Efficacy of LHRHa treatment in luminal type breast cancer and in vivo estrogen-independent breast cancer cell lines

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Background

Hormone receptor-positive (ER+/PR+), HER2-negative (luminal type) breast cancer accounts for 75.6% of all cases. For premenopausal patients, luteinizing hormone-releasing hormone agonists (LHRHa) suppress ovarian estrogen and progesterone production and are often combined with antiestrogen therapies like tamoxifen or aromatase inhibitors. Clinical trials, such as SOFT and TEXT, demonstrate that LHRHa improves outcomes. However, challenges include side effects (e.g., menopausal symptoms, bone density loss) and identifying which patients benefit most. It is important to personalize treatment strategies to optimize LHRHa use.

Moreover, we performed orthotopic injection of MCF7 cells into bilaterally ovariectomized mice (estrogen depleted). After 2-4 months of observation in the estrogen-depleted state, multiple in vivo-estrogen-independent breast cancer cell lines (iEIBCCs) were established (Figure 1). This may be a model that approximates luminal-type recurrent breast cancer under anti-estrogen therapy.

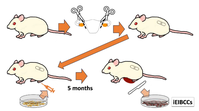


Figure 1. Establishment of iEIBCCs

Here, we investigated the effects of LHRHa combination therapy in luminal type breast cancer patients and iEIBCC cell lines.

Methods and Materials

The clinicopathological factors and prognosis of patients with Stage I-III Luminal breast cancer from 2016 to 2021 who were treated at Chiba University hospital were analyzed. We selected 152 analytic cohort patients and split them into two groups by treatment with or without LHRHa.

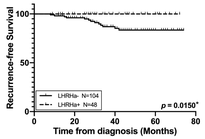
Two kinds of iEIBCCs, 1LR and E10, and parental MCF-7 cells were transplanted into ovariectomized SCID mice, LHRHa-treated mice, and wild-type mice.

Furthermore, we conducted RNA sequencing to define the differential gene expression between 1LR, E10, and parental MCF-7.

Results

- **Real-world data of LHRHa treatment for luminal breast cancer patients from 2016 to 2021**

The 5-year RFS of the patients treated with LHRHa (N=48) was statistically higher than without LHRHa (N=104) ($p = 0.0150$).



Kaplan–Meier survival curves were used to show the 5-year RFS rate of patients with or without LHRHa treatment.

Figure 2. Comparison of 5-year RFS rate for patients with or without LHRHa treatment

Results

- **In vitro results of iEIBCCs**

In vitro proliferation assay shows that iEIBCCs have different proliferation rates. 1LR grew the slowest, whereas E10 grew the fastest.

According to Western Blot, ER expression was low in 1LR but high in E10.

We hypothesize that 1LR and E10 are the most different type of iEIBCCs and transplanted them into ovariectomized SCID mice, LHRHa-treated mice, and wild-type mice.

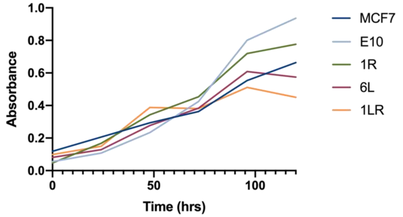


Figure 3. Proliferation of iEIBCCs.

In vitro proliferation assay of 4 types of iEIBCCs and parental MCF-7 was performed. Cell proliferation was evaluated every 24 hours.

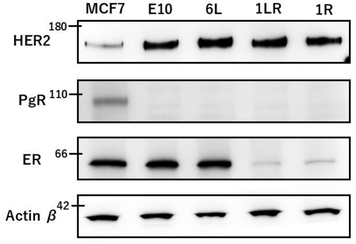


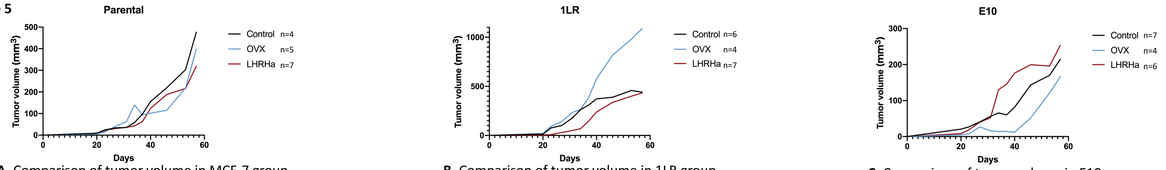
Figure 4. Western Blot of iEIBCCs.

