

Identification of biomarkers and effective treatments against Herceptin-resistant breast cancer

I. Introduction

Breast cancer is the leading cause of cancer-related deaths in American women. Overexpression of HER2 is observed in 20-25% of all breast cancers, with Herceptin resistance being one of the many clinical challenges. In Herceptin-sensitive cells, FOXO3a regulates specific miRNAs to modulate the expression of IGF2. This is attributed to the expression regulation of PPP3CB, which restricts phosphorylation of FOXO3a. There is a lack of efficient biomarkers for Herceptin response indication. Novel therapy implementing PPP3CB/FOXO3a/IGF2 signaling can be promising for the reduction/elimination of mortality related to Herceptin-resistant breast cancer.

II. Hypothesis/Objective

In conjunction with the preliminary information, the main aim of the study was to identify biomarkers and develop epigenetic approaches with therapeutic potential for treatment of Herceptin-resistant breast cancer.

III. Methods and Materials

Cells and Culture Conditions:

Herceptin-resistant cultures of HR20 and Pool2 were developed from BT474 and SKBR3, respectively. BT474 and SKBR3 are two well-known HER2-positive breast cancer cell lines sensitive to Herceptin.

1) Cell Proliferation (MTS) Assay

Cell sensitivity to Herceptin was determined using the CellTiter 96® Aqueous One Solution Cell Proliferation Assay kit. The cells were seeded onto 96-well plates at 4000 cells/well with varying concentrations of Herceptin, Panobinostat, and Combination. The cell viability was determined the following day via incubation and recorded absorbance with MTS.

2) Western Blot Assay

The total protein was extracted from the cells using RIPA buffer, Halt Protease and Phosphatase Inhibitor Cocktail, and measurements from BCA Protein Assay Kit. Gels were run and a transfer process was performed. The membranes were blocked with 5% non-fat milk in Tris-buffered saline, and incubated with a primary and secondary antibody. Signal detection was performed via enhanced chemiluminescence western blot detection kit.

