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Functional Characterization and Therapeutic Response Differences Between Orthotopic and Subcutaneous Patient-Derived Xenograft Models

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ABSTRACT

Subcutaneous patient-derived xenografts (PDX) have provided the research community with dynamic and robust preclinical model systems for which to study cancer biology and pharmacogenomic associations. Orthotopic patient-derived xenografts (O-PDX) provide an even more clinically relevant model that recapitulates tumor environment aspects of the human disease. Here, we compare *in vivo* pharmacological response between the two model systems and identify several functional characterizations that explain pharmacological response discordance between subcutaneous and orthotopic xenograft models.

METHODS

Patient biopsies were surgically implanted into rear flanks of female NOG mice and serially passaged orthotopically. Animals were imaged with the M3™ compact MRI from Aspect Imaging to monitor tumor growth. Drugs were formulated and administered per manufacturer's instructions or past publications. For the humanized study, 1x10^6 donor peripheral blood mononuclear cells (PBMCs) were inoculated intravenously via tail vein 6 days before tumor implantation. Tumors were formalin-fixed, paraffin-embedded, sectioned, and stained with hematoxylin and eosin. Tissue slides were digitally scanned using the 3DHistech Panoramic Scan II. For RNA-Seq analysis, mouse contamination was removed (Xenome) and aligned to Human GRCh38 genome using STAR/RSEM, and differential gene expression was performed using edgeR against matched normal tissue from the Genotype-Tissue Expression (GTEx) project. Gene set enrichment analysis (GSEA) was performed to find enriched pathways (KEGG). For tumor-infiltrating lymphocyte (TIL) analysis, tumors were removed and dissociated using the Miltenyi gentleMACS™. Immune populations were analyzed using the Cytek™ Biosciences Auroa 3 spectral flow cytometer.

CONCLUSIONS

O-PDX implantation effects *in vivo* pharmacological response and gene expression. Discordance in functional pathways may explain differences in pharmacological efficacy. O-PDX models can predict effective treatment strategies for individual patients and forecast tumor recurrence after therapy.

CITATIONS & ACKNOWLEDGEMENTS

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RESULTS

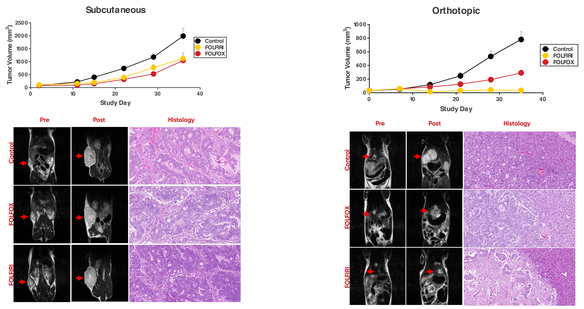


Figure 1. Subcutaneous and O-PDX implantation affect *in vivo* pharmacological response. A colorectal adenocarcinoma PDX (CRT_256) generated from a liver metastasis responds differently to chemotherapy depending on location (n=8).

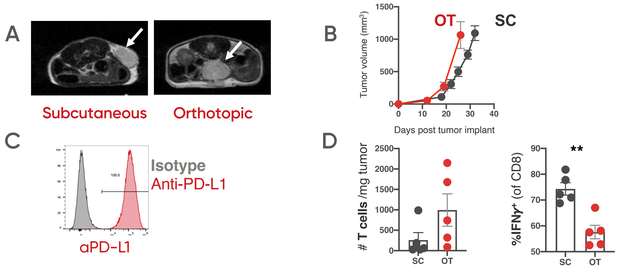


Figure 2. Subcutaneous and orthotopic implantation effect *in vivo* PBMC recruitment. A. MRI images and **B.** Growth curves of a liposarcoma PDX model (CRT_395) implanted in the rear flank or abdomen of PBMC-humanized animals. **C.** Confirmation of PD-L1 status by spectral flow cytometry. **D.** Differential TIL recruitment and immunophenotypes of untreated tumors in locations (n=8).

