ASPH blockade abrogates doxorubicin induced chemoresistance in chondrosarcoma

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Significance & Hypothesis/Techniques:

Chondrosarcoma (CS) is notable for being resistant to cytotoxic chemotherapy, which contributes to poor survival. Hence, there is a need for strategies that can reverse resistance. Molecular mechanisms underpinning CS chemoresistance and methods of reversal are unclear. Aspartyl/asparaginyl beta-hydroxylase (ASPH) is an oncofetal cell surface protein overexpressed in CS that is predictive of survival. ASPH augments Notch signaling, and Notch is implicated in many of the hallmarks of cancer, including chemoresistance. We hypothesized that ASPH inhibitor MO-I-1182 would sensitize CS cells to doxorubicin. We used these drugs to treat CS cell lines that were engineered with loss of ASPH or Notch expression to analyze the mechanisms of the drug effects. Cell viability and invasion assays, and molecular analyses were performed.

Innovation:

Our findings show conceptual and pre-clinical innovation by identifying synergy between ASPH inhibition and doxorubicin. We linked this to mechanisms involving ASPH-Notch signaling and multidrug resistance protein 1 (MRP1/ABCC1) expression. These innovations represent new directions for chondrosarcoma treatment.

Approach:

CS-1 and JJ012 CS were used with C28/I2 chondrocytes for control. ASPH knockout was performed with CRISPR/Cas9 and Notch1 knockdown with siRNA. Cell viability was measured using MTT assay and nuclear staining with Hoechst 33342. Gene and protein expression were analyzed with qPCR and Western blotting. Dose-response curves were compared using nonlinear mixed models. Compusyn software was used for synergy analysis. Other comparisons were made with Student's t-test, $p \leq 0.05$ for significance.

Results

CS cells express more ASPH than C28/I2 cells and MO-I-1182 decreased viability and invasion more so in CS cells compared to C28/I2 cells (not shown). Combination treatment with MO-I-1182 plus doxorubicin showed reduced doxorubicin EC50 in chondrosarcoma cells but not in C28/I2 cells (1A). Combination treatment was synergistic at all doses in CS but not in C28/I2 cells (1B).

ASPH or Notch1 knockdown reduced ASPH and Notch1 target gene expression (not shown), decreased sensitivity to MO-I-1182, and increased sensitivity to doxorubicin (2A). Notch1 KD had a dose-dependent effect on doxorubicin sensitivity (2B).

In CS cells, doxorubicin increased ASPH, Notch1, and ABCC1 transcripts, and all were mitigated by MO-I-1182 (3A). Doxorubicin increased protein levels of ASPH (3B), activated Notch1 (N1ICD) and ABCC1 in CS cells (3C), and these increases were blocked by MO-I-1182 (3BDE), blocked in a dose-responsive manner (3F), and were dependent on ASPH expression as shown using ASPH KO cells (3G).

Discussion & Impact:

ASPH inhibition with MO-I-1182 synergistically increased the effect of doxorubicin on CS cell viability. Mechanistically, we observed a link between ASPH and Notch signaling resulting in increased expression of drug efflux transporter ABCC1, and positive feedback induced by doxorubicin. MO-I-1182 inhibits this pathway and counteracts doxorubicin induced expression of ASPH, Notch, and ABCC1 (3H). Notch signaling inhibition has been a longstanding goal in cancer therapeutics, but clinical trials have failed due to off target effects. ASPH expression is restricted to cancer cells, making it a more therapeutically appropriate target. Our findings support development of ASPH inhibition in combination with cytotoxic chemotherapy to convert chondrosarcoma to a chemosensitive tumor.

