Beneficial effect of estrogen on lymphatic system is inhibited by hormone therapy to promote lymphedema.

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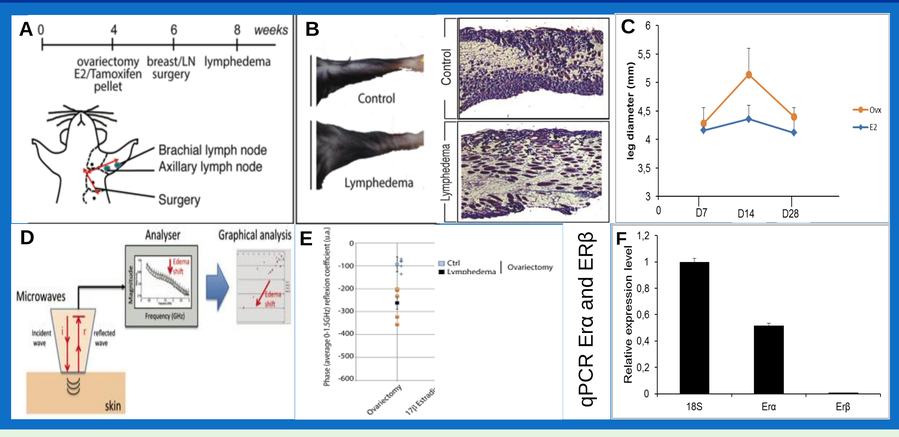
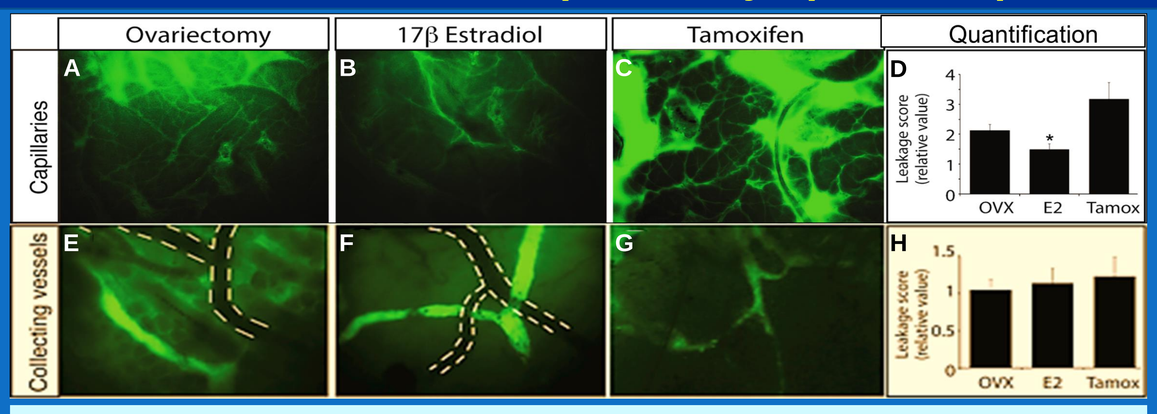
More than 10 percent of breast cancer survivors develop secondary lymphedema within weeks and months after surgery and radiotherapy. Lymphedema refers to a condition of lymphatic dysfunction, which results in a massive fluid and fat accumulation. Although it is a common disabling disease, treatment for this chronic pathology remains limited and largely ineffective. Here, we developed a new model of mice secondary lymphedema consisting in a mastectomy associated to axillary and brachial lymph nodes dissection. We observed lymphedema formation after 2 weeks associated with a massive dermal lymphatic leakage and lipid accumulation. To study the effect of estrogen on lymphatic endothelium, mice were ovariectomized and treated with constant delivery of 17bEstradiol (80mg/kg/d). Surprisingly, we found that estradiol protects from edema and restore lymphatic flow. Estradiol insures a functional lymphatic network by promoting hyaluronan synthesis in the skin in vivo and inducing lymphatic endothelial cells migration, tubulogenesis and filopodia formation in vitro. We found that the effect is dependent of the nuclear receptor ERalpha, but not beta. To evaluate the effect of hormone therapy on lymphedema, mice were treated with tamoxifen, an estrogen receptor antagonist and the major therapy for breast cancer. We found that the protective effect of estradiol on lymphatic endothelium is abolished in tamoxifen-constant delivery treatment, but is not affected by bolus injections. We observed a massive disruption of the lymphatic network associated with an inhibition of the lipid drainage function. More importantly, we found that this deleterious effect is associated with a modification of the extracellular matrix content and the lymphatic endothelial cell adhesion and filopodia formation. In conclusion, this study demonstrates for the first time the beneficial effect of estradiol on lymphatic endothelium. We showed that the hormone therapy abolished this effect by disrupting the lymphatic network and modifying the microenvironment. Our work should thus bring new insights for a better understanding on the lymphedema prevalence and constitute a first step to the treatment of secondary lymphedema.

