

Targeting Tumor Metabolism through Lactate Dehydrogenase A Inhibits Tumor Progression in Anaplastic Thyroid Carcinoma

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Introduction

Anaplastic thyroid carcinoma (ATC) is one of the most lethal and aggressive human malignancies with a high mortality rate and a median survival duration of 4 to 6 months. Current therapeutic approaches to ATC have limited efficacy, heightening the urgency for additional therapeutic options for this disease. Lactate dehydrogenase A (LDHA) is a critical and essential glycolytic enzyme responsible for conversion of pyruvate into lactate in anaerobic and aerobic glycolysis (Warburg effect). *LDHA* overexpression is a hallmark of aggressive malignancies and has been linked to tumor initiation, reprogramming, and progression in multiple tumor types¹⁻³. We previously showed that the conversion of pyruvate into lactate by LDH represents a critical metabolic nexus that contributes to tumor cell management of oxidative stress⁴. Both conventional chemotherapy (e.g., cisplatin, doxorubicin) and external beam radiotherapy (EBRT) generate transient fluctuations in cellular oxidative stress that are balanced through differential metabolic flux through LDH⁵. This phenomenon suggests that LDHA plays a critical role in not only tumorigenesis, but also treatment response. We established an inducible *LDHA* knockdown cell line to investigate the actual contribution of *LDHA* to tumorigenesis *in vitro* and *in vivo*.

Methods

Anaplastic thyroid cancer (ATC) cell lines were authenticated by short tandem repeat profiling. Inducible *LDHA* knockdown cell lines were established by using pINDUCER-EmGFP-sh*LDHA* lentivirus system⁶. *LDHA* expression were determined by immunoblotting. LDHA enzyme activity and lactate production were determined by biochemical assay kits. Functional validation of *LDHA* knockdown was measured kinetically using hyperpolarized ¹³C-Pyruvate MRI (HP-MRI). We assessed the functional contribution of *LDHA* in cell proliferation and colony formation following exposure to doxycycline (DOX) to induce *LDHA* knockdown. We assessed the anti-tumor effects of *LDHA* suppression in an orthotopic xenograft tumor mouse model. Xenograft tumor growth was monitored by serial bioluminescence imaging and physical measurement.

