Intraductal xenografts show lobular carcinoma cells rely on their own extracellular matrix and LOXL1

George Sflomos[1], Laura Battista[1], Patrick Aouad[1], Fabio De Martino[1], Valentina Scabia[1], Athina Stravodimou[2], Ayyakkannu Ayyanan[1], Assia Ifticene-Treboux[2], RLS[3], Philipp Bucher[1], Maryse Fiche[3,4], Giovanna Ambrosini[1] & Cathrin Brisken[1]

https://www.epfl.ch/labs/brisken-lab/ - **Name of the presenter**: George SFLOMOS (georgios.sflomos@epfl.ch)

Overview - Abstract

Invasive lobular carcinoma (ILC) is the most frequent special histological subtype of breast cancer, typically characterized by loss of E-cadherin. It has clinical features distinct from other estrogen receptor-positive (ER+) breast cancers but the molecular mechanisms underlying its characteristic biology are poorly understood
because we lack experimental models to study them. Here, we recapitulate the human disease, including its metastatic pattern, by grafting ILC-derived breast cancer cell lines, SUM-44 PE and MDAMB-134-VI cells, into the mouse milk ducts. Using patient-derived intraductal xenografts from lobular and non-lobular ER+ HER2+ tumors to compare global gene expression, we identify extracellular matrix modulation as a lobular carcinoma cell-intrinsic trait. Analysis of TCGA patient datasets shows matrisome signature is enriched in lobular carcinomas with overexpression of elastin, collagens, and the collagen modifying enzyme LOXL1. Treatment with the pan LOX inhibitor BAPN and silencing of LOXL1 expression decrease tumor growth, invasion, and metastasis by disrupting ECM structure resulting in decreased ER signaling. We conclude that LOXL1 inhibition is a promising therapeutic strategy for ILC.

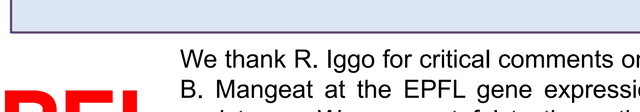

Figure 1: *Experimental Design - A Simplified Flow Chart. Dissociated surgical specimen of ILCs is lentiviral infected with GFP-luc2 and injected into the milk ducts. Retransplanted tumors were follow up and treated with endocrine therapies. Along with the clinical samples, MDA-MB-134VI (model for ILC) and MCF7 (model for IDC) breast cancer cell lines used for the development of in vivo xenografts.*

Keywords: Lobular Carcinoma, Microenvironment, Preclinical Models, Patient-derived Xenograft

References

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Results

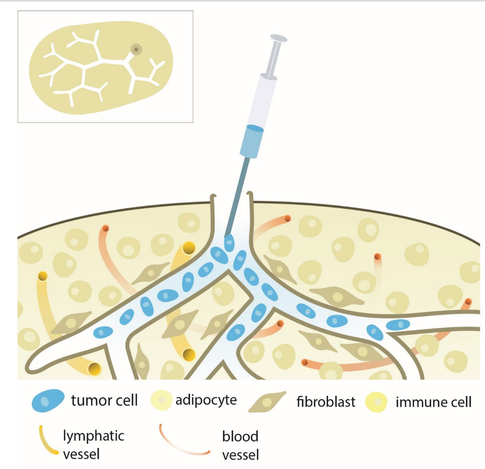

Figure 2: *The MIND transplantation technology is based on mammary intraductal injections of ILC derived cells into the mouse milk ducts via the teat*

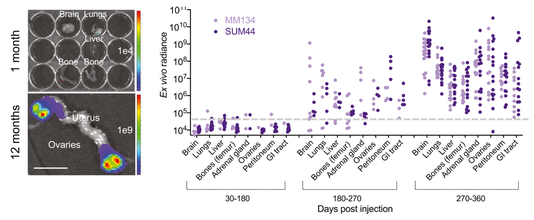

Figure 4: *Ex vivo luminescence images of bones, brain, lungs, and liver from MM134 engrafted mice 1 month post-injection (top) and ovaries (bottom) 12 months post-injection. Scale bar, 1 cm. Dot plot showing ex vivo bioluminescence of different organs from mice xenografted with MM134 and SUM44 cells plotted over organ and time of analysis.*