FIGURE 1

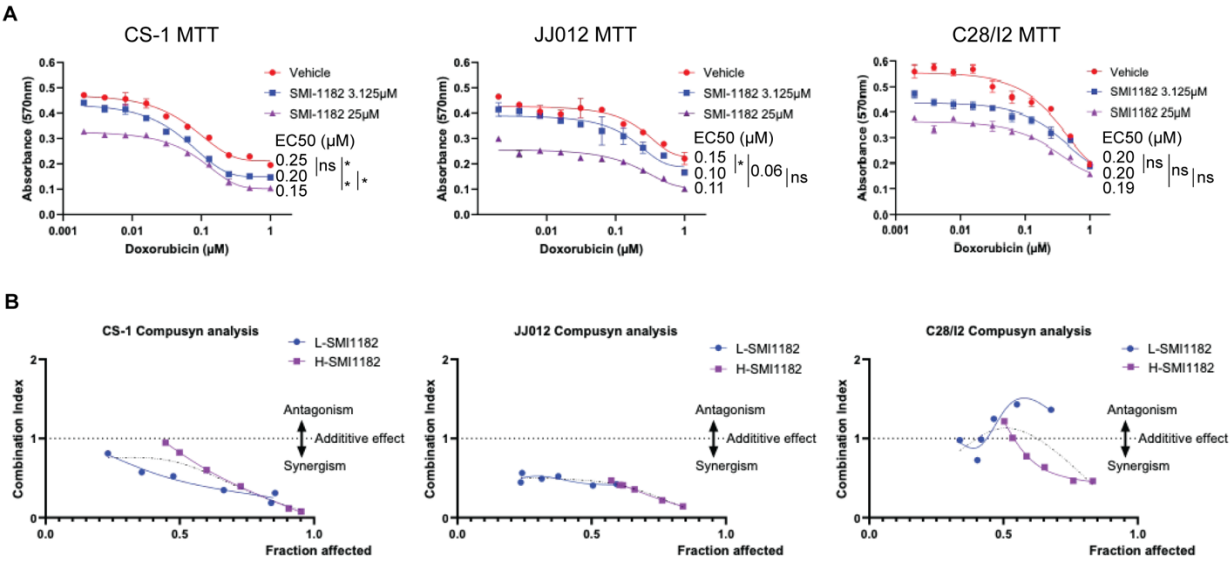

A
CS-1 MTT
JJ012 MTT
C28/I2 MTT
Absorbance (570nm)
Doxorubicin (μM)
Vehicle
SMI-1182 3.125μM
SMI-1182 25μM
SMI1182 3.125μM
SMI1182 25μM
EC50 (μM)
0.25
0.20
0.15
ns
*
*
*
0.15
0.10
0.11
*
0.06
ns
0.20
0.20
0.19
ns
ns
ns
B
CS-1 Compusyn analysis
JJ012 Compusyn analysis
C28/I2 Compusyn analysis
L-SMI1182
H-SMI1182
Combination Index
Antagonism
Additive effect
Synergism
Fraction affected

