

Wnts as Self-Renewal Factors: Mammary Stem Cells and Beyond

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Adult stem cells hold great promise for regenerative medicine, yet it is difficult to expand such cells. In this issue of *Cell Stem Cell*, Zeng and Nusse (2010) demonstrate that Wnt3A keeps mammary stem cells multipotent over multiple passages in vitro.

A surprisingly small number of signaling pathways determine the outcome of developmental decisions throughout the animal kingdom. One of these, the canonical Wnt signaling cascade, controls cell-fate determination during animal development in animals as diverse as the sea anemone *Nemostella*, *C. elegans*, *Drosophila*, and vertebrates. More recently, it has become evident that activities of Wnt are not just restricted to cell-fate decisions during embryogenesis but that the pathway also controls prominent biological phenomena in adult mammals. In these latter cases, Wnt signaling often mediates the in vivo maintenance or expansion of stem cells. The two best understood examples of Wnt-controlled stem cells are the self-renewing crypt compartment of the intestinal epithelium and the bulge of the hair follicle (reviewed in Reya and Clevers, 2005). All stem cell activity halts in these compartments when Wnt signals are blocked. Conversely, elevated levels of Wnt signaling lead to excessive stem cell division. Moreover, deregulated Wnt signaling in stem cell compartments has a prominent role in human disease. Colorectal adenocarcinoma, one of the commonest human cancers, is almost invariably caused by mutation of the negative Wnt pathway regulator APC (reviewed in Polakis, 2007).

The best-characterized adult stem cell, the bone marrow stem cell, has resisted all attempts to induce ex vivo expansion, essentially limiting clinical bone marrow transplantation to the absolute quantity of stem cells obtained from the donor. In general, it has been surprisingly difficult to increase the numbers of any adult stem cell type outside the body of the

donor. The only clinically applied example involves the expansion of epidermal progenitors from biopsies of burn patients. Yet, one may argue that these are not typical stem cells given that they produce only a single cell type, the keratinocyte. Many other examples of Wnt-dependent embryonic and adult stem cell types have since been reported (reviewed in Reya and Clevers, 2005). Yet, in most of these cases, the Wnt signals can enhance stem/progenitor activities, but they are dispensable for base-line proliferation.

Given the prominent role of Wnt signaling in stem cell biology, it is striking that few attempts have been published that use Wnt factors for in vitro stem cell expansion. This experimental gap may be because of the challenging biochemical properties of Wnt proteins. They are very cysteine-rich and—as shown by the Nusse lab—are lipid-modified (Willert et al., 2003). It is telling that 25 years after cloning of the first Wnt protein, the field still awaits an X-ray structure of these important signaling molecules.

Previously, the Nusse lab pioneered the purification of small amounts of highly biologically active Wnt proteins (Willert et al., 2003). Zeng and Nusse (2010) have now used purified Wnt3A to design a culture system that allows long-term expansion of mammary gland stem cells in vitro. In vivo expansion of any stem cell population has been fraught with challenges, most notably the maintenance of self-renewal properties and potency. The mammary stem cells have been no exception. Isolation of a mammary stem cell-enriched pool (MaSC), using a combination of cell surface markers (Lin⁻CD29^{hi}CD24⁺), has

allowed scientists to directly transplant single stem cells and reconstitute the mammary gland (Shackleton et al., 2006; Stingl et al., 2006). Short-term culturing of these mammary cells permits mammosphere formation, yet after repeated passaging, the resulting cells are compromised in their ability to self-renew and initiate differentiation (Dontu et al., 2003).