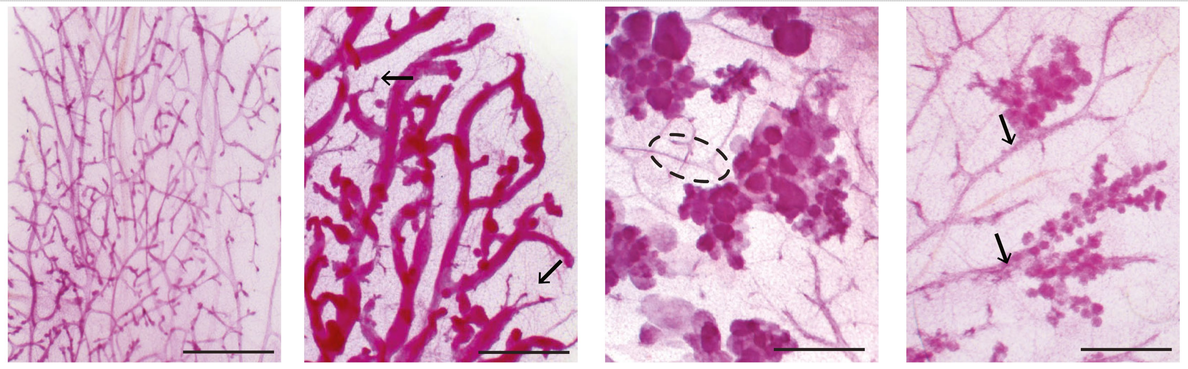

Figure 3: *Whole mount stereo-micrographs of carmine alum stained mammary glands either uninjected, 2 months after intraductal injection with MCF7, arrows point to undilated ductal tips, MM134, dotted line highlight the subtending duct, or SUM44 cells, (n > 6) scale bar, 1 mm.*

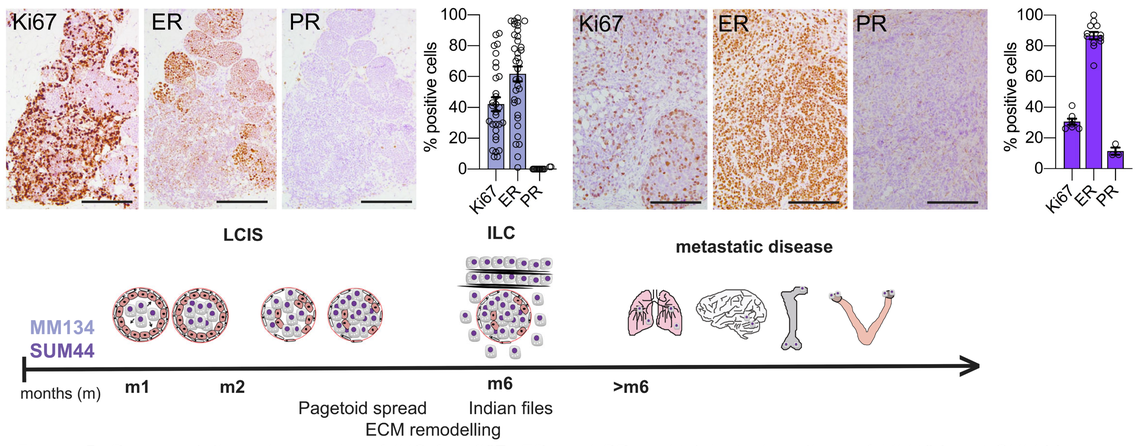

Figure 5: *Representative micrographs of IHC for Ki67, ER, and PR on histological sections of MM134 and SUM44 xenografts 5 months after intraductal injection counterstained with hematoxylin. Scale bars, 200 um. Bar plots represent means SEM of measurements and indicate the percentage of positive cells for > 1,000 cells counted on three glands.*

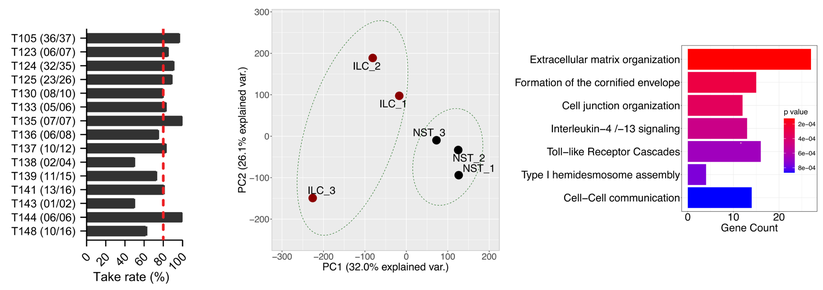

Figure 6: *Right: Bar graph showing take rates in % of PDXs from the treatment naive patient ILCs. In parentheses number of injected glands (n) with growth over total n of injected glands. Red dotted line shows average take rate. Middle: Principal component analysis plot of global gene expression profiles of ILC and non-ILC ER+ HER2 (NST) PDX cells purified by FACS sorting based on GFP expression, n=3. Right: Barplot showing Reactome pathway enrichment analysis of differentially upregulated genes.*

