Aberrant DDR1 Expression in Liposarcoma and the Therapeutic Potential of its Targeted Small Molecule Inhibition

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INTRODUCTION

Discoidin domain receptor family member 1 (DDR1) is a collagen-activated receptor tyrosine kinase with roles in proliferation, apoptosis, invasion, and cell survival in various cancers.

Several studies to date have successfully abrogated DDR1 expression and subsequent tumorigenesis via small molecule inhibition. However, there is currently no work exploring this strategy in liposarcomas.

We sought to confirm DDR1 expression and the potential of targeted anti-DDR1 therapy via CRISPR-Cas9 knockout, specific DDR1 siRNA, and small molecule inhibitor 7RH in metastatic human liposarcoma cells.

METHODS

Tissue Microarray

A tissue microarray of 53 liposarcoma patient specimens was analyzed by immunohistochemistry to measure DDR1 expression. Relative Expression of DDR1 was compared between liposarcoma and liposarcoma subtypes. The survival curves were produced by Kaplan Meier methods.

CRISPR-Cas9 plasmid design and purification

The CRISPR-Cas9 sgRNA design, transfection optimization, gene editing, sequencing validation, and liposarcoma and cell line DDR1 knockouts CRL-3043 and SW872 were purchased from Synthego, Inc.

Protein preparing and Western blotting

The expression of DDR1 protein in CRL-3043 and SW872 DDR1 knockouts and wild-type cells was evaluated by Western blotting following silencing via CRISPR-Cas9, siRNA, and small molecule inhibitor 7RH.

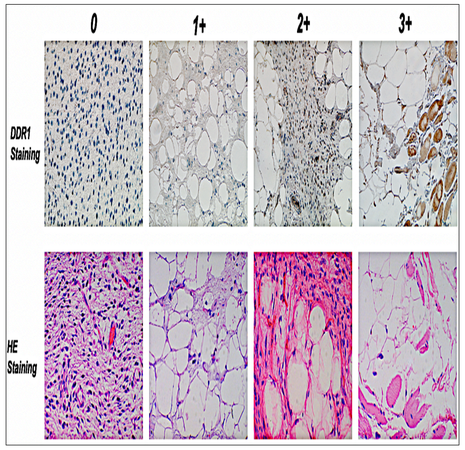
Wound healing assay and cell invasion assay

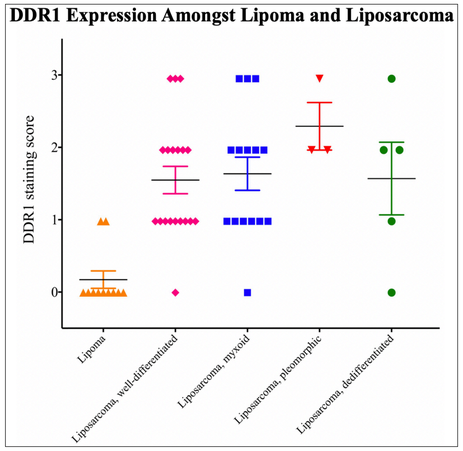
Cell migration activity was evaluated by wound healing assay. Cell invasion activity was evaluated by transwell invasion chamber assay.

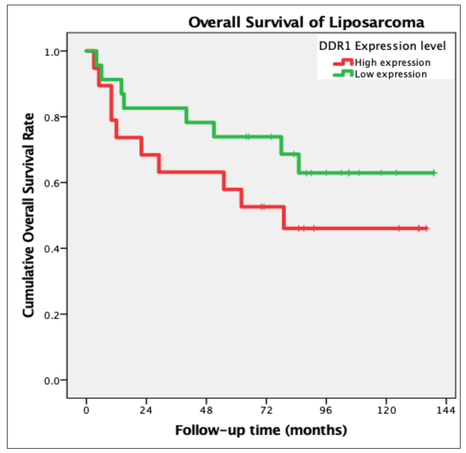
Determination of DDR1 knockout on liposarcoma cell spheroid formation in 3-D culture

Cell spheroid formation was performed following HDP 1096 Perfecta3D 96-Well Hanging Drop Plates Protocol (3D Biomatrix).

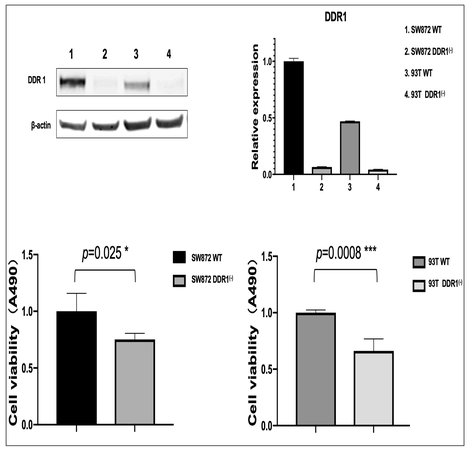
DDR1 STAINING IN A LIPOMATOUS TISSUE MICROARRAY