IV. Results: MTS Assay

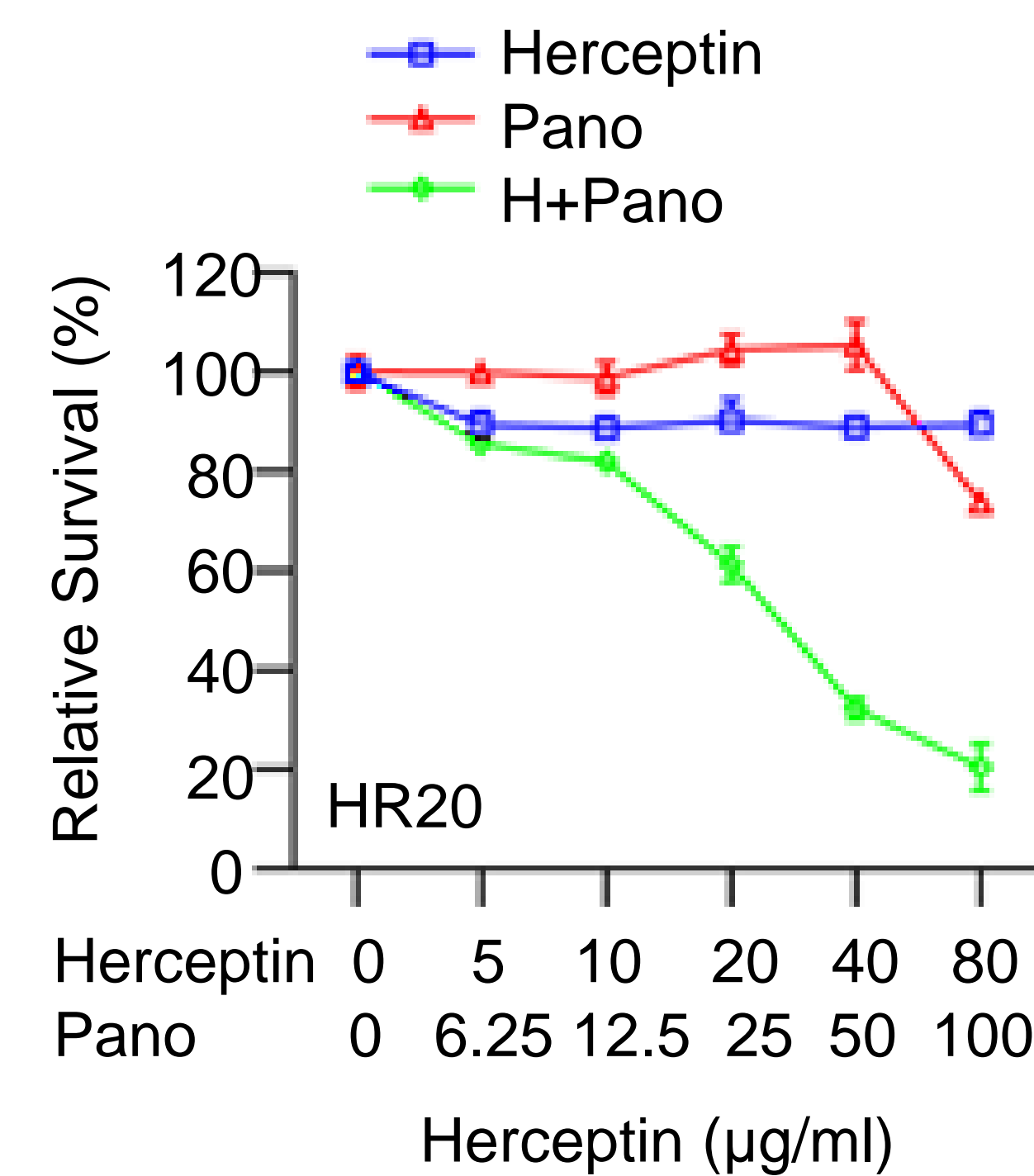


Figure 1. Cell viability analyzed via MTS Assay. The combination of H+Pano was shown to have positive effects in cell growth inhibition.

