Regulating the regulator: Numb acts upstream of p53 to control mammary stem and progenitor cell

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In this issue, Tosoni et al. (2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201505037) report that cell fate determinant and tumor suppressor Numb imposes asymmetric cell divisions in mammary stem cells by regulating p53. Numb thereby restricts mammary stem cell expansion and controls the proliferation and lineage-specific characteristics of their progeny.

The mammary epithelium contains an outer layer of basal cells and an inner layer of luminal cells. The basal cell layer harbors bipotent stem cells able to give rise to basal and luminal progeny (Rios et al., 2014; Wang et al., 2015). In addition to these cells, both mammary epithelial cell layers contain their specific lineage-restricted stem cells (Van Keymeulen et al., 2011; van Amerongen et al., 2012; Prater et al., 2014). The unipotent stem cells residing in the luminal layer are also referred to as luminal progenitors (Rios et al., 2014). Mammary stem cells have been identified on a functional basis by transplantation, clonogenicity assays, and lineage tracing, but their exact localization remains unknown, making it difficult to monitor their mode of division in situ.

Asymmetric stem cell division (ACD) yields a stem cell, ensuring self-renewal, and a progenitor cell that is destined to proliferate and give rise to differentiated progeny. In contrast, symmetric cell division (SCD), with both daughter cells adopting a stem cell fate, expands the stem cell pool. Control over the mode of stem cell division is thus essential for normal tissue homeostasis. Although ACDs serve for maintenance of the stem cell number, unlimited SCDs may lead to cancer (Knoblich, 2010). Both extrinsic and intrinsic cues determine whether stem cells divide symmetrically or asymmetrically, and Numb has been identified as an intrinsic cell fate determinant partitioned asymmetrically between daughter cells during ACD (Knoblich, 2010; Williams and Fuchs, 2013). In the mammary epithelium, the Notch antagonist Numb is expressed throughout the luminal layer and in rare basal cells (Tosoni et al., 2015). Numb has been found to protect the tumor suppressor p53 from degradation, and its expression is often lost in breast cancer (Colaluca et al., 2008).

In this issue, Tosoni et al. (2015) report elegant studies of the mode of stem cell division in an ex vivo setting. The stem cells from mouse mammary glands were retrospectively identified on the basis of their capacity to give rise to clonal mammospheres (colonies of proliferating cells in suspension) under nonadherent conditions, and to retain a lipophilic fluorescent dye, PKH26, as a result of their return to quiescence after the first mitosis. Stem cell divisions were considered to be asymmetric if, after the initial division, only one of the two daughter cells continued to divide (with the dye being progressively diluted and lost in the progeny), the other cell remaining quiescent, i.e., having adopted a stem cell fate.

This team previously used the same experimental approach to show that p53 imposes ACD and restricts the expansion of mammary stem cells (Cicalese et al., 2009). In this new study, Tosoni et al. (2015) demonstrate that, during ACD, Numb is retained in the daughter cell that adopts a stem cell fate, i.e., stops dividing after the initial mitosis (Fig. 1). As Numb is known to stabilize p53, the researchers assessed p53 activity using a p53-GFP reporter and found that cells inheriting Numb also displayed a Numb-dependent increase in p53 activity. However, during subsequent divisions, Numb again accumulated in the dividing daughter cells, suggesting a possible additional role for this protein in progenitors and differentiated cells. The researchers indeed observed that suppression of Numb expression by knockdown in isolated mammary cells or by conditional gene deletion in the mouse mammary epithelium favored SCD, leading to an increase in the number and proliferative capacity of stem and progenitor cells. In addition, as transplantation of Numb-depleted cells in mice yielded the formation of tumors, Tosoni et al. (2015) further dissected the tumorigenic potential of these cells. They found that Numb-deficient mammary glands displayed preneoplastic lesions and signs of aberrant lineage specification, showing that loss of Numb is associated with aberrant mammary morphogenesis in vivo. Moreover, loss of Numb led to the induction of Snail, Slug, and Sox9 (Tosoni et al., 2015), transcription factors known to trigger epithelial-mesenchymal transition (EMT) and to reprogram progenitors and differentiated cells to a stem cell–like state (Guo et al., 2012). These data indicated that Numb exerts a role at the progenitor compartment level, where Numb appears to suppress EMT and to ensure proper maturation and luminal cell fate specification. In addition, Numb deficiency in the mammary epithelium phenocopied p53 deficiency, with the activation of the Notch pathway observed in Numb or p53 mutant cells (Cicalese et al., 2009; Tao et al., 2011; Chiche et al., 2013). Tosoni et al. (2015)