Results

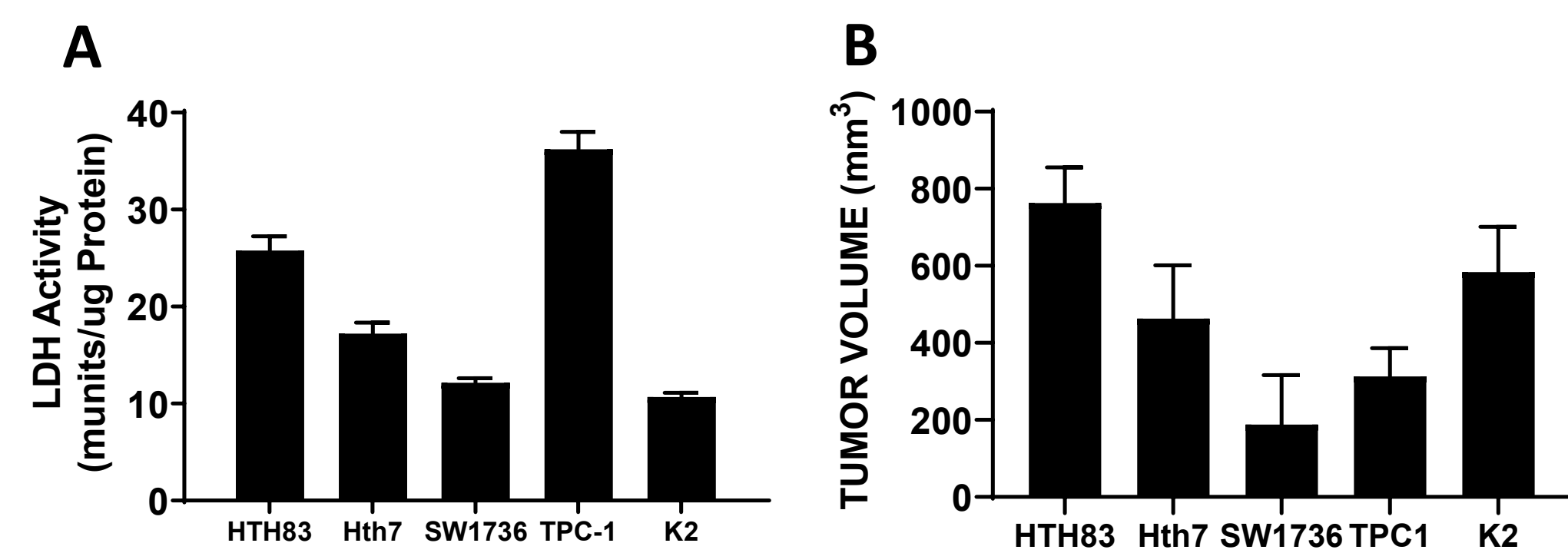


Figure 1. LDH activity and tumorigenic potential on thyroid carcinoma. (A) LDH activity under baseline growth conditions in thyroid cancer cell lines. (B) Baseline tumorigenic potential of thyroid cell lines in the orthotopic model. Thyroid cancer cells demonstrated increased LDH enzyme activation. However, LDH activity was highly variable in thyroid cancer cell lines. LDH activity did not correlate with tumorigenesis in the orthotopic murine models of thyroid cancer.

