydroxylases (12). Furthermore, metabolic alterations that result in depletion of oxoglutarate facilitate stabilization of HIF-1 α . Our metabolomic data indicates that MUC1 expression decreases the intracellular levels of 2-oxoglutarate, which may be the reason for increased stabilization of HIF-1 α under conditions of MUC1 expression. Treatment of S2-013.Neo or S2-013.MUC1 cells with excess 2-oxoglutarate diminishes MUC1-dependent increase in the levels of HIF-1 α (Fig. S8).

Previous studies have demonstrated that MUC1 itself is a target of HIF-1 α (18). Thus, increased stabilization of HIF-1 α might be a part of a positive feedback loop that may serve to promote tumorigenesis and metastasis in cancer cells. Our studies are in contrast to the previous studies by Yin et al. in HCT116 and ZR-75-1 cells, in which the authors demonstrate PHD3 down-regulation-mediated decreased stabilization of HIF-1 α by MUC1 (19). Interestingly, even Yin et al. identified that MUC1 causes a decrease in the expression of PHD2 (19), the major prolyl hydroxylase for HIF-1 α in most cell types. However, the decreased stabilization of HIF-1 α by MUC1 in their studies could reflect cell type-specific dependence of HIF-

1 α on PHD3. Regardless of which PHD is the major regulator of HIF-1 α stabilization, 2-oxoglutarate is a critical substrate for the reaction. Hence, our metabolomic studies indicating a decrease in the levels of 2-oxoglutarate by MUC1 clearly suggest that MUC1 causes a decrease in PHD activity that results in HIF-1 α stabilization in pancreatic cancer cells. Our mRNA expression data indicating an increase in the levels of PHD1-2 as a result of MUC1 suggests that the cells try to recover from the decreased PHD activity by increasing the mRNA levels (Fig. S6). A similar phenomenon is observed under hypoxic conditions in which PHD activity is significantly depleted as a result of a lack of oxygen substrate.

Furthermore, MUC1 physically occupies the promoter regions of and selectively enhances the transcription of some of the glycolytic genes in a HIF-dependent manner. Our studies indicate that MUC1 co-occupies promoter elements of glycolytic genes along with HIF-1 α . Also, MUC1 modulates glycolytic promoter occupancy by HIF-1 α and p300 and thus H3K9 acetylation status and HIF-1 α transcriptional activity in pancreatic cancer. There are at least two potential mechanisms whereby MUC1 could modulate the effects of hypoxia signaling through the HIF transcription factors: by regulating HIF stability and interaction partners and thereby directly modifying HIF signaling; and by direct signaling through the MUC1.CT, in a manner in which MUC1 directly associates with transcription factors (e.g., p53, β -catenin, and ER α) (2). Our studies demonstrate that MUC1 regulates HIF stability, HIF activity, p300 recruitment, and histone acetylation status on HIF-inducible promoters and that MUC1 can induce the expression of hypoxia-inducible genes in an HIF-dependent manner. The MUC1-dependent increase in p300 recruitment and H3K9 acetylation at HRE elements of glycolytic gene promoters may explain MUC1-mediated increase in HRE-luciferase activity under expression of constitutively stabilized HIF-1 α .

Our NMR-based metabolomics studies indicate that MUC1 facilitates glucose and amino acid metabolism in pancreatic cancer cells. We observed decreased accumulation of glucose, glycolytic intermediates, and lactate in S2-013.MUC1 cells, in contrast to S2-013.Neo cells. Metabolomic studies indicate the

