

Treating MCF-7 Human Breast Cancer Cells with Bisphenol A to Examine Effects on Inflammatory Pathways

Rachael Reesman and Dr. Winnifred Bryant | Biology Department, University of Wisconsin Eau Claire



INTRODUCTION

This project aims to examine the effects of the environmental estrogen, bisphenol A (BPA), on inflammatory pathways in human breast cancer cells. Environmental estrogens are found in a wide variety of plastics, food, and pesticides, so there are human health implications for clarifying the link between environmental estrogens and cancer cell inflammation and growth. Environmental estrogens mimic the effects of 17 β -estradiol (E_2) as they interact with the same receptor proteins. These interactions cause growth, differentiation, and proliferation in their target cells. One pathway that plays a regulatory role in inflammation-associated cancer development is the Mitogen-activated Protein Kinase pathway (MAPK). The MAPK pathway is activated by growth factors, inflammatory cytokines, and stress. These stimuli activate regulatory proteins that mediate cell growth, differentiation, and proliferation. We are curious if BPA acts as an activator of the MAPK pathway specifically in cancer cells. To study this, MCF-7 human breast cancer cells were grown, treated with BPA, and lysed. Protein lysates were collected, quantified, and analyzed via chemiluminescent array to detect protein markers in the MAPK pathway.

MATERIALS AND METHODS

CELL CULTURING

MCF-7 Human Breast Cancer Cells were grown at 37 °C in 5% CO_2 in Eagle's Minimum Essential Media (EMEM) supplemented with 20% Fetal Bovine Serum, 0.1% non-essential amino acids, and 1% Penicillin-Streptomycin.

BPA TREATMENTS

MCF-7 cells were plated to 1.25 million cells per well in 6 well plates with culture media. Plates were incubated overnight at 37°C in 5% CO_2 .

The following day, cells were challenged with MeOH (vehicle) for two minutes, 10 nM E_2 for two minutes, or 1 μ M BPA for two or five minutes.

Cells were washed once with 1X PBS buffer.

HUMAN CELL STRESS ARRAY

A Human Cell Stress Array (R&D Systems) was used to monitor changes in the expression of 26 proteins involved in the inflammatory stress response. Protein lysates were quantified by BCA assay. 200-300 μ g of protein lysate were incubated with membranes containing antibodies against proteins of interest. Proteins were visualized by chemiluminescent detection. Digital images of membranes were generated using an AnalytikJena UVP GelStudio Imager.

IMAGING AND ANALYSIS

ImageJ software was used to quantify pixelation between treatment groups. Pixelation is given in Raw Integrated Density (Schieder).