V. Results: Western Blot

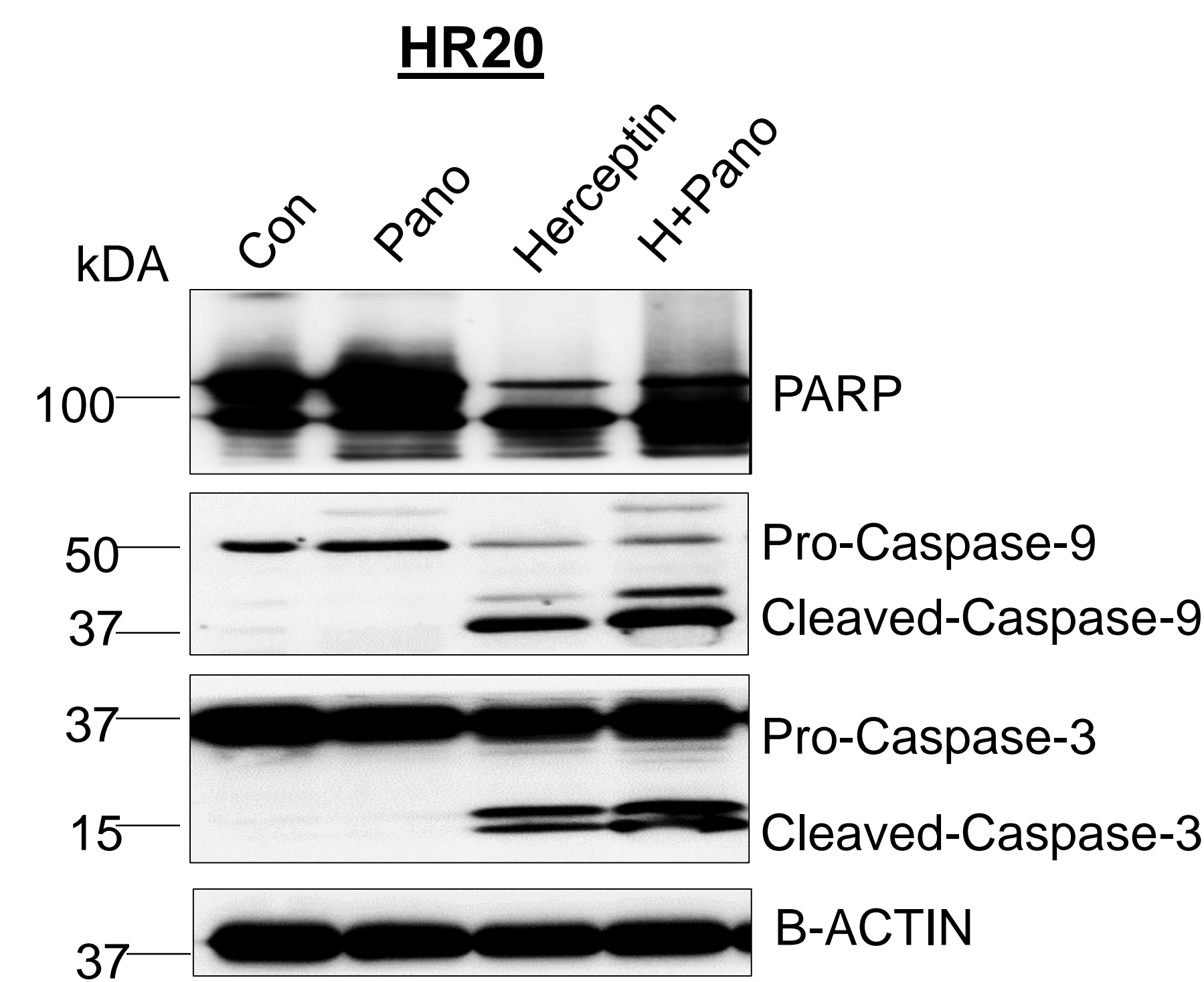


Figure 2. Protein expression of HR20 cells was analyzed via Western Blot assays. Cleaved lines represent the apoptotic effects. The combination of H+Pano was shown to have additive effects on induction of apoptosis.