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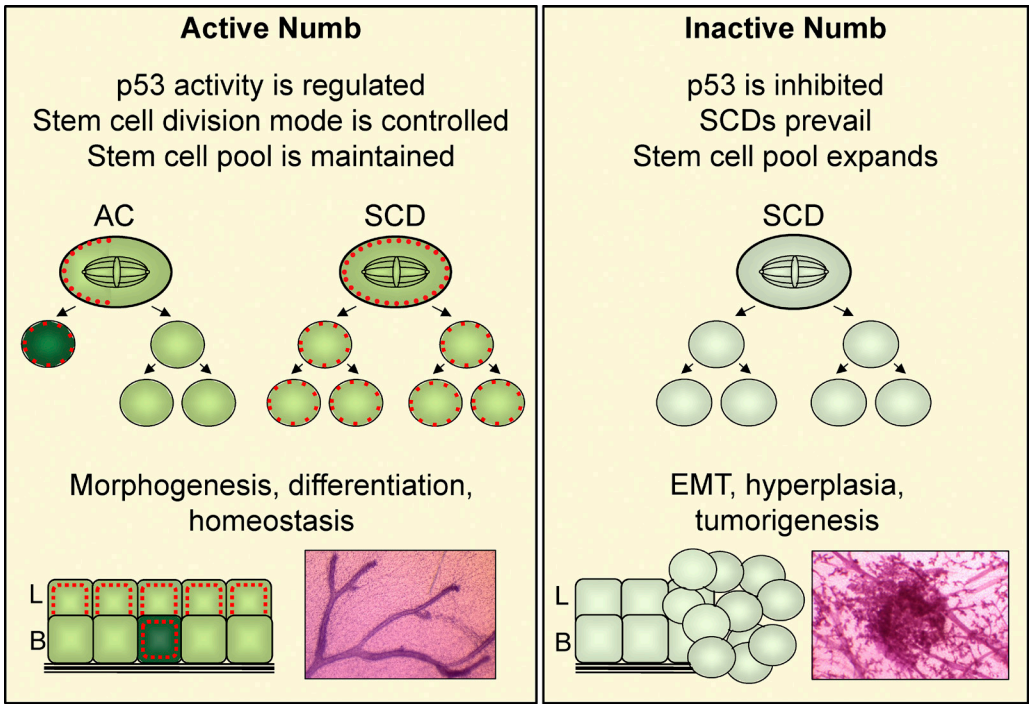

Figure 1. **Role of Numb in the maintenance of the stem and progenitor cell pools in the mammary epithelium.** (left) In the mammary epithelium, Numb (red dots) is expressed in all luminal (L) and rare basal (B) cells. During ACD, Numb is retained in one daughter cell, where it stabilizes p53 (dark green), leading to quiescence and the adoption of a stem cell fate. In normal mammary epithelium, ACDs prevail, maintaining the stem cell pool and gland homeostasis. Luminal progenitor cells divide symmetrically. Numb is partitioned equally between the two daughter cells, which have identical proliferative and developmental characteristics. Numb restricts proliferation in the luminal layer by stabilizing p53 and, conceivably, by regulating Notch. (right) Numb is inactivated by mutation or gene deletion. In the absence of Numb, p53 activity is decreased favoring SCD, stem, and progenitor pool expansion, and activation of the EMT program leading to hyperplasia and tumorigenesis. The micrographs show a representative image of normal mouse mammary ducts (left) and an example of a hyperplastic lesion (right).

tested whether impairment of p53 activity could be responsible for the tumorigenicity of Numb-depleted cells. Remarkably, Nutlin3, an inhibitor of p53 ubiquitination/degradation, rescued the abnormal phenotype of Numb-deficient cells, confirming that Numb acts at least partly by regulating p53 activity in mammary epithelial cells.

Interestingly, although mice deficient for Numb in the mammary epithelium did not spontaneously develop breast tumors, when isolated from the tissue and cultured in mammospheres, Numb-null mammary epithelial cells became tumorigenic and produced neoplastic outgrowths upon transplantation into mouse mammary fat pads (Tosoni et al., 2015). One remarkable finding of this study is that treatment of mice carrying grafted Numb-null mammary tumors with Nutlin3 significantly decreased the tumorigenicity in subsequent retransplants. A plausible interpretation of this result is that restoration of p53 activity affects cancer stem cells by imposing ASD, confirming that the Numb–p53 pathway acts as a barrier against the development of cancer stem cells in the mammary epithelium.