FIGURE 2

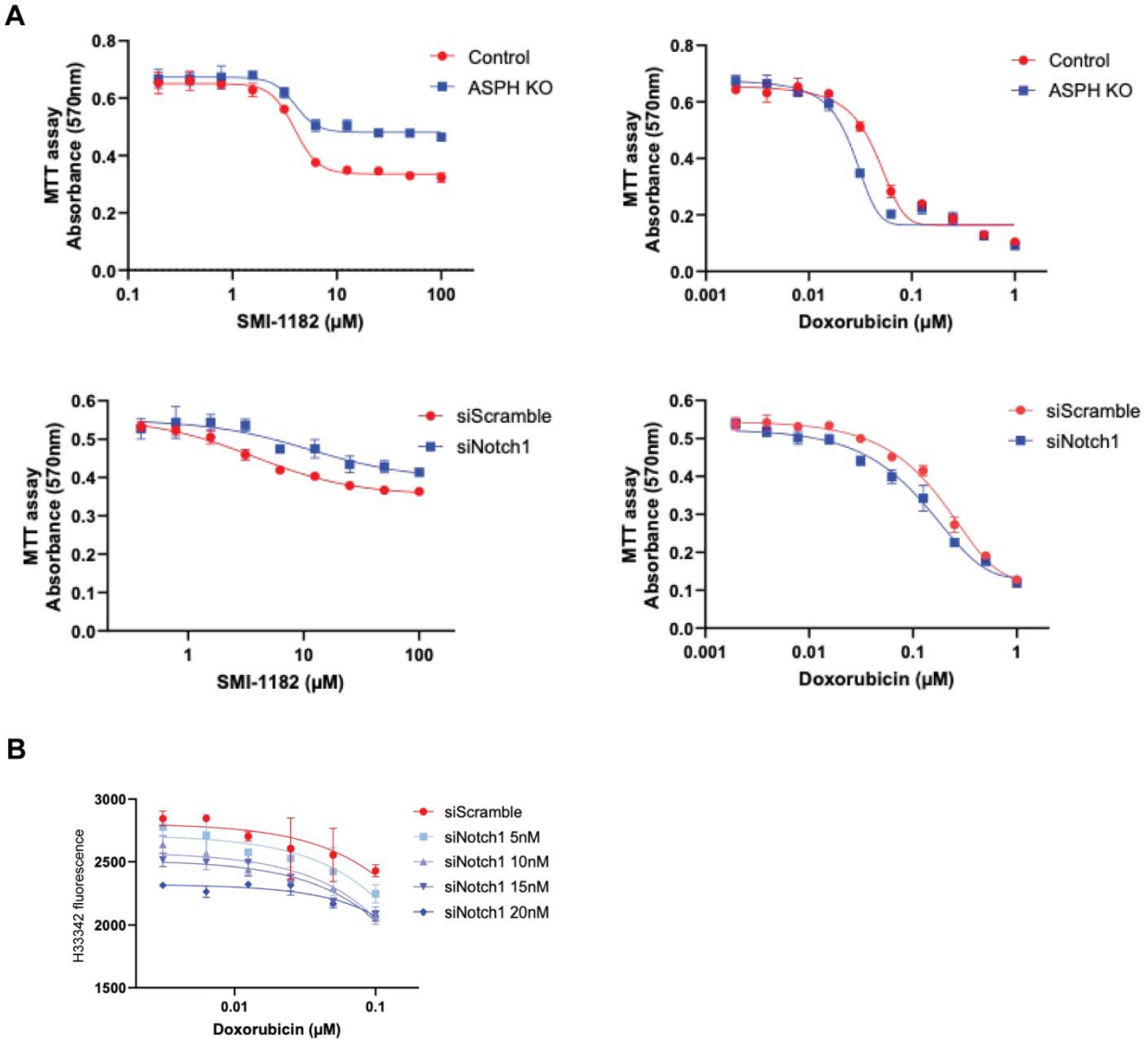
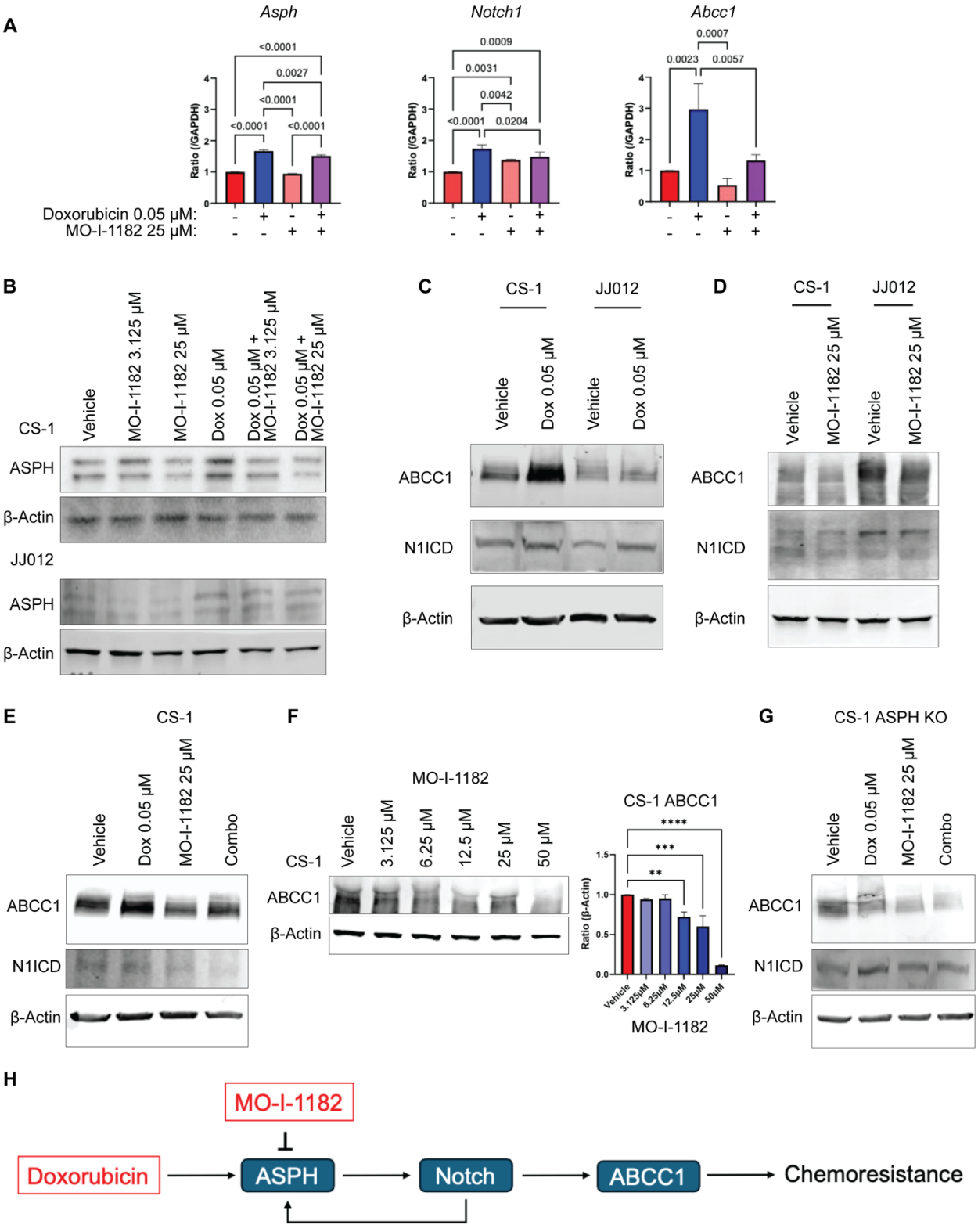

