

## Desmoid Invasiveness Correlates with *in vitro* Resistance to Doxorubicin

<sup>1</sup>Joyner, DE; <sup>1</sup>Trang, SH; <sup>2</sup>Aboulafia, AJ; <sup>3</sup>Damron, TA; <sup>1</sup>Cummings, JE; <sup>1</sup>Randall, RL

<sup>1</sup>SARC™ Laboratory, Sarcoma Services, Department of Orthopaedics & Huntsman Cancer Institute, University of Utah, Salt Lake City, UT

<sup>2</sup>Sinai Hospital Cancer Institute, Baltimore, MD

<sup>3</sup>Department of Orthopaedics, SUNY Upstate Medical University, Syracuse, NY

Senior author David E. Joyner@hci.utah.edu

### PURPOSE:

Desmoid tumors are locally invasive myofibroblastic lesions that arise predominantly in the abdominal wall or shoulder girdle but can develop in the extremities, chest wall and head/neck region. They are prone to aggressive local recurrences without metastases. Treatment of primary lesions often involves resection, although adjuvant radiation and/or low-dose chemotherapy have been used alone or in conjunction with resection. We compared the *in vitro* drug response of cells derived from a highly invasive Desmoid tumor (SS07-038) against the *in vitro* drug response shown by cells from two less invasive Desmoid tumors (SS07-037 and ss07-008).

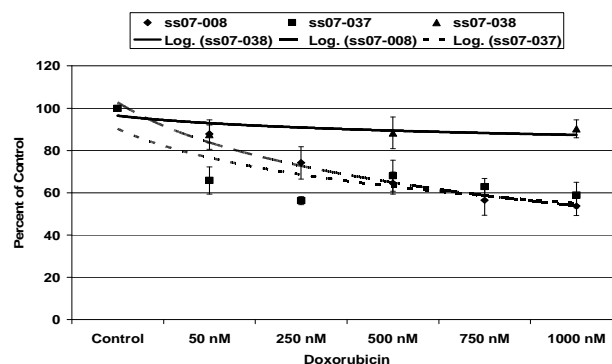
### METHODS AND MATERIALS:

**Desmoid cell lines:** Three Desmoid tumors resected at the University of Utah according to IRB approved and HIPAA compliant protocols were enzymatically dissociated for 2 h at 37° C in Collagenase 1A (Sigma C9891;100 collagen digestion units/mL; St. Louis, MO). Desmoid cell cultures, maintained in DMEM culture medium containing 15% fetal bovine serum, were grown to near-confluency, and then subdivided for continued *in vitro* culture or cell freeze.

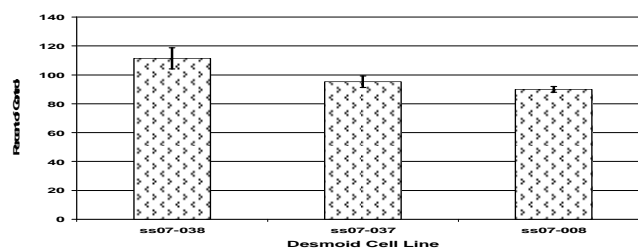
***In Vitro* doxorubicin experiments:** Desmoid cultures were exposed to Doxorubicin (Sigma D-1515) for 24 h, under serum-free conditions or in culture medium containing 15% fetal bovine serum (complete medium), and then assayed for cell survival by trypan blue dye exclusion. To identify the genes responsive to Doxorubicin, cell cultures were exposed to 1 μM Doxorubicin under serum-free conditions for 3 h, and then processed for microarray analysis 24 h later.

**Microarrays:** Following treatment with Doxorubicin, total RNA was extracted from the cells using an RNeasy Mini Kit according to the manufacturer's protocol (Qiagen). One microgram of total RNA was used for each microarray analysis. Prior to dye labeling and hybridization, total RNA was quantified using a NanoDrop Spectrophotometer ND-1000 and RNA quality assessed by capillary electrophoresis (Agilent 2100 Bioanalyzer, RNA nanochip). Three independent hybridizations per cell line were created on a Human 44K gene expression catalog microarray (Agilent Technologies, Santa Clara, CA). The slides were scanned using a G2505B microarray scanner (Agilent Technologies) set at a 5 μm resolution for simultaneous Cy5-3 and Cy5-5 signal detection. The .txt file extracted from each microarray hybridization was uploaded into Genesifter™ (VizX Labs, Seattle, WA) for data analysis. Pairwise Analysis was used to screen for genes impacted by Doxorubicin treatment. Student's t-test employing the Benjamini-Hochberg correction was used to test for statistical significance ( $p \leq 0.01$ ). The preliminary microarray data in Table 1 are derived from a single microarray submission for each cell line.

**RESULTS:** Cells derived from the highly invasive SS07-038 Desmoid tumor were significantly more resistant to Doxorubicin toxicity than were cells originating from the less invasive SS07-037 and ss07-008 Desmoid tumors, under both complete medium (Fig. 1) and serum-free (Fig. 2) culture conditions. GeneSifter™ analysis yielded 21 "stress" genes that were significantly ( $p \leq 0.01$ ) upregulated or downregulated in response to Doxorubicin in the SS07-038 cell line. In contrast, no genes were identified in the SS07-037 cell line when the .txt files were evaluated at a probability value of 0.01. However, when evaluated at a value of 0.05 rather than 0.01, nine of the 21 stress genes highlighted by GeneSifter™ for the SS07-038 cell line were also identified as having been altered by Doxorubicin in the SS07-037 cell line. Furthermore, an additional two "drug" genes identified as having been significantly altered by doxorubicin in the SS07-038 cell line were not altered in SS07-037 cultures.



**Figure 1.** The invasive ss07-038 Desmoid cell line is more resistant to doxorubicin toxicity than are two less invasive Desmoid cell lines (ss07-037 and ss07-008) when assayed under complete medium culture conditions.



**Figure 2.** The invasive ss07-038 Desmoid cell line is also more resistant to doxorubicin toxicity when assayed under serum-free culture conditions than are two less invasive Desmoid cell lines (ss07-037 and ss07-008)

**Table 1.** The invasive SS07-038 Desmoid cell line displayed a more diversified genetic response to Doxorubicin under serum-free culture conditions than did cells from the less invasive SS07-037 Desmoid.

GENE (Stress)	SS07-038 ( $p = 0.01$ )	SS07-037 ( $p = 0.05$ )
<i>BLM</i>	Down 1.92	Down 2.1
<i>ABL1</i>	Up 1.77	n/a
<i>CHAF1B</i>	Up 1.5	n/a
<i>CCNA2</i>	Down 1.87	n/a
<i>CDKN1A</i>	Up 1.52	n/a
<i>DTL</i>	Down 6.39	Down 3.93
<i>ESCO2</i>	n/a	Down 3.86
<i>EXO1</i>	Down 2.26	Down 1.91
<i>FANCA</i>	Down 2.45	Down 2.16
<i>FANCD2</i>	n/a	Down 1.61
<i>FANCI</i>	Down 1.54	n/a
<i>HSPA4L</i>	Up 1.53	n/a
<i>HIST1H2BC</i>	Up 1.68	n/a
<i>HIST1H2BJ</i>	Up 1.73	Up 1.63
<i>HIST1H2BE</i>	Up 1.56	n/a
<i>POLE2</i>	Down 1.72	n/a
<i>PCNA</i>	Up 1.51	n/a
<i>RAD51A1</i>	Down 2.36	Down 2.17
<i>RAD51</i>	Down 2.18	Down 1.6
<i>RAD54L</i>	Down 2.27	Down 1.97
<i>TYMS</i>	Up 1.54	n/a
<i>TOP2A</i>	Down 3.2	n/a
<i>UHRF1</i>	Down 2.29	Down 1.97
GENE (Drug)	SS07-038 ( $p = 0.01$ )	SS07-037 ( $p = 0.05$ )
<i>CENPF</i>	Down 2.55	n/a
<i>RAD54L</i>	Down 2.27	Down 1.97
<i>SLC47A1</i>	Up 1.53	n/a

**CONCLUSIONS:** Cell cultures derived from the highly invasive SS07-038 Desmoid tumor were more resistant to Doxorubicin toxicity and displayed a more diversified genetic response to Doxorubicin than did cells from less invasive Desmoid tumors.

**Acknowledgments:** Supported by research grants from the Desmoid Tumor Research Foundation and the Huntsman Foundation.