The kisspeptin signalling pathway and its role in breast cancer biology

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INTRODUCTION

Kisspeptin is a neuropeptide encoded by the metastasis suppressor gene, *KISS1*. It functions through its cognate receptor, KISS1R. The activation of KISS1R by kisspeptin results in ERK1/2 activation, protein kinase B activation, calcium mobilization and inositol-1,4,5-triphosphate accumulation. Kisspeptin and KISS1R expression/activity inhibits metastasis in melanoma, pancreatic, lung, bladder, and ovarian cancers. However, in breast cancer, controversy remains with contradictory reports assigning pro- or anti-metastatic properties to kisspeptin. This study aims to shed light on the reasons for the contradictory role of kisspeptin/KISS1R in breast cancer by investigating the intracellular signalling pathways that are activated in response to kisspeptin signalling in breast cancer cell lines and determining their role in cell migration and invasion.

METHODS

KISS1R expression: Expression of KISS1R in two triple-negative breast cancer cell lines, BT-20 and MDA-MB-231, was determined through Western blotting, using an anti-KISS1R antibody. β-tubulin was used as a loading control.

ERK1/2 phosphorylation: BT-20 and MDA-MB-231 cell lines were stimulated with kisspeptin (KP-10; 100 nM) for 5, 10, 30, 45 or 60 min, at 37ºC. Vehicle -treated negative controls were included. Following stimulation, cells were lysed and ERK1/2 phosphorylation was assessed through Western blotting, using an anti-ERK1/2 antibody. Measurement of total-ERK1/2 was used as an internal control. For measurement of the effects of β-arrestin inhibition, cells were pre-treated for 30 mins in the presence/absence of a range of concentrations of a β-arrestin 1/2 inhibitor (barbadin) prior to stimulation with KP-10 for 60 min.

Cell migration: A confluent monolayer of BT-20 and MDA-MB-231 cell lines was scratched with a 200 µl pipette tip and then treated with media only, vehicle or 100 nM KP-10 and incubated for 18 hrs at 37ºC. Images of the scratch was taken on the day the scratch was created (0 hrs) and 18 hrs later using a Zeiss Axiovert microscope.

RESULTS

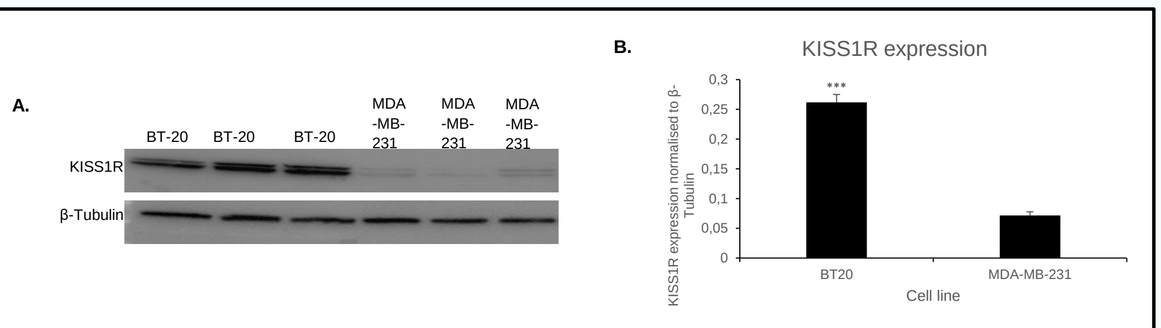

Figure 1: **The triple-negative breast cancer cell lines, BT-20 and MDA-MB-231, express endogenous KISS1R, with BT-20 cells having the highest level of expression**. **A**. Representative Western blot image (from three biological repeats) showing expression of KISS1R protein in BT-20 and MDA-MB-231 cells. **B**. A composite graph from three biological repeats showing KISS1R protein expression relative to β-Tubulin. *** p< 0.001, Student's t-test for comparison between both cell lines.