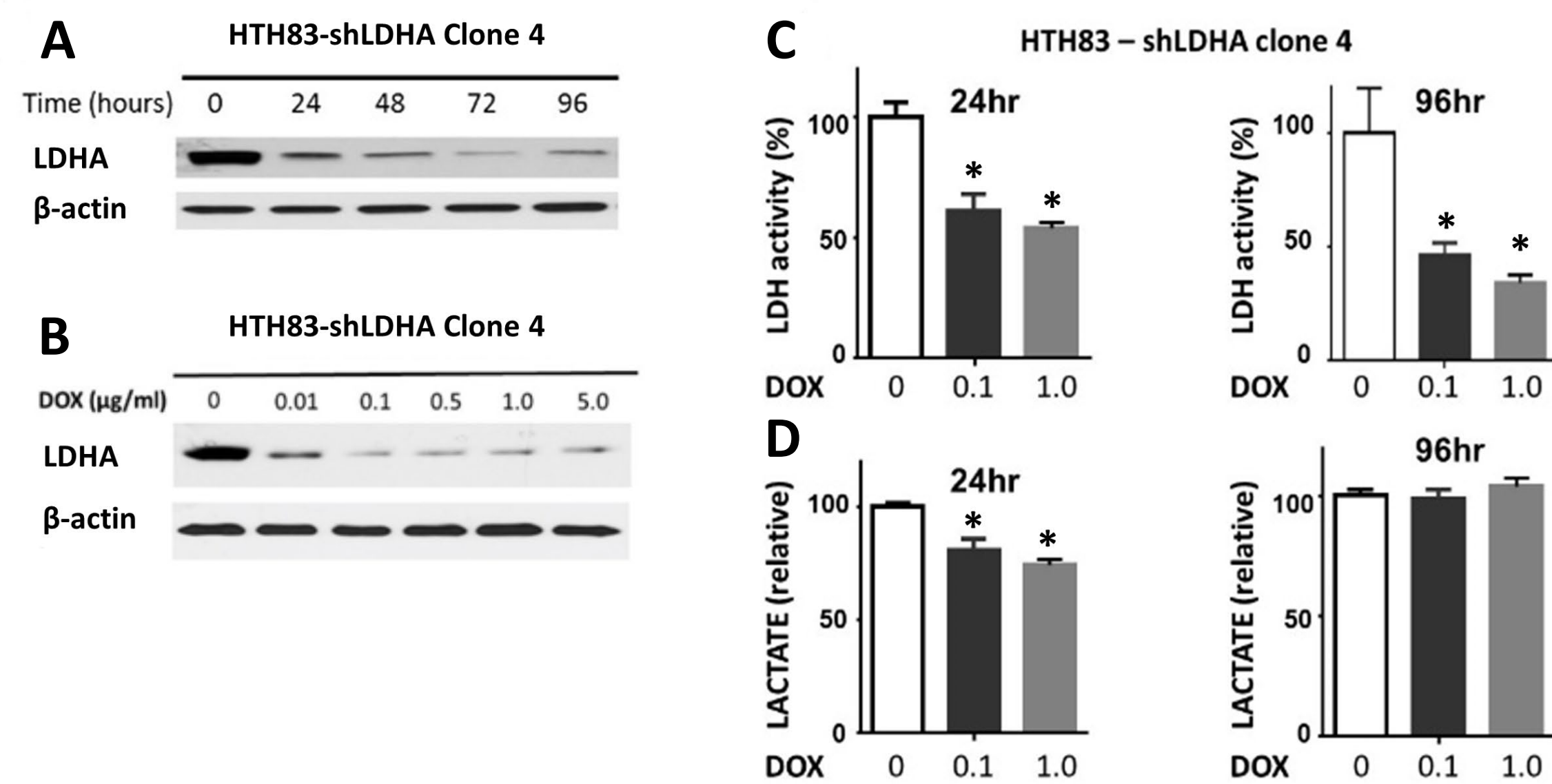


Figure 2. Inhibition of *LDHA* by inducible shRNA led to decreased LDH enzyme activation and lactate production. (A) HTH83 cells were infected with lentivirus containing sh*LDHA* constructs. Following exposure to DOX for 24-96 hours, activation of sh*LDHA* suppressed *LDHA* protein levels within 24 hours of exposure. (B) *LDHA* was suppressed by DOX(0, 0.01, 0.1, 0.5, 1, and 5 μg/mL) for 48 hours, resulting in decreased *LDHA* protein levels. (C) Reduced *LDHA* expression led to decreased LDH activity and lactate production within 24 hours of activated suppression. LDH activity continued to drop significantly at both 24 and 96 hours after activation. (D) However, although LDH activity remained suppressed, lactate levels equilibrated at 96 hours after activation. All experiments were performed at least triplicate, and every condition was tested at least in triplicate. Error bars represent standard deviation. *p<0.05 compared with control condition.