1/ Estradiol protects against secondary lymphedema. This effect is dependent of Erα



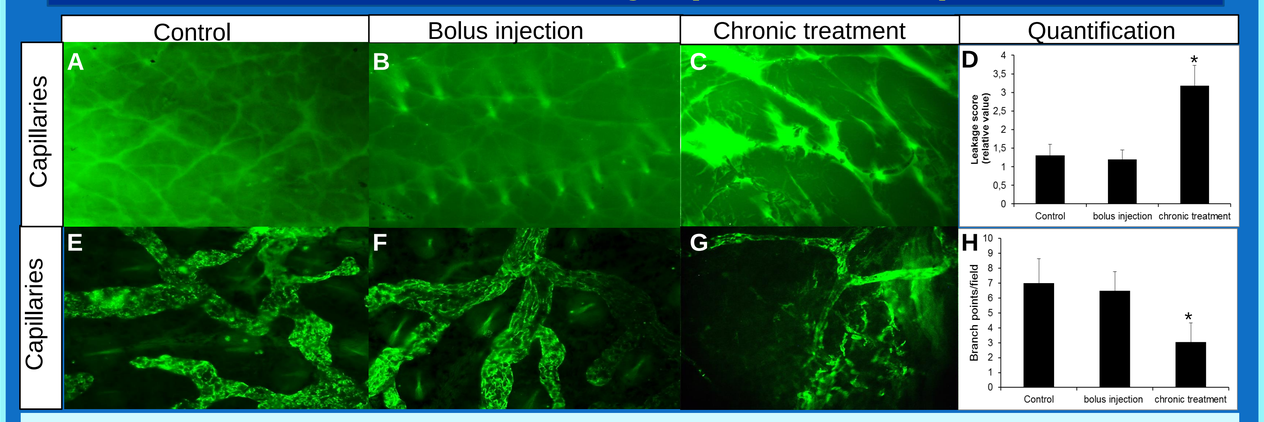
To induce secondary lymphedema, we designed a mice model in which we performed a skin flap surgery associated with axillary and brachial lymph nodes dissection [A] . Two weeks after surgery we observed an increase of leg size [B] reduced by a treatment with 17β Estradiol (E2) [C]. In our model we observe also an increase in interstitial fluids after the surgery (D,E). By qPCR analysis we also observed that only Erα is expressed in lympatic cells (HDLEC) [F].

2/ Protective effect of estradiol is reversed by a tamoxifen-induced disruption of lymphatic capillaries



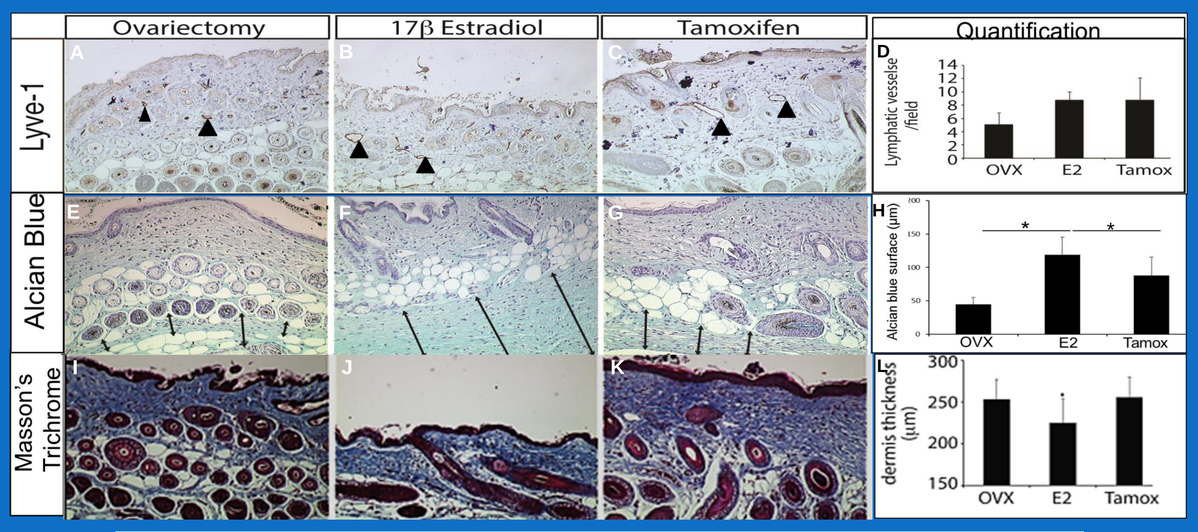
Tamoxifen inhibits drainage of extra-tissular fluids by disrupting the lymphatic cappilaries [A,D] but not collecting vessels [E,H].

