Effects of Estrogen Removal by Ovariectomy on Growth of Human MCF-7 Breast Cancer Cells in Bone

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Introduction

Hormone receptor positive breast cancers commonly metastasize to bone. Hormonal regulation in primary tumor and in bone is well established but less studied in the context of bone metastases.

Aim of the Study

To study the effects of hormone deprivation on growth of bone metastases in the early phases of outgrowth and on already growing metastases in bone.

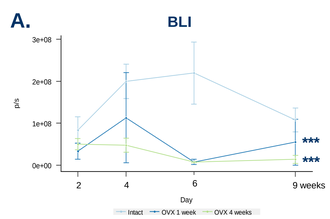
Materials and Methods

Estrogen responsive, luciferase labelled MCF-7 human breast cancer cells (ATCC, labelled at DKFZ, Germany) were inoculated into the tibia of 7-8 week old female athymic nude mice (Envigo, Netherlands). The mice were ovariectomized (OVX) one week before or four weeks after the cancer cell inoculation. Mice in the control group were left intact. Tumor growth and tumor-induced bone changes were studied by bioluminescence imaging (BLI) and X-ray imaging, respectively, at 2, 4, 6 and 9 weeks after the cancer cell inoculations. Changes in bone mineral density (BMD) was studied at endpoint by dual X-ray absorptiometry (DXA). Decalcified bone sections were evaluated by histology.

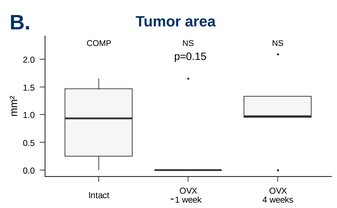
Study Design

Figure 1: Timeline of the study. Hormone responsive MCF-7 human breast cancer cells were inoculated into tibial bone marrow cavity of nude mice at day 0. OVX operations were performed one week before and 4 weeks after cancer cell inoculation to groups 2 and 3, respectively. X-ray and BLI imaging was performed at 2, 4, 6 and 9 weeks. DXA imaging was performed at sacrifice.

Results



Group	2 weeks	4 weeks	6 weeks	9 weeks
Intact	8/9, 89%	6/9, 67%	9/9, 100%	9/9, 100%
OVX -1 weeks	9/9, 100%	5/9, 56%	8/9, 89%	1/9, 11%
OVX 4 weeks	8/8, 100%	8/8, 100%	8/8, 100%	2/8, 25%



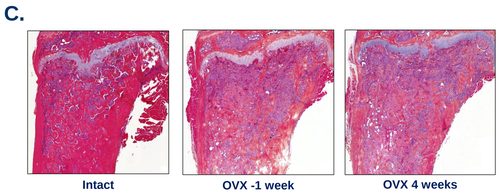


Figure 2: A) BLI was performed to follow tumor growth in bone at 2, 4, 6 and 9 weeks after cancer cell inoculations. The lines present mean ± SD of each study group (8-9 animals in each group). ***, p<0.001. Summary of mice with BLI signal (n, %) during the timecourse of the study is presented in the Table below. B) Tumor area (mm²) was quantified from HE-stained histological sections. The box plots present median±IQR25%±min/max, n=6/group. Outliers are presented as individual dots. NS = not significant compared with the intact group (COMP). C) Representative HE-stainings from the tibial tumor area after 9 weeks.

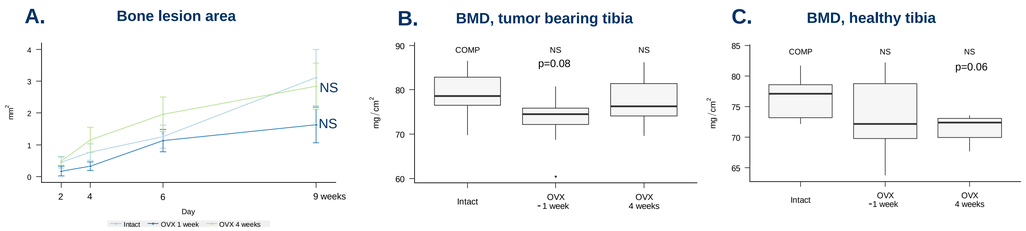


Figure 3: A) Growth of bone lesions was followed by X-ray imaging at 2, 4, 6 and 9 weeks after cancer cell inoculations. The lines present mean ± SD of each study group (8-9 animals in each group). Bone mineral density (BMD, mg/cm2) was analyzed in tumor-bearing tibia (B) and in intact leg (C). The box plots present median ± IQR25% ± min/max. NS = not significant compared with the intact group (COMP).

Summary

- The increase in BLI signal was seen in intact mice up to 6 weeks after cancer cell inoculations, and after that the signal decreased. The decrease was most propably due to tumor-induced bone formation, which required space from tumor cells to grow in the marrow.
- The BLI signal decreased in the OVX groups and by 9 weeks after tumor cell inoculation, the signal was diminised from some mice and was only seen in 11% and 25% of the mice ovariectomized one week before and four weeks after the cancer cell inoculations.
- The tumor take rate was verified by histology and was 83%, 17% and 83% in intact mice and in mice ovariectomiced one week before and four weeks after cancer cell inoculations. The tumors in mice ovariectomized one week before cancer cell inoculation were markedly smaller compared to intact mice.
- The bone lesion area was comparable in all study groups but lowest in the mice ovariectomized one week before cancer cell inoculation.
- OVX was associated with a trend towards decreased BMD seen in tumor-bearing and healty tibias.

Conclusions

Hormone regulation in bone metastasis is complex, consisting of hormone related changes in bone combined with tumor-induced changes. In the presence of endogenous estrogen, the tumors grew and induced major osteoblastic bone reaction. In the absence of estrogen the tumor cells disappeared in some, but not all cases from the bone marrow by time.

In order to establish more predictive preclinical models for advanced breast cancer drug development, influence of hormonal microenvironment in the bone should be taken into account.

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