Commentary

Lactate dehydrogenase acetylation adds another piece to the puzzle of metabolic reprogramming in pancreatic cancer

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A recent study by Zhao and collaborators has unraveled the key role of post-translational modification of lactate dehydrogenase (LDH) by acetylation in the control of metabolisms and proliferation of pancreatic cancer cells (1).

Unlike normal cells, most invasive tumor phenotypes show a metabolic switch, named the "Warburg effect", changing energy supply from oxidative phosphorylation to an increased glycolysis (2,3). This switch ensures that glucose provides cells with sufficient energy supply and thus high vitality in the hypoxic environment characterizing several tumor types, such as pancreatic cancer (4).

LDH is an oxidoreductase, which constitutes the major checkpoint for the switch from aerobic to anaerobic glycolysis by catalyzing pyruvate reduction into lactate (5). LDH is a tetrameric enzyme that is composed of three different monomeric subunits: LDH-A, LDH-B and LDH-C. The C subunit is only part of homotetrameric enzyme, LDH-C, which plays a role in male fertility, while the A and B subunits are mainly present in skeleton muscles/liver and heart, respectively. An overexpression of LDH-A subunit has been found in several tumor cells, including primary pancreatic cancer cultures (6).

Pancreatic cancer is the fourth most common cause of cancer-related death. Prognosis is very poor with survival rates that have not improved over the past 40 years, and fewer than 5% patients alive five years after diagnosis. The incidence of these tumors has increased steadily in the last decade and recent epidemiological studies predict rising mortality rates (7). The main reasons for the dismal prognosis of PDAC include the lack of effective biomarkers for screening/diagnosis/prognosis (8), as well as the early metastatic spread and the intrinsic resistance to most currently available systemic treatments (9). Surgical resection is the only curative modality, but less than 20% of patients have resectable disease at the time of diagnosis. However, only a subset of resected PDAC patients benefit from chemotherapy, and novel prognostic biomarkers are urgently needed (9).

Serum/plasma and tissue LDH-A levels are prognostic factors in several tumor types (10), and LDH-A levels resulted as a significant predictor for survival in a multicenter study on locally advanced and metastatic pancreatic cancer patients (11). Importantly, LDH-A levels are not necessarily correlated to nonspecific cellular damage. Rather they can be caused by overexpression induced by malignant tumor phenotypes, as shown in several cancer cells, including PDAC cells (6). Lactate production contributes also to extracellular acidosis, thus supporting tumor invasivity and exerting immunosuppressive effects (1,12). Moreover, lactate can be taken up by other tumor cells as well as by stromal cells to regenerate pyruvate that can be used for oxidative phosphorylation (13), promoting the cell's antioxidant defenses against chemotherapeutic agents (14). This arrangement generates an ecosystem in which cancer and stromal cells use complementary metabolic pathways, recycling products of anaerobic metabolism to sustain cancer cell survival and invasion.

Against this background, LDH-A has been identified as an attractive biomarker and potential target for therapies tackling tumor metabolism (6,15). This raised the interest on the mechanisms underlying LDH-A regulation in cancer cells. LDH-A has been identified as a direct target of the c-myc and HER2/neu oncogenes, as well as of hypoxia-inducible factor (HIF-1α), which is a pivotal transcription

factor in hypoxic adaptation (14,16).

However, a large number of proteins undergo critical post-transcriptional modification, and the study by Zhao and collaborators (1) evaluated for the first time the acetylation of LDH-A and its role in pancreatic tumorigenesis. Through mass-spectrometry analysis they identified eight putative acetylation sites, which were then examined by functional studies revealing that acetylation at lysine 5 (K5), inhibited LDH-A catalytic activity. Conversely sirtuin 2 (SIRT2) decreased LDH-A acetylation and increased LDH-A catalytic activity. Moreover, K5 acetylation reduced LDH-A protein levels, because the K5 acetylated LDH-A is recognized by the HSC70 chaperone promoting its lysosomal degradation, within the processes of chaperone-mediated autophagy.

