Validation of In Vitro Assays for Identifying Compounds That Affect Breast and Prostate Cancer Cells

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Introduction

Breast and prostate cancer are among the most common types of cancer that metastasize to bone. Skeletal metastases can lead to severe pain, pathological fractures, paralysis and eventually increased morbidity. Although there is no curative medication for bone metastases, there are many treatments that can be used to treat the symptoms. Bone resorption inhibitors can be used to inhibit osteolysis, whereas chemotherapy drugs can be used to prevent cancer for spreading. However, the responses to treatments depend on the patient and the type of underlying cancer, and it is therefore of importance to know the direct response of treatment on cancer cells.

Aim of the Study

Our goal was to optimize in vitro cancer cell culture models for testing the effects of new chemotherapy agents.

Materials and Methods

Cell cultures
MDA-MB-231 and MCF-7 breast cancer cells and PC-3 and LNCaP prostate cancer cells metastasizing to bone were used in the study. Seven known clinically used chemotherapy drugs with different modes of actions, including an alkylating agent Tri-ethylenemelphosphoramide (ThioTEPA, Sigma-Aldrich), a pyrimidine analog fluorouracil (Sigma-Aldrich), an antimetabolic agent Cytosine arabinoside (Ara-C, Sigma-Aldrich), a topoisomerase I inhibitor topotecan (Sigma-Aldrich), an anthracycline antibiotic doxorubicin (Sigma-Aldrich), a mitotic inhibitor paclitaxel (Pharmachemie) and a nucleoside analog gemcitabine (Lilly), were tested as reference compounds with the range of 1, 10, 100 and 1000 nM concentrations. The cells were cultured for 5 days and the effects of the agents on bone metastatic cancer cells.

Statistical analysis

The results of all other groups (p<0.05 between all groups) were compared separately with the results of the baseline group using linear model with a fixed effect for the treatment, a continuous covariate for the day and an interaction term for the treatment and the day. In the figures, the p-values are indicated only for the inhibitors whose response is below the control and the difference between the inhibitor and the control is statistically significant (p < 0.05).

Effects of different chemotherapy agents

Figure 1: Effects of seven different chemotherapy agents on the proliferation of (A) MDA-MB-231, (B) MCF-7, (C) PC-3 and (D) LNCaP cells. * p<0.05, ** p<0.01, *** p<0.001 compared to control group.

Summary

➢ The most potent inhibitors of cancer cell proliferation were paclitaxel and gemcitabine, which were potent inhibitors already at 10 nM concentration.

➢ No major differences of treatment responses between the cell lines were observed, with the exception that gemcitabine was more potent to breast cancer cells than to prostate cancer cells.

➢ ThioTEPA and fluorouracil showed no effect or only mild effects at 1000 nM concentration on the tested cell lines.

➢ Ara-C was a potent inhibitor at 1000 nM concentration, whereas topotecan and doxorubicin were potent inhibitors already at 100 nM concentration.

Conclusions

These results suggest that paclitaxel, gemcitabine, doxorubicin, topotecan and Ara-C inhibit cell proliferation of breast and prostate cancer cells in this cancer cell culture model.

We conclude that this culture system can be used as a screening tool for finding new chemotherapy agents on bone metastatic cancer cells.

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