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Lysine-5 Acetylation Negatively Regulates Lactate Dehydrogenase A and Is Decreased in Pancreatic Cancer

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SUMMARY

Tumor cells commonly have increased glucose uptake and lactate accumulation. Lactate is produced from pyruvate by lactate dehydrogenase A (LDH-A), which is frequently overexpressed in tumor cells and is important for cell growth. Elevated transcription by c-Myc or HIF1α may contribute to increased LDH-A in some cancer types. Here, we show that LDH-A is acetylated at lysine 5 (K5) and that this acetylation inhibits LDH-A activity. Furthermore, the K5-acetylated LDH-A is recognized by the HSC70 chaperone and delivered to lysosomes for degradation. Replacement of endogenous LDH-A with an acetylation mimetic mutant decreases cell proliferation and migration. Importantly, K5 acetylation of LDH-A is reduced in human pancreatic cancers. Our study reveals a mechanism of LDH-A upregulation in pancreatic cancers.

INTRODUCTION

Alteration in cell metabolism is a common event in tumorigenesis, as indicated by the dramatic increase of glucose utilization. However, the increased glucose uptake in tumor cells often does not lead to a corresponding increase in oxidative phosphorylation even in the presence of sufficient oxygen supply. Instead, glycolysis is highly elevated in most cancer cells. This metabolic alteration, known as the Warburg effect (Warburg, 1956), is believed to benefit tumor cells not only by conditioning the microenvironment, but also by increasing the levels of glycolytic intermediates, many of which also serve as precursors for anabolic biosynthesis, to support increased cell growth (Koppenol et al., 2011; Vander Heiden et al., 2009). The fact that tumor cells have a dramatically increased glucose uptake has provided the basis for ^{18}F-fluorodeoxyglucose-positron emission tomography technology, which is widely used for detecting tumors.

The last step of glycolysis is catalyzed by pyruvate kinase (PK), which converts phosphoenopyruvate to pyruvate. In normal nonproliferating cells, most, if not all, of pyruvate enters mitochondria, where it is converted to acetyl-CoA by the pyruvate dehydrogenase complex to fuel the tricarboxylic acid (TCA) cycle and oxidative phosphorylation for efficient energy production. In contrast, in cancer cells, and probably other highly proliferating cells, the influx of pyruvate into mitochondria and the

Significance

This study uncovers a critical role and the mechanism of acetylation in the regulation of lactate dehydrogenase A (LDH-A), which is elevated in cancer cells. Lysine-5 acetylation inhibits LDH-A by two mechanisms: decreasing enzymatic activity and increasing degradation by a chaperone-mediated autophagy. Moreover, LDH-A lysine-5 acetylation inversely correlates with pancreatic cancer initiation. Therefore, acetylation plays an important role in the regulation of cell growth and cancer metabolism.

TCA is not proportional to the increased glucose uptake; instead, more pyruvate is converted to lactate by lactate dehydrogenase (LDH). Therefore, a high conversion rate of pyruvate to lactate, hence high LDH, is commonly observed in cancer cells.

LDH is a homo- or hetero-tetrameric enzyme composed of two subunits, M and H, encoded by two highly related genes, *LDH-A* (also known as *LDHM*, *LDH1*, *GSD11*, and *PIG19*) and *LDH-B* (also known as *LDH-H, H-LDH*, and *LDH2*), resulting in five different isozymes depending on the ratio of the M and H subunits (M4, M3H1, M2H2, M1H3, and H4). LDH enzyme catalyzes the reversible conversion of pyruvate to lactate using NAD+ as a cofactor. Although the physiologic significance of lactate accumulation in tumor cells, a dead-end product in cellular metabolism, is currently a topic of debate, it has long been known that many tumor cells express a high level of LDH-A (Goldman et al., 1964), including non-small cell lung cancer (Koukourakis et al., 2003), colorectal cancer (Koukourakis et al., 2006), and breast and gynecologic cancers (Koukourakis et al., 2009). In many tumors, elevated LDH-A levels have been correlated with poor prognosis and resistance to chemotherapy and radiation therapy. Further evidence linking an LDH-A increase to tumorigenesis comes from the findings that the *LDH-A* gene is a direct target of both Myc and HIF transcription factors (Lewis et al., 1997; Semenza et al., 1996; Shim et al., 1997). Inhibition of LDH-A by either RNA interference or pharmacologic agents blocks tumor progression in vivo (Fantin et al., 2006; Le et al., 2010; Xie et al., 2009), supporting an important role of elevated LDH-A in tumorigenesis and LDH-A as a potential therapeutic target.

We and others have recently discovered that a large number of non-nuclear proteins, especially those involved in intermediate metabolism, are acetylated (Choudhary et al., 2009; Kim et al., 2006; Wang et al., 2010; Zhao et al., 2010). In this report, we investigated LDH-A acetylation and its functional significance in tumorigenesis.

