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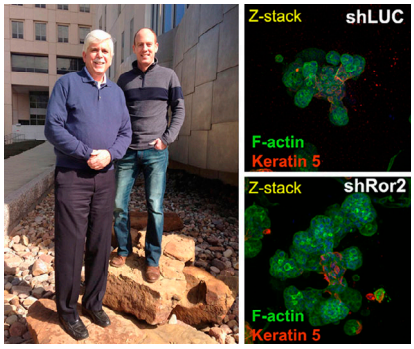
A Ror recruit to mammary gland development

Study shows how noncanonical Wnt signaling promotes differentiation and morphogenesis of mammary epithelia.

All signaling pathways have their complexities, but Wnt signaling may be more complex than most. There are 19 different Wnt ligands in mammals that bind to multiple types of receptors, some of which activate a canonical pathway that stabilizes the transcriptional coactivator β-catenin, and some of which signal via noncanonical, β-catenin–independent pathways. Roarty et al. begin to unpick the cross talk between Wnt ligands and receptors during mammary gland development and reveal a crucial role for the noncanonical receptor Ror2 (1).

Mammary gland development mainly occurs postnatally. The ducts formed by the bilayered mammary epithelium extend and branch at puberty and then, during pregnancy, differentiate into milk-producing alveoli. Multiple Wnt ligands are expressed in the mammary gland (2, 3), and β-catenin's transcriptional activity is required for these developmental processes and for mammary stem cell self renewal (4, 5). "But no one really knew what the role of noncanonical receptors and ligands were," explains Jeffrey Rosen from Baylor College of Medicine in Houston, Texas.

Rosen and colleagues, led by postdoc Kevin Roarty, initially found that the noncanonical receptors *Ror1* and *Ror2*, and their ligands *Wnt5a* and *Wnt5b*, were expressed in mouse mammary epithelium (1). *Ror1* and *Ror2* were expressed in both the basal and luminal cell layers but their distribution was distinct from that of canonical Wnt signaling. A reporter of β-catenin transcriptional activity showed that canonical Wnt signaling was primarily active in the Cap cells in the terminal end buds of mammary epithelial ducts, which are thought to be enriched in mammary stem cells. This activity declined in the more differentiated basal myoepithelial cells further along the ducts, which, in contrast, expressed increased levels of *Ror2*. "[Canonical and noncanonical signaling] seemed to be inversely correlated," says Roarty. "This provided evidence for us to pursue some functional studies."



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Focal Point

Jeffrey Rosen (left), Kevin Roarty (right), and colleagues (not pictured) examine the role of the noncanonical Wnt receptor Ror2 in mammary gland development. In mammary epithelial ducts, the pattern of *Ror2* expression is inversely correlated to canonical Wnt signaling and, at least in vitro, the receptor inhibits activation of the canonical Wnt effector β-catenin. Knocking down Ror2 in vivo disrupts epithelial cell differentiation and stimulates excessive branching morphogenesis, a phenotype that can be recapitulated in organoid cultures in vitro (right) that show defects in the organization of F-actin (green).

> "There was a striking increase in branching in the absence of Ror2."

Ror2 and its ligand Wnt5a have been shown to antagonize canonical Wnt signaling during embryogenesis (6), and the inverse relationship between *Ror2* expression and β-catenin activity suggested that the pathways might operate similarly in mammary gland development. Indeed, in primary mammary epithelial cells in vitro, Wnt5a inhibited β-catenin activation by the canonical ligand Wnt3. Overexpressing *Ror2* enhanced this antagonism, whereas knocking down the receptor blocked Wnt5a's inhibitory effect.

To investigate Ror2's function in vivo, Roarty et al. transplanted Ror2-deficient cells into mouse fat pads and followed their development into mammary ducts. "There was a striking increase in branching in the absence of Ror2," says Roarty. The actin cytoskeleton plays a key role in epithelial branching morphogenesis, and Roarty et al. found that the expression of several actin regulatory proteins, including Rho GTPases, was altered upon Ror2 knockdown. Accordingly, F-actin was disorganized in Ror2-deficient cells undergoing branching morphogenesis in vitro.

In addition to causing excessive branching, depleting Ror2 also altered epithelial cell differentiation in vivo. "In the luminal compartment, we observed a precocious differentiation into a pregnancy-like phenotype," Roarty explains. "We saw an accumulation of alveolar progenitors, which are primed for milk production. In the basal compartment we determined that Ror2 is required for the proper differentiation of myoepithelial cells."

Surprisingly, however, and in contrast to the researchers' in vitro results, canonical Wnt signaling wasn't enhanced in the absence of Ror2. β-catenin activity was in fact lower in Ror2-deficient ducts, suggesting that the integration of multiple Wnt signals is more complex in vivo. One possibility is that defects in epithelial cell differentiation result in changes to the stem cell niche, indirectly inhibiting canonical Wnt signaling. "That's why it's important to do these studies in vivo," says Rosen. "The whole microenvironment plays a big role in normal development."

The researchers now want to understand in more detail how the different Wnt signals are coordinated in vivo and to determine the intracellular signaling pathway downstream of Ror2. "There's evidence that Ror receptors are misregulated in breast cancers," says Rosen, "so we'd like to know more about what this pathway does."

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