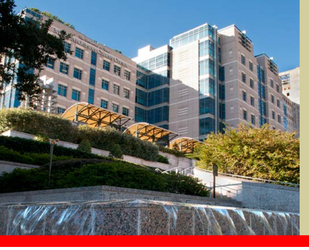

Targeting Low Molecular Weight Cyclin E in ER-positive Breast Cancer

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Background

Cyclin E is an important cell cycle regulator controlling the G1/S checkpoint. In many cancer types, cyclin E overexpression causes dysregulation of the G1/S checkpoint and cell cycle progression[1,2]. Low molecular weight cyclin E (LMW-E) has been associated with breast cancer tumorigenesis, genomic instability, and resistance to aromatase inhibitors[3,4]. No approved therapeutic agents exist to target ER-positive breast cancer patients that overexpress LMW-E[5]. The purpose of this research is to **1)** study the underlying mechanism in which ER-positive breast cancer cells overexpressing LMW-E become resistant to endocrine therapy and **2)** test the efficacy of various cell cycle inhibitors on full length (FL) and LMW-E expressing ER-positive breast cancer cell growth.

Hypothesis

We hypothesize that cell cycle inhibitory drugs affect FL and LMW-E expressing cells differentially and are dependent on the presence of estrogen (E2).

Methods

Cell lines

MCF7 cell lines were used in this study as a model for ER-positive breast cancer. Cells were stably transfected to express full length cyclin E (EL), LMW-E (T1), or empty vector (4.0) and were grown in the presence and absence of estrogen for different lengths of time. MDA-MB-231 cells were used as a model for triple negative breast cancer (TNBC). These cells were used as a negative control for MCF7 ER-positive breast cancer cells.

Dose response assays

MCF7 cells expressing 4.0, EL, and T1 were cultured in 96-well plates for 14 days with various cell cycle drugs in IMEM cell media without E2 or with 1 nM E2. Media and drug were refreshed every other day before cell confluency was measured using an IncuCyte ©. After 14 days, cell confluency was measured using a crystal violet stain.

Immunoblot assays

1x10^6 cells were sonicated and centrifuged and protein concentration was measured via BSA assay. Samples were run on SDS-PAGE gels and then transferred to nitrocellulose membranes overnight at 4℃. The membranes were blocked for in 5% BSA and incubated with indicated primary antibody overnight at 4℃. Membranes were then incubated with anti-mouse or anti-rabbit secondary antibody. Films were developed using the Renaissance chemiluminescence system.

Results

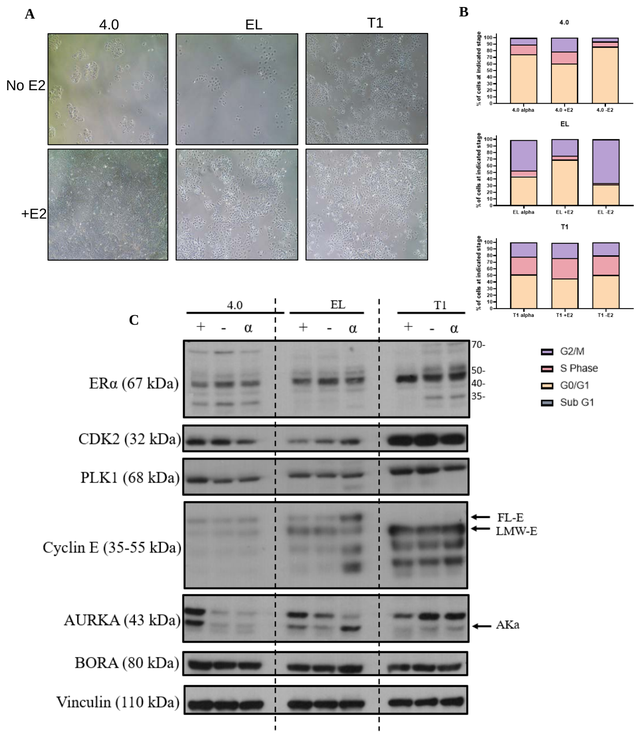

Figure 1. **(A)** Cell images of 4.0, EL, and T1 MCF7 cells cultured in the presence or absence of E2 for 13 days. **(B)** Cell cycle flow cytometry of 4.0, EL, and T1 MCF7 cells grown in the presence and absence of 1 nM E2 for 48 hours. Cell cycle phase was quantified using FlowJo and analyzed with GraphPad Prism. **(C)** Western blot analysis of 4.0, EL, and T1 MCF7 cells grown in the presence and absence of 1 nM E2 for 24 hours. Vinculin was used as a positive loading control.

