Amplified luminal progenitors in prostates of prolactin-transgenic mice

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The epithelial cell hierarchy in the prostate remains insufficiently understood and is an area of active research. Evidence reported by different groups has proved to be highly dependent on the model chosen and/or the methodological approach employed. In addition, pathophysiological conditions such as varying developmental stages, inflammatory status, or regeneration after androgen deprivation appear to greatly impact epithelial cell lineage plasticity. In a recent report, we have described the amplification of basal/stem and putative luminal progenitor cells in adult Pb-PRL mice (a mouse model of prostate-specific prolactin expression). These two epithelial populations are proposed players in prostate cancer initiation/progression