The ER, PgR, and HER2 expression of 4 types of iEIBCCs and parental MCF-7 was evaluated by Western Blot. Actin β was used as the loading control.

- **The sensitivity to LHRHa treatment of mice transplanted 1LR, E10, and parental MCF-7**

In the parental MCF-7 group, compared to wild-type mice (N=4), the tumor growth was inhibited by both ovariectomize (N=5) and LHRHa-treated (N=7).
In 1LR group, the tumor growth was inhibited by LHRHa-treated (N=7) but was not inhibited by ovariectomized (N=4).
In E10 group, the tumor growth was inhibited by ovariectomized (N=4) but was not inhibited by LHRHa-treated (N=6).

Figure 5



A. Comparison of tumor volume in MCF-7 group.

B. Comparison of tumor volume in 1LR group.

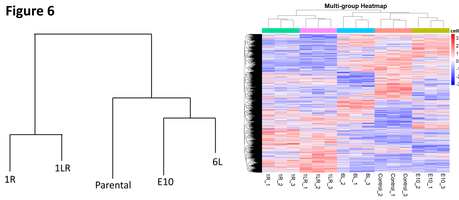
C. Comparison of tumor volume in E10 group.

Tumor volume of ovariectomized SCID mice, LHRHa-treated mice, and wild-type mice which were transplanted parental MCF-7 tumor cells (A), 1LR tumor cells (B), and E10 tumor cells (C) were evaluated for 1 month after tumor cell injection.

- **Parental and derivative cell line relationship and differential gene expression between 1R, 1LR, 6L, E10, and parental MCF-7 cell lines**

There are some differential gene expressions between the 1R, 1LR, 6L, E10, and parental MCF-7 cells.

Figure 6

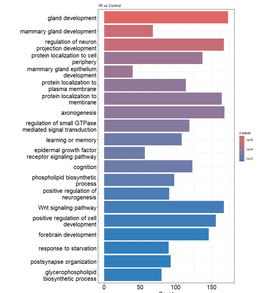


A. Hierarchical clustering of cell lines.

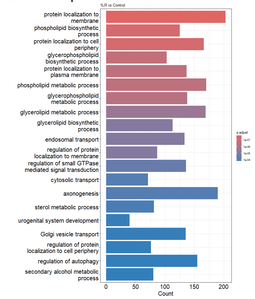
B. Heatmap of cell lines.

Hierarchical clustering based on rlog-transformed gene expression data.The branch length represents the degree of similarity in expression patterns among samples (A). Heatmap was used to show the different gene expression of 1R, 1LR, 6L, E10, and parental MCF-7 cell lines (B).

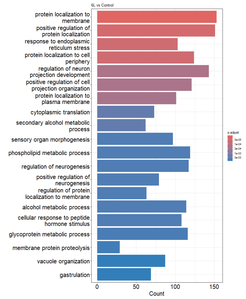
GO pathways analysis was used to show the different pathways enrichment between 1R and MCF-7(C), 1LR and MCF-7 (D), 6L and MCF-7 (E), E10 and MCF-7 (F), E10 and 1LR (G).



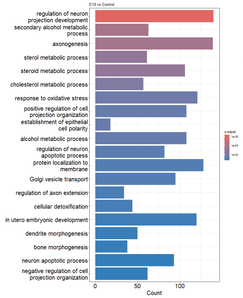
C. GO Pathways: 1R vs Parental.



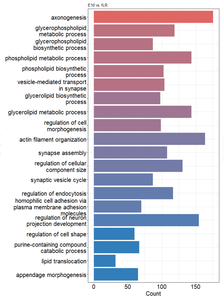
D. GO Pathways: 1LR vs Parental.



E. GO Pathways: 6L vs Parental.



F. GO Pathways: E10 vs Parental.



G. GO Pathways: E10 vs 1LR.

Conclusion

- LHRHa treatment is effective in patients with luminal type breast cancer.
- The differential gene expression between 1LR, E10 and parental MCF-7 cell and the resistance and sensitivity in 1LR, and E10 provide crucial context for interpreting how LHRHa impacts cancer cells.
- We hypothesize that differential signaling pathways contribute to this resistance and sensitivity.
These insights underscore the complexity of cancer cell responses to hormone depletion therapies like LHRHa. Understanding the specific adaptations that confer resistance or sensitivity in these cell lines can guide the development of combination therapies.

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