A
MTT assay Absorbance (570nm)
SMI-1182 (μM)
Control
ASPH KO
Doxorubicin (μM)
siScramble
siNotch1
B
H33342 fluorescence
siNotch1 5nM
siNotch1 10nM
siNotch1 15nM
siNotch1 20nM

FIGURE 3


A
Asph
Notch1
Abcc1
Ratio (/GAPDH)
<0.0001
0.0027
<0.0001
<0.0001
<0.0001
0.0009
0.0031
0.0042
<0.0001
0.0204
0.0007
0.0023
0.0057
Doxorubicin 0.05 μM:
MO-I-1182 25 μM:
B
CS-1
Vehicle
MO-I-1182 3.125 μM
MO-I-1182 25 μM
Dox 0.05 μM
Dox 0.05 μM + MO-I-1182 3.125 μM
Dox 0.05 μM + MO-I-1182 25 μM
ASPH
β-Actin
JJ012
C
CS-1
JJ012
Vehicle
Dox 0.05 μM
ABCC1
N1ICD
β-Actin
D
CS-1
JJ012
Vehicle
MO-I-1182 25 μM
E
CS-1
Vehicle
Dox 0.05 μM
MO-I-1182 25 μM
Combo
ABCC1
N1ICD
β-Actin
F
MO-I-1182
Vehicle
3.125 μM
6.25 μM
12.5 μM
25 μM
50 μM
CS-1 ABCC1
****
***
**
Ratio (β-Actin)
MO-I-1182
G
CS-1 ASPH KO
H
MO-I-1182
Doxorubicin
ASPH
Notch
ABCC1
Chemoresistance