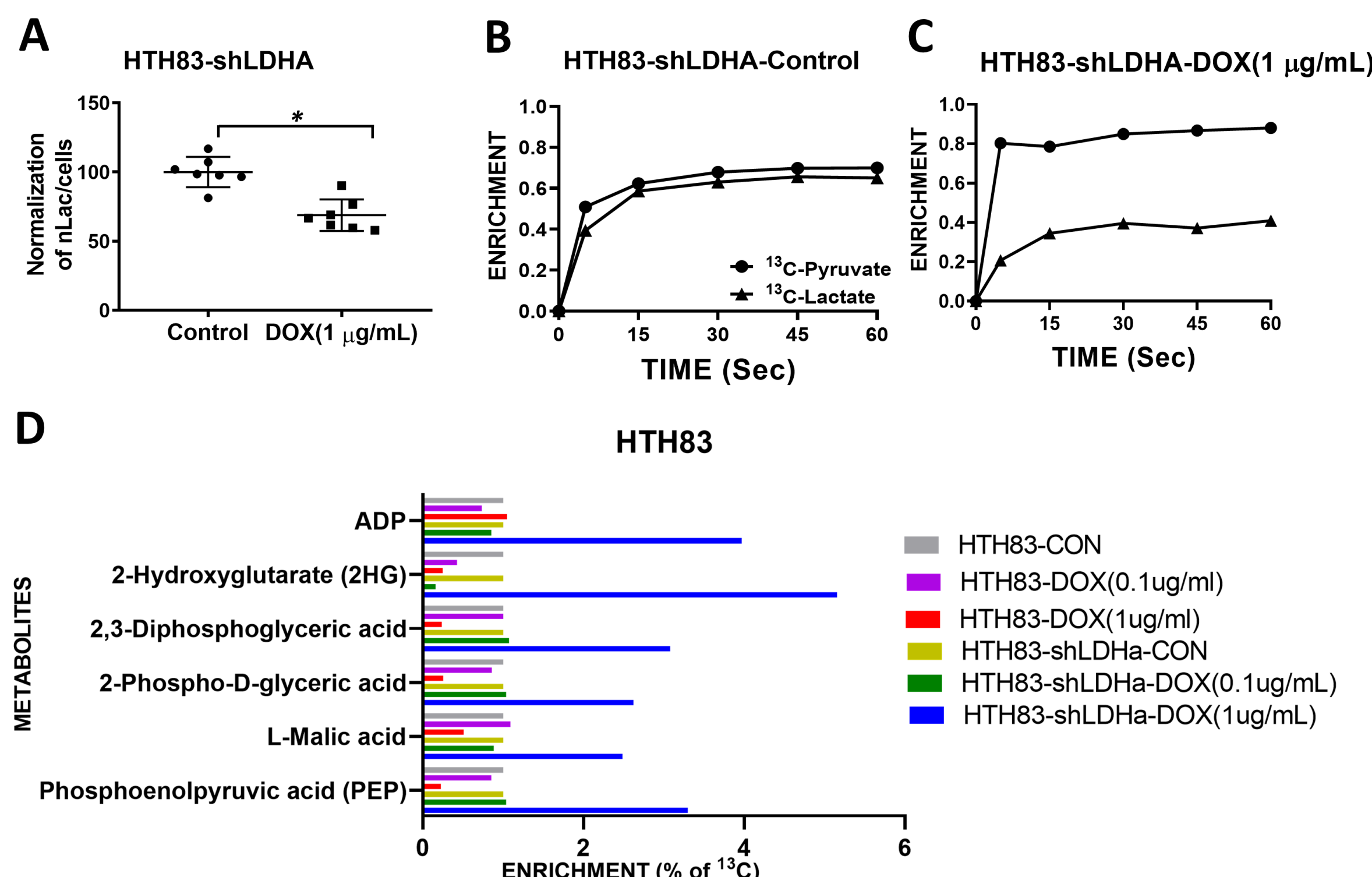


Figure 3. *LDHA* suppression resulted in decreased nLac values and conversion ratio of ¹³C-pyruvate to lactate as measured by HP-MRI (A) Activation of sh*LDHA* constructs suppressed LDH activity as measured kinetically using HP-MRI for ¹³C-labeled pyruvate and its labeled byproduct lactate in HTH83-sh*LDHA* clone 4. (B) Mass spectrometry-based analysis of intracellular pool of pyruvate and lactate in HTH83-sh*LDHA* clone 4 using ¹³C-labeled pyruvate as tracer. (C) Ion chromatography high-resolution accurate-mass mass spectrometry analysis of intracellular tricarboxylic acid and glycolysis pathways metabolites to determine these pathways' incorporation of ¹³C3-pyruvate. All experiments were performed at least triplicate, and every condition was tested at least in triplicate. Error bars represent standard deviation. *p<0.05 compared with control condition.