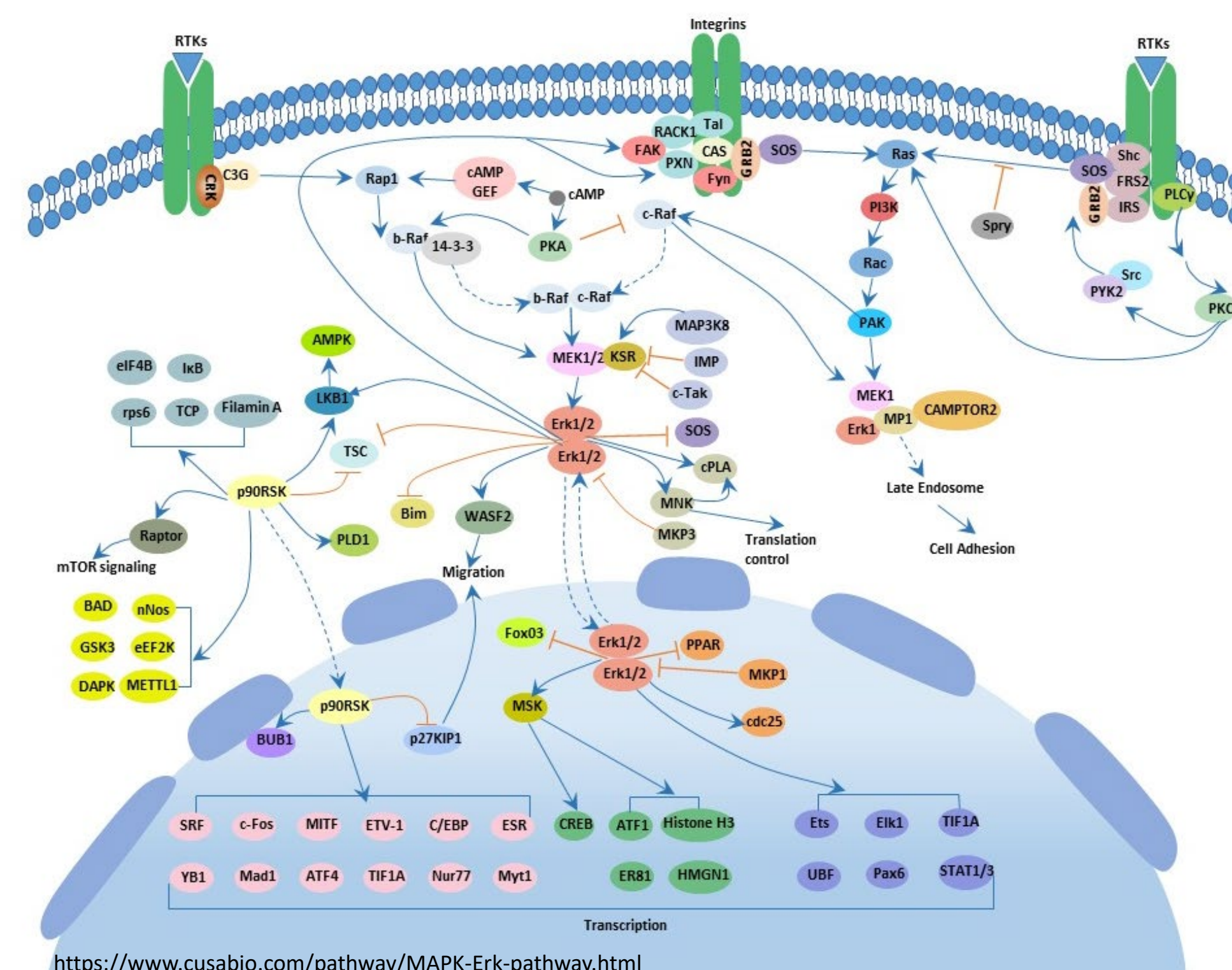


Figure 1: The MAPK pathway. The major functions of this signaling pathway involve the regulation of cell proliferation, transformation, differentiation and apoptosis. This signaling pathway is activated by a variety of hormones, growth factors, and differentiation factors.

