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RESEARCH HIGHLIGHT

A developmental stage-dependent switch of the mechanisms for prostate epithelial maintenance

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Previous studies demonstrated that adult murine prostate basal and luminal cells are independently self-sustained, but prostate basal cells possess the potential to differentiate into multiple lineages upon induction by embryonic urogenital sinus mesenchyme. Nevertheless, it is unknown how prostate epithelia mature during the postnatal stage. Recently, Ousset *et al.* showed that some prostate basal cells in neonatal mice still possess the capacity for multilineage differentiation, while luminal cells have committed to become unipotent. This study demonstrates a developmental stage-dependent switch of the mechanisms for prostate epithelial maintenance, and implies a critical role of the stromal environment in regulating epithelial maintenance.

During the last four decades, much progress has been made in understanding how the prostate develops and how prostate epithelial homeostasis is maintained. Recently, using an elegant lineage tracing approach, Ousset *et al.*[1] determined how prostate epithelia develop at the postnatal stage. This study corroborated *in vivo* and *in situ*, many previous studies showing that prostate basal cells possess the stem cell capacity for multilineage differentiation. In addition, together with other recent studies,[2–4] this study also reveals a developmental stage-dependent switch of the mechanisms for epithelial maintenance.

There are three major types of epithelial cells in the prostate.[5] Luminal cells express low-molecular weight cytokeratins (K8/18) and form a single continuous layer that surrounds glandular lumen filled with secretions. Basal cells express high molecular weight cytokeratins (K5/14) and are aligned between the luminal cells and the basement membrane. Neuroendocrine cells are very rare and are distributed sporadically within prostate acini. Classic work by Isaacs *et al.*[6] showed that prostate epithelia can undergo extensive turnover in response to fluctuating serum testosterone levels. Because basal cells are independent of androgen for their survival, it has been hypothesized that basal cells contain the stem cells that can functionally regenerate prostate glands.

This hypothesis was proven correct from the results of a prostate regeneration assay.[7,8] It was demonstrated that a fraction of dissociated prostate epithelial cells are capable of forming clonogenic glandular structures that contain all three types of epithelial cells, when they are mixed with embryonic urogenital sinus mesenchymal cells and transplanted under renal capsules of immunodeficient host mice.[9,10] With this assay, we and a few other groups showed that only basal cells in human and rodents are capable of regenerating prostate glands.[9–13] One group reported that cells from castrated mice that display a luminal cell phenotype can also regenerate.[14]

Subsequently, our group used a lineage tracing approach to further investigate *in vivo* and *in situ* how adult prostate epithelial homeostasis is maintained.[2] We used a K14-CreER and a K8-CreERT2 model,[15] in combination with a fluorescent reporter mouse line, to specifically label prostate basal and luminal cells, respectively. We followed the fate of the labeled cells under physiological conditions, as well as under conditions when acute regeneration is induced by androgen deprivation and replacement. Surprisingly, we found that adult murine prostate basal and luminal cells are self-sustained independently. This result raises the question of whether the regenerative capacity of the basal cells in the prostate regeneration assay represents an obligate function of the prostate basal cells in adults *in vivo*.

The study by Ousset *et al.*[1] provided novel insights for this. The major focus of this study was to examine how prostate epithelia develop at the postnatal stage. Upon birth, male fetal prostate epithelial cells have undergone substantial lineage commitment. Many cells are phenotypically either K5/K14 positive basal cells or K8 positive luminal cells. There are also some intermediate cells that express both basal and luminal cell markers. Ousset *et al.*[1] used a K14rtTA/TetOCRE/RosaYFP triple transgenic model through which basal cells can be specifically marked with yellow fluorescent protein upon doxycycline induction. The authors treated newborn mice with doxycycline and demonstrated that only K14-expressing basal cells were labeled with YFP. Interestingly, after a 4-week chase, they identified YFP-expressing basal, luminal and neuroendocrine cells, demonstrating that the newborn fetal basal cells can generate all three different cell lineages. The authors obtained the same results when they performed similar lineage tracing on the K5-expressing basal cells using a K5CreER/RosaYFP bigenic model that enables fluorescent labeling of K5-expressing basal cells upon tamoxifen induction.

By titrating doxycycline or tamoxifen, the authors were able to label and trace basal cells at clonal levels. They found that while some fluorescently labeled basal cells can generate foci that contain both basal and luminal cells, other foci contained only basal cells or luminal cells. This observation suggests that at this stage some basal cells have already committed to become unipotent basal or luminal progenitors. The other important conclusion procured from this experiment is that intermediate cells were only found in foci

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containing both basal and luminal cells, which suggests that they are strongly associated with basal-luminal differentiation.

Finally, the authors utilized a K8CreER/RosaYFP bigenic model to label K8-expressing luminal cells in newborn fetuses or 2-week-old pups and determined their fate after a 4-week chase. Interestingly, they found that even at these stages luminal cells are unipotent, and can only generate luminal cells.

Collectively, this study demonstrated that at the postnatal developmental stage, some prostate basal cells possess multilineage differentiation capacities. They may give rise to luminal cells through the generation of unipotent luminal progenitors, or by a linear differentiation scheme through K5 and K8 dual positive intermediate cells. In contrast, luminal cells have largely completed lineage commitment at this stage and only possess unipotent potential. Interestingly, our previous study showed that in adult prostates, both basal cells and luminal cells are independently self-sustained by either unipotent progenitors or by self-duplication.[2] These studies imply a developmental stage-dependent switch of the mechanisms for epithelial maintenance, which has been observed previously in the mammary gland.[3,4]

It is reasonable to hypothesize that the stromal environment plays a critical role in this process. Neonatal prostate stromal cells may differ from adult prostate stromal cells in that they can provide signals that sustain the multipotent potential of basal cells. This is in agreement with previous studies showing that embryonic urogenital sinus mesenchyme can reprogram adult murine prostate basal cells and restore their capacities for multilineage differentiation.[9–13]

One caveat for this study[1] as well as other studies using rodents as model systems is that fundamental differences between human and rodent exist in spite of many microscopic similarities between their glandular structures. Most notably, human prostate basal cells form a continuous layer and form tight junctions between each other. In contrast, rodent prostate basal cells form a punctuated layer.[16] Therefore, there is a possibility that knowledge obtained from rodent studies may not always apply to the human condition.

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