Modeling Bone Sarcomas: a 3D Spheroid Culture Model

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INTRODUCTION: Primary bone sarcomas account for less than 1% of all diagnosed cancers each year, yet are associated with high morbidity and mortality (Ferguson, et. Al., 2018). Osteosarcoma (OS) is the most common primary bone sarcoma and disproportionately affects children and adolescents as the 8th most common pediatric cancer (Mirabello, et al., 2009). Ewing's sarcoma is the second most common form of primary bone sarcoma, comprising approximately 1/3rd of cases, and shares similar characteristics to OS including primarily affecting children and adolescents (Ferguson, et. al., 2018). Historically, treatment of these cancers has relied heavily on ablative surgery, and often amputation to resect the primary tumor (Anderson, 2016). Fortunately, the development and use of neoadjuvant and adjuvant chemotherapy, combined with surgery, has raised survival rates for localized disease above 70% with 90-95% of patients no longer requiring amputation (Ferguson, et. al., 2018). Unfortunately, the development of metastasis lowers the survival rate of these cancers to less than 20% (Hattinger, et al., 2021). Investigations into further chemotherapeutic alterations have failed to improve these outcomes for decades (Gill, et al., 2021). Even modern immunotherapies have had disappointing treatment results in OS (Wedekind, et al., 2018). Clinically, chemotherapeutic resistance is a major barrier to the successful treatment of these cancers (Prudowsky, et al., 2020). For this reason, a research approach that appropriately captures the complexity of bone sarcomas is required. Traditional 2D cell culture methodologies poorly replicate the *in-vivo* tumor microenvironment and structure of sarcomas, such as osteoid formation. Here, we utilize a 3D hydrogel culture that reliably produces high viability "spheroids" which replicate key sarcoma physiology toward the goal of understanding mechanisms of resistance in OS.

METHODS: Patient-derived clinical cell isolates from multiple sarcoma types were used to develop and optimize our methods. Tumor-derived sarcoma cells were first expanded in monolayer culture for 7-10 days prior to hydrogel encapsulation. Expanded cells were encapsulated in spheroids of a methacrylated collagen and thiol-modified hyaluronan scaffold (Advanced Biomatrix). Chemical cross-linking of the components was performed via the photoinitiator Irgacure and UV light exposure. Spheroids were cultured for 7 days to facilitate cell proliferation. Cultured spheroids were evaluated for viability and morphology using CellTiter-Glo 3D Viability Assay (Promega), Live/Dead fluorescent staining (Invitrogen), Z-stack confocal microscopy using a Zeiss 710 Laser Scanning Microscope (Carl Zeiss, Inc.), and hematoxylin and eosin (H&E) staining. In addition to evaluating the viability of these organoids, a subset were treated with chemotherapeutic agents for 48 hours and treatment outcomes were analyzed via Invitrogen LIVE/DEAD staining and Promega CellTiter-Glo 3D analysis. Statistical analysis (one-way ANOVA, non-linear regression) was done using GraphPad Prism 10 software.

RESULTS: Patient derived clinical cell isolates exhibited high viability over time following the brief UV light exposure that took place during hydrogel encapsulation (Fig 1A). This viability was unaffected by the seeding concentration of the hydrogels (Fig. 1B, P=0.6662). Patient derived Ewing's sarcoma spheroids treated with Panobinostat ranging in concentration from 12.21 nM – 3125 nM for 48 hours resulted in an IC50 of 59.07 nM (Fig 2). Patient derived sarcoma spheroids treated with doxorubicin respond with cell death and morphological changes as seen by Live/Dead staining (Fig 3. A-B).

DISCUSSION: We have developed 3D hydrogel cultures of patient-derived tumor cells from several different types of sarcoma tumors. Our results indicate the presence of key sarcoma features that do not occur in monolayer cultures. These 3D sarcoma spheroid cultures also exhibited high proliferation and viability and a quantifiable dose-response to chemotherapeutic drug treatment. In future studies this methodology will be used on clinical tumor cell isolates to test chemo-responsiveness and compare those findings with clinical treatment outcomes, while also investigating the molecular mechanisms of chemoresistance.

SIGNIFICANCE/CLINICAL RELEVANCE: We have developed and validated a method for 3D culture of patient derived sarcoma cells that allows for formation of key sarcoma tumor morphology and testing of treatment methods. The ultimate goal for this work is an in-vitro model used to evaluate treatment methods that lowers the burden of chemotherapy on patients while simultaneously improving treatment effectiveness and outcomes.

REFERENCES: Ferguson, et al., (2018) Am Fam Physician 98(4) 201-213.; Mirabello, et al., (2009) Cancer 115(7) 1531-43. PMC2813207.; Anderson, (2016) Orthop Clin North Am 47(1) 283-92.; Hattinger, et al., (2021) Cancers (Basel) 13(12). PMC8228414.; Gill, et al., (2021) Nat Rev Clin Oncol 18(10) 609-624.; Wedekind, et al., (2018) Pediatr Blood Cancer 65(9) e27227.; Prudowsky, et al., (2020) Cancers (Basel) 13(1). PMC7795058.

IMAGES/TABLES:

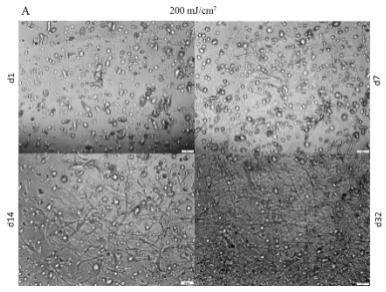

Figure 1: High proliferation over time of organoids after 200mJ/cm2 UV exposure (A). Cell seeding concentration had no impact on viability after UV exposure (B).

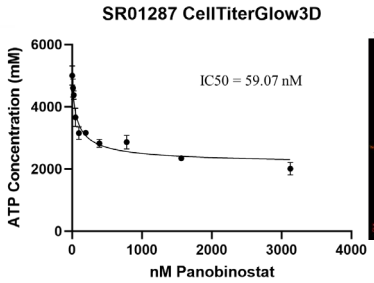

Figure 2: Dose response curve of Ewing's sarcoma 3D organoids treated with 12.21 nM – 3125 nM concentrations of Panobinostat.

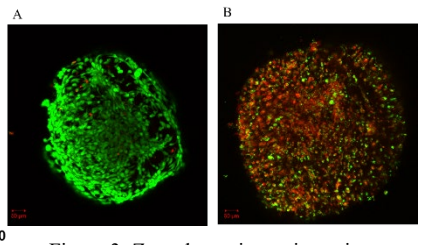

Figure 3: Z-stack maximum intensity projections from Live/Dead stained 3D sarcoma organoids: control (A), and 48-hour 10μM doxorubicin treated (B).