Membrane ERα modulates the properties of mammary stem cells

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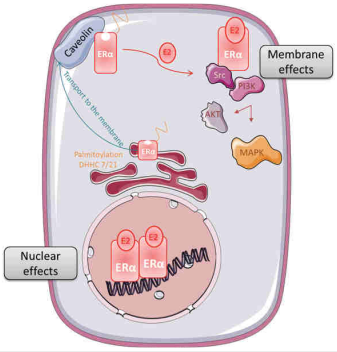
Abstract -

Although it is well established that the female sex hormones, such as 17β-estradiol, is a major regulator of breast development and have an important role in breast carcinogenesis, the mechanisms by which they exert their effects are not completely understood. Estradiol classically binds to Estrogen Receptor (ERα) that mediates nuclear actions via activation of gene transcription while a fraction of ERα expressed at the membrane elicits rapid signaling. Using a mouse with a mutation of the palmitoylation of ERα (C451A-ERα) given a specific membrane-specific loss of function of ERα, our goal is to understand how these membrane actions of ERα interact with developmental signaling pathways in the breast to control growth and differentiation.

On C451A-ERα mice, we observed a complete fat pad filling with a decrease of the secondary branching. To assess whether this defect is due to defect on either the mammary gland epithelium or stroma or even to the disturbance of hormonal levels in the mouse, we grafted C451A-ERα/GFP mammary ductal epithelium on WT mice. Surprisingly, we showed a total absence of development of the mutant mammary gland. Quantification of mammary luminal and myoepithelial cells by flow cytometry using specific markers indicate that there is an increase of luminal cells but a reduction of myoepithelial cells on intact 3-months old C451A mice. Moreover, transplantation of an equal number of myoepithelial cells ($CD29^{high}$, $CD24^{low}$) isolated from intact C451-ERα mice into the cleared inguinal glands of 3 weeks old C57BL/6 showed that this population of myoepithelial cells contains a lower frequency of basal stem cells that can repopulate the total fat pad.

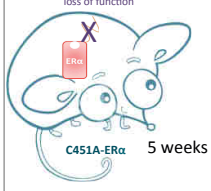
Meanwhile, we conducted the same experiment with mice supplemented with estradiol and progesterone.

These data indicate that membrane ERα controls the properties of mammary stem cells, affecting development of mammary gland development.

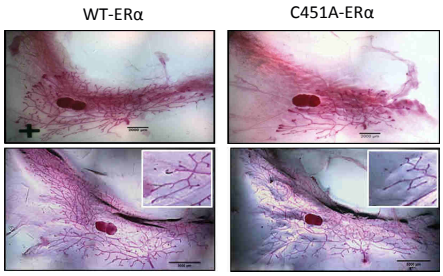


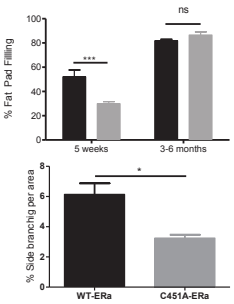
1. C451A-ERα mutation alters mammary gland development in 3-months-old mice

Mice model of Membrane ERα loss of function

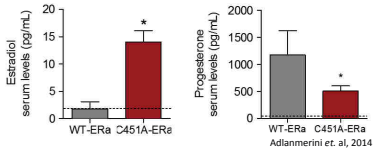


A. Whole-mounts of mammary glands



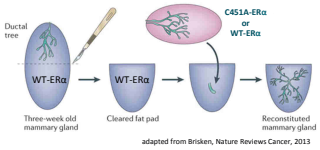


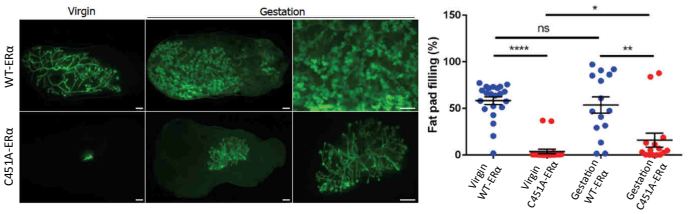
B. Circulating hormones levels



- At 5 weeks, there is a delay in mammary epithelium development.
- At 6 months, mammary fat pad filling of the C451A-ERα mice was fully developed.
- The ductal side branching is largely reduced in C451A-ERα, probably due to alteration of circulating progesterone levels.

