Cell Injection Technique Impacts the Shape and Rate of Tumor Growth in a Syngeneic Tumor Mouse Model

Tina Gonzales, M.S., Cody Pyles, B.S., Elisa Dunkin, B.S., Kelsey Mayer, B.S., Liana Loos-Austin, M.S.,
Erin Slosarek, Ph.D., M.B.A., Craig S. Atwood, Ph.D. and Sivan V. Meethal, Ph.D.
Heartland Vivarium, Madison, WI 53711

ABSTRACT

Syngeneic mice are a widely used model of carcinogenesis for drug testing. In this study, we examined how cell injection and tumor measurement techniques impact the growth curves and tumor ulceration of a breast cancer cell line in a syngeneic model. Female BALB/cJ mice (n = 40) were injected with EMT6 breast cancer cells (1 million cells/mouse) into the sub-dermis of the right flank by four animal technicians (n = 10 each) and tumor size and ulceration determined on post-injection days 2-32 using the same calipers (Mitutoyo Absolute AOS Digimatic model CD-6" ASX). Tumors formed in all mice. Spherical tumors were observed in animals injected by highly skilled cell injectors, and they showed exponential growth (R^2 = 0.99) with the lowest standard deviations (from the mean tumor volume) when measured by four animal technicians, contrary to the dispersed tumors that gave high standard deviations and slow growth in animals injected by a first-time injector, suggesting that injection technique greatly impacts the growth of resulting tumors as well as measurement precision. Besides, mice injected by the first-time injector revealed less ulceration likely due to the spreading of cells into different sub-dermal areas. In animals injected by the highly skilled injectors tumors were localized to one area, and 100% of tumors became ulcerated by day 18. A simple correlation analysis indicated that enhanced growth rate increased ulceration rate. Together, these results suggest that the cell injection technique greatly impacts the shape of the tumor, its exponential growth, ease of measurement, and ulceration. Future studies using various animal species with varying injecting cell numbers, cell types, sites of injection, calipers or personnel on the rate of tumor growth and the timing of ulceration are warranted.

OBJECTIVES

To determine if:

1) cell injection technique has an impact on tumor growth and shape?

2) cell injection technique has an impact on tumor ulceration?

3) tumor growth rate is correlated to ulceration?

INTRODUCTION

- Syngeneic mouse models are widely used for testing of cancer drugs.
- An exponential cell growth curve prior to ulceration/necrosis is ideal for many tumor therapeutic studies.
- In this study, we determine the impact of cell injection technique on 1) tumor growth, 2) tumor measurements (using calipers) and, 3) tumor ulceration.

METHODS

Objective 1: 8-week-old female BALB/cJ mice (n = 40) were injected with EMT6 breast cancer cells (1 million cells/mouse) into the sub-dermis of the right flank by four animal technicians (Injectors A (highly skilled, B (skilled), C (skilled) and D (first-time); n = 10 each) with different skill levels and tumor growth measured over time by all four animal technicians using the same calipers (Mitutoyo Absolute AOS Digimatic model CD-6" ASX).

Objective 2: The number of ulcerated tumors that developed in the animals injected by the four technicians was determined to assess the relationship between tumor growth rate and ulceration.

Objective 3: Curve fitting algorithms were performed on the data to determine the growth properties and correlation analyses was done to determine whether tumor growth is correlated to tumor ulceration.

RESULTS

Individual Variation in Tumor Cell Injections Impact Tumor Growth and Measurement Precision

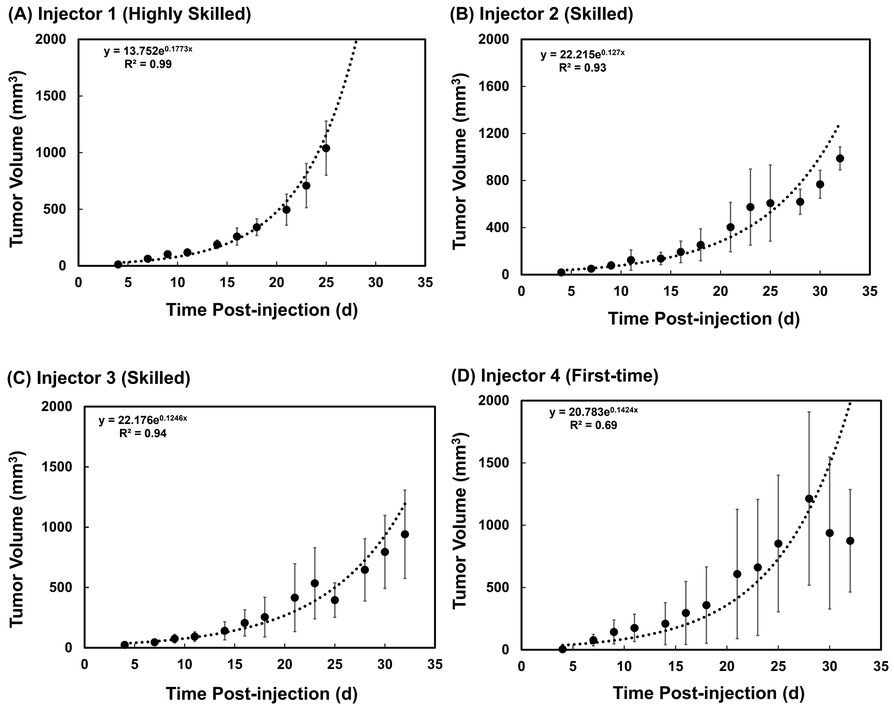
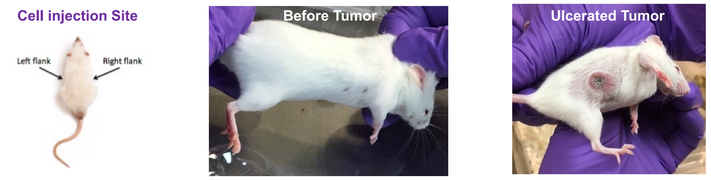


