Blocking Amphiregulin in the presence of WNT5B can block the Luminal and HER2 Breast Cancer Cell lines Migration

Sarah Alkabbani[1], Rachid Kaddoura[1], Masa Al Ashkar[1], Murtadha Resen[1], Fatma Alqutami[1], Mahmood Al Mashhadani[1]

[1] College of Medicine , Mohammed Bin Rashid University Of Medicine and Health Sciences

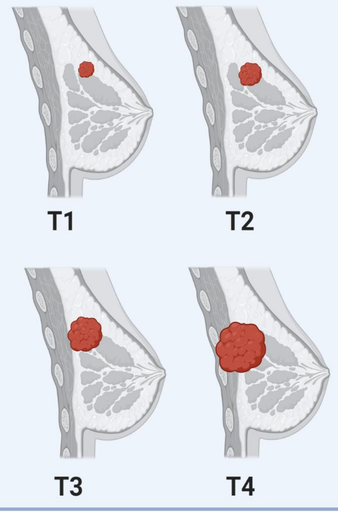
Introduction

Breast Cancer

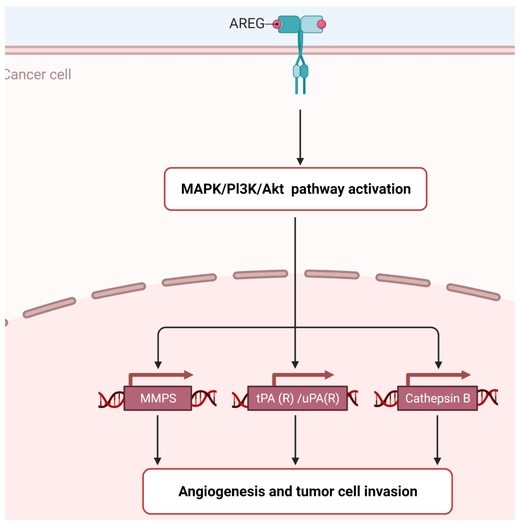
Is the most common cancer among women

1.67 million women diagnosed each year

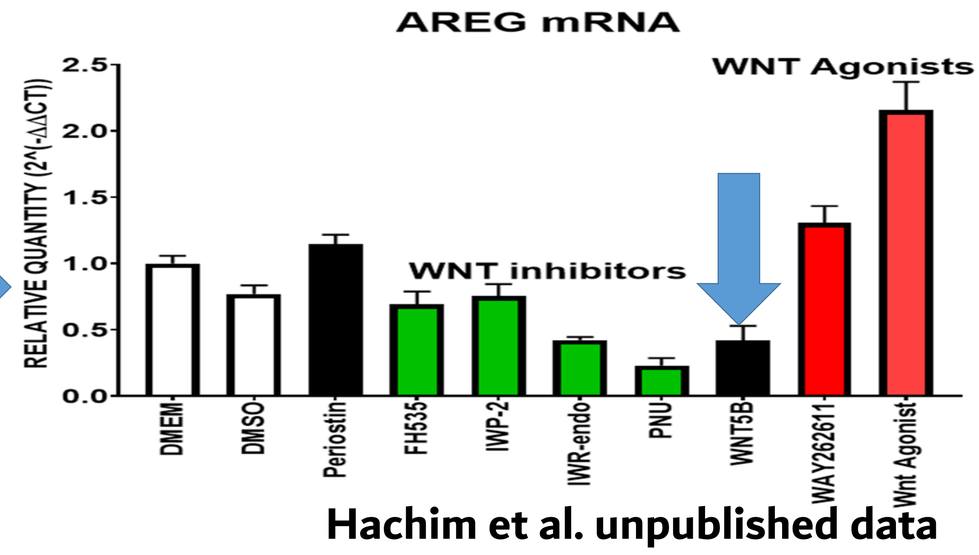
Incidence of breast cancer would inflate to 85 per 100000 women by 2021



Amphiregulin (AREG)



WNT5B Blocks AREG



Hachim et al. unpublished data

Figure 1. The Relationship between breast cancer, Amphiregulin (AREG) and WNT5B.