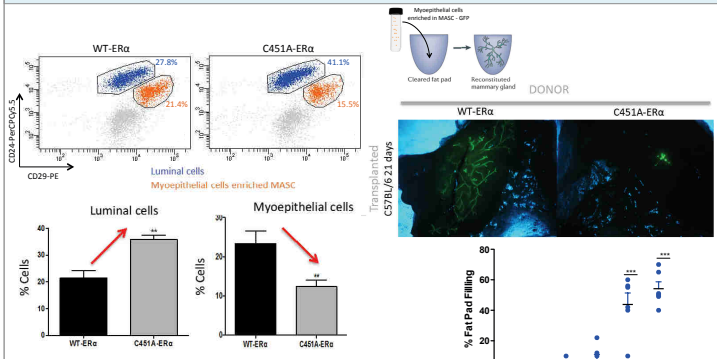
2. The membrane ERα in the epithelium is required for mammary ductal elongation





- In virgin mice, almost any of mutant grafts showed ductal outgrowth.
- Pregnant recipients showed a very limited development (10% in average) and absence of development of alveolar lobules.

3. C451A-ERα mutation changes the profiles of mammary cell epithelial populations and their capacities



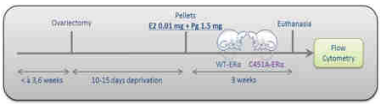
In the C451A-ERα 3 months-old mice :

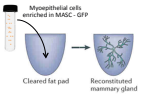
- The number of luminal cells ($CD29^{low}CD24^{+}$) is increased.
- The number of myoepithelial cells enriched in mammary stem cells (MASC- $CD29^{high}CD24^{+}$) is decreased.
- When we transplanted in equal numbers the C451A-ERα and WT-ERα myoepithelial cells enriched in MASC, the repopulating ability is strongly altered by the loss of membrane ERα .

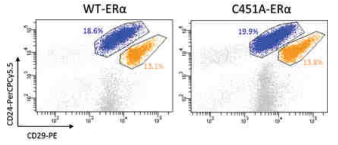
Number of transplanted cells	WT-ERα	C451-ERα
50	0/7	0/7
150	0/7	0/7
300	1/6	0/6
500	3/6	0/6
1000	6/6	0/6
2000	6/6	2/6
MASC frequency	1/682	1/11070

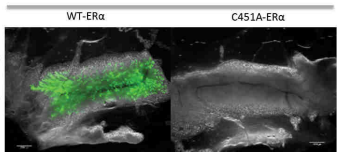
x16 ***

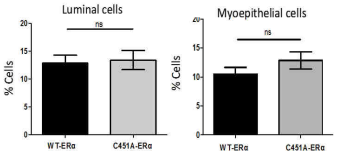
4. Estradiol and Progesterone treatment restores C451A-ERα mammary cells balance but not transplantation

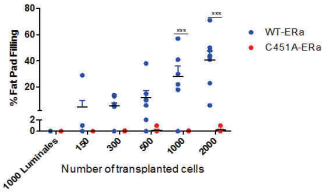












- The hormonal co-treatment restores the proportion of luminal cells and myoepithelial cells enriched in MASC in the C451A-ERα.
- When we transplanted in equal numbers the myoepithelial cells enriched in MASC, the repopulating ability remains altered in the C451A-ERα mice.

Number of transplanted cells	WT-ERα	C451-ERα
150	2/6	0/6
300	4/7	1/7
500	5/6	1/6
1000	5/6	1/6
2000	8/8	2/6
MASC Frequency	1/380	1/4183

x11 ***

Conclusion

- Physiological mammary gland development is altered due to alteration of the circulating hormone levels.
- The transplantation of C451A-ERα ducts into WT mice abolished mammary gland development.
- Membrane ERα regulated the balance of epithelial cell populations, altering the number of cells enriched in stem cells and modifying their repopulating abilities.
- The estradiol and progesterone treatment restores the balance between luminal and myoepithelial cells.
  But it is not sufficient to restore the C451A-ERα mammary stem cells properties.

Our perspectives are now to characterize the membrane ERα signalling pathways involved in invasion and migration of these mammary cells.

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