RESULTS

LDH-A Is Acetylated at Lysine 5

Eight putative acetylation sites were identified in LDH-A by mass spectrometry (Figure S1A available online; Choudhary et al., 2009). Western blotting with anti-acetyllysine antibody showed that LDH-A was indeed acetylated and its acetylation was enhanced approximately 3.5-fold after treatment with trichostatin A (TSA), an inhibitor of histone deacetylase HDAC I and II (Ekwall et al., 1997; Furumai et al., 2001), and nicotinamide (NAM), an inhibitor of the SIRT family of deacetylases (Avalos et al., 2005) (Figure 1A).

We then mutated each of eight putative acetylation sites individually to glutamine (Q), and examined their acetylation. Mutation of either K5 or K318, but not other lysine residues, to glutamine resulted in a significant reduction in LDH-A acetylation (Figure S1B). Arginine substitution of K5, but not K318, dramatically decreased the LDH-A acetylation by approximately 70% (Figure 1B; data not shown), indicating that K5, which is evolutionarily conserved from *Caenorhabditis elegans* to mammals (Figure S1C), is a major acetylation site in LDH-A.

We generated an antibody specifically recognizing the K5-acetylated LDH-A. The specificity of the anti-acetyl-LDH-A (K5) antibody was verified as it recognized the K5-acetylated peptide but not the unacetylated control peptide (Figure S1D). Western blotting using this antibody detected ectopically expressed wild-type, but only weakly recognized the K5R mutant LDH-A (Figure 1C). Moreover, this antibody detected the acetylated but not the unacetylated LDH-A that was expressed and purified from bacteria (Figure 1I). These characterizations demonstrate the specificity of our anti-acetyl-LDH-A(K5) antibody in recognizing the K5-acetylated LDH-A.

We used the anti-acetyl-LDH-A (K5) antibody to determine acetylation of endogenous LDH-A. Acetylation of LDH-A could readily be detected by the antibody. This signal was diminished by LDH-A knockdown and was completely blocked by the preincubation with the antigen peptide (Figure 1D), confirming the specificity of the anti-acetyl-LDH-A(K5) antibody. Treatment of cells with deacetylase inhibitors TSA and NAM strongly increased K5 acetylation of both endogenously (Figure 1E) and the ectopically expressed LDH-A (Figure S1E). To quantify LDH-A acetylation, we employed IEF (isoelectric focusing) to separate the acetylated protein based on the loss of positive charge due to lysine acetylation. The spot with highest pI, spot 0, showed the lowest relative acetylation, while the lowest pI spot 4 had the highest acetylation, indicating that the change of LDH-A pI is at least in part due to acetylation (Figure 1F). Assuming that spot 0 represented the unacetylated LDH-A while spot 4 represented the fully acetylated LDH-A, we estimated that approximately 20% of the LDH-A is acetylated on lysine 5. Therefore, a substantial fraction of endogenous LDH-A could be acetylated.

K5 Acetylation Inhibits LDH-A Enzyme Activity

To test the effect of K5 acetylation, the activity of LDH-A^{K5R} and LDH-A^{K5Q} mutants was compared with that of wild-type LDH-A. We found that LDH-A^{K5Q} displayed only 18% of the wild-type activity, while the LDH-A^{K5R} mutation had a minor effect on the LDH-A activity (Figure 1G). Consistent with an inhibitory effect of acetylation on LDH-A activity, inhibition of deacetylases by NAM and TSA treatment significantly decreased LDH-A enzyme activity by more than 60% (Figures 1H and S1F). Moreover, treatment of NAM and TSA had little effect on the activity of either LDH-A^{K5Q} or LDH-A^{K5R} mutants (Figure 1H).

To definitively demonstrate the effect of K5 acetylation on LDH-A activity, we employed the system of genetically encoding Nε-acetyllysine to prepare recombinant proteins in *Escherichia coli* (Neumann et al., 2008, 2009). This expression system produced LDH-A proteins with 100% acetylation at K5 due to the suppression of the K5-TAG stop codon by the Nε-acetyllysine-conjugated amber suppressor tRNA. We prepared both unacetylated and K5-acetylated LDH-A and compared their enzymatic activity. As shown in Figure 1I, K5-acetylated LDH-A showed significantly lower activity when compared with the unacetylated LDH-A. Collectively, these results demonstrate that acetylation at lysine 5 inhibits LDH-A activity.

SIRT2 Decreases LDH-A Acetylation and Increases Its Enzyme Activity

To identify the deacetylase responsible for LDH-A regulation, we first determined how inhibition of either SIRT or HDAC could affect LDH-A acetylation at lysine 5. Treatment of cells with