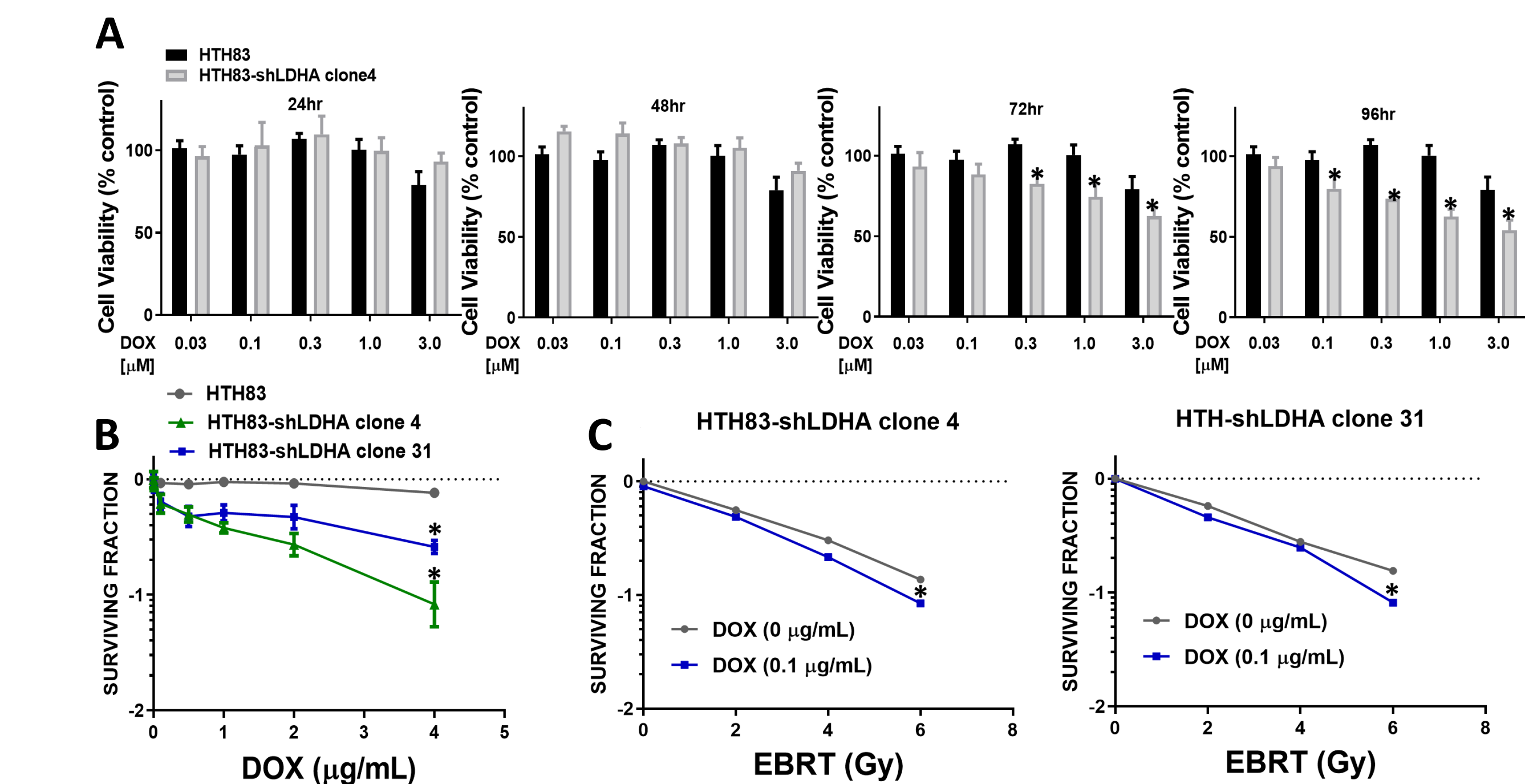


Figure 4. Decreased *LDHA* activation decreased cell proliferation and colony formation following DOX treatment. (A) Suppression of *LDHA* triggered a time- and dose-dependent decrease in viability. (B) Suppression of *LDHA* significantly decreased the clonogenic surviving fraction, suggesting the *LDHA* is essential for cell survival. (C) Suppression of *LDHA* modestly increased radiation effectiveness, measured using clonogenic surviving fraction. All experiments were performed at least triplicate, and every condition was tested at least in triplicate. Error bars represent standard deviation. *p<0.05 compared with control condition.

