

**Precision Medicine. METHODS/STUDY POPULATION:** Transurethral resection of bladder tumors were selected for testing based on availability and tissue composition. A wash step was used to generate daughter aliquots composed of dislodged cells and a solution with prior contact to the parent tissue. This wash step served two purposes: 1) reduce the amount of contaminating material from spreading to other cases, a problem known to be associated with this type of specimen; and 2) create aliquots from which additional informative data could be generated. These daughter aliquots were then examined to determine their value as a source for exosome profiling, metabolomic studies, molecular characterization and organoid development. The parent tissue was not compromised, was able to undergo conventional processing and yielded results equivalent to unwashed specimens. **RESULTS/ANTICIPATED RESULTS:** Exosomes secreted by the tumor cells were identified to be present in the daughter aliquots by a combination of their isolation using CD31 and detection of miR-21 expression. These exosomes were confirmed to be not related to fragmented cells from testing for beta-tubulin. A global/discovery-based approach using mass spectrometry provided insights into early characterization of metabolomic profiles present in these tumor cells. Ample amounts of high quality DNA (226 ng/ul concentrations; 11.3 ug total) were recovered from the dislodged, excess cells in the wash for molecular studies. Finally, from viable cells recovered in one of the daughter wash aliquots, the ability to grow organoids was proven to be possible and reproducible. **DISCUSSION/SIGNIFICANCE:** Based on these results, the value of the clinical specimen can be markedly expanded for utilization in research and possible clinical use without detracting from the parent tissue. This non-destructive, easy to adopt wash procedure can potentially lead to an influx of data that may ultimately prove useful in improving patient care.

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### WDR5 represents a therapeutically exploitable target for cancer stem cells in glioblastoma

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**OBJECTIVES/GOALS:** Glioblastomas (GBMs) are heterogeneous, treatment-resistant tumors that are driven by populations of cancer

stem cells (CSCs). In this study, we perform an epigenetic-focused functional genomics screen in GBM organoids and identify WDR5 as an essential epigenetic regulator in the SOX2-enriched, therapy resistant cancer stem cell niche. **METHODS/STUDY POPULATION:** Despite their importance for tumor growth, few molecular mechanisms critical for CSC population maintenance have been exploited for therapeutic development. We developed a spatially resolved loss-of-function screen in GBM patient-derived organoids to identify essential epigenetic regulators in the SOX2-enriched, therapy resistant niche. Our niche-specific screens identified WDR5, an H3K4 histone methyltransferase responsible for activating specific gene expression, as indispensable for GBM CSC growth and survival. **RESULTS/ANTICIPATED RESULTS:** In GBM CSC models, WDR5 inhibitors blocked WRAD complex assembly and reduced H3K4 trimethylation and expression of genes involved in CSC-relevant oncogenic pathways. H3K4me3 peaks lost with WDR5 inhibitor treatment occurred disproportionately on POU transcription factor motifs, required for stem cell maintenance and including the POU5F1(OCT4)::SOX2 motif. We incorporated a SOX2/OCT4 motif driven GFP reporter system into our CSC cell models and found that WDR5 inhibitor treatment resulted in dose-dependent silencing of stem cell reporter activity. Further, WDR5 inhibitor treatment altered the stem cell state, disrupting CSC in vitro growth and self-renewal as well as in vivo tumor growth. **DISCUSSION/SIGNIFICANCE:** Our results unveiled the role of WDR5 in maintaining the CSC state in GBM and provide a rationale for therapeutic development of WDR5 inhibitors for GBM and other advanced cancers. This conceptual and experimental framework can be applied to many cancers, and can unmask unique microenvironmental biology and rationally designed combination therapies.

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### A Novel Animal Model of Radiation-Induced Heart Disease Using Photon Radiation

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**OBJECTIVES/GOALS:** The purpose of this study is to develop a clinically relevant mouse model of Radiation-Induced Heart Disease (RIHD) and characterize the resulting phenotype to find biomarkers and therapeutic targets as well as to understand the changes in cellular and molecular mechanisms of bioenergetics. **METHODS/STUDY POPULATION:** We used a two-beam method in the axillary region targeting the heart to irradiate male BALB/c mice at an iso-dose of 22, 16 and 8 Gray (Gy). We examined cardiac damage (i.e., vacuolization), inflammation, and DNA damage at 10 days post irradiation using histology and immunohistochemistry of heart tissue and cardiac function at day 35 by echocardiography. Additionally, cardiac tissue of mice irradiated at 22 Gy was collected at day 10 and day 35 post irradiation and sent for RNA sequencing. Data from RNA sequencing was analyzed using gProfiler, GSEA, and Cytoscape to enrich and visualize differentially expressed genes. RT-qPCR was performed to validate findings of significantly differentially expressed genes. **RESULTS/ANTICIPATED RESULTS:** Significantly increased phosphorylation of H2A.X indicated that irradiated mice were undergoing DNA double strand break repair indicating cardiac damage. Additionally, we found that regulators of mitochondrial function were decreased in the heart at day 10 for all doses. We found