Effect of E2 deprivation on cell growth

Differences in cell growth, cell cycle status, and protein expression were observed for 4.0 and EL cells grown in E2 media compared to E2-deprived media (Fig. 1). These differences were seen at one week (Fig. 1A), 48 hours (Fig. 1B) and 24 hours of E2 deprivation (Fig. 1C). No significant differences were observed in T1 cells.

Results (continued)

Dose response assay of various cell cycle inhibitors

Treatment of MCF7 cells with 4 novel CDK inhibitors showed no specificity for T1 cells in either E2 media or E2-deprived conditions (**Fig. 2**). An inhibitor of CDK1/CDK2 was found to specifically target T1 cells in E2 media. Drug specificity was lost in E2 deprived media (**Fig. 3**). Overall, data shows altered specificity to T1 over EL and 4.0 amongst the various tested CDK inhibitors.

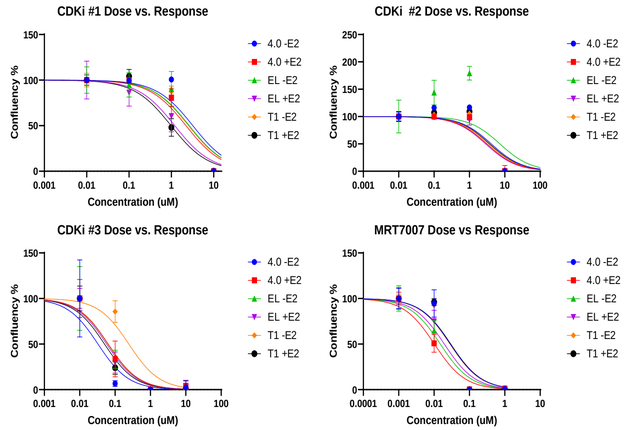

Figure 2. MCF7 cells expressing FL-E, LMW-E, or empty vector were grown for 14 days with various CDK inhibitors in the presence and absence of 1 nM E2.

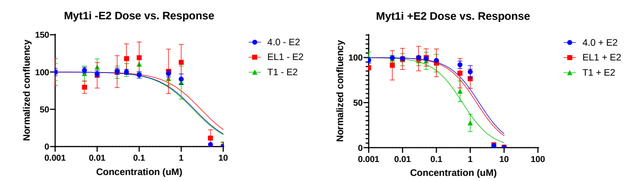

Figure 3. MCF7 cells expressing FL-E, LMW-E, or empty vector were treated for 20 days with a CDK1/CDK2 inhibitor in the presence and absence of 1 nM E2.

A potent PLK1 inhibitor, Volasertib, has recently been shown in our lab to inhibit 4.0, EL and T1 cells growth at 1nM. Western blot analysis of cells treated for 48hr with Volasertib at 1nM revealed differential patterns of ERα and Cyclin E expression (**Fig. 4).**

Results (continued)

Despite the changes in cell growth, T1 exhibited no changes in protein expression

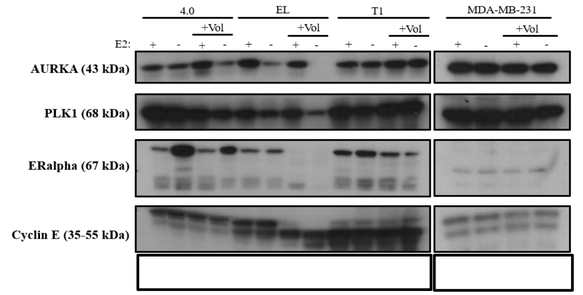

Figure 4. Western blot analysis of 4.0, EL, and T1 MCF7 cells grown in the presence and absence of 1 nM PLK1i (Vol) and E2 for 48 hours. ERα, PLK1, Cyclin E (FL-E and LMW-E), and Aurora Kinase A were measured.

Discussion

The data suggest a unique mechanism that differentiates cells that express FL-E in E2 containing and E2-deprived media. CDK inhibitors fail to specifically inhibit growth of T1-expressing MCF7 cells in both E2 media and E2-deprived media. However, a CDK1/CDK2 inhibitor specifically inhibits T1-expressing MCF7 cells in E2-media. Efficacy of this drug in E2 media, alongside fundamental changes shown between E2-deprivation and E2-growth suggests that combination with endocrine therapy may be most effective to inhibit growth of these cells. Further studies are needed to solidify the mechanism in which cell cycle proteins such as PLK1, AURKA, and ERα are regulated in different estrogen conditions.

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