DEVELOPING MOUSE-TUMOR MODEL FOR HIGH INTENSITY FOUCSED ULTRASOUND (HIFU) ABLATION PROCEDURES

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INTRODUCTION

High Intensity Focused Ultrasound (HIFU) stands out as a non-invasive modality gaining prominence for the localized treatment of deep-seated malignant tumors. To enhance the effectiveness and validate the applicability of HIFU procedures, researchers have devised diverse *in vitro* [1, 2] and *in vivo* models [2, 3]. The literature reveals a spectrum of experiments involving *in vitro* models employing tissue mimicking materials (TMM) and turkey/chicken breasts, progressing to *in vivo* models featuring mice. Currently, the majority of HIFU research revolves around mouse tumor models.

In the area of cancer research, an array of mouse strains is readily available, primarily sourced from Jackson Laboratory in Bar Harbor, ME. The judicious selection of mouse strain is important for seamless experimental flow in tumor studies. Cincinnati Children's Hospital and Medical Center (CCHMC) locally houses three mouse strains, all originating from Jackson Laboratory: NOD/SCID GammaC-/- (NSG), NSG-SGM3 (NSGS), and Homozygote J:NU (Nude). These three types of mice were assessed in this study for developing a mouse tumor model.

The NSG mice, with their severe immunodeficiency due to scid and IL2rgnull mutations, have become pivotal in cancer research. Their compromised immune system allows efficient engraftment of human cells, facilitating studies on tumor biology and the testing of potential cancer therapies. The NSGS mice, an advancement in cancer research models, express human IL3, GM-CSF, and SCF. This modification enhances engraftment of myeloid lineages and regulatory T cells, making them particularly relevant for immuno-oncology studies. These mice provide a superior platform for investigating complex interactions between the immune system and cancer. Nude mice, characterized by athymia and immunodeficiency, have been fundamental in cancer research for decades. Their lack of T cells enables successful transplants of tumor cell xenografts, contributing to studies on tumor growth and drug testing in oncology. While not capturing full immune complexity, Nude mice remain relevant in specific contexts, offering insights into tumor biology and early-stage drug evaluations.

A human PC3 (CRL-1435) prostate cancer cell line used for developing the tumor model in mice is provided by American Type Culture Corporation (ATCC). The PC3 cell line is initiated from a bone metastasis of grade IV prostatic adenocarcinoma from a 62-year-old, White, male.

METHODS

The experimental cohort was comprised of two male mice from each of the three selected strains, totaling six mice. All animal studies were approved by the internal ethics and the local government committees. To establish the cell line-derived xenograft model, 6 to 8 weeks old male mice with a bodyweight around 30 g were engrafted subcutaneously with 1×10^7 PC3 cells into the right flank. Mice were group-housed under pathogen-free and controlled environmental conditions (21 ± 1.5 °C temperature, $55 \pm 10\%$ humidity and a 12 h light–dark cycle).

The PC3 cells were procured from ATCC, and the thawed contents of the vial were transferred to a 75 cm^2 tissue culture flask. A growth medium was formulated using F-12K as the base medium, supplemented with 10% fetal bovine serum (FBS) and 1% Penicillin-Streptomycin (Pen-Strep). Rigorous filtration was employed to eliminate potential contaminants. The cells were cryopreserved using DMT solution.

For cell culture, a mixture of 9 ml of the prepared medium and approximately 1 ml of frozen cells were placed in a 50 ml centrifuge tube and spun at room temperature. Cell counting, facilitated by a cell counter, revealed a visible white cell pellet at the tube's base. The culture was then incubated at 37°C, with a waiting period of 2-3 days for confluence. Subsequently, subculturing ensued to attain the desired cell quantity. Ethanol was used to sterilize all equipment before subculturing, and 0.25% (w/v) Trypsin-0.53 mM EDTA solution aided

in cell detachment. The cells were observed under an inverted microscope until the cell layer dispersed, typically within 5 to 15 minutes. The addition of 6 to 8 ml of complete growth medium facilitated aspiration, and sub cultivation was carried out from 1 culture vessel to 3 culture vessels. Medium renewal occurred 2 to 3 times per week, maintaining consistency across subsequent passages.

For the injection procedure, PBS served as the medium, and a 0.2 ml solution containing the requisite cell count was injected using a 27-gauge needle. Post-injection, all mice underwent observation for abnormal behavior. Tumor monitoring involved regular measurements 2 to 3 times weekly over a 5 to 7 week growth period. The area surrounding the tumor was consistently shaved to facilitate clear observation. The tumor measurement was done using digital calipers.

The tumor was assumed as an ellipsoid and the following formula was used to calculate the surface area of the ellipsoid.

$$SA = \left(\frac{4\pi((ab)^{1.6}+(ac)^{1.6}+(bc)^{1.6})}{3}\right)^{1/1.6} \quad (1)$$

The following formula was used to calculate the volume of ellipsoid.

$$V = \frac{4}{3}\pi abc \quad (2)$$

where a, b and c are the three dimensions of the ellipsoid.