RESULTS

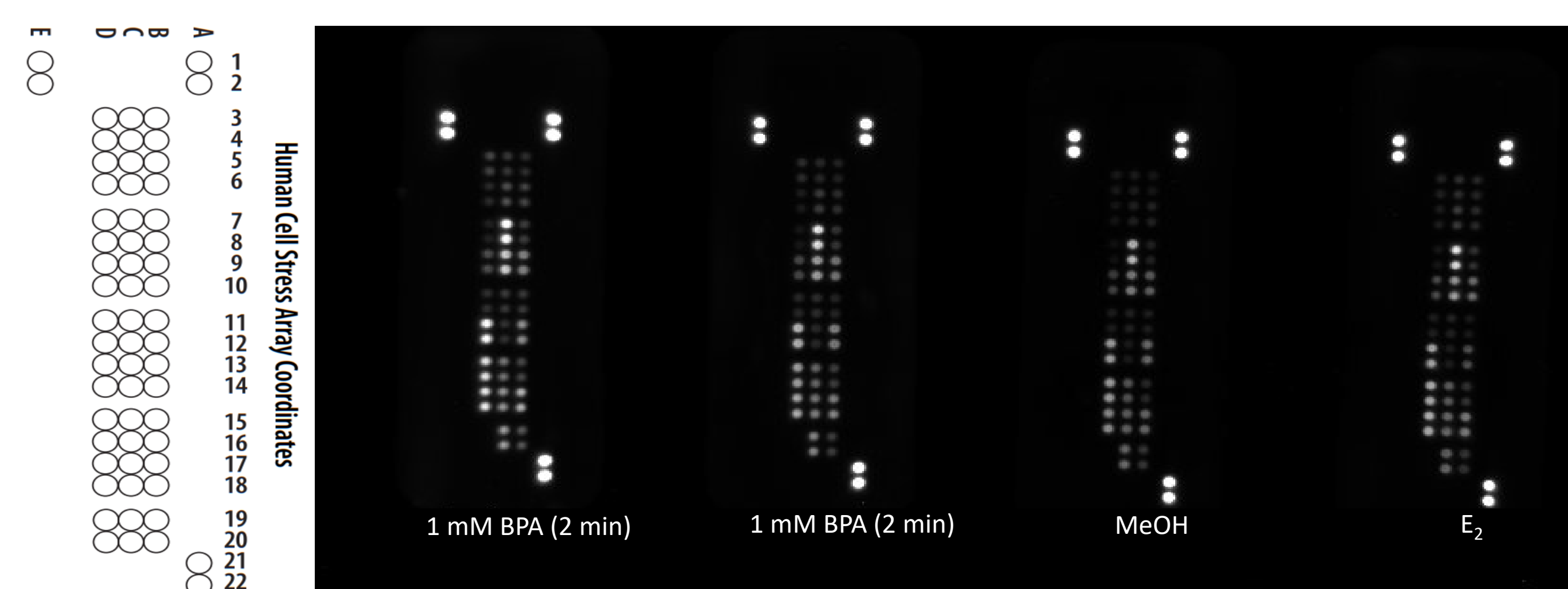


Figure 2: Representative blots showing expression of 26 proteins involved in the MAPK pathway. Protein lysates were obtained from cells treated with MeOH (vehicle), 10 nM E_2 or 1mM BPA for two or five minutes. Thioredoxin-1 (Trx-1, positions D13 and D14), SIRT2 (positions D15 and D16), superoxide dismutase (SOD2, positions D17 and D18), and p27 (positions C19 and C20) exhibited the most distinct differences in expression (as compared to control) after two minutes of BPA treatment.