Remarkably, further functional experiments using an acetylation mimetic mutant, demonstrated that K5 acetylation impairs the activity of LDH-A in supporting the proliferation of BxPC-3 pancreatic cancer cells and xenografts. K5 acetylation also reduced pancreatic cancer cells migration, suggesting the role of this protein modification in the control of the invasive behaviour of pancreatic cancer.

In order to validate these preclinical observations, Zhao and collaborators collected more than 100 pancreatic cancer tissues and performed immunohistochemical analyses using both anti-LDH-A and a specific anti-acetyl-LDH-A-K5 antibody. These analyses found higher LDH-A protein levels in tumors compared to normal tissues in 37 out of 39 paired cases. These tumors also showed decreased acetylation at K5, paralleled by increased expression of SIRT2, supporting the direct and inverse correlation of this deacetylase with LDH-A and LDH-A-K5 levels, respectively.

However, no significant differences in LDH-A-K5 expression levels were observed in pancreatic tumors at different stages, suggesting the role of this potential novel biomarker for early diagnosis, but not for the study of pancreatic cancer progression. Future studies in larger cohorts of patients as well as in patients with clinically annotated data are needed to evaluate the prognostic role of LDH-A-K5 expression levels.

The identification of new prognostic factors for survival can indeed be critical for better clinical management for subsets of pancreatic cancer patients. The most biologically aggressive pancreatic cancers, such as those that recur soon after resection, should be treated initially with systemic therapy, as opposed to major surgery, which exposes the patients to substantial operative risk with little expected benefit. On the other hand, patients with indolent cancers may benefit from a more aggressive surgical approach (17). Moreover, prognostic biomarkers provide mechanistic insights into cancer progression, and might unravel molecular targets for novel treatment strategies.

Recent studies evaluated the pharmacological activity of novel LDH inhibitors in pancreatic cancer cells (6,18). These compounds were especially effective against pancreatic cancer cells under hypoxic condition and their combination with gemcitabine was synergistic. This synergistic effect was associated with increase in apoptosis and inhibition of cell migration. Importantly, inhibition of LDH-A is unlikely to give rise to major side effects in humans since hereditary LDH-A deficiency does not provoke any symptoms under ordinary circumstances, and only causes myoglobinuria after intense anaerobic exercise (5). Therefore, compounds that inhibit LDH-A enzymatic activity should be safe agents that can interfere selectively with tumor growth, invasiveness and chemoresistance.

The findings of Zhao and collaborators open new avenues for the development of targeted agents against the metabolic reprogramming of pancreatic cancer, supporting the study of novel drugs that stimulate LDH-A acetylation by targeting the LDH-A deacetylase SIRT2. However, SIRT2 has been reported as a tumor suppressor gene in knockout mouse models, with females primarily developing mammary tumors, and males developing more hepatocellular carcinoma (19). These data raise questions about the differential biological role of SIRT2 in pancreatic tumors and other cancers. Further studies should therefore evaluate if SIRT2 function in cancer development might be tumor-dependent.

Similarly, the results of Zhao and collaborators add to the varied and often contradictory results regarding the activity of autophagy and its regulation in pancreatic cancer. Both decreased and increased autophagy showed to be related to pancreatic cancer, and several experimental evidence pointed at autophagy as a mechanism to protect pancreatic cancer cells under adverse environmental conditions, while other studies showed that autophagy is detrimental to pancreatic cancer cells (20). From these conflicting data one could infer that the high inter- and intra-tumor heterogeneity in terms of complexity of genetic and metabolic aberrations, which is one of the main hallmarks of pancreatic cancer, might cause a range of different events involved in autophagy-mediated survival or death. Thus, future studies aimed at inhibiting the LDH-A activity or restore its acetylation should also investigate how the different autophagic processes might affect the proliferation of pancreatic cancer cells.

In conclusion, the study by Zhao and collaborators not only provided new insights on the regulation of LDH

activity, unraveling the key role of acetylation of this protein, but will also prompt important studies on the potential prognostic and therapeutic value of K5 acetylated LDH-A.

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