3/ Chronic treatment but not bolus injection of tamoxifen induces lymphatic disruption



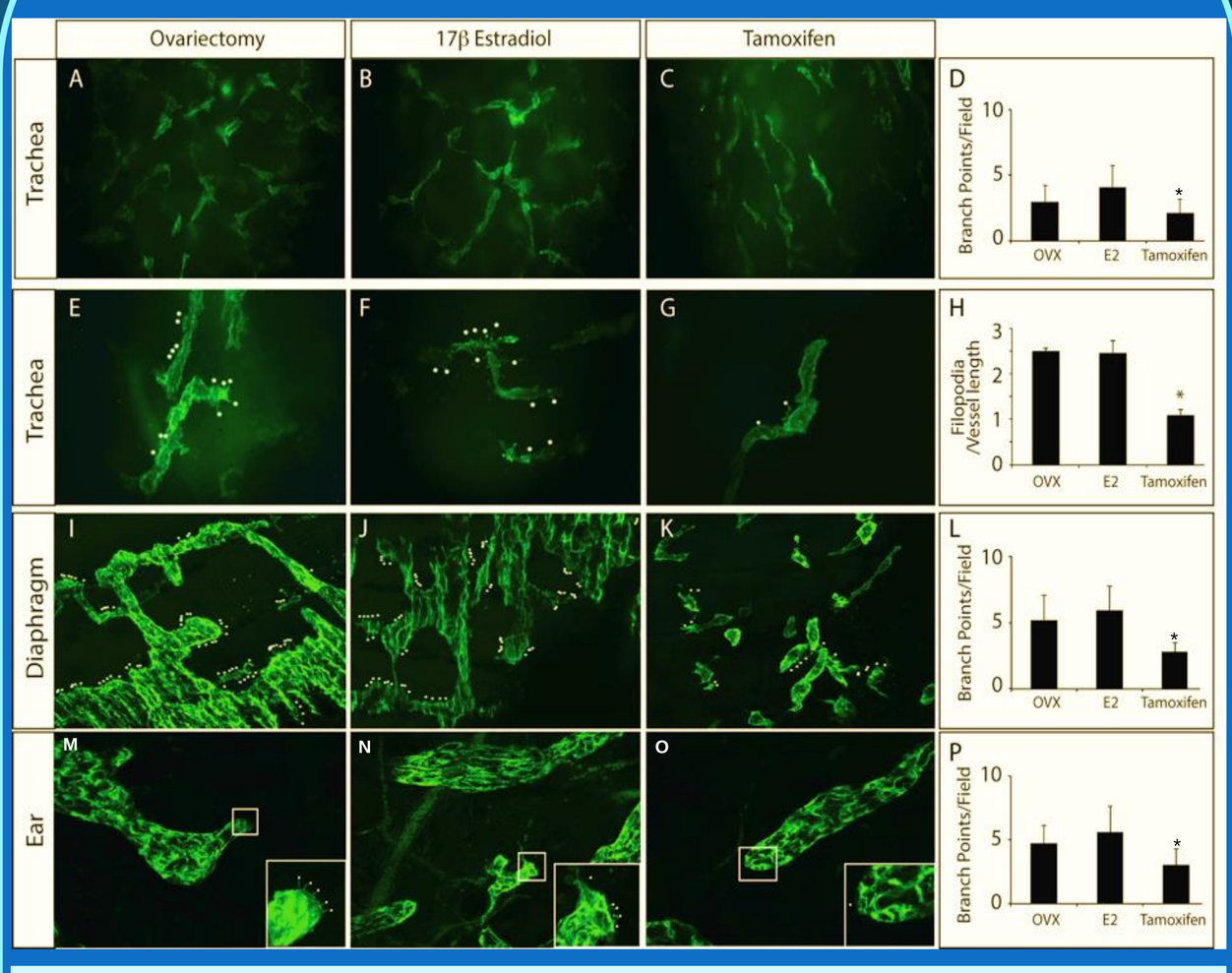
Bolus IP injections of tamoxifen have no effect on lymphatic network compared to a chronic treatment [A,H]

