

# BIO299: EBP1 Overexpression and Its Impact on HER2 Signalling in Breast Cancer Cells

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## EBP1 background

**EBP1** (encoded by the gene *PA2G4*), is a ubiquitously expressed protein which is involved in cell proliferation, survival and differentiation.

EBP1 can function as a tumour suppressor and oncogene in cancer depending on the tissue. In breast cancer, both activity have been described (1, 2).

## Aim

Rationale: overexpression of EBP1 was shown to decrease cell proliferation in the breast cancer cell line AU565 (3, 4), via a decrease in the levels of tyrosine receptor human epithelial receptor (HER2).

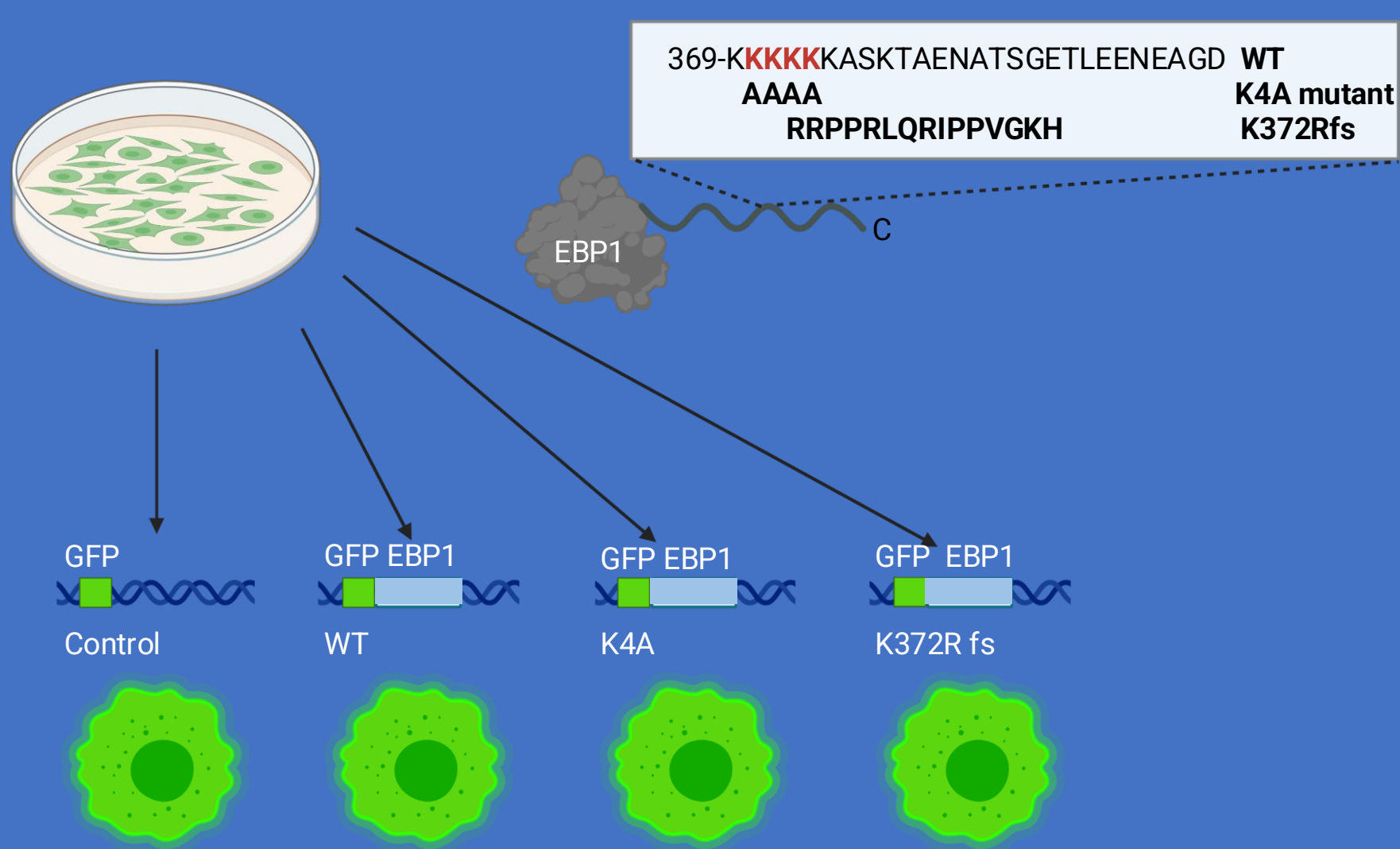
The aim is to test the effect of EBP1 overexpression on *HER2* downstream signalling by looking at MAPK ERK1/2.

