Developing an immunocompetent mouse model of renal cell carcinoma bone metastasis

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Introduction

Renal cell carcinoma (RCC) is an adenocarcinoma of the tubules of the kidney. It accounts for 3% of all cancers but 90% of kidney cancers. The treatments for RCC include surgical kidney removal, chemotherapy, radiation, and cryotherapy. Most commonly, RCC metastasizes to the lungs, bone, liver, and lymph nodes. RCC bone metastasis (RCCBM) can impair patient mobility and lead to worse outcomes.

Renal cell carcinoma metastasis

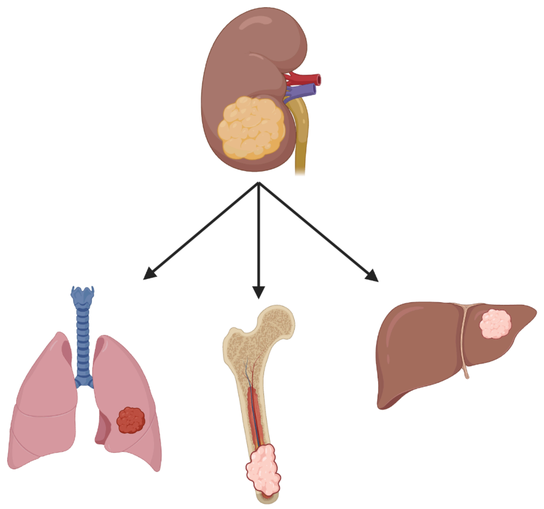

Figure 1. Renal cell carcinoma most common sites of metastasis (lung, bone, liver)

RCCBM cause osteolytic lesions. In previous work, we showed that Beta ig-h3 protein (BIGH3) also known as transforming growth factor beta-induced protein (TGFBI) promotes osteolytic lesions and inhibit the differentiation of osteoblasts, which are cells that construct new bone.

Introduction cont.

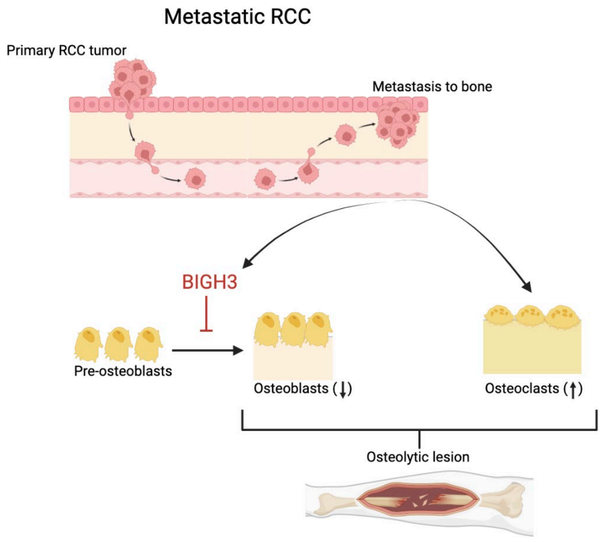

Figure 2. Proposed mechanism of osteolytic lesion formation in RCCBM.

Purpose

Our previous studies of RCCBM used severe compromised immunodeficiency (SCID) mice. **The purpose of this research is to develop an immunocompetent mouse model for the study of novel avenues for bone metastasis prevention in RCC.** This model will allow us to investigate the effects that the immune system have on bone metastasis progression and to better relate our RCCBM mouse model to humans.

Methods

RT-qPCR and DNA gel electrophoresis will be done using four sets of primers to detect relative mRNA expression of BIGH3 in K7 cells, an immunocompetent mouse RCC cell line.

Ex vivo study

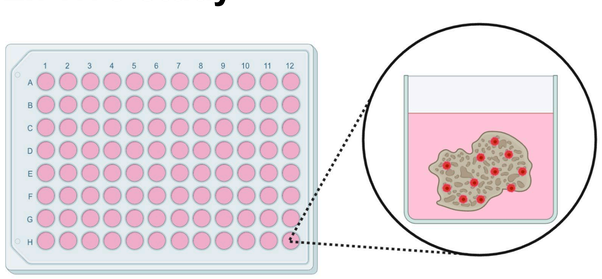

Figure 3. *Ex vivo* study visual representation

Methods cont.

Ex vivo study cont.

Cortical bone fragments will be taken from the femur and tibia of two C57BL6 mice. **K7 cells with and without a luciferase tomato (LT) gene will be seeded on the bone fragments** and placed in DMEM/F12, containing 5% p/s, 0.5% FBS, and $NaHCO_3$ on a low-attachment 96-well plate. IVIS Lumina imaging will be used to detect the amount of K7/LT cells in each well.

In vivo study

SCID mice will be injected with K7 and K7/LT cells intracardially. After aggressive metastatic cancer is detected, the mice will be euthanized and bone marrow will be collected and cultured to grow bone-derived K7 cells. IVIS Lumina imaging will be used to detect the amount and location of K7/LT cells in the mice. The bone-derived K7 cells will be further selected using the intracardiac injection in SCID mice to get cells that specifically target bone. The specific bone-derived K7 cells will then be administered to immunocompetent C57BL6 mice as the new mouse model for RCCBM.

Potential Results and Conclusions

- If K7 cells express BIGH3 similar to previous human renal cell carcinoma cell lines, this supports using human anti-BIGH3 antibody as a potential avenue for treatment and prevention of bone metastasis in RCC.
- We expect the K7 cells to attach to and grow on bone up to fourteen days. Bone-derived K7 cells will result in better attachment.

Potential Results and Conclusions cont.

- We anticipate the K7 cells to migrate mostly to the lungs, liver, spine, and femur in the live mice.
- The future use of K7 cells in the RCCBM immunocompetent mouse model will depend on how rapidly the mice acquire aggressive metastatic cancer.

Responsible Conduct of Research

We will minimize the number of mice ordered for the project while also having enough mice to obtain significant results. For the *in vivo* study, we ordered 15 SCID mice in total where 5 will be injected with PBS as a negative control, 5 will be injected with K7 cells, and 5 will be injected with K7/LT cells. Only two C57BL6 mice will used for the *ex vivo* bone fragment study.

Acknowledgements

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Reference

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