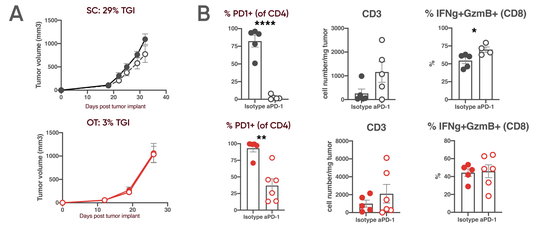
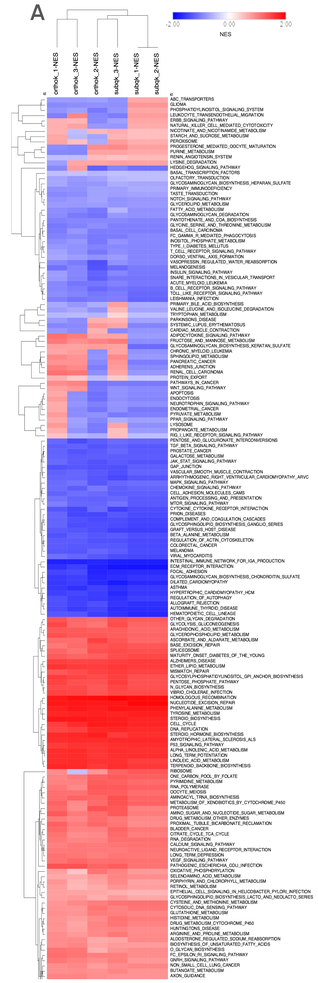
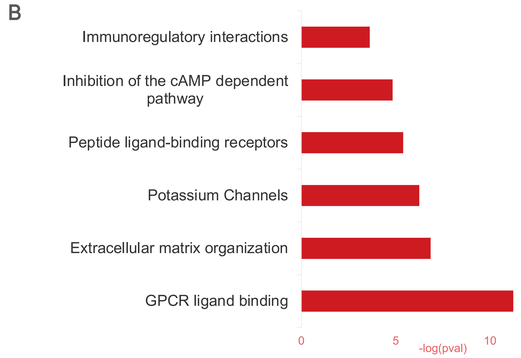


Figure 3. Subcutaneous and orthotopic implantation effect *in vivo* efficacy and immunophenotypes when treated with pembrolizumab. A. Growth curves of subcutaneous and orthotopic CRT_395 under treatment show enhanced efficacy in the subcutaneous setting. **B.** Differential PD1 binding, TIL recruitment, and immunophenotypes of treated tumors between subcutaneous and orthotopic implantation (n=6).





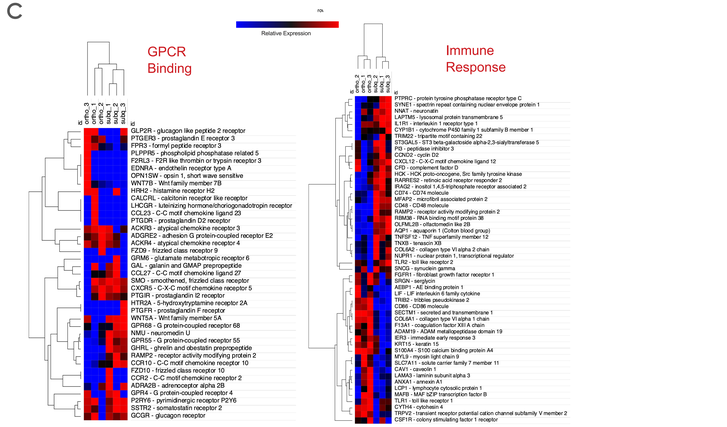


Figure 4. Differential gene expression between subcutaneous and orthotopic PDX identifies significant enrichment of GPCR and immune regulatory genes. A. Gene expression analysis (GSEA) against matched normal tissue from the Genotype-Tissue Expression (GTEx) project identifies enriched cancer-related pathways (Cell Cycle, P53 Signaling). B. Pathway enrichment analysis of top differentially expressed genes between subcutaneous and orthotopic tissue of PDX models show pathways that may functionally contribute to differences in therapy response. C. Upregulated (red) and downregulated (blue) genes compared to matched normal (GTEx) highlight gene expression dysregulation between subcutaneous and orthotopic PDX in GPCR binding and immune response.