## Methods

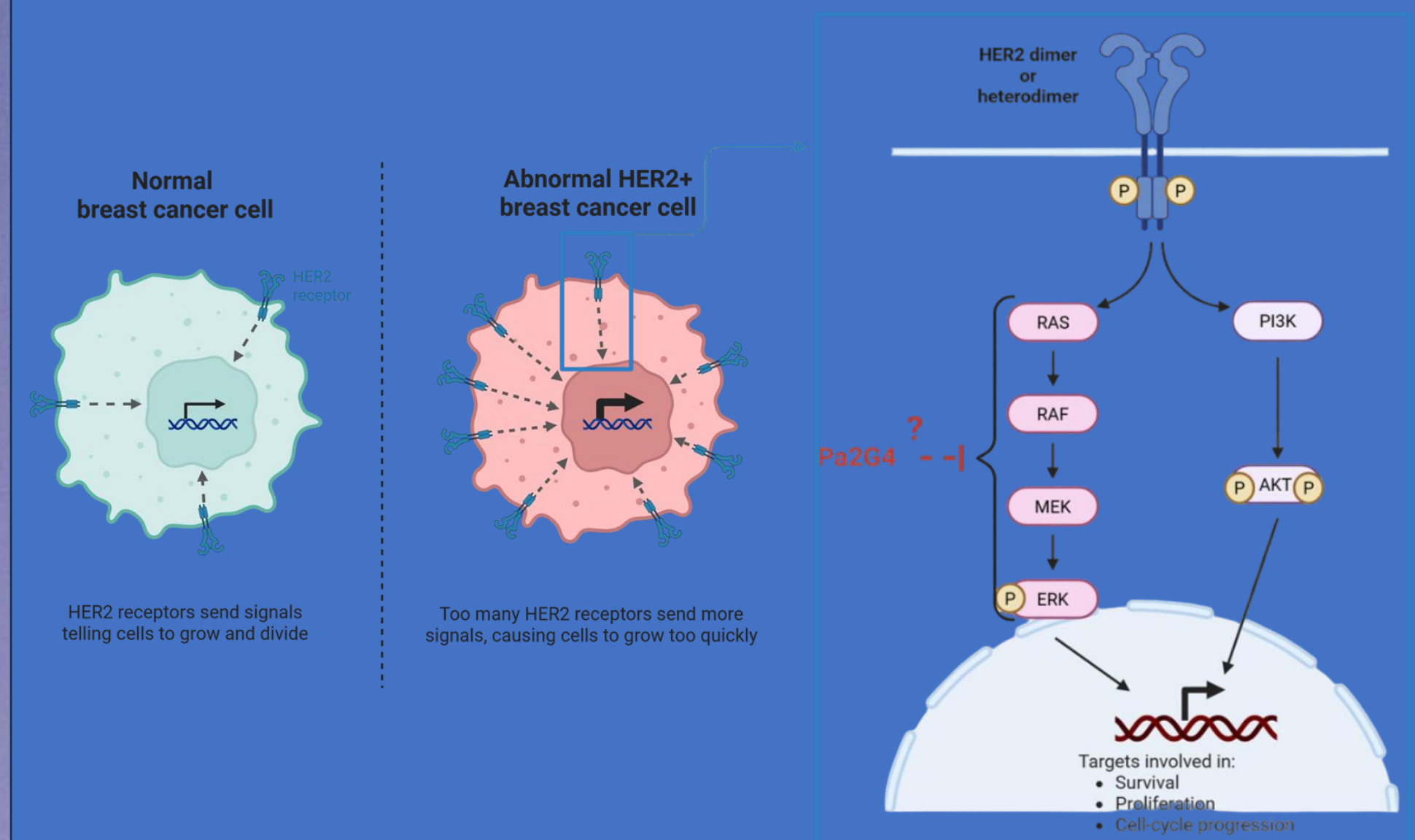


- Cell line: AU565 (*HER2*-positive breast cancer) Stably overexpressing EGFP-EBP1 WT, K4A mutant, K372Rfs mutant, GFP control
- K372Rfs mutation has been reported in patient samples from pancreas, colorectal, ovarian, and stomach cancers.
- Techniques: Western blot, RT-PCR, agarose gel