4/ Tamoxifen induces extracellular matrix remodeling not lymphangiogenesis



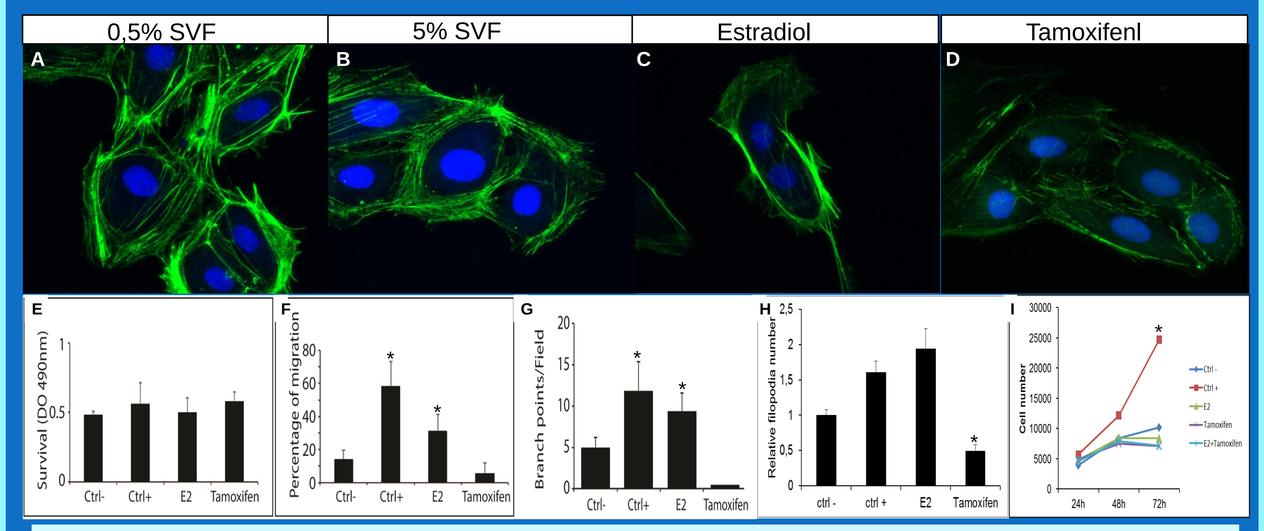
Neither estradiol nor tamoxifen induce lymphangiogenesis [A,D] but tamoxifen decreases estradiol-induced hyaluronan synthesis [E,H] and also increases skin thickness and fibrosis [I,L]

4/ Tamoxifen disrupts lymphatic network by inhibiting filopodia formation *in vivo*



Whole-mount LYVE-1 staining shows lymphatic disruption in tamoxifen-treated mice trachea [A-G] and diaphragm [I-K], two organs highly vascularised by lymphatics. Tamoxifen inhibits the formation of branch points and filopodia [D-L] and disorganizes the structure of the lymphatic network.

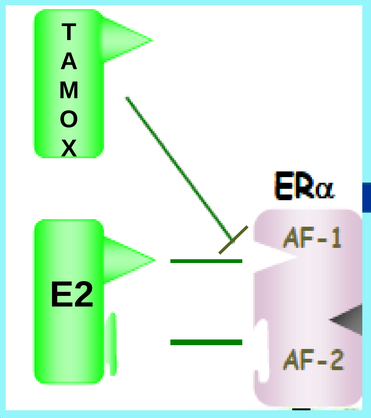
5/ Tamoxifen inhibits filopodia formation *in vitro* and reversed estradiol-induced migration and tubulogenesis



In vitro, tamoxifen inhibits filopodia formation [A-D,H] and estradiol-induced tubulogenesis and migration [F,G] but has no effect on survival and proliferation [E,I].

Conclusion

Tamoxifen : A risk factor for lymphedema



Lymphatic dysfunction → **Secondary Lymphedema**

→ **A public health challenge**