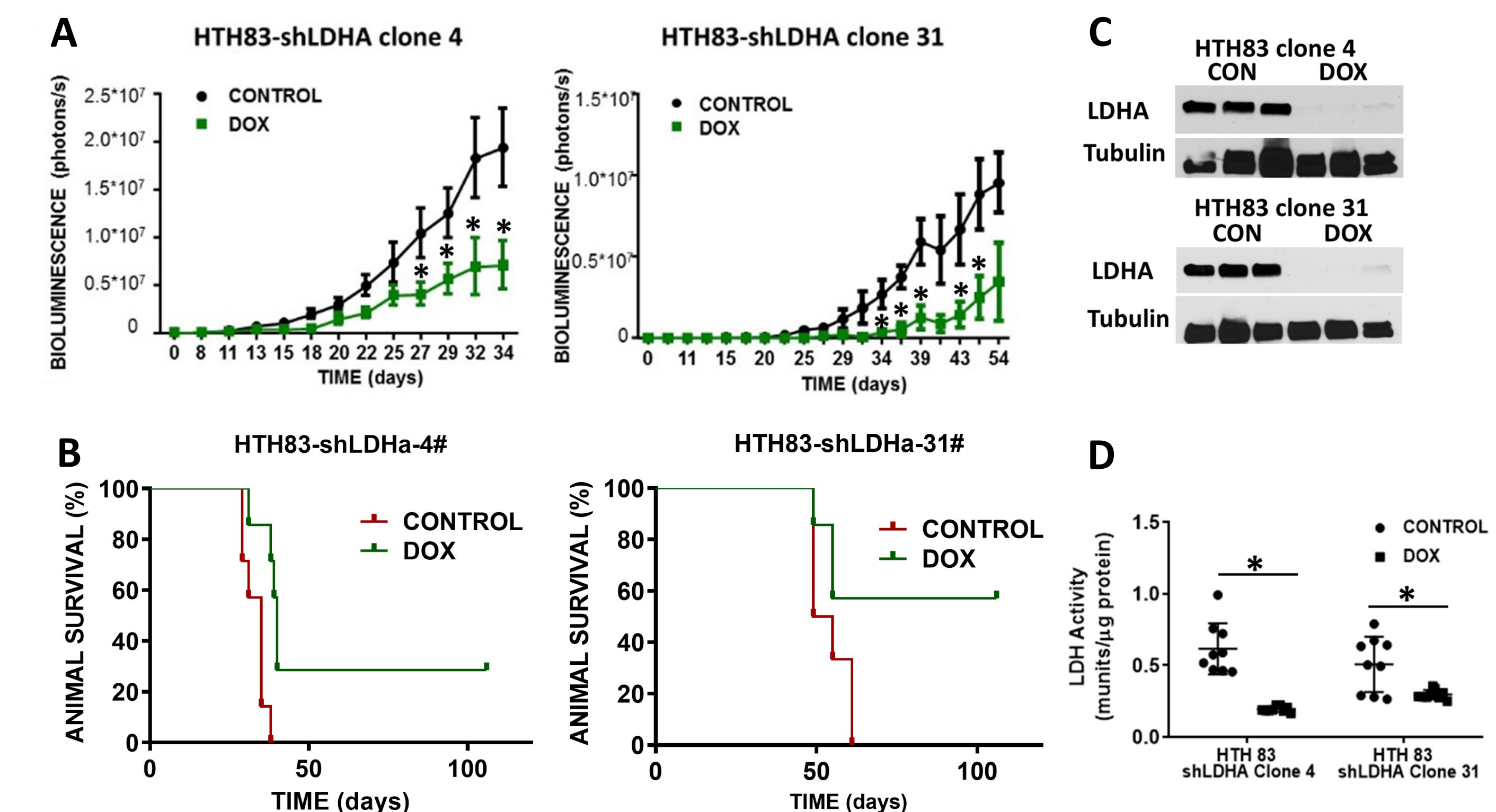


Figure 5. *LDHA* suppression significantly reduced ATC orthotopic tumor growth and prolonged median survival time. (A) HTH83 clones demonstrated that suppression of *LDHA* significantly decreased bioluminescence activity and suppressed tumor-growth compared with growth in control mice, which is consistent with relative *LDHA* activity levels. (B) Suppression of *LDHA* significantly prolonged median survival time; the control group (n=11) had a median survival of 56 days, while mice treated with DOX-water (n=11) had a median survival more than 80 days and were subsequently euthanized to complete this study. (C) Western blots demonstrating suppression of *LDHA* protein levels in tumors following treatment with DOX. (D) Biochemical analysis of LDH activity was confirmed significantly decreased LDH activity in the tumors undergoing *LDHA* suppression. Data for A, B, and D are presented as means with error bars representing standard deviations. *p<0.05 compared with control condition.

Conclusion

- This study, we developed a systematic approach to investigate a critical metabolic enzyme (*LDHA*) that is essential to the Warburg effect in order to identify some potential limitations to our current metabolic targeting approaches.
- We employed an inducible shRNA Lentiviral Tet-On system and established stable ATC cell lines with inducible downregulation of *LDHA* expression by DOX.
- Suppression of *LDHA* led to decreased LDH enzyme activation and lactate production.
- Suppression of *LDHA* resulted in decreased nLac values and conversion ratio of pyruvate to lactate.
- Suppression of *LDHA* inhibits maximal cancer cell proliferation and colony formation of ATC cell lines.
- Suppression of *LDHA* radiosensitizes ATC cells.
- Partial adaptation to *LDHA* inhibition is driven by redirection of carbon flux.
- LDHA* activity is required for tumor proliferation and its suppression resulted in significant tumor growth delay and prolonged median survival time. Targeting of *LDHA* in ATC affected tumor function and growth *in vitro* and *in vivo*.
- Understanding the functional role of *LDHA* supports the development of new anti-metabolic therapeutic strategies to treat aggressive carcinomas.

References

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