## Model: stable AU565 breast cancer cell lines



## HER2 overexpression and signalling in breast cancer cells



## HER2 mRNA levels Results

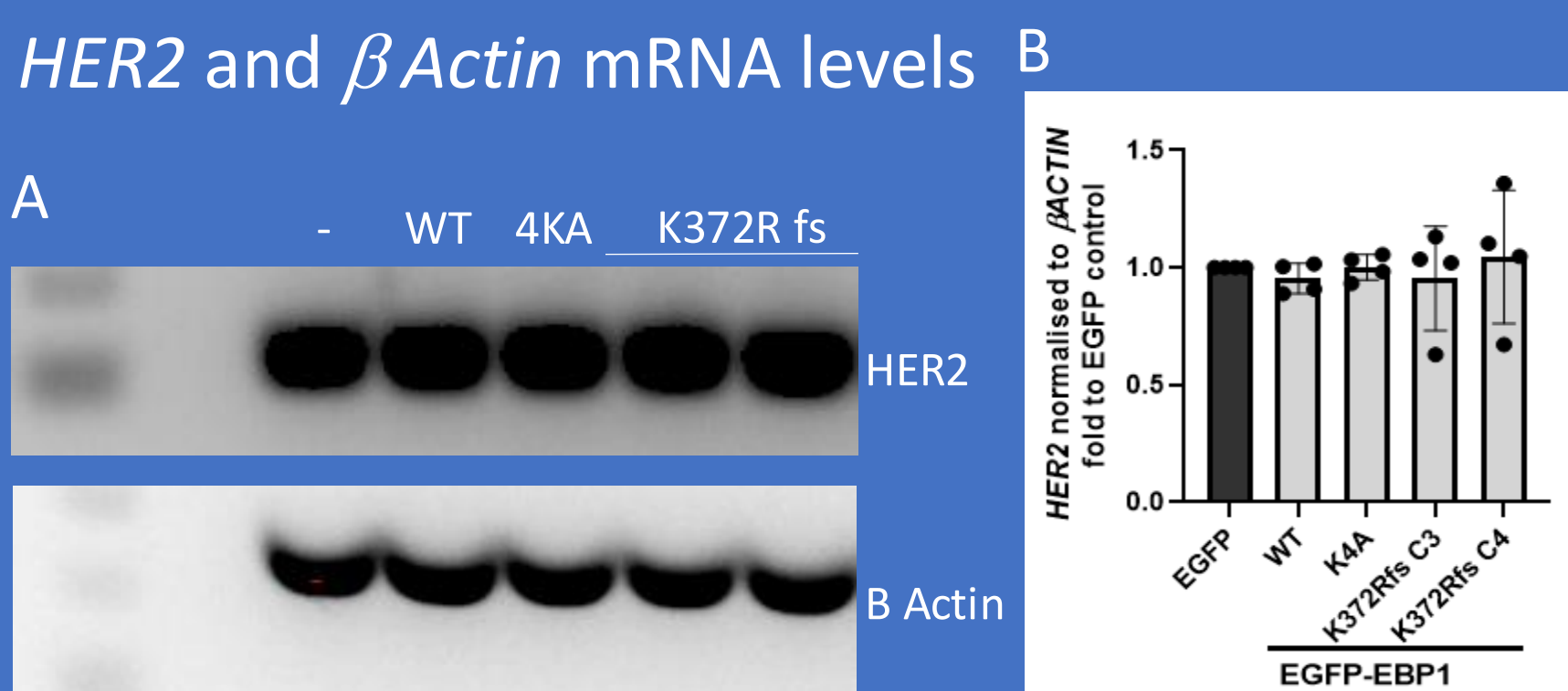


Figure 1 – mRNA levels of *HER2* and  $\beta$ -Actin in AU565 cell lines  
A) *HER2* and  $\beta$ -Actin RT PCR levels showed on a representative agarose gel.  
B) Quantification of *HER2* mRNA levels normalized against  $\beta$ -Actin n= 4.