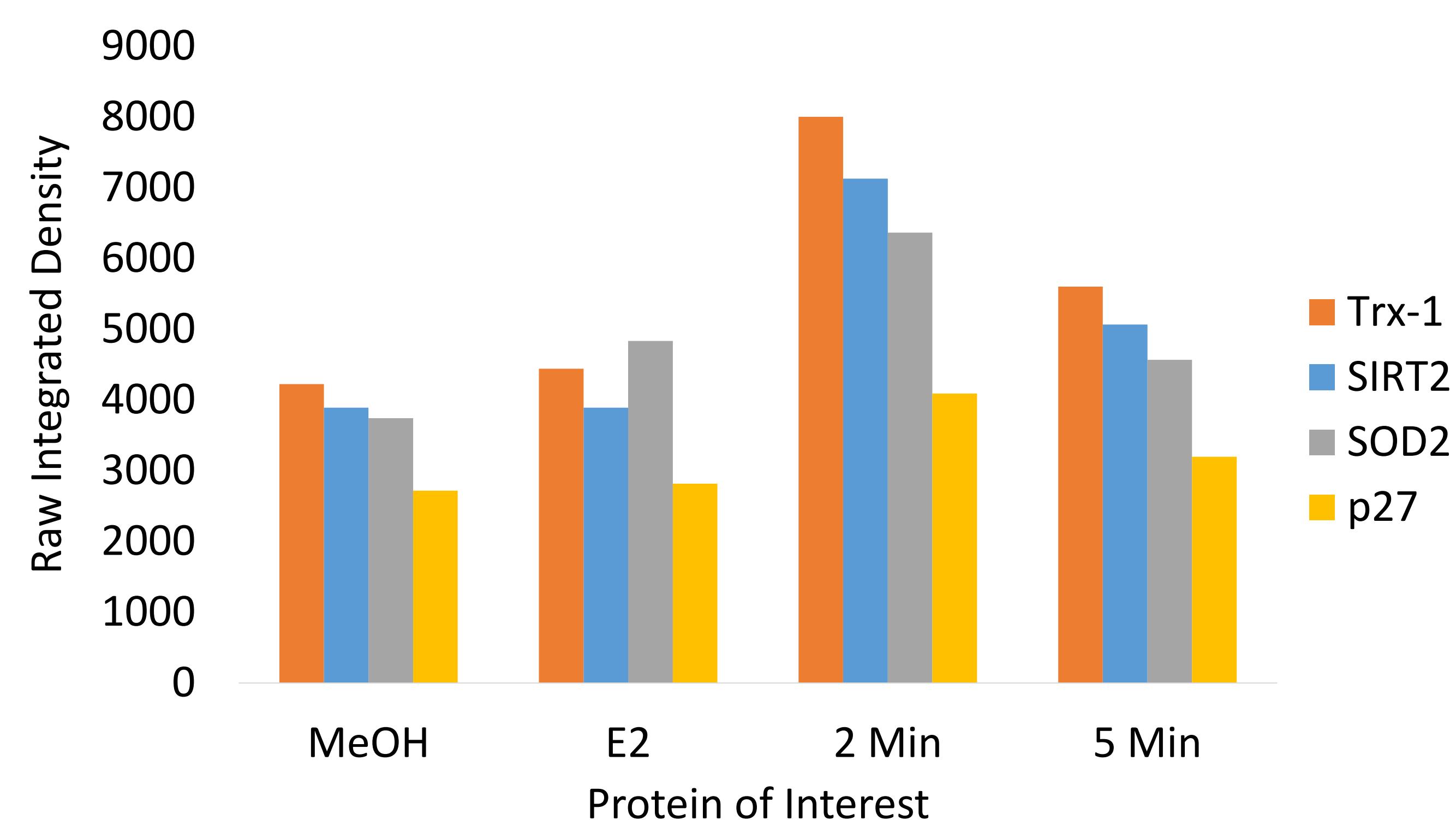


Figure 3: Raw Integrated Density Data from ImageJ from four different proteins. In MCF-7 Human Breast Cancer Cells, BPA works faster to activate the MAPK pathway as compared to MeOH and E_2 .

DISCUSSION

In these experiments, we seek to determine if BPA activates the MAPK pathway in MCF-7 cells. We focused on Trx-1, SIRT2, SOD2, and p27, components of the MAPK signaling pathway that demonstrated pronounced changes in expression following BPA treatment.

Thioredoxin-1 (Trx1) is an oxidoreductase enzyme that plays crucial roles in cell growth and inhibition of apoptosis. Overexpression of Trx1 can suppress inflammation within the cell (Yeo, E. J). This overexpression of Trx1 facilitates cancer cell survival.

Sirtuin 2 (SIRT2) is a deacetylase that participates in mitotic cell death. Downregulation of SIRT2 caused apoptosis in cancer cells (Li, Y.). This suggests that overexpression of SIRT2 would cause proliferation in cancer cells.

Superoxide dismutase 2 (SOD2) is an enzyme that ameliorates the effects of oxidative stress. Elevated content of SOD2 is correlated with increased tumor cell invasion, metastasis, proliferation, and resistance to apoptosis (Yi, L.). The overexpression of SOD2 facilitates cancer cell survival.

p27 is a protein that inhibits cyclin dependent kinases involved in the regulation of the cell cycle (Abbastabar). Overexpression of p27 facilitates cancer cell growth.

Our results suggest that the environmental estrogen, BPA, stimulates the MAPK pathway acutely in breast cancer cells and may contribute to cancer cell survival and proliferation. These results reinforce the need for understanding the relative risk BPA presents to human health.

FUTURE DIRECTIONS

Future studies include replicating these results, examining the effect of BPA on the MAPK pathway using an even shorter time course, and examining the effect of BPA on other pathways that involve Trx-1, SOD2, p27 and SIRT2.

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