The efficacy of RJ-22, RJ-23, and AT-13 against the Y537S mutation of ERα - positive breast cancer cells

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Introduction

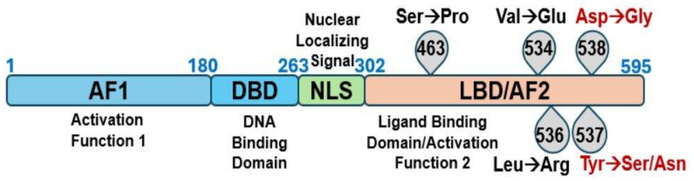
Estrogen Receptor alpha (ERα) is expressed in 70% of breast cancers. ERa binds to estrogen and is the trigger for growth in the tumor. ERa cancer cells are treated with endocrine therapies. Endocrine therapies include selective estrogen receptor modulators (SERMs), selective estrogen degraders (SERDs), and aromatase inhibitors (AIs). These treatments prevent the binding of the estrogen receptor and estrogen. Although these treatments are effective, in approximately 20% of cases, the mutations in *ESR1* gene is seen that renders endocrine therapy ineffective. This is due to the mutation in ERα no longer requiring the binding of estrogen for its activity. Y537S is the most common mutation accounting for about 60% of mutated cases.

Previously in this lab, compounds RJ-22, RJ-23, and AT-13 showed efficacy in reducing the proliferation of wild type MCF7 (Michigan Cancer Foundation) by decreasing transcription of ERα gene *ESR1*. Our hypothesis is that these drugs can be applied to the ERα mutated cell for a similar decrease in the proliferation of MCF7-Y537S cells. The effect of the compounds on levels of target proteins and other associated proteins involved in *ESR1* transcription is explored and the results presented here.

Methods

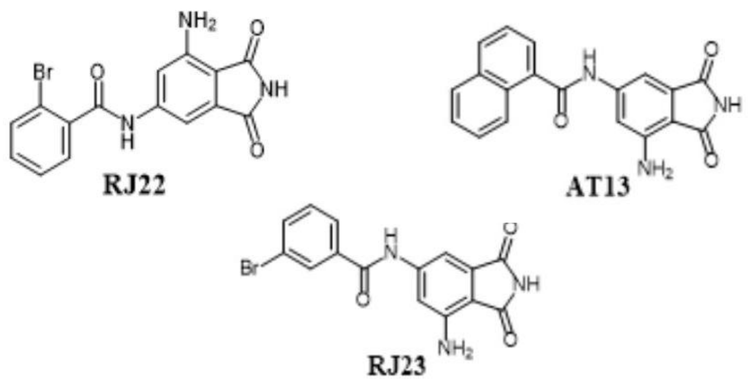
The MCF7-Y537S cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS and Gentamycin. For Compounds treatment, cells were hormone depleted for 3 days with 5% charcoal-depleted FBS before compounds or DMSO vehicle control treatment for 24 hours. Western blot analysis was done in a 10% acrylamide concentration gel. For colony forming assay, cells grown in charcoal depleted medium were seeded onto 6-well plates (500 cells/well) for treatment with vehicle control and different concentrations of compounds for 2 weeks by changing the medium every three days.

Results

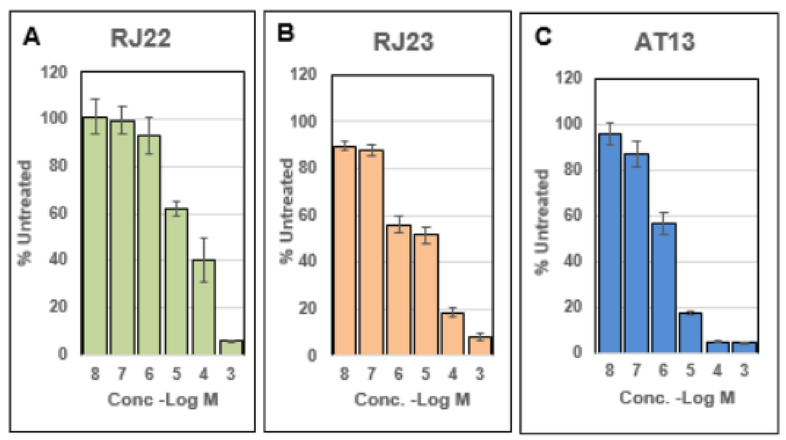


ERα domain structure illustrating the identified mutations in the LBD. Prevalent mutations are colored red.

