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A novel role for β-adrenergic receptor in mammary branching morphogenesis and its implication in breast cancer

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The mammary gland develops from embryogenesis to infancy-puberty and adulthood, by the input of circulating hormones. At birth the gland is composed of a rudimentary ductal system that grows allometrically until puberty (4-weeks in mice). Later on, estrogens, growth hormone and insulin like growth factor induce expansive proliferation. Normal breast has three types of lobules, type 1, 2 and 3. Full term pregnancy and lactation results in the development of lobule type 3 into type 4, preparing the gland for lactation. This transformation is known to inhibit carcinogenic initiation through the induction of differentiation.

β_2-adrenergic receptors (β_2-AR) have been well characterized in several human breast cell lines (normal and tumor) and in tissue samples. Recently, we have demonstrated that β_2-AR expression and stimulation are associated with a benign breast tumor cell phenotype, reducing proliferation and cell migration, and increasing adhesion, suggesting that this receptor might be an important factor during tumor progression. In order to understand the implications of β_2-AR on breast development and cancer, we evaluated β-activation in experimental models of normal and tumor breast, using cell lines, cultures 3D and *in vivo* approaches.

Non tumor breast cell line MCF-10A cultured during 15 days on matrigel, formed gland-like organoids. Tubular structures mimicking mammary ducts were recognized, with type 1 and 2 lobules. No sign of lumen was observed. When treated with the β-agonist Isoproterenol (1 μM ISO) MCF-10A cells showed an increase in type 2 and 3 lobules ($p<0.0001$) and the presence of lumen was observed. Furthermore, breast cancer cells MCF-7 growing in 3D, formed unorganized espheroid structures. When treated with ISO these structures acquire a polarized phenotype and some of them develop lumen.

Later, Balb/c female virgin mice, weaning and 2 months old were treated daily with ISO 1 mg/Kg during 15 days. Control groups received saline solution. Mice were

sacrificed and the 4th mammary glands were removed for *Whole Mount* and hematoxylin and eosin staining.

Interestingly, ISO induced a statistically significant difference in mammary gland branching in weaning (150±32 *vs* 57±21 branches/field in controls, $p<0.05$) as well as in puberal mice (196±16 *vs* 94±14, $p<0.01$). It is well established that branching is controlled by progesterone receptor (PR), estrogen receptor alpha (ERα), FGF2, FGF10 and FGFR2, hence we studied their involvement in β-AR effect. First, ISO action was assessed on PR knockout mice in which branches were also significantly increased ($p<0.05$). After that, we addressed ER related signaling comparing ovariectomized (OVX) or ER depleted (by fulvestrant administration) mice treated or not with ISO. In both cases, despite the administration of ISO, branching was not induced. These results suggest that estrogen receptor and its classical ligand, estrogen, are required for ISO-induced branching. In addition, ISO induced a significant increase of FGF2, FGF10 and FGFR2 expression in both *in vitro* treated cells and in ISO treated mammary glands ($p<0.05$).

Finally, we tested ISO in a mouse model of mammary ductal carcinoma. Surprisingly, tumors exhibited newly rough lumens and changes in cell polarity towards them, reinforcing the differentiating role of ISO also in a pathological context.

In conclusion, β-AR stimulation seems to be involved in normal mammary development, leading to a terminal stage phenotype. In agreement with this, the analysis of a publicly available MicroArray dataset (GSE8191) showed an increase in β-AR expression levels during lactation. This study highlights a possible physiological role of β-AR in the development of mammary gland. Understanding the function of β-AR in normal development is crucial to elucidate its role in breast cancer and its possible use as a therapeutic target.