OVERVIEW

Tumours exploit and manipulate for their benefit the same mechanisms that regulate homeostasis in healthy tissue. In the Transformation and Metastasis Group, we aim to understand normal mammary gland development and the key events that lead to tumour initiation, progression, and metastasis, and to identify novel therapeutic targets to combat breast cancer. We use complementary tools, including primary cell cultures and organoids, lineage tracing mouse models, and clinical samples with the goal of translating basic knowledge into clinically relevant findings.

“Luminal Rank loss impairs lactation, awakening basal bipotency to restore functional milk production in parous glands.”

“RANK pathway inhibitors can restore sensitivity to CDK4/6i and prevent acquired resistance in breast cancer.”
**RESEARCH HIGHLIGHTS**

**RANK is a poor prognosis marker and a therapeutic target in ER-negative postmenopausal breast cancer**

Analyses of RANK and RANKL expression in more than 2000 breast tumours revealed that tumour RANK expression associated with poor prognosis in ER-negative breast cancer and in postmenopausal breast cancer patients. Gene set enrichment analysis (GSEA) showed that RANK protein expression in tumour cells in postmenopausal E- negative breast tumours was associated with multiple immune and metabolic pathways, suggesting that RANK signalling increases after menopause. Our results demonstrate that RANK expression is an independent biomarker of poor prognosis in postmenopausal patients with ER-negative breast cancer and support the therapeutic benefit of RANK pathway inhibitors in breast cancer patients with RANK-positive, ER-negative tumours after menopause. (Ciscar M et al., *EMBO Mol Med* 2022).

**Luminal Rank loss decreases cell fitness leading to basal cell bipotency in parous mammary glands**

The Rank signalling pathway regulates mammary gland homeostasis and epithelial cell differentiation. By combining temporal/lineage specific Rank genetic deletion with lineage tracing techniques, we found that loss of luminal Rank reduces the luminal progenitor pool and leads to aberrant alveolar-like differentiation with high protein translation capacity in virgin mammary glands. These Rank-deleted luminal cells are unable to expand during the first pregnancy, leading to lactation failure and impairment of protein synthesis potential in the parous stage. The unif parous Rank-deleted luminal cells in the alveoli are progressively replaced by Rank-proficient cells early during the second pregnancy, thereby restoring lactation. Transcriptomic analysis and functional assays point to the awakening of basal bipotency after pregnancy through the induction of Rank/NF-κB signalling in basal parous cell to the awakening of basal bipotency at 60 days after the second pregnancy. (Suárez E# (2023). *Nat Commun*).

**Microglia Rank signalling regulates GnRH function and the hypothalamic-pituitary-gonadal axis**

We have demonstrated a novel role of hypothalamic microglia in controlling reproductive hormones through Rank signalling. Congenital and microglial Rank deletion leads to severe hypogonadotropic hypogonadism (CHH) in males and females, resulting from a direct alteration in gonadotropin-releasing hormone (GnRH) regulation. In addition, we identified rare sequence mutations of RANK in patients with congenital hypogonadotropic hypogonadism (CHH). Moreover, inducible Rank deletion during puberty and adulthood also leads to HH. Transcriptional profiling at single-cell level of hypothalamic microglia revealed the importance of Rank signalling in the maintenance of a functionally active homeostatic microglia. Our data have revealed the crucial role of microglia, mediated by Rank signalling, in regulating GnRH function and the hypothalamic-pituitary-gonadal (HPG) axis, which is essential for reproductive maturation and fertility. (Collado et al., under review in *Nature*).

**FIGURE 1** Mammary gland at G9.5 showing in pink luminal cells and in cyan PR expression.

**FIGURE 2** Defective HPG axis in microglia Rank null models.