## HER2 Pathway Results

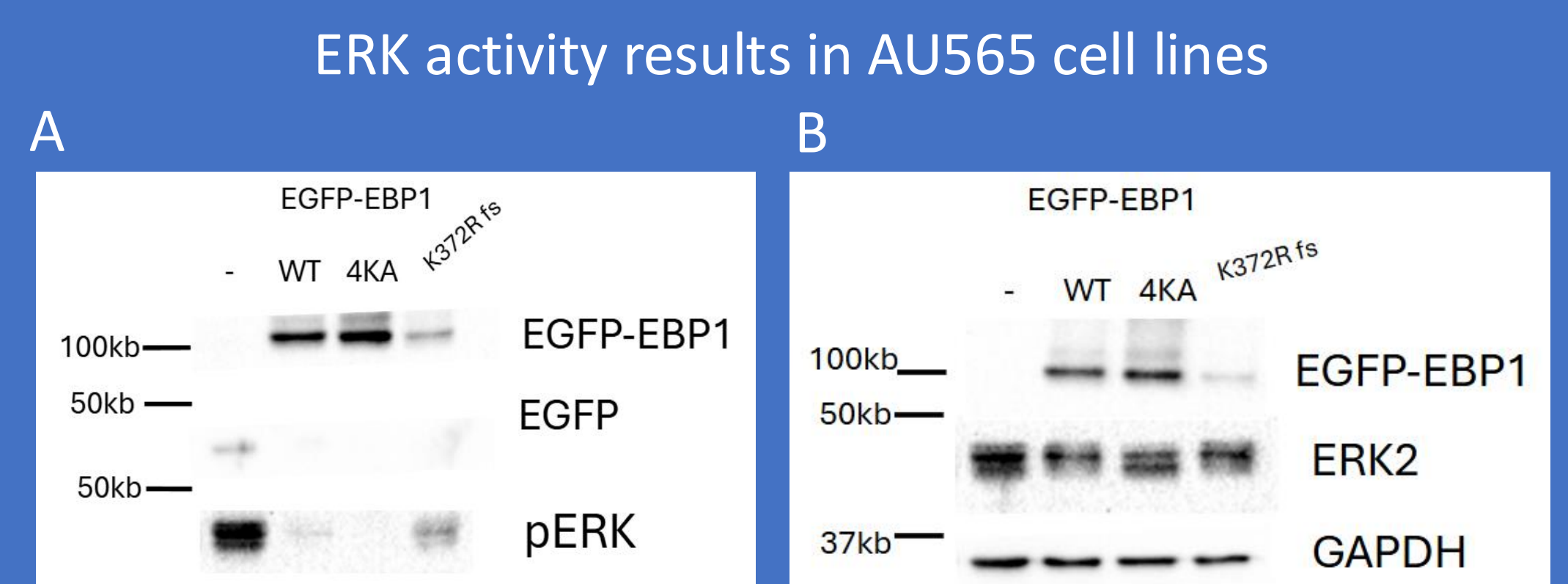


Figure 2 - ERK activity in AU565 cell lines stably over expressing EGFP-EBP1. Western immunoblotting showing EGFP and EGFP-EBP1 levels together with pERK (A) and total ERK2 levels (B) and EBP1 levels. GAPDH was used as a loading control.

## Conclusions

- Overexpression of EBP1 WT or mutants does not affect *HER2* mRNA levels. This suggests that EBP1's reported effects on cell proliferation may not involve transcriptional regulation of *HER2*. Protein expression detection does not reveal major changes, but further study might clarify whether EBP1 influences *HER2* signalling.
- Overexpression of EBP1 led to a decrease in pERK levels, indicating that EBP1 may act downstream from *HER2* receptor activation.

## References

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