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Evaluation of zinc treatment on spheroids and adherent cells of the MCF7 breast cancer cell lineage

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ABSTRACT

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Breast cancer is a heterogeneous and complex disease that affected more than two million women in 2020. Among the modifiable risk factors, nutrition has a fundamental role. Micronutrients such as zinc are fundamental in the body, as their catalytic, structural, and regulatory properties impact various cellular processes, including breast development. However, the mechanisms associated with breast zinc homeostasis and its function in breast cancer are still unclear. **Objectives:** To implement the culture of tumor spheroids using cells of the MCF7 lineage, as well as to investigate the impact of zinc sulfate treatment on non-invasive breast cancer cells. Parameters such as cell viability, proliferation, apoptosis, and intracellular zinc levels were analyzed. Additionally, the study aimed to explore the mechanisms underlying zinc's effects by examining the expression of the ZIP6 and ZnT2 genes. **Methods:** Mammary spheroids of MCF7 cells were cultured in a serum-free medium using an ultra-low attachment plate. A range of zinc concentrations was tested to determine optimal dosages. Cell viability was evaluated using alamarBlue, while cell proliferation was measured using a BrdU Cell Proliferation kit. Programmed cell death was evaluated using the Alexa Fluor 488 annexin V/Dead Cell Apoptosis Kit. The expression of ZnT2 and ZIP6 genes was analyzed through RT-qPCR. **Results:** The findings point to a dose-dependent effect of zinc on cell viability, indicating a possible influence on tumor proliferation of breast cancer spheroids. Treatment with zinc at 75 µM (****$p<0.0001$) and 100 µM (**$p=0.0211$) significantly stimulated proliferation by 120% and 59%, respectively. For both culture platforms (2D and 3D), higher zinc concentrations reflected an increase in ZnT2 gene expression. In adherent cells, a considerable increase in ZnT2 expression was observed in cells treated with 100 µM (77.26%), despite the absence of statistical significance ($p=0.3316$). In spheroids, a prominent up-regulation in ZnT2 expression was also observed when 200 µM (267.56%) (**$p=0.0016$) was used, with no changes for the other treatments. As for the ZIP6 gene, it showed significantly lower expression only in MCF7 spheroids at 100 µM (*$p=0.0256$) and 200 µM (***$p=0.0004$), resulting in a decrease in gene expression by 31.9% and 53.1%, respectively. **Conclusions:** In summary, this study provides an initial investigation of the effects of zinc on adherent cells and in breast cancer spheroids. The results suggest that

zinc stimulates tumor proliferation in spheroids, while differentially regulating the expression of genes associated with zinc homeostasis, potentially facilitating cell survival and tumor progression. Importantly, these effects appear to be specific to certain cell subtypes, highlighting the need for further studies with other models to increase understanding of zinc function in breast cancer.

Keywords: breast cancer, spheroids, MCF7, zinc transporters, zinc;