VI. Results: Comparison

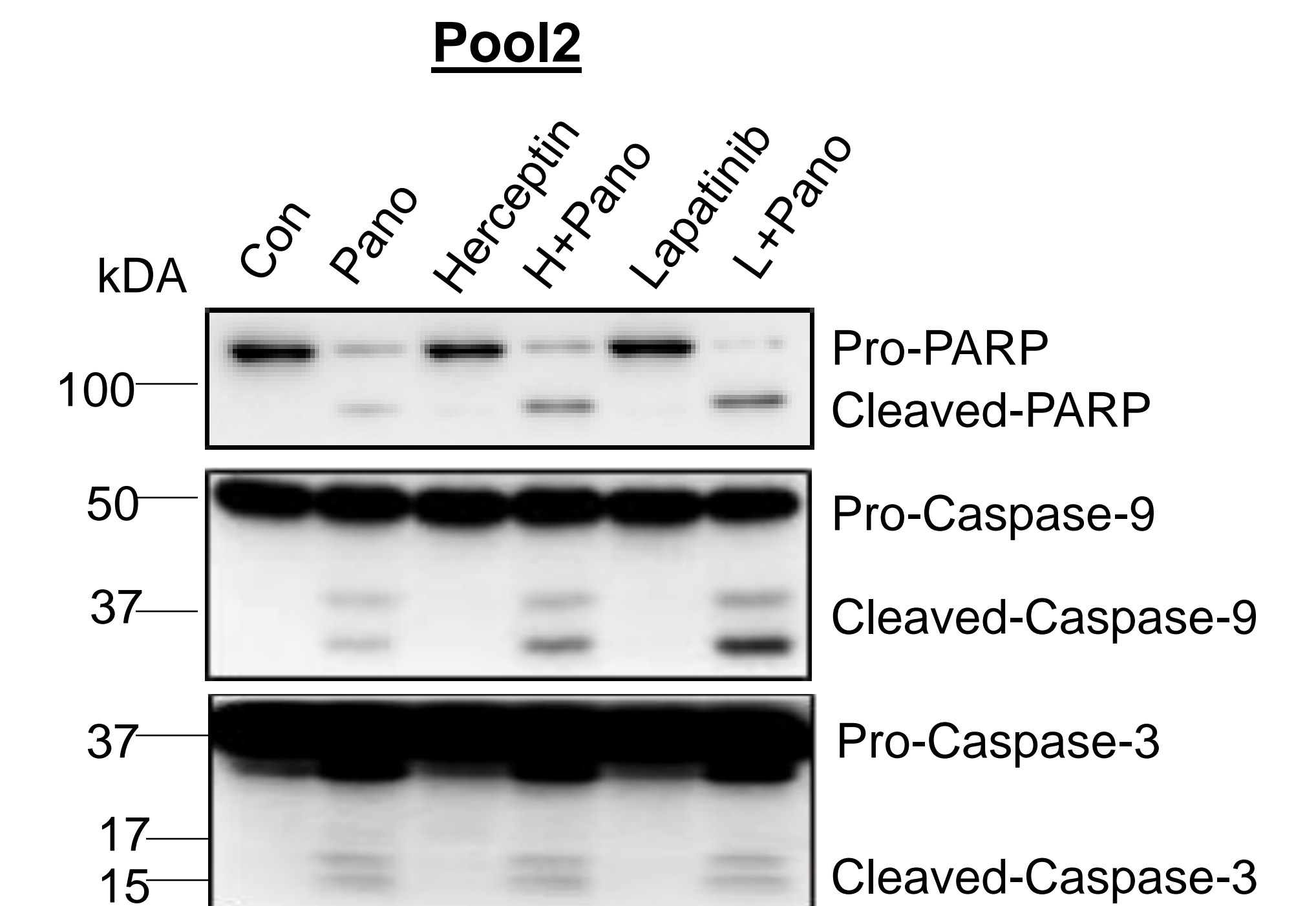


Figure 3. Protein expression of Pool2 cells was analyzed via Western Blot assays. Cleaved lines represent the apoptotic effects. The combination of L+Pano was shown to have additive effects on induction of apoptosis.

VII. Possible Target: HDAC

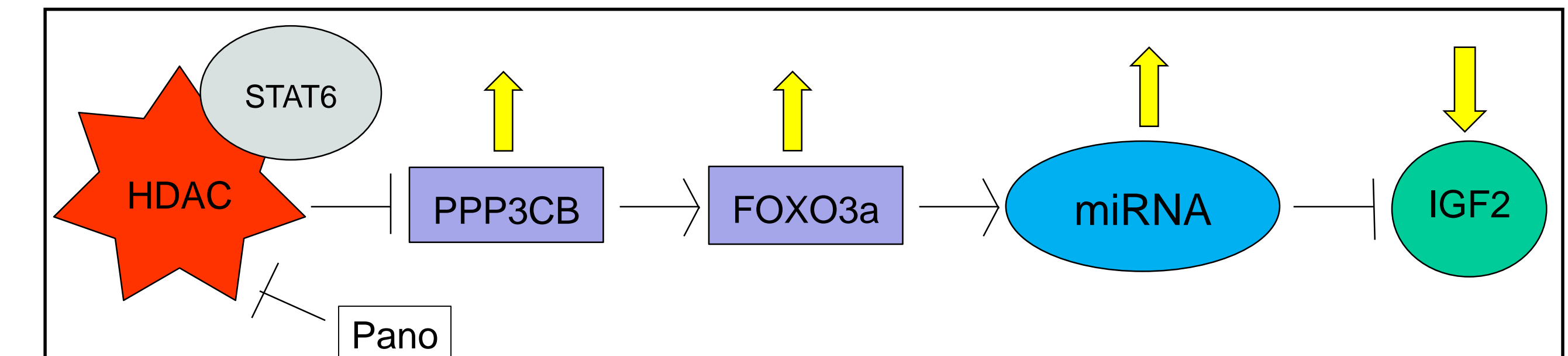


Figure 4. PPP3CB/FOXO3a/IGF2 signaling contributed to a positive response of Herceptin treatments. Panobinostat increased PPP3CB, which was inhibited by histone deacetylase (HDAC).

VIII. Conclusion and Future Studies

While this study does show promise for possible treatments, the precise mechanism remains elusive. We suggest that further evaluations using more clinical cases should be performed. Our data provide new avenues to identify effective biomarkers for predictive indication of overcoming Herceptin resistance.