Characterizing differential efficacy and phenotypic response to proteasome and survivin inhibitors in colorectal cancers using a high throughput organoid assay.

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Background: Conventional monolayer cell cultures and xenograft models, while useful and economical in early drug discovery, cannot predict clinical efficacy. Further, preclinical screening assays that rely on differential metabolic activity between separate control and treated wells are incapable of capturing phenotypic response and could overstate efficacy for cells with high rates of proliferation. Consequently, over 95% of anticancer agents that show efficacy in preclinical studies, fail in clinical trials. Recently, patient-derived organoid (PDO) models have been utilized in developing platforms to predict clinical efficacy of preclinical formulations. If successful, such predictive *ex vivo* technologies could revolutionize cancer treatment by reducing cost and time-to-market for new, more effective therapeutics. Objective: Characterize a novel bioprinted organoid tumor (BOT) high-throughput screening *ex vivo* platform for drug response prediction (DRP) with known proteosome and survivn inhibitors in colorectal cancer. **Methods:** Bioink for 3D printing BOTs was prepared with HT-29 cells, an established NCI-60 human colorectal adenocarcinoma cell line with known sensitivity to proteosome and survivin inhibitors. Bioink was deposited layer-by-layer on multiple substrates, in various geometrical configurations, and cured in stages to allow cells and matrix to self-assemble with limited degrees of freedom. BOTs were screened 24h and 48h after printing with proteosome inhibitor Bortezomib and survivin inhibitor YM-155. BOTs were evaluated 48h and 72h after treatment using immunofluorescence live/dead assay. Morphological phenotypic changes resulting from treatment were also recorded. **Results:** Proteasome and survivin inhibitors have been reported to inhibit proliferation and induce cell death in colorectal cancer cells. A dose dependent response was observed for both agents in our novel BOT HTS thereby validating the platform. In addition, characteristic self-assembly of HT-29 cells was observed to be disrupted at effective doses and at certain concentrations below the effective dose. Traditional ATP assays are incapable of recording such phenotypic modulation. Further, a higher proliferation profile was observed in untreated BOTs suggesting that use of independent control wells in traditional assays could overstate efficacy of treatment. **Conclusions:** Functional high-throughput *ex vivo* DRP technologies have the potential to transform cancer treatment – from bench to bedside – along the drug discovery to market roadmap for much needed novel anticancer agents. Research Sponsor: NSF.