

Proteomics-based analysis of Wilms tumor patient-derived xenografts identifies critical components of tumor microenvironment

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Abstract:

Purpose: Preclinical studies in high-risk Wilms tumor (WT) are limited by difficulty of establishing cell lines and WT patient-derived xenografts (WTPDX) after dissociation to single cells. This contrasts with other pediatric solid tumors which are cultured and engraft more readily. We have observed that intact tumor tissue exhibited high WTPDX engraftment rate (>70%), while single cells and tumor-derived spheroids exhibit unreliable engraftment (delayed to none). We therefore sought to identify key mediators of WTPDX cell survival and tumorigenesis to better optimize *in vivo* model development and highlight potential therapeutic targets.

Methods: Six different WTPDX were studied in different conditions (intact tissue, single cells (SC), and 3D tumor-derived spheroids) via tandem mass tag (TMT)-based quantitative proteomic analysis. Principal component (PCA), differential expression, and enrichment analyses were performed. Selected differentially-expressed proteins were validated with Western blot. For one WTPDX model (KT-47R1), representative immunofluorescence (IF) images were taken in 2D adherent and 3D cultures. Pilot experiments were performed to compare tumor engraftment and growth from intact tissue, SC, and 2D/3D cultures.

Results: TMT-proteomics identified 10,523 proteins across all samples (Fig 1A). On PCA of top 100 differentially expressed proteins, samples clustered together by condition rather than WTPDX model (Fig 1B). Differential expression and enrichment analyses identified two major clusters; (1) 42 proteins uniquely up in spheroids predominantly related to cytoplasmic translation, receptor-mediated endocytosis, and platelet granulation; and (2) 109 proteins uniquely up in tissues predominantly related to extracellular matrix (ECM) organization, integrin cell surface interactions, and regulators of angiogenesis (Fig 1C). Increased levels of leucine-rich proteoglycans in tissues were confirmed via Western blot. Temporary culture of 2D adherent KT-47R1 WTPDX cells in nonadherent condition followed by reattachment increased levels of lumican and adhesion molecules on IF; SIX2 and CITED1 were most strongly expressed in 3D spheroids relative to organoids and adherent-to-nonadherent cells (Fig 1D). Injection of KT-47R1 intact tissue or single cells formed tumors in NSG mice but injection of 2D adherent cells or 3D organoids or spheroids was insufficient to form tumors.

Conclusions: This proteomic approach identified candidate stromal/extracellular matrix organization and cell adhesion molecules implicated in tumor engraftment for WT patient-derived xenograft tissues and *in vitro* cultures. 2D and 3D *in vitro* WTPDX tissues have

variable gene expression patterns and morphologies but fail to recapitulate intact tissue tumor formation/engraftment. These critical components of the extracellular matrix/tumor microenvironment could inform future therapies.

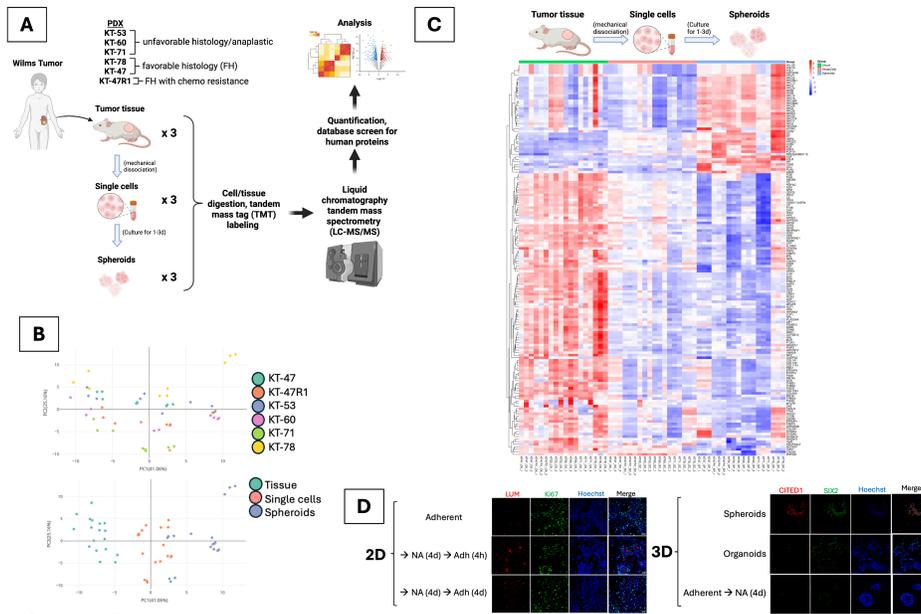


Figure 1. A: Proteomics experimental design flowchart; three conditions of 6 different Wilms Tumor patient-derived xenografts (WTPDX) were analyzed via liquid chromatography tandem mass spectrometry (LC-MS/MS) with technical triplicates for each condition of each WTPDX. B: Principal component analysis (PCA) of top 100 variable proteins by TMT proteomics, with samples colored by WTPDX model (top) and condition (bottom), showing samples grouped by condition. Heatmap of differentially-expressed proteins by TMT-proteomics (FDR < 0.05, |Log2FC| > 1). D: Representative immunofluorescence images of KT-47R1 2D cultures before and after temporary nonadherent (NA) condition (left) and 3D cultures (right) for lumican (LUM) and proliferative marker Ki-67 in 2D and blastemal markers CITED1 and SIX2.