Fig. 1. Tumor cell injection technique impacts tumor growth and tumor measurements using calipers. Exponential curve fitting of the average tumor volume measured using the same calipers by four observers in animals injected with cancer cells by four animal technicians (n = 10 animals/each person) with varying cell injection skills (A-D). The values are mean ± SD.

- Discrete cell injection by highly skilled injector made round tumors amenable to precise measurements (using calipers) by multiple observers resulting in lower standard deviations (A).
- Dispersed cell injection by first-time injector made irregular tumors that were difficult to measure precisely by multiple observers resulted in dispersed values with very high standard deviations (D).
- Exponential growth curve with an R^2 = 0.99 was observed when cells were injected by highly skilled technician (A) and measured by all the four observers in contrast to that observed for the first-time cell injector (D; R^2 = 0.69).

These data demonstrate that cell injection technique impacts tumor growth and measurement precision.



Cell Injection Technique Impacts Rate of Tumor Growth that Directly Correlates with the Rate of Tumor Ulceration

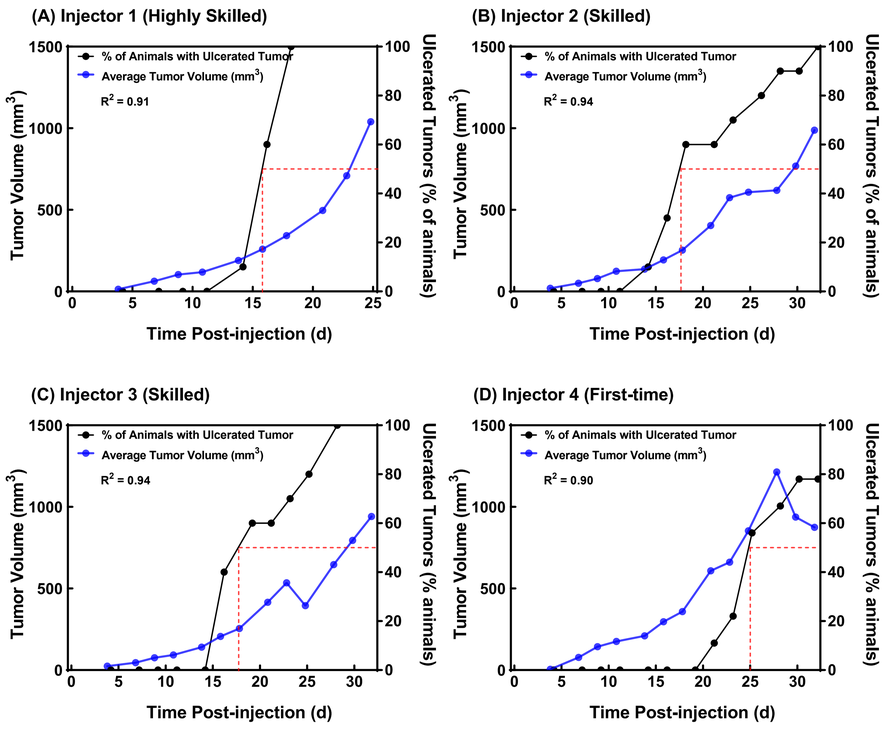


Fig. 2. Tumor volume vs percent of animals with ulcerated tumors following tumor cell injection. Tumor growth following cancer cell injection by technicians (n = 10) plotted (Y-axis) against time after cell injection (in days; X-axis). The righthand Y-axis represents the corresponding percentage of animals with ulcerated tumors (in the same group) following cell injection. Red lines indicate time to 50% ulceration.

- The rate of tumor growth correlates with tumor ulceration rate.

These data reveal that cell injection techniques that promote rapid tumor growth enhance the rate of tumor ulceration.

CONCLUSIONS

- Cancer cell injection technique is critical for tumor growth and precise tumor measurements.
- Cell injection technique greatly impacts the shape of tumor growth, its exponential growth, and ulceration.
- The impact of cell injection technique on the shape of the tumor affects its measurements using calipers.
- Faster exponential tumor growth and early tumor ulceration is observed in animals injected by highly skilled personnel injecting cell bolus in one discrete location.
- Disperse sub-cutaneous cell injections is a technique to avoid ulceration.
- **Take away: Optimizing cell injection technique for desired rate and type of tumor growth allows for standardized testing of cancer drugs.**