Zeng and Nusse (2010) now show that Wnt signals allow long-term culture of MaSC that retain their multipotency following many generations in culture. The actual role of Wnt signaling in regulating the behavior of MaSC is not well understood even though Wnts are clearly implicated in many aspects of mammary growth and development, as well as in mammary cancers in the mouse. The authors investigated the role of Wnt in mammary development by first isolating cells expressing the Wnt-responsive *Axin2^{lacZ}* reporter (Lustig et al., 2002). *Axin2^{lacZ/+}*-positive cells were only found among the purified Lin⁻CD29^{hi}CD24⁺ cells enriched for MaSC. Transplantation of these *Axin2^{lacZ/+}*-positive cells resulted in more efficient repopulation than their lacZ-negative counterparts. The *Axin2^{lacZ}* insertion inactivates the *Axin* gene, leading to slightly elevated levels of Wnt responsiveness in these cells. In an elegant assay to determine the properties of cells carrying homozygous inactivation of *Axin*, Zeng and Nusse performed mammary gland repopulation assays with cells of different genotypes that were marked with either GFP or DsRed. GFP-marked *Axin2^{lacZ/lacZ}* cells, which can respond more potently to Wnt signals, consistently outcompeted wild-type DsRed-expressing cells in these

assays. These results suggested that Wnt-responsiveness plays a role in MaSC behavior and provides the cells with essential signals to generate mammary glands.

To further study the effect of Wnt on MaSC, Zeng and Nusse plated isolated Lin⁻CD29^{hi}CD24⁺ cells and exposed them to purified Wnt3A, vehicle control, or the Wnt inhibitor Dkk1. In this first generation, there was no noticeable effect following Wnt3A treatment. However, subsequent serial colony formation (following colony dissociation and replating) was significantly increased in Wnt3A-treated cells. This effect was amplified in later serial passages. The increased clonogenicity of the Wnt3A-treated cells did not result from higher proliferation rates or from changes in apoptosis. Furthermore, *Axin^{lacZ/lacZ}* cells exhibited even more robust repopulation as compared to wild-type cells. These findings imply that the clonogenicity of MaSC can be greatly influenced by their exposure and responsiveness to Wnt proteins. Most strikingly, the clonally

propagated cells retained their full developmental potential. Wnt-treated colonies could robustly reconstitute mammary glands in cleared fat pad assays, while vehicle-treated colonies failed. Significantly, the effectiveness of Wnt-treated cells did not decrease following further passages. Furthermore, cessation of Wnt treatment prevented efficient mammary gland reconstitution, showing that MaSC require exposure to Wnt signals to initiate proper development of mammary structures. This finding has allowed the researchers to overcome the technical hurdles faced in the past, notably the inability to maintain stem cell cultures long term in their undifferentiated state.

With this paper, the study of Wnt's role in the mammary gland has come full circle: the first Wnt gene to be cloned, *Wnt1*, was identified as a prominent insertion site for mouse mammary tumor virus (Nusse and Varmus, 1982). And the current study implies a prominent role for Wnt factors in the normal physiology of the mammary gland stem cell.

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The Silence of the LADs: Dynamic Genome-Lamina Interactions during ESC Differentiation

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In a recent issue of *Molecular Cell*, Peric-Hupkes et al. (2010) use DamID to map the interactions between chromatin and the nuclear lamina (NL) in differentiating embryonic stem cells. NL-mediated locking/unlocking of genomic regions during differentiation provides an additional facet of transcription regulation.

The nuclear lamina (NL) is considered to be a principal guardian of the eukaryotic cell nucleus. It underlies the inner nuclear membrane and is comprised of a fibrous meshwork of proteins, mostly A- and B-type lamins. Lamins provide a mechanical scaffold for the nucleus, are required for communication between the nucleus and the cytoplasm, are associated both directly and indirectly with chromatin, and are involved in most nuclear activities

(Prokocimer et al., 2009). The nuclear lamina of essentially all mammalian cell types contains B-type lamins. In contrast, A-type lamins are absent from the inner-cell mass (ICM) of the early mouse embryo and appear only at a later stage of development in more differentiated cells. It is therefore no surprise that embryonic stem cells (ESCs), which are derived from the ICM at the blastocyst stage, lack any lamin A expression (Mattout and Meshorer,

2010). Interestingly, in undifferentiated ESCs, lamin B is significantly more dynamic than in differentiated cell types (Bhattacharya et al., 2009), constituting a more “fluid” nuclear lamina and mirroring the hyperdynamic state of chromatin proteins in ESCs (Meshorer et al., 2006).

In human fibroblasts, Lamina Associated Domains (LADs) were previously mapped genome-wide using the DamID technique (Figure 1A). LADs are mostly