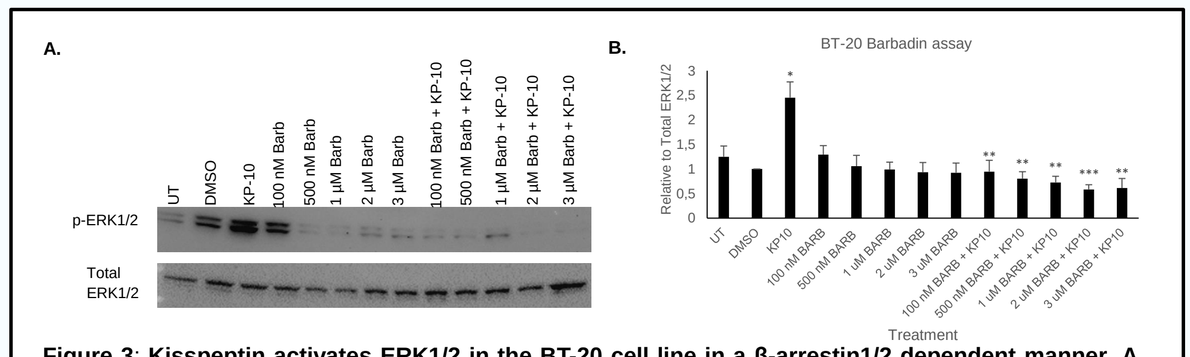

Figure 3: **Kisspeptin activates ERK1/2 in the BT-20 cell line in a β-arrestin1/2 dependent manner**. **A**. Representative Western blot image (from three biological repeats) measuring ERK1/2 phosphorylation in BT-20 cells following pre-treatment in the absence or presence of a range of concentrations of barbadin (Barb) before stimulation with either vehicle or 100 nM KP-10. Untreated cells (UT) served as a negative control. **B.** A composite graph from three biological repeats showing the expression of phosphorylated ERK1/2 relative to total ERK1/2. *p<0.05, **p<0.01, ***p<0.001 Student's t-test for comparison between UT and KP-10 and Barbadin concentration and between KP-10 and each Barbadin + KP-10 treatment.

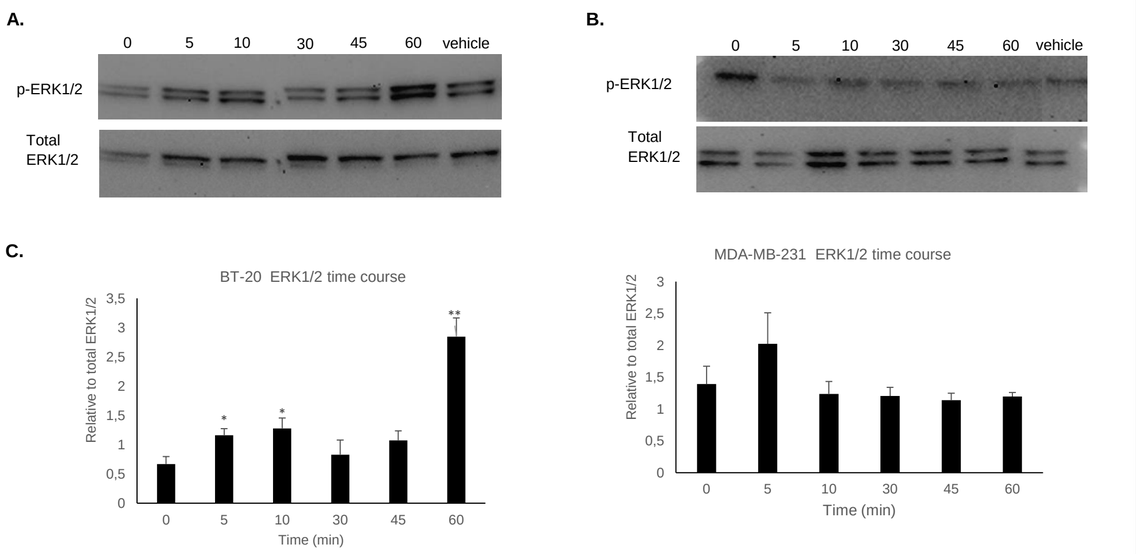

Figure 2: The ERK1/2 signalling pathway is activated by KP-10 in the BT-20 but not the MDA-MB-231 cell line, with an early activation phase occurring at 5-10 mins of stimulation and a later, more robust, phase after 60 min of stimulation Representative Western blot images (from three biological repeats) measuring ERK1/2 phosphorylation in BT-20 (**A**) or MDA-MB-231 (**B**) cell lines stimulated with 100 nM KP-10 for 0, 5, 10, 30, 45 and 60 min or with vehicle for 60 min. **C** and **D.** Composite graphs from three biological repeats showing the phospho-ERK1/2 expression in the BT-20 and MDA-MB-231 cell lines, respectively, relative to total-ERK. * p< 0.05, **<0.01 Student's t-test for comparison between 0 min and each time point.