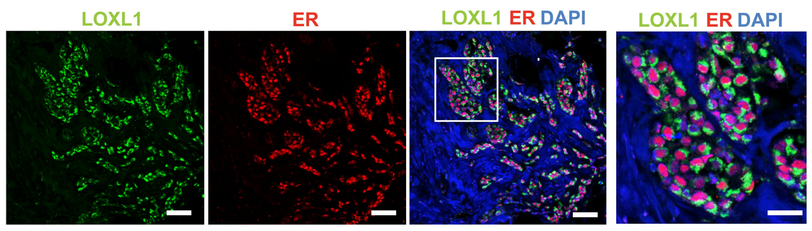

Figure 7: *Representative immunofluorescence image of primary ILC (n = 3) stained with anti-LOXL1 (green) and anti-ER (red) antibodies counterstained with DAPI (blue). Scale bars; 50 um and right inset 30 um.*

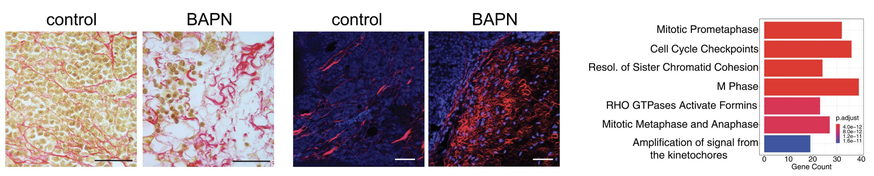

Figure 8: Left: *Representative micrographs showing picrosirius red staining of sections from MM134 xenografts treated with PBS (left) or BAPN (right), n = 4. Scale bars, 100 um.* ***Middle****: Representative second-harmonic generation (SHG) image on SUM44 xenografts treated with vehicle (left) or BAPN (right), n = 3. Collagen fibers in red and DAPI nuclear stain (blue). Scale bar, 50 um.* ***Right****: Barplot showing reactome pathway enrichment analysis of differentially down-regulated genes. GSEA plots showing decreased early and late estrogen response and MYC targets. P-values are computed by nonparametric permutation tests.*

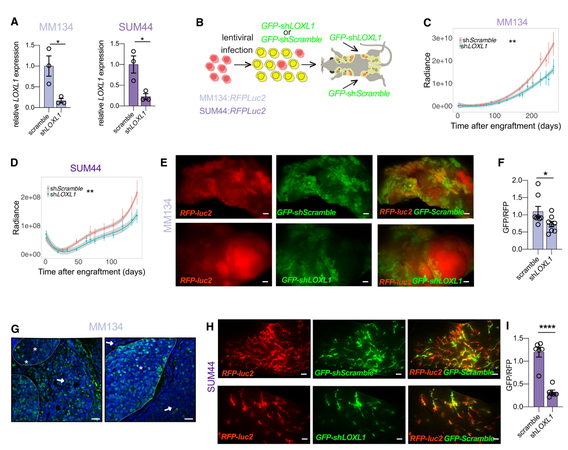

Figure 9*: LOXL1 knockdown and its effects on ILC progression. A. Bar plot showing LOXL1 transcript levels as measured by semi qRT–PCR normalized to 36B4 of scramble and shLOXL1 expressing ILCs cells in vitro. B. Scheme describing the experimental approach used to generate shLOXL1 expressing and control ILC xenograft models. ILC:RFP-luc2 cells were transduced with GFP+ sh-scramble or GFP+ shLOXL1 expressing lentiviruses, selected by FACS and injected into the into contralateral 3rd thoracic and the 4th inguinal gland. Xenografted mice were monitored for tumor growth by bioluminescence imaging.*

Conclusions

Here, lobular breast cancer cells either from cell lines or from patient tumors are grafted directly to the milk ducts of immunocompromised female mice. We show that in these models, the tumor cells grow, invade, and metastasize in a similar way as they do in patients. Molecular analysis of purified lobular carcinoma cells from intraductal xenografts reveals that these cells actively modulate their extracellular environment. Blocking an enzyme that is critical for this modulation interferes with tumor growth and progression, suggesting that this can be exploited for new therapies.

Perspectives

The new models for lobular carcinoma we have developed and characterized will improve our understanding of the disease. The finding that the lobular tumor cells are highly dependent on the proteins that surround them and that they themselves secrete proteins and enzymes that control this matrix opens new strategies for therapy.

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