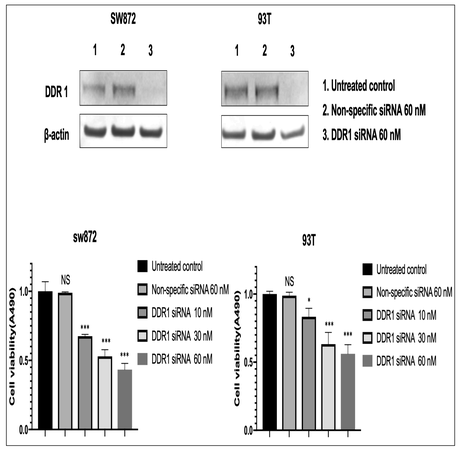


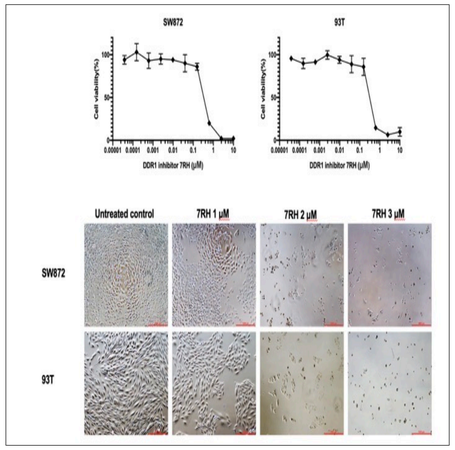




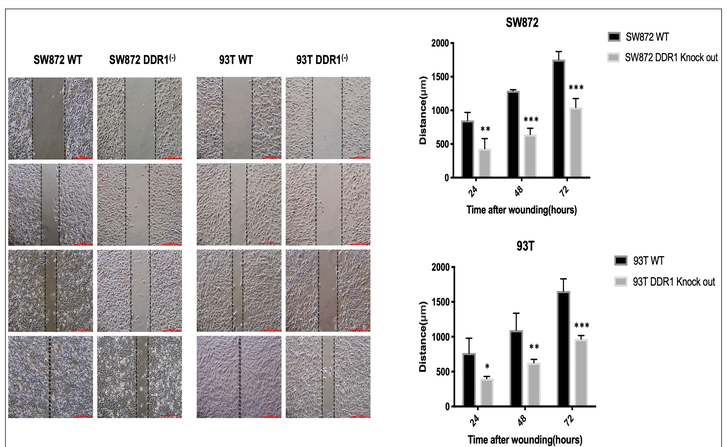
DDR1 SILENCING VIA CRISPR-CAS9, SIRNA, SMALL MOLECULE INHIBITOR



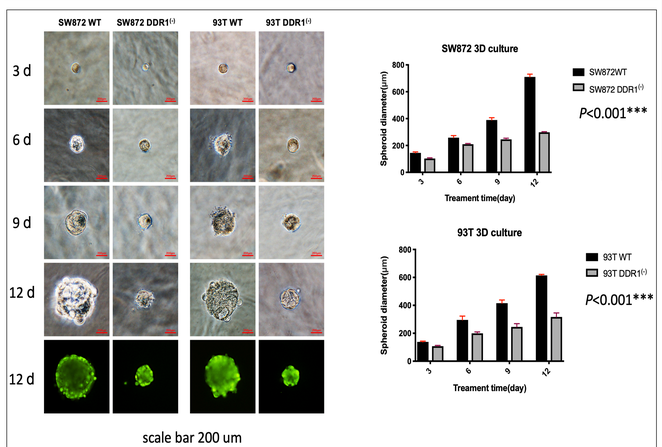




DDR1 KO MIGRATION ASSAY



DDR1 KO 3D CELL CULTURE



RESULTS

DDR1 was expressed in 92.9% of the liposarcomas and *highly* expressed in 54.8% of the liposarcomas. None of the lipoma tissues showed high DDR1 expression

DDR1 is principally localized to the cell membrane. It was highly expressed in all tested human liposarcoma cell lines.

The expression of DDR1 in highly metastatic human liposarcoma cell lines was successfully silenced by CRISPR-Cas9, DDR1 siRNA, and small molecule inhibitor 7RH.

When DDR1 was silenced, the proliferation and spheroid formation of cells was inhibited under 3-D culture conditions. Furthermore, the migratory and invasive functions were also impaired in these highly metastatic liposarcoma cell lines.

Inhibition of DDR1 via DNA knockout reduced prevented liposarcoma cell growth and proliferation in a dose-dependent manner.

SIGNIFICANCE

Ours is the first study to verify the expression of DDR1 in liposarcoma tissues and cell lines and its successful knockdown at the DNA, RNA, and protein levels.

The function of DDR1 was evaluated *in vitro* and *ex vivo*, showing DDR1 inhibition decreases cell growth and proliferation, while inducing apoptosis in liposarcoma cells.

Collectively, these results suggest DDR1 is a critical regulator of liposarcoma progression and a promising target of future *in vivo* studies.

ACKNOWLEDGEMENTS

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