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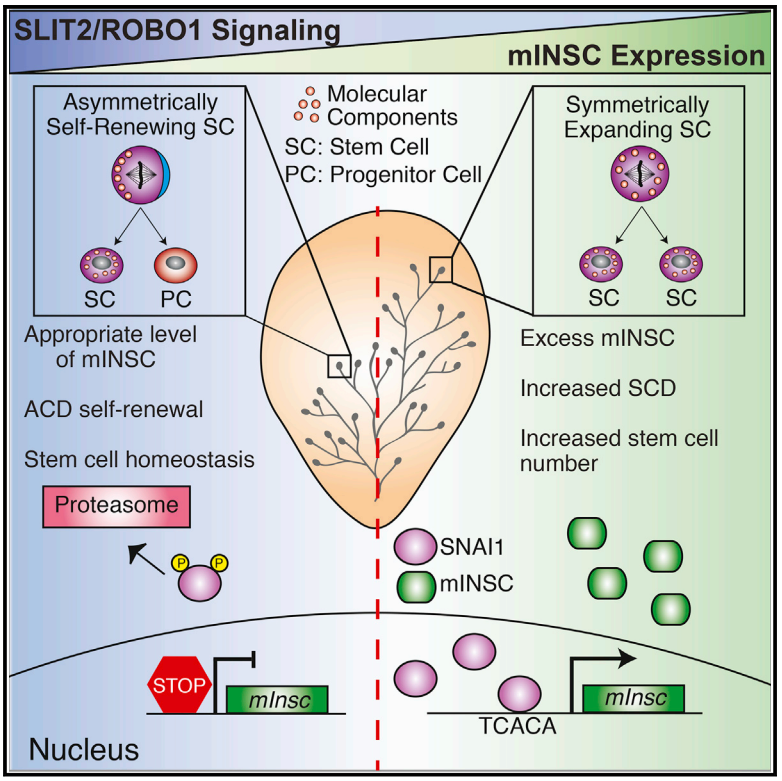
Mammary Stem Cell Self-Renewal Is Regulated by Slit2/Robo1 Signaling through SNAI1 and mINSC

Graphical Abstract



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In Brief

Tissue homeostasis depends on the balanced self-renewal of stem cells. Ballard et al. describe a mechanism whereby the extracellular cue SLIT2 signals through receptor ROBO1 to regulate the asymmetric self-renewal of basal stem cells during mammary gland development by controlling *Inscuteable* expression through the transcription factor Snail.

Highlights

- Mammary stem cells undergo classic asymmetric cell division
- SLIT2/ROBO1 govern stem cell division type by regulating *mInsc* levels through SNAI1
- Excess mINSC drives symmetric cell division
- Excess mINSC leads to more stem cells and enhanced mammary outgrowth

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Mammary Stem Cell Self-Renewal Is Regulated by Slit2/Robo1 Signaling through SNAI1 and mINSC

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SUMMARY

Tissue homeostasis requires somatic stem cell maintenance; however, mechanisms regulating this process during organogenesis are not well understood. Here, we identify asymmetrically renewing basal and luminal stem cells in the mammary end bud. We demonstrate that SLIT2/ROBO1 signaling regulates the choice between self-renewing asymmetric cell divisions (ACDs) and expansive symmetric cell divisions (SCDs) by governing *Inscuteable* (*mInsc*), a key member of the spindle orientation machinery, through the transcription factor Snail (SNAI1). Loss of SLIT2/ROBO1 signaling increases SNAI1 in the nucleus. Overexpression of SNAI1 increases *mInsc* expression, an effect that is inhibited by SLIT2 treatment. Increased *mInsc* does not change cell proliferation in the mammary gland (MG) but instead causes more basal cap cells to divide via SCD, at the expense of ACD, leading to more stem cells and larger outgrowths. Together, our studies provide insight into how the number of mammary stem cells is regulated by the extracellular cue SLIT2.

INTRODUCTION

Stem cells use division type, symmetric cell division (SCD) versus asymmetric cell division (ACD), to balance stem cell expansion with self-renewal and generate daughter cells with different cell fates. This balance is critical to maintaining tissue homeostasis, as illustrated by a study in which the overexpression of ErbB2 resulted in the increased proliferative capacity of murine mammary tumors by favoring SCD (Cicalese et al., 2009). The distinction between these division types depends on the equal (SCD), or unequal (ACD), partitioning of molecular components between the daughter cells, with SCDs generally resulting in two equivalent daughter cells and ACDs resulting in daughter cells with two different fates. However, when a daughter is placed in a different niche, an SCD can yield daughters with different cell fates, even though cellular components are symmetrically partitioned. Orientation of the mitotic spindle can play an important role in this process: for example, when perpendicular orientation of a cell undergoing mitosis places the daughter cell in another environment where extrinsic cues promote a different cell fate. This type of division, resulting in asymmetric fate outcomes through symmetric cell division, is sometimes referred to as extrinsic ACD (Williams and Fuchs, 2013). Extrinsic ACDs have been observed in the MG with misregulation of aurora A kinase and huntingtin proteins, both of which change spindle pole orientation in basal cells, thereby promoting Notch signaling in displaced daughter cells, which subsequently acquire luminal cell fates (Regan et al., 2013; Elias et al., 2014).

In contrast to SCDs, classic ACDs involve the unequal partitioning of cellular components along with spindle reorientation. Classic ACDs are regulated by the formation of a NuMA/LGN complex above one mitotic spindle pole, whereas this does not occur during SCDs, not even during SCDs in which the spindle reorients (i.e., extrinsic ACDs). mINSC serves as a link between the apically localized complex (PAR3/PAR6/aPKC) and the microtubule-associated complex (NuMA/LGN/Gαi). Recent biochemical studies showed that mINSC and NuMA bind to the same site on LGN (Culurgioni and Mapelli, 2013). mINSC, recruited by the PAR complex, initially engages LGN before handing this adaptor protein off to NuMA, resulting in the co-localization of LGN and NuMA at the apical pole, facilitating spindle pole tethering, and contributing to the unequal distribution of cell fate determinants. In its role as a molecular baton, mINSC has the potential to be a very specific regulatory target, capable of governing the balance between classic ACD and SCD.

One consequence of these divisory events during classic ACD is that stem cell self-renewal occurs by generating daughter cells that are molecularly distinct from each other. This distinction may be only in potency, renewing the basal stem cell and generating a basal progenitor cell, or the distinction may additionally involve a change in cell lineage, renewing the basal stem cell while generating a luminal progenitor. The former is an example of unipotent self-renewal, whereas the latter is an example of bipotent self-renewal. Recently in the MG, lineage-tracing studies have provided evidence for mammary stem cells that renew via both mechanisms (Van Keymeulen et al., 2011; de Visser et al.,