kinetics of metabolic reactions in the cells. Hence, the decreased accumulation of glucose, glycolytic intermediates, and lactate, despite higher glucose uptake rate and increased secretion of lactate in the culture media, indicates that S2-013.MUC1 cells have faster glucose turnover, in contrast to S2-013.Neo cells. It is evident that glucose and amino acid metabolic pathways are critical for the production of biosynthetic intermediates, and thus for rapid tumor cell growth (20). We observed increased flux through glycolysis and pentose phosphate pathways. Increased levels of ribose 5-phosphate may account for increased nucleotide biosynthesis in S2-013.MUC1 cells in comparison with the S2-013.Neo cells. Also, we observed increased levels of amino acids, including glutamine, which may explain MUC1-dependent increase in cell growth even under conditions of glutamine deprivation. In fact, our analysis of Ki67 levels in tumor sections suggest that MUC1-facilitated HIF-1 α stability/activity promotes cancer cell growth under hypoxic conditions in the pancreatic tumor model. These findings are critical because HIF-1 α is a key modulator of glycolysis in cancer and HIF-1 α has been implicated in tumorigenesis. Furthermore, compared with the normal tissues, which are well oxygenated, metastasizing tumor cells intermittently switch between hypoxic and aerobic conditions. This leads to stabilization and activation of HIF-1 α , which in turn facilitates aerobic glycolysis in tumors.

In summary, we have presented findings that MUC1 serves as a metabolic regulator that facilitates the transcription of multiple genes involved in the glucose metabolic pathways. Also, we present a mechanistic relationship between MUC1 and HIF-1 α , whereby MUC1 may increase the stability of HIF-1 α by diminishing the levels of 2-oxoglutarate. We also present evidence that MUC1 physically interacts with HIF-1 α and p300 in a hypoxia-dependent manner, and enhances promoter occupancy of the HIF-1 α and p300 on glycolytic gene promoters. The interrelationship between MUC1–HIF-1 α oncogenic signaling networks serves to facilitate tumor growth and metastasis and could present a potential therapeutic target for the treatment of malignant diseases that rely upon MUC1 and HIF-1 α .

Materials and Methods

Cells and Reagents. Cell culture media and conditions are described in *SI Materials and Methods*. DMOG (Cayman Chemical) was dissolved in DMSO, and CoCl₂ (Sigma) was dissolved in DMEM.

ChIP and qPCR Assays. ChIP assays and qPCR assays were performed as described in *SI Materials and Methods*. The qPCR primer sequences are given in Table S1. For ChIP qPCR analysis, values were normalized to a genomic region located within the β -glucuronidase (*GUSB*) gene and expressed as fold increase vs. enrichment detected by using IgG as published previously (7). The average expression \pm SEM was reported.

Immunoblotting, Immunoprecipitation, and PLAs. Western blotting and immunoprecipitations were performed as previously described (21, 22). The membranes were probed with primary antibodies against HIF-1 α (Santa Cruz Biotechnology), MUC1 (Abcam), GLUT1 (Abcam), HK2 (Cell Signaling Technology), and LDHA (Abcam). In situ PLA experiments were performed as described in *SI Materials and Methods*.

Animal Studies, Immunohistochemistry, and Hypoxia Imaging. Animal studies, immunohistochemistry and hypoxia imaging were performed as described in *SI Materials and Methods*.

Metabolomic Analysis. S2-013.Neo or S2-013.MUC1 cells (1.5×10^7) were used for NMR-based metabolomic analysis. The details of metabolite extraction and one-dimensional ¹H NMR spectra and 2D ¹H-¹³C HSQC spectra analysis are described in *SI Materials and Methods*. The peaks were assigned to individual metabolites by using the chemical shift references from the Human Metabolomics Database (23). KEGG (24) database was used for drawing the metabolite network.

Statistical Analysis. Nonparametric Kruskal–Wallis tests were used to compare differences between cell lines. If the overall Kruskal–Wallis test indicated a difference in cell lines, a Wilcoxon rank-sum test was performed for each pairwise comparison of interest. Student *t* test was used when appropriate. *P* < 0.05 was considered significant.

