Murine Xenograft Model to Test Efficacies of Chemodrugs

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[Abstract] This experiment is used to test the efficacies of chemo treatments or gene therapy in an *in vivo* system. In this protocol, the mouse xenograft model is used.

Materials and Reagents

1. Severe combined immunodeficiency (SCID) mice
2. NaCl
3. Pluronic acid
4. Phosphate buffered saline (PBS)
5. HBSS solution

Equipment

1. 1 ml syringe with 22-24 gauge of needls
2. Bioluminescent imaging instrument (in university core facility)

Procedure

A. For single tumor cell (or any kind of tumor cells)

1. Six-week-old female in-bred Fox Chase SCID mice were obtained from Charles River Laboratories (Hartford, CT, USA). Animals were handled according to a protocol approved by the Institutional Animal Care and Use Committee at our university.
2. Mice were allowed to acclimate to animal housing, and xenografts were developed by subcutaneously injecting 5 x 10^6 cancer cells in murine flank bilaterally. Twice weekly, tumor volume was determined using digital caliper measurements and the formula:

$$\frac{\text{large diameter}^2 \times \text{small diameter}}{2}$$

3. After 14 days, all mice had measurable tumours and were sorted into treatment and control groups with equal number of animals (n=5). Treatment group mice received some

dose of chemodrugs dissolved in vehicle (0.1 M NaCl, 0.05% pluronic acid in PBS) per treatment while control mice received vehicle only.

4. All mice received 10 intraperitoneal injections over a 14-day period (cycle: Five treatment days followed by two non-treatment days). After 14 days (2 cycles), mice were killed. So the whole experiment lasts 28 days.
5. The tumor sample can be used either for extract RNA, protein or immunohistochemistry staining.

B. For interaction of tumor and Stella cells (for cancers containing big volume of stella cells) --using pancreatic cancer model.

1. A xenograft nude mouse model of pancreatic cancer using BxPC3 pancreatic tumor cells labeled with firefly luciferase (BxPC3-FL).
2. All mice were divided into groups receiving intrapancreatic injections of
   a. BxPC3-FL alone, either 0.5×10^6 or 1×10^6 per mouse.
   b. BxPC3-FL with immortalized HPSCs (human pancreatic stellate cells), in varying tumor-to-stroma ratios (1:1, 1:1, or 1:5).
   c. Immortalized HPSCs alone (0.5×10^6 or 1×10^6). All cell suspensions, including the mixture of BxPC3 and HPSCs, were injected in a 50 µl volume of HBSS.
3. Bioluminescent imaging was done weekly to follow the luciferase signal from BxPC3 cells.
4. Mice were sacrificed and tumors were harvested and measured.
5. Statistical analysis was done using GraphPad Prism (GraphPad). Comparisons were made using two-tailed Student's t test and significant difference was defined as $P < 0.05$. Data are shown as mean ±SE.

Acknowledgments

This protocol was adapted from Hwang *et al.* (2008).

References

1. Hwang, R. F., Moore, T., Arumugam, T., Ramachandran, V., Amos, K. D., Rivera, A., Ji, B., Evans, D. B. and Logsdon, C. D. (2008). Cancer-associated stromal fibroblasts promote pancreatic tumor progression. *Cancer Res* 68(3): 918-926.