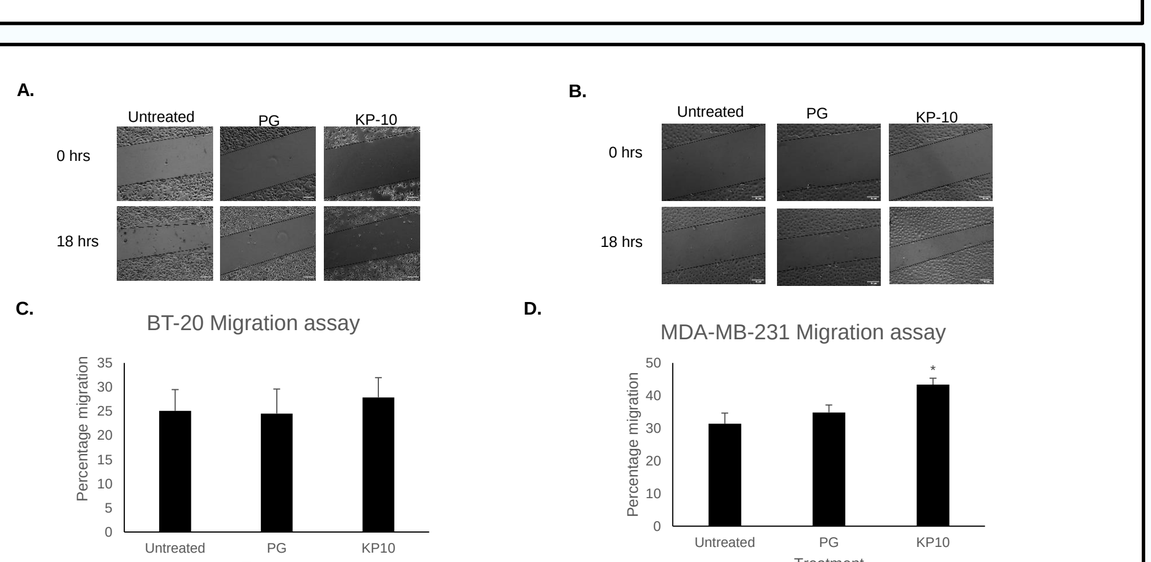

Figure 4: **Migration is increased in the MDA-MB-231 cell line stimulated with kisspeptin, but not the BT-20 cell line**. BT-20 (**A**) or MDA-MB-231 (**B**) cell lines were grown to confluence and a scratch created. The cells were then treated with either untreated or treated with 0.002% propylene (PG), or 100 nM KP-10. Images of the scratches were taken upon creation (0 hrs) and 18 hrs later using a Zeiss Axiovert microscope. **C** and **D.** Composite graphs showing the percentage increase in migration of BT-20 and MDA-MB-231 cells, respectively *p<0.05 Student's t-test for comparison between untreated and KP-10 treatment.

DISCUSSION AND CONCLUSIONS

The breast cancer cell lines, BT-20 and MDA-MB-231, which are both triple negative breast but non-metastatic and metastatic, respectively, express endogenous KISS1R. However, the BT-20 cell line had a much higher level of expression and KP-10 was able to activate ERK1/2 phosphorylation in this cell line but not the MDA-MB-321 cells. The late phase of ERK1/2 phosphorylation in the BT-20 cells is dependent on beta-arrestin1/2, as shown by decrease in ERK1/2 phosphorylation in the cells co-treated with Barbadin and KP-10. Finally, even though the MDA-MB-231 cell line was unable to activate ERK1/2 phosphorylation, there was an increase in the migration of MDA-MB-231 cells (but not BT-20 cells) when stimulated with KP-10. This data suggests that there could potentially be another signalling pathway in the MDA-MB-231 cells that is responsible for the increase in migration as shown by the decrease in the gap of KP-10 treated cells compared to the untreated cells.

Our previous data showed that the decrease in the gap is not due to cell growth.