Lead Compounds Structure

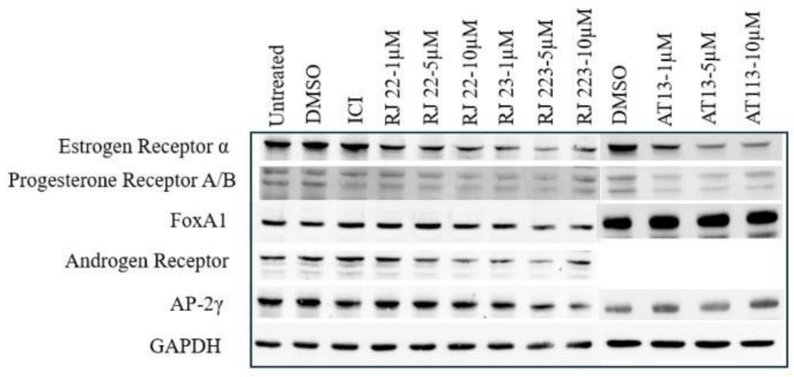


Proliferation Assay



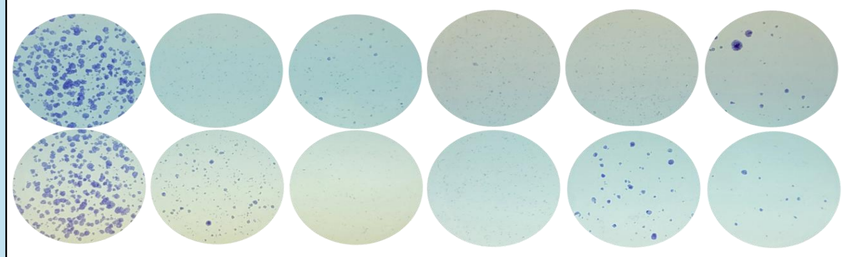
MCF7-Y537S cells treated with varying concentrations of (A) RJ22, (B) RJ23, and (C) AT13. The experiments were run in quadruplicates, repeated thrice and shown as mean ± S.D.

Western Blot



Western blot of MCF7-Y537S cells treated with 1, 5 and 10 μM concentrations of RJ22, RJ23, and AT13, ICI (10^{-7}M) for 24 hrs and probed with ERα66 (SC-787, Santa Cruz Biotech), and Progesterone Receptor A/B (8757s, Cell Signaling), Androgen receptor (AR, Cell Signaling), AP2gamma (AP2γ, sc-12762, Santa Cruz), GAPDH (2118, Cell Signaling) and FoxA1 (ab23738, ABCAM) antibodies

Colony Forming Assay



Top row left to right: untreated, ICI (10^{-7}M), RJ-22 (5μM), RJ-23 (1μM), RJ-23 (10μM), AT-13 (5μM)
Bottom row left to right : DMSO, RJ-22 (1μM), RJ-22 (10μM), RJ-23 (5μM), AT-13 (1μM), AT-13 (10μM)

Results and Discussion

The efficacy of the compounds was assessed on MCF7 cells bearing CRISPR-mediated mutations at the *ESR1* locus, resulting in MCF7 Y537S. Derivative cell lines were exposed to multiple concentrations of the compounds RJ22, RJ23 and AT13, resulting in dose responsive growth inhibition. Additionally, the compounds showed enhanced efficacy in combination with the clinical therapeutic ICI. The colony forming assay also exhibited the decrease in cell colonies in RJ22, RJ23 and AT13 treated cells. The ERα levels were markedly decreased for the cells treated with the compounds RJ22, RJ23 and AT13, similar to our observation in parental MCF7 cells as ascertained by western blot. Similarly, disruption of ERα target gene expression was observed, with abrogation of Progesterone Receptor A/B (8757s, Cell Signaling), Androgen receptor (AR, Cell Signaling), AP2gamma.

Conclusion

Our data and results show that RJ-22, RJ-23, and AT-13 decrease the proliferation of MCF-7-Y537S cells. Currently, there is no drug like this on the market and this mutation is very difficult to treat. However, these drugs are potential therapeutics for treating Y537S mutation in breast cancer.

References

Harrod, A.; Lai, C. F.; et al. , Genome engineering for estrogen receptor mutations reveals differential responses to anti-estrogens and new prognostic gene signatures for breast cancer. Oncogene 2022, 41 (44), 4905-4915.

Acknowledgements

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