Preliminary exploration of molecular mechanism of the anticancer activity of a novel lead Lactate Dehydrogenase inhibitor against Pancreatic Cancer

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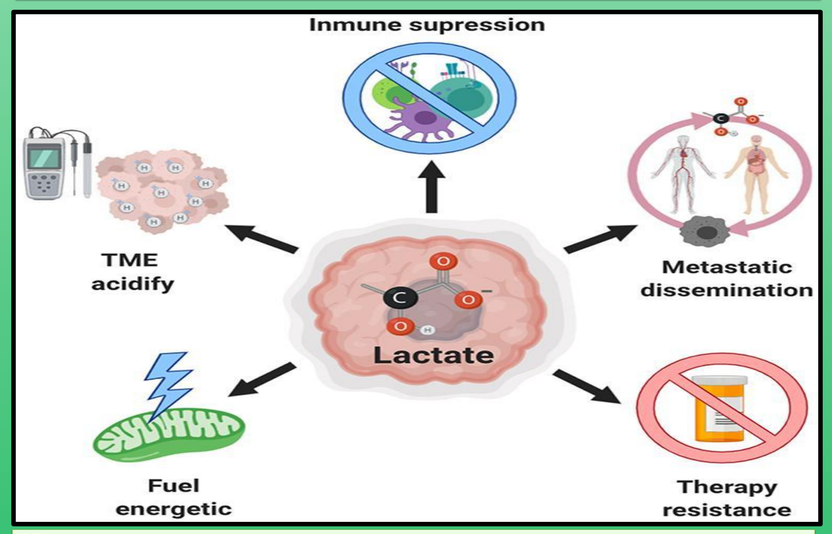
BACKGROUND

Pancreatic ductal adenocarcinoma (PDAC) is a recalcitrant cancer and a leading cause of cancer-related death in the United States, with a long-term survival rate of just under 10% [1]. Chemotherapy is one of the standard treatment options for pancreatic cancer; however, rapid development resistance to chemotherapeutic agents represents a significant bottleneck. Therefore, identifying novel biological drug targets and exploring biomolecular mechanisms that lead to chemoresistance in pancreatic cancer is urgently required. Lactate dehydrogenase A (LDHA): the primary metabolic enzyme that facilitates the glycolytic process by rapidly converting pyruvate to lactate. The overexpression of LDHA is common in malignant cancers. [2,3]. Multiple studies have shown that LDHA plays an important role in the growth, invasion and metastasis of these malignant tumors [4]. As LDHA expression increases, lactate concentration in the tumor microenvironment (TME) increases as well. By exploiting aerobic glycolysis, cancer cells secrete high amounts of lactate that is then used for diverse functions that support cell proliferation and resistance [5].



[4] **FIGURE 1: Lactate produces many intermediates that promotes cancer cell growth. The increased production of lactate promotes acidosis in TME which favors angiogenesis and metastasis. It favors immunosuppression by inhibition of T cell lymphocytes. Lactate can enter the TCA cycle where it participates in generation of many TCA intermediaries that can then be used as fuel.**

OBJECTIVES

- To understand the molecular mechanisms underlying the anticancer activity of a novel LDHA inhibitor, compound 23, in pancreatic cancer cell lines.
- To determine if LDHA inhibitor, compound 23 promotes cancer cell death via inducing apoptosis.

METHODS

Cell Culture: MIA PaCa-2 cell were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum and 2.5% , horse serum at 37°C in a 5% CO_2 atmosphere.

FIGURE 2: Culturing of MIA PaCa-2

FIGURE 3: Cell Treatment Methods: MIA PaCa-2 cells were seeded in 6-well plates overnight, next day medium was replaced with serum free medium, and cells were treated with 50uM concentration of the LDH-A-inhibitor for 24 hours.

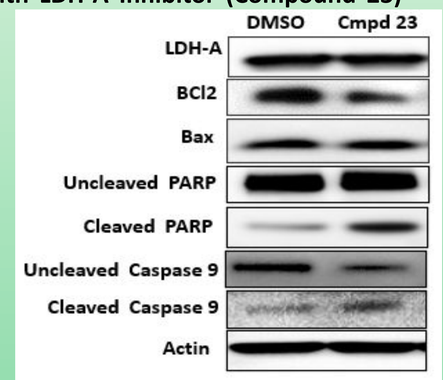
Protein extraction:
Cells were lysed using ice-cold RIPA buffer supplemented with protease inhibitors by using gentle scrapping. Lysate was incubated (4°C, 20 min), and then centrifuged (15,000g, 20 min, 4°C) to collect the total proteins. Total protein in cell lysates was measured by the bicinchoninic acid method.

Immunoblotting:
Equal amounts of protein from each sample were loaded on a NuPAGE Bis-Tris Gels (4-12% gradient gel) and transferred to polyvinylidene fluoride membrane. The membranes were incubated with appropriate primary and secondary antibodies. Immunoreactive proteins were detected using Luminata Forte Western HRP Substrate and quantified. . Antibodies for LDHA (#3582), Actin (#3700), and apoptosis sampler kit (#9915.) were purchased from Cell Signaling Technology.

RESULTS

Treatment of Mia-PaCa2 cells with LDH-A Inhibitor (Compound 23) Promotes Apoptosis:

Figure 4: Mia-Paca2 Cells were treated with 50 µm concentration of LDH-A inhibitor. DMSO was used as the control. The cells were lysed, and the expression of proteins related with apoptotic pathway was examined by western blot analysis.



- We evaluated the mechanisms behind the anticancer activity of a novel LDHA inhibitor 23 in pancreatic cancer cell lines.
- We performed Western blot analysis of Mia-Paca2 cells treated with compound 23 for 24 h.
- We found that the LDHA-inhibitor, inhibited the activity of LDHA(data not shown), without affecting its the expression.
- Western blot analysis revealed activation of proapoptotic proteins, like cleaved poly (ADP-ribose) polymerase (PARP), cleaved Caspase 9 while the expression of the anti-apoptotic protein, B-cell lymphoma 2 (Bcl-2) was decreased by the LDH-A-inhibitor.
- Actin was used as a loading control.

CONCLUSIONS

- Our initial findings suggest that the antiproliferative effect of LDHA inhibitor 23 in pancreatic cancer cells are due to induction of apoptosis.

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