Objectives and Design

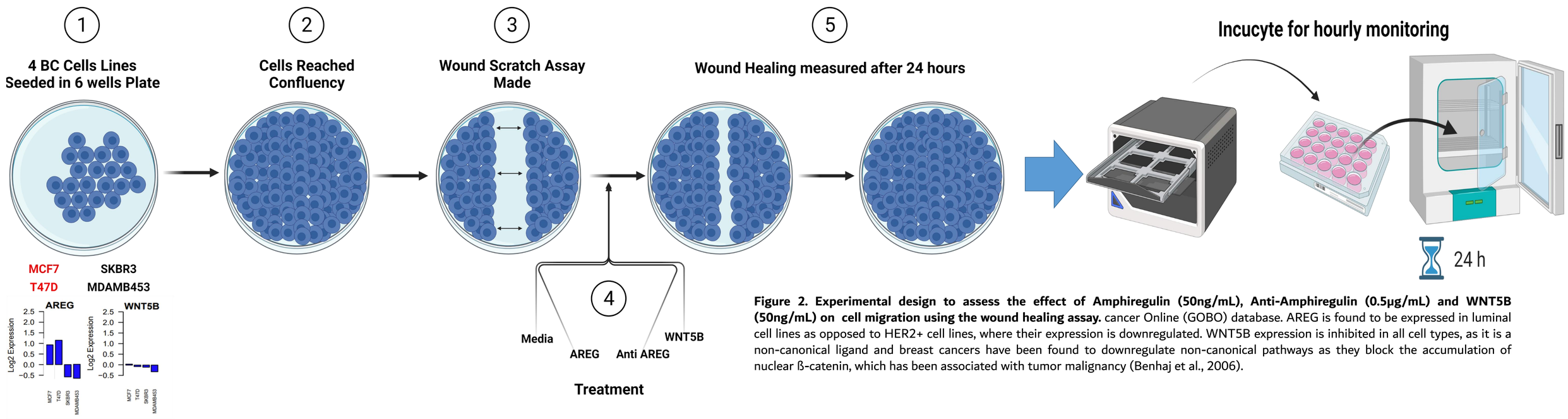


Figure 2. Experimental design to assess the effect of Amphiregulin (50ng/mL), Anti-Amphiregulin (0.5µg/mL) and WNT5B (50ng/mL) on cell migration using the wound healing assay. cancer Online (GOBO) database. AREG is found to be expressed in luminal cell lines as opposed to HER2+ cell lines, where their expression is downregulated. WNT5B expression is inhibited in all cell types, as it is a non-canonical ligand and breast cancers have been found to downregulate non-canonical pathways as they block the accumulation of nuclear ß-catenin, which has been associated with tumor malignancy (Benhaj et al., 2006).

Benhaj, K., Akcali, K. C., & Ozturk, M. (2006). Redundant expression of canonical Wnt ligands in human breast cancer cell lines. Oncol Rep, 15(3), 701-707. https://www.ncbi.nlm.nih.gov/pubmed/16465433