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- Besmer DM, et al. (2011) Pancreatic ductal adenocarcinoma mice lacking mucin 1 have a profound defect in tumor growth and metastasis. *Cancer Res* 71:4432–4442.
- Singh PK, Hollingsworth MA (2006) Cell surface-associated mucins in signal transduction. *Trends Cell Biol* 16:467–476.
- Singh PK, et al. (2007) Platelet-derived growth factor receptor beta-mediated phosphorylation of MUC1 enhances invasiveness in pancreatic adenocarcinoma cells. *Cancer Res* 67:5201–5210.
- Tsutsumida H, et al. (2006) RNA interference suppression of MUC1 reduces the growth rate and metastatic phenotype of human pancreatic cancer cells. *Clin Cancer Res* 12: 2976–2987.
- Qu CF, et al. (2004) MUC1 expression in primary and metastatic pancreatic cancer cells for in vitro treatment by (213)Bi-C595 radioimmunoconjugate. *Br J Cancer* 91: 2086–2093.
- Ho JJ, Chung YS, Yuan M, Henslee JG, Kim YS (1992) Differences in expression of SPan-1 and CA15-3 antigens in blood and tissues. *Int J Cancer* 52:693–700.
- Behrens ME, et al. (2010) The reactive tumor microenvironment: MUC1 signaling directly reprograms transcription of CTGF. *Oncogene* 29:5667–5677.
- Kohlgraf KG, et al. (2003) Contribution of the MUC1 tandem repeat and cytoplasmic tail to invasive and metastatic properties of a pancreatic cancer cell line. *Cancer Res* 63:5011–5020.
- Satoh S, et al. (2000) Enhancement of metastatic properties of pancreatic cancer cells by MUC1 gene encoding an anti-adhesion molecule. *Int J Cancer* 88:507–518.
- Hidalgo M, Maitra A (2009) The hedgehog pathway and pancreatic cancer. *N Engl J Med* 361:2094–2096.
- Neesse A, et al. (2011) Stromal biology and therapy in pancreatic cancer. *Gut* 60: 861–868.
- Semenza GL (2010) HIF-1: Upstream and downstream of cancer metabolism. *Curr Opin Genet Dev* 20:51–56.
- Bertout JA, Patel SA, Simon MC (2008) The impact of O₂ availability on human cancer. *Nat Rev Cancer* 8:967–975.
- Rundqvist H, Johnson RS (2010) Hypoxia and metastasis in breast cancer. *Curr Top Microbiol Immunol* 345:121–139.
- Brahimi-Horn MC, Chiche J, Pouyssegur J (2007) Hypoxia signalling controls metabolic demand. *Curr Opin Cell Biol* 19:223–229.
- Semenza GL, et al. (1996) Hypoxia response elements in the aldolase A, enolase 1, and lactate dehydrogenase A gene promoters contain essential binding sites for hypoxia-inducible factor 1. *J Biol Chem* 271:32529–32537.
- Kelly BD, et al. (2003) Cell type-specific regulation of angiogenic growth factor gene expression and induction of angiogenesis in nonischemic tissue by a constitutively active form of hypoxia-inducible factor 1. *Circ Res* 93:1074–1081.
- Aubert S, et al. (2009) MUC1, a new hypoxia inducible factor target gene, is an actor in clear renal cell carcinoma tumor progression. *Cancer Res* 69:5707–5715.
- Yin L, Kharbanda S, Kufe D (2007) Mucin 1 oncoprotein blocks hypoxia-inducible factor 1 α activation in a survival response to hypoxia. *J Biol Chem* 282:257–266.
- DeBerardinis RJ, Lum JJ, Hatzivassiliou G, Thompson CB (2008) The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. *Cell Metab* 7:11–20.
- Wen Y, Caffrey TC, Wheelock MJ, Johnson KR, Hollingsworth MA (2003) Nuclear association of the cytoplasmic tail of MUC1 and beta-catenin. *J Biol Chem* 278: 38029–38039.
- Singh PK, et al. (2008) Phosphorylation of MUC1 by Met modulates interaction with p53 and MMP1 expression. *J Biol Chem* 283:26985–26995.
- Wishart DS, et al. (2009) HMDB: A knowledgebase for the human metabolome. *Nucleic Acids Res* 37(Database issue):D603–D610.
- Kanehisa M, et al. (2008) KEGG for linking genomes to life and the environment. *Nucleic Acids Res* 36(database issue):D480–D484.