Regulation of division mode and of mitotic spindle orientation is a key question in stem cell biology, and only few studies addressed this question in regard to the mammary epithelium (Taddei et al., 2008; Cicalese et al., 2009; Regan et al., 2013; Elias et al., 2014; Ballard et al., 2015). The findings of Tosoni et al. (2015) add a key piece to the puzzle and raise additional questions about the coordination of the molecular pathways interacting with Numb in the regulation of stem cell division mode in the contexts of normal mammary epithelium and cancer. Unfortunately, the ex vivo model used in this study precludes consideration of the specific bilayer organization of the mammary epithelium and of the position of the stem cells relative to other cells and to the extracellular matrix. This setting excludes the niche, and although Numb is known to be an intrinsic cell fate determinant, its asymmetric distribution is regulated by a mechanism involving aPKC, whose cellular localization in mammary epithelial cells is dependent on extrinsic cues (Akhtar and Streuli, 2013). The bilayer organization of the mammary epithelium adds another level of complexity to the evaluation of stem cell division outcome, including the developmental characteristics of the daughter cells. If Numb expression labels basal stem cells, are these cells bipotent or basal lineage restricted? It will be important to determine whether the asymmetric distribution of Numb during ACD plays a role in the orientation of the basal stem cell division axis and whether the progeny of Numb-expressing basal stem cells expands within the basal layer only or contributes to the luminal layer as well.

Another question raised by this study concerns the role of the Notch–Numb–p53 cross talk in the regulation of mammary lineages. Cells simultaneously expressing basal and luminal markers were found in Numb-deficient epithelium, indicating a role for Numb in the regulation of the luminal and/or basal cell phenotypes. Numb is a well-known inhibitor of Notch, and Notch activation has been found to specify mammary luminal fate (Bouras et al., 2008; Yalcin-Ozuysal et al., 2010). A high Notch activity is characteristic of luminal progenitors, rather than mature luminal cells (Bouras et al., 2008). Thus, Numb, in concert with p53, may differentially regulate Notch activity in the two luminal cell subsets. In the basal compartment, with

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few Numb-expressing cells, Notch activity could be inhibited by a different mechanism (Yalcin-Ozuysal et al., 2010). Conditional deletion of Numb targeted to specific mammary epithelial cell populations followed by the analysis of the resulting phenotypes at different developmental stages could help to address these questions.

This interesting and important study provides new insight into the molecular mechanisms controlling the growth and homeostasis of the mammary epithelium. However, the importance of this work is not limited to fundamental aspects of mammary gland biology. This work provides data suggesting that restoration of p53 activity in breast cancers displaying inactive Numb and low p53 levels may inhibit the formation/proliferation of tumor-initiating cells, which are responsible for tumor recurrence. Additional studies are required to explore possibilities for targeting cancer stem cells and making them switch to an asymmetric mode of division, resulting in their quiescence and differentiation and limiting their proliferation in an effort to limit tumor expansion.

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References

Akhtar, N., and C.H. Streuli. 2013. An integrin-ILK-microtubule network orients cell polarity and lumen formation in glandular epithelium. *Nat. Cell Biol.* 15:17–27. http://dx.doi.org/10.1038/ncb2646

Ballard, M.S., A. Zhu, N. Iwai, M. Stensrud, A. Mapps, M.P. Postiglione, J.A. Knoblich, and L. Hinck. 2015. Mammary stem cell self-renewal is regulated by Slit2/Robo1 signaling through SNAI1 and mINSC. *Cell Reports.* 13:290–301. http://dx.doi.org/10.1016/j.celrep.2015.09.006

Bouras, T., B. Pal, F. Vaillant, G. Harburg, M.L. Asselin-Labat, S.R. Oakes, G.J. Lindeman, and J.E. Visvader. 2008. Notch signaling regulates mammary stem cell function and luminal cell-fate commitment. *Cell Stem Cell.* 3:429–441. http://dx.doi.org/10.1016/j.stem.2008.08.001

Chiche, A., M. Moumen, V. Petit, J. Jonkers, D. Medina, M.A. Deugnier, M.M. Faraldo, and M.A. Glukhova. 2013. Somatic loss of p53 leads to stem/progenitor cell amplification in both mammary epithelial compartments, basal and luminal. *Stem Cells.* 31:1857–1867. http://dx.doi.org/10.1002/stem.1429

Cicalese, A., G. Bonizzi, C.E. Pasi, M. Faretta, S. Ronzoni, B. Giulini, C. Brisken, S. Minucci, P.P. Di Fiore, and P.G. Pelicci. 2009. The tumor suppressor p53 regulates polarity of self-renewing divisions in mammary stem cells. *Cell.* 138:1083–1095. http://dx.doi.org/10.1016/j.cell.2009.06.048

Colaluca, I.N., D. Tosoni, P. Nuciforo, F. Senic-Matuglia, V. Galimberti, G. Viale, S. Pece, and P.P. Di Fiore. 2008. NUMB controls p53 tumour suppressor activity. *Nature.* 451:76–80. http://dx.doi.org/10.1038/nature06412