RESULTS

Figure 1 illustrates the temporal progression of *tumor surface area* growth. Data collection commenced on the tenth day post-tumor cell injection, considering the initial small size of the tumors. This figure depicts the mice's tumor surface growth fitted to a parabolic profile. The initial tumor growth is characterized by a slow pace. Notably, NSGS mice exhibit a larger tumor surface area until approximately day 26, after which NSG mice surpass them, and the gap in surface area widens over time. Beyond day 40, NSG mice demonstrate a surface area of approximately 17 cm², NSGS mice exhibit around 13 cm², and Nude mice present approximately 11 cm². This highlights that, by the end of day 40, NSG mice possess a tumor surface area: about 31% larger than NSGS mice and 55% larger than Nude mice.

Figure 2 illustrates the temporal evolution of *tumor volume* growth. Similar to the surface area data, the figure displays the tumor surface area growth of mice fitted to a parabolic profile. Until approximately day 24, NSGS mice exhibit the largest tumors among the three strains; however, from that point onward, NSG mice surpass the others in tumor size. By day 40, a discernible distinction in tumor size emerged, with NSG mice boasting the largest tumor volume, Nude mice presenting the smallest, and NSGS mice fell in between the two. Specifically, the tumor volumes at day 40 are approximately 0.8 cm² for NSG mice, 0.52 cm² for NSGS mice, and 0.4 cm² for Nude mice. This indicates that, by the end of day 40, NSG mice have a tumor volume about 54% larger than NSGS mice and 100% larger than Nude mice.

In summary, both the tumor surface area and volume results consistently indicate that NSG mice exhibit the most robust tumor growth among the three experimental groups. This is evident from their larger tumor volume and surface area.

DISCUSSION

This research establishes that the NSG mice are better suited for tumor research based on tumor growth. NSG mice have the larger tumor surface area and volume compared to the other two over the established growth period. One can select this strain for HIFU research involving mice. One secondary factor that could have been considered is the Nude mice having no fur. They are very easy to handle as one must shave the tumor area of NSG and NSGS mice. As the most important parameter for developing a model is tumor size, the tumor surface area and volume of Nude mice is extremely low compared to the other two strains. Hence, they were not considered suitable for development of mouse tumor model.

Limitations: There are other strains of mice provided by Jackson Laboratory and those could be considered as well. This research only focuses on the three strains that are available at CCHMC. Only two mice of each category were taken for observation. More mice from each category can be observed in the future to strengthen the current results. Digital calipers were used for tumor measurement. While it is relatively easy to measure the two dimensions of the tumors with calipers, it was somewhat complex to accurately measure the depth of the tumor.

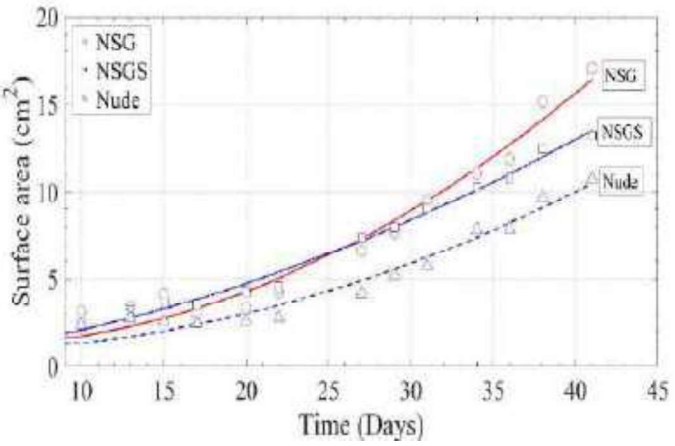

Figure 1: Tumor surface area growth with time

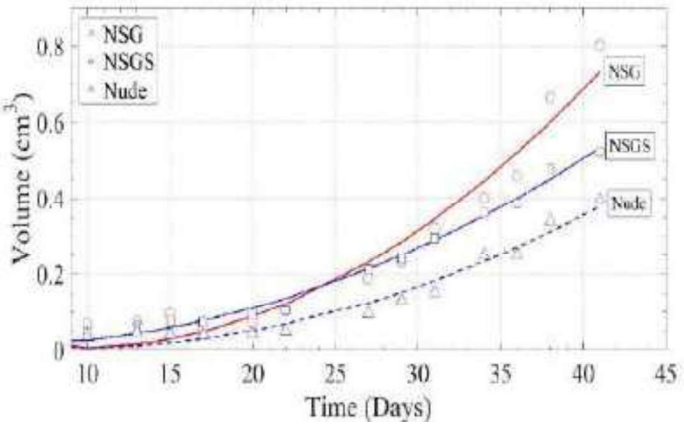

Figure 2: Tumor volume growth with time

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REFERENCES

[1] Dibaji et. al., J. of Nanotechnol. Eng. Med. Nov 2013, 4(4): 040902.
[2] Devarakonda et. al., Nano Lett. 2017, 17, 4, 2532-2538.
[3] Devarakonda et. al., ACS Biomater. Sci. Eng. 2019, 5, 8, 4102-4111.