Syngeneic Bone Metastasis Models for Testing Efficacy of Novel Therapies

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Introduction

Bone metastases count 30-70% of metastases in breast, lung and bladder cancer, and 80% of multiple myeloma patients have bone disease. Despite recent progress in cancer treatment, bone metastases still remain incurable. In this study we established and validated syngeneic mouse models with a focus on bone metastasis.

Materials and Methods

Mouse breast cancer (4T1-GFP, ATCC) cells were inoculated intracardially to Balb/c mice (Envigo), and mouse multiple myeloma (5TGM1, University of Texas Health Science Center at San Antonio) cells were inoculated into the tail vein of C57BL/Ka mice (Envigo). The mice were treated with cyclophosphamide (100 mg/kg, Baxter) or zoledronic acid (0.1 mg/kg, Q1W, Hamelin Pharmaceuticals) in the 4T1 model, and with bortezomib (1 mg/kg, Q3D, LC Laboratories) in the 5TGM1 model. In the 4T1 model, tumor burden was assessed by ex vivo GFP imaging (Lightsheet Research) and in vivo 5TGM1 model by measuring serum IgG2b paraprotein levels (Bethyl Laboratories Inc). Mouse bladder (MBT-2, Riken Cell Bank) and lung (KLN-205, Riken Cell Bank) cancer cells were inoculated into tibia bone marrow of CHS or DBA mice (Envigo), respectively. The effect of anti-PD-1 treatment (200 μg/dose, Q3D, BiodexCell) was evaluated in the MBT-2 model. Tumor-induced changes in bone were followed by X-ray imaging (Faxitron) in all models. Hind limbs were analyzed by histology and immunohistochemistry for tumor-infiltrating lymphocytes (TLs: CD3, T cells, Spring Bioscience; CD4, helper T cells, Sino Biological; CD8, cytotoxic T cells, Bioss).

Breast cancer - 4T1 intracardiac model

Bladder cancer - MBT-2 intratibial model

Lung cancer - KLN-205 intratibial model

Multiple myeloma - 5TGM1 intravenous model

Summary

4T1 model: Osteolytic bone lesions were formed within 13 days from inoculation of cancer cells. Cyclophosphamide decreased tumor burden and the area of osteolytic bone lesions. Zoledronic acid decreased osteolytic lesion area but had no effect on tumor burden. CD3+ and CD8+ TILs were absent or low in number in tumors growing in bone. In addition to tumors growing in bone, about 50% of the mice had metastases in lungs, ovaries, kidneys and adrenal glands based on GFP imaging.

5TGM1 model: Osteolytic lesions were observed and the study was ended at day 35. Bortezomib decreased serum paraprotein levels. Moderate number of CD3+ TILs were observed in the tumors. Cachexia and paraplegia were occasionally observed, and soft tissue metastasis to ovaries, kidneys and adrenal glands in about 30% of the mice.

MBT-2 and KLN-205 models: MBT-2 and KLN-205 tumors induced large osteolytic lesions within 25 days, and in the KLN-205 model also lung metastases were observed. Anti-PD-1 treatment decreased osteolytic tumor area in the MBT-2 model. Moderate number of CD3+ and low number of CD4+ and CD8+ TILs were observed in the tumors growing in bone or in lung metastases.

Conclusions

A high incidence of bone metastases was observed in all models. The use of systemic models allows studying the effects of test compounds on prevention or treatment of metastases. Intratibal models can be used when the primary interest is in tumor growth in bone microenvironment.

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