Results

Figure 3

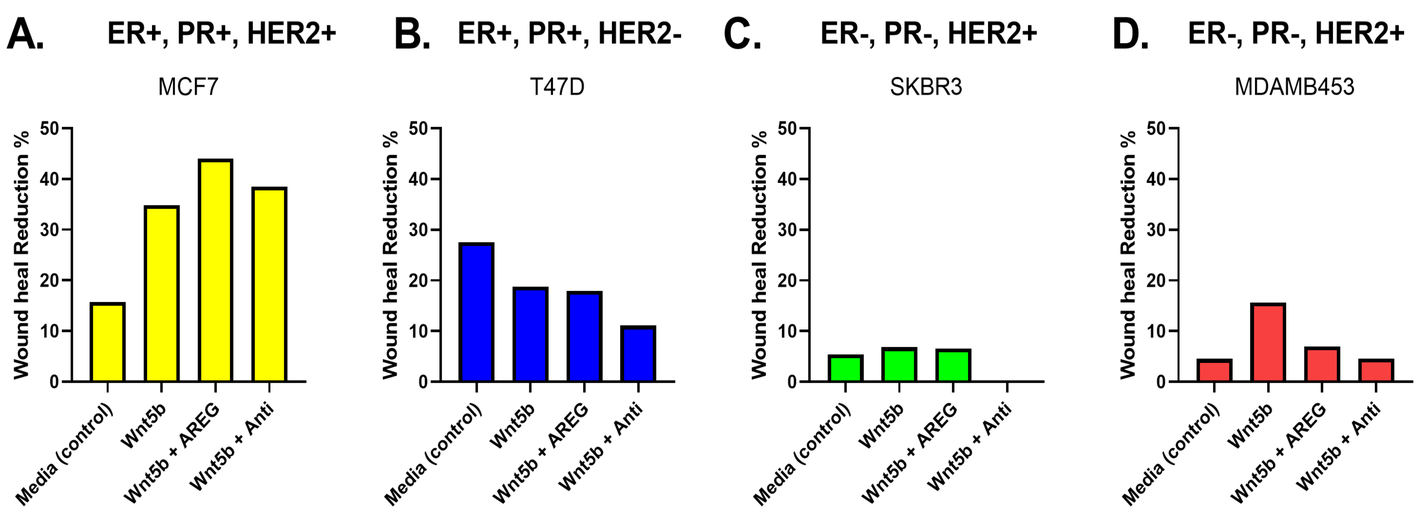


Figure 3: The effect of Amphiregulin (AREG), Anti-Amphiregulin (ANTI) and WNT5B on the migration of the cell lines MCF7, T47D, SKBR3 and MDAMB453 in a wound healing assay using a 24-well plate. (A) The addition of WNT5B with and without AREG increased cell migration in MCF7 cells, but the blocking of AREG with ANTI reduced some of the effect of endogenous AREG. (B) T47D cells reacted negatively to the addition of WNT5B, with the combination of WNT5B and ANTI causing the least amount of wound closure. (C) The addition of WNT5B did not induce an affect different from the control in SKBR3, but the combination with ANTI halted cell migration as there was no change in the wound size. (D) MDAMB453 cells were positively stimulated with the addition of WNT5B, but the addition of either AREG or ANTI has arrested cell migration as observed with the lower percentage in wound size reduction.

Figure 4. Further analysis of the effect of Amphiregulin (AREG), Anti-Amphiregulin (ANTI) and WNT5B on the migration of MCF7 cells in a 96-well plate observed at hourly intervals for 25 hours using the Incucyte® Live-Cell Analysis Systems revealed that AREG is important for wound healing and cell migration. The addition of AREG (Red) on it's own did not effect the migration of the cells, however inhibiting the endogenous AREG with Anti-AREG (Green) resulted in reduction in wound closure. WNT5B (Purple) on it's own or with the addition of Anti-AREG (Black) resulted in a decrease of cell migration, this is observed where both WNT5B and WNT5B + Anti-AREG had the lowest percentage of wound size reduction. When combining both WNT5B with AREG (Orange), the AREG rescued the cells from the inhibition caused by WNT5B. Additionally, most of the effect caused by WNT5B and Anti-AREG was observed during the first 4 hours, after, which their inhibiting effect has been reduced. These findings indicate that blocking AREG reduces cell migration and that WNT5B work opposite of AREG.

Figure 5. The migration rate was calculated, and a one-way ANOVA statistical test was performed for the identification of the degree of significance between conditions. Compared to the control, AREG and WNT+AREG showed no statistical significance (p-value > 0.05), as opposed to Anti-AREG ($P \leq 0.01$), WNT5B and WNT5B + Anti-AREG ($P \leq 0.0001$). Whereas comparing AREG to Anti-AREG ($P \leq 0.001$), WNT5B and WNT5B + Anti-AREG ($P \leq 0.0001$), there was a reduction in the migration rate . There was a significant difference on the migration rate between Anti-AREG and WNT5B ($P \leq 0.01$), WNT5b+AREG ($P \leq 0.001$), and WNT5b + Anti-AREG ($P \leq 0.01$). Whereas WNT5B showed significant difference when compared to WNT5b+AREG ($P \leq 0.0001$). These results indicate that WNT5B and Anti-Amphiregulin work against AREG, blocking and reducing cell migration.

Figure 4

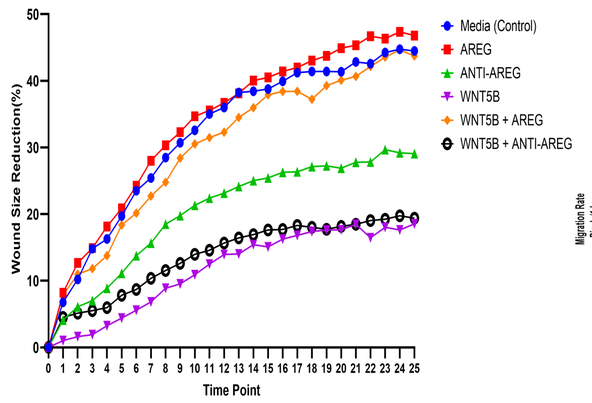


Figure 5