Elias, S., M.S. Thion, H. Yu, C.M. Sousa, C. Lasgi, X. Morin, and S. Humbert. 2014. Huntingtin regulates mammary stem cell division and differentiation. *Stem Cell Rep.* 2:491–506. http://dx.doi.org/10.1016/j.stemcr.2014.02.011

Guo, W., Z. Keckesova, J.L. Donaher, T. Shibue, V. Tischler, F. Reinhardt, S. Itzkovitz, A. Noske, U. Zürrer-Härdi, G. Bell, et al. 2012. Slug and Sox9 cooperatively determine the mammary stem cell state. *Cell.* 148:1015–1028. http://dx.doi.org/10.1016/j.cell.2012.02.008

Knoblich, J.A. 2010. Asymmetric cell division: recent developments and their implications for tumour biology. *Nat. Rev. Mol. Cell Biol.* 11:849–860. http://dx.doi.org/10.1038/nrm3010

Prater, M.D., V. Petit, I. Alasdair Russell, R.R. Giraddi, M. Shehata, S. Menon, R. Schulte, I. Kalajzic, N. Rath, M.F. Olson, et al. 2014. Mammary stem cells have myoepithelial cell properties. *Nat. Cell Biol.* 16:942–950: 1–7. http://dx.doi.org/10.1038/ncb3025

Regan, J.L., T. Sourisseau, K. Soady, H. Kendrick, A. McCarthy, C. Tang, K. Brennan, S. Linardopoulos, D.E. White, and M.J. Smalley. 2013. Aurora A kinase regulates mammary epithelial cell fate by determining mitotic spindle orientation in a Notch-dependent manner. *Cell Reports.* 4:110–123. http://dx.doi.org/10.1016/j.celrep.2013.05.044

Rios, A.C., N.Y. Fu, G.J. Lindeman, and J.E. Visvader. 2014. In situ identification of bipotent stem cells in the mammary gland. *Nature.* 506:322–327. http://dx.doi.org/10.1038/nature12948

Taddei, I., M.A. Deugnier, M.M. Faraldo, V. Petit, D. Bouvard, D. Medina, R. Fässler, J.P. Thiery, and M.A. Glukhova. 2008. Beta1 integrin deletion from the basal compartment of the mammary epithelium affects stem cells. *Nat. Cell Biol.* 10:716–722. http://dx.doi.org/10.1038/ncb1734

Tao, L., A.L. Roberts, K.A. Dunphy, C. Bigelow, H. Yan, and D.J. Jerry. 2011. Repression of mammary stem/progenitor cells by p53 is mediated by Notch and separable from apoptotic activity. *Stem Cells.* 29:119–127. http://dx.doi.org/10.1002/stem.552

Tosoni, D., S. Zecchini, M. Coazzoli, I. Colaluca, G. Mazzarol, A. Rubio, M. Caccia, E. Villa, O. Zilian, P.P. Di Fiore, and S. Pece. 2015. The Numb/p53 circuitry couples replicative self-renewal and tumor suppression in mammary epithelial cells. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201505037

van Amerongen, R., A.N. Bowman, and R. Nusse. 2012. Developmental stage and time dictate the fate of Wnt/β-catenin-responsive stem cells in the mammary gland. *Cell Stem Cell.* 11:387–400. http://dx.doi.org/10.1016/j.stem.2012.05.023

Van Keymeulen, A., A.S. Rocha, M. Ousset, B. Beck, G. Bouvencourt, J. Rock, N. Sharma, S. Dekoninck, and C. Blanpain. 2011. Distinct stem cells contribute to mammary gland development and maintenance. *Nature.* 479:189–193. http://dx.doi.org/10.1038/nature10573

Wang, D., C. Cai, X. Dong, Q.C. Yu, X.O. Zhang, L. Yang, and Y.A. Zeng. 2015. Identification of multipotent mammary stem cells by protein C receptor expression. *Nature.* 517:81–84. http://dx.doi.org/10.1038/nature13851

Williams, S.E., and E. Fuchs. 2013. Oriented divisions, fate decisions. *Curr. Opin. Cell Biol.* 25:749–758. http://dx.doi.org/10.1016/j.ceb.2013.08.003

Yalcin-Ozuysal, O., M. Fiche, M. Guitierrez, K.U. Wagner, W. Raffoul, and C. Brisken. 2010. Antagonistic roles of Notch and p63 in controlling mammary epithelial cell fates. *Cell Death Differ.* 17:1600–1612. http://dx.doi.org/10.1038/cdd.2010.37

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