**Utilizing Metabolomics to Understand Novel Anti-Desmoid Tumor Drugs**

Metabolomic Analysis: NIH Eastern Regional Comprehensive Metabolomics Resource Core (RTI RCMRC)

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IRB Number(s):

**Abstract:**

Desmoid tumors (DT) are locally invasive soft tissue growths with no directed therapies currently. While two genes (β-catenin and adenomatous polyposis coli) have been found in patients who develop desmoids, it is unclear how these mutations and other downstream mechanisms lead to desmoid tumorigenesis. Extensive research has been explored in the molecular biology of desmoids; however, the use of metabolomics to understand the how the low molecular weight complements of cells, tissues, and biological fluids are perturbed by this highly localized disease. Additionally, the Desmoid Collaboration for a Cure has identified 45 active drugs against primary cell lines. It is unclear how these therapies perturb the metabolome, outside the Wnt and notch pathways. This pilot study will use broad spectrum metabolomics to study the tumorigenesis process of fibroblasts to desmoids by investigating paired desmoid and fibroblast cell lines, in addition to unaffected fibroblast cells. Additionally, this pilot study will explore the effects of three of the active drugs identified on the desmoid and fibroblast cells.

**Sample Description:**

Monolayer cell cultures of desmoid tumors and normal fibroblasts from desmoid patients and an unaffected fibroblast cell line were grown in DMEM supplemented with 5% fetal bovine serum and maintained at 37°C in 5% CO2. Cells were divided when confluent and experiments were performed between the third and sixth passages. Approximately 10 x 106 cells were treated with 1.0uM Dasatinib (Selleck, Houston, USA) dissolved in DMSO, or 0.5uM FAK Inhibitor 14 (Cayman Chemicals Company, Michigan, USA) dissolved in water. Cells were incubated in fresh media containing the inhibitors, or vehicle, for 24 hours. An aliquot of the media following treatment was collected, and the remainder of the media was aspirated. The cells were washed with PBS twice, and quenched with 8mL ice-cold isotonic 0.9% (w/v) saline for 2 minutes. Total cellular content was then extracted with 1.7mL ice-cold acetonitrile/water (50:50, v/v) solution. Cell extracts were collected using a cell scraper and quickly transferred to MagNA Lyser Green Beads tubes (Roche, Indianapolis, USA) and stored in -80°C. Media was added to empty plates and incubated together with the cells for the duration of the experiment served as a blank.

A total of 48 coded media samples were shipped to the NIH RTI-RCMRC on dry ice and immediately stored at -80 °C after being logged in for metabolomics analysis.

The data obtained for the NMR metabolomics analysis can be found in the accompanying files:

Procedures: 1. Mercier Desmoid Media Metabolomics Procedures.docx

Study Design Tables: 2. Mercier Desmoid Media Metabolomics Study Design Table.xls

Metadata: 3. Mercier Desmoid Media Metabolomics METADATA.xlsm

Processed Data: 4. Mercier Desmoid Media Metabolomics Normalized Binned Data.xlsx

Raw Data: 5. Mercier Desmoid Media Metabolomics NMR Raw Data.zip

**Notes:**

Full sample preparation and analysis procedures are available in the accompanying document entitled **1. Mercier Desmoid Media Metabolomics NMR Procedures**.

Descriptions of abbreviations for factors are available in the Variable Dictionary in the accompanying file no. **2. Mercier Desmoid Media** **Metabolomics NMR Study Design Table.xls**.

The phenotypic and normalized data are available in the accompanying files: **4. Mercier Desmoid Media** **Metabolomics NMR Normalized Binned Data.xlsx** for normalized binned NMR data. Sample ID and factors can be found in the first 5 columns and other columns in the spreadsheet contain sample metadata and the normalized binned data. If the statistical program does not allow variable names to begin with a number then add a prefix to the column names, for example, bin\_8.98 instead of 8.98.

The Sample ID serves as the unique identifier (Graphical ID) of the individual samples and is used as the NMR folder name in the raw NMR data file **5. Mercier Desmoid Media** **Metabolomics NMR Raw Data.zip**.