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The establishment of tumor model in vivo

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MTS



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Abstract

This protocol encompasses in vivo tumorigenicity assays and LDTIA in immunocompromised mice, utilizing MTS derived from both established tumor cell lines and freshly resected clinical tumor specimens.

3: The establishment of tumor model in vivo

- 1 All animal studies and procedures were conducted according to a protocol approved by the Animal Care and Use Committee, Suzhou Institute of Nano-Tech and Nano-Bionics, Chinese Academy of Sciences (SINANO/EC/2023-030).

3.1 The generation of xenograft and metastasis mouse models using M231 and MTSs-M231 cells

- 2 To investigate the tumorigenic and metastasis advantage of MTSs-M231 in vivo, 1×10^6 MTSs-M231 or parental M231 cells suspended in 100 μ L with Matrigel (Corning) were orthotopically injected into the mammary fat pad of female nude mice (6 weeks old, $n=6$ /group). After a period of 40 days post-implantation, the mice were sacrificed and the primary tumors were excised, weighed, and imaged. Tumor volume was measured and calculated according to the formula: tumor volume (TV) = $0.55 \times \text{length} \times \text{width}^2$. The organ index was calculated using the formula: organ index = organ weight (mg)/body weight (g). Lung, liver, spleen, heart, and kidney tissues were collected from two groups of mice and subjected to H26E staining to evaluate the in situ metastasis of breast cancer.
- 3 For the tail vein metastasis assay, 1×10^6 MTSs-M231-Luc or parental M231-Luc cells in 100 μ L PBS were intravenously injected into Nude mice ($n=5$ /group). The in vivo luminescence imaging was conducted at 14-, 28-, and 42-days post-injection to monitor cellular colonization. After 42 days, the mice were euthanized, and subjected to luminescence imaging of major organs, including lungs, liver, spleen, heart, and kidneys.

3.2 Patient-derived MTSs-based xenograft generation and drug testing

- 4 MTSs achieve amplification of more than 7 passages to get enough cells for xenograft generation. For the generation of xenograft models, 1×10^6 trypsinized cells from patient-derived MTSs were suspended in 100 μ L PBS mixed with 100 μ L Matrigel and orthotopically transplanted into the mammary fat pad of female NOD SCID mice (6 weeks old, $n=12$). Once the tumor volume in mice reached approximately 400 mm^3 , they were randomly divided into 4 groups ($n = 3$ /group): 1) saline (control), 2) Epirubicin hydrochloride (2.5mg/kg body weight (BW)) + Cyclophosphamide (20 mg/kg BW) + Docetaxel (10 mg/kg BW) group, 3) Doxorubicin Hydrochloride liposome (2.5mg/kg BW) + Cyclophosphamide (20 mg/kg BW) + Docetaxel (10 mg/kg BW) group and 4) Docetaxel (10 mg/kg BW) + Epirubicin hydrochloride (2.5mg/kg BW) + Cyclophosphamide (20 mg/kg BW) + Trastuzumab and Pertuzumab (30 mg/kg) group. The drugs were intraperitoneally injected daily. After 46 days of treatment, mice were euthanized and tumors were harvested for analysis. Tumor growth inhibition (TGI) was calculated using the formula: $\text{TGI} = [1 - (T_i - T_{0_i}) / (C_i - C_{0_i})] \times 100\%$. Where, T_i is the mean tumor



volume (TV) of the treatment group on the initial measurement day, T_0 is the mean TV of the treatment group at the first day of dosing, C_i is the mean TV of the control group at the initial measurement day, and C_0 is the TV of the control group at the first day of dosing.

3.3 In vivo limiting dilution tumor initiation assay (LDTIA) for the calculation of tumor-initiating cell frequency

- 5 In vivo LDTIA was performed to assess tumor-initiating cell (TIC) or CSC frequency of MTS-cells. MTS-cells and dissociated bulk tumor cells derived from BRC Patient-derived tumors were transplanted into mice via mammary fat pad injection at cell doses of 500,000, 50,000, 5,000, and 500 per mouse in a volume of 100 μ L (50 μ L Matrigel + 50 μ L cell suspension). Tumor formation was monitored for 5 weeks, with xenografts defined as tumors exceeding 2 mm in diameter, according to an established criterion. The frequencies of CSC in the tumors from clinical BRC patient were calculated using the tool at <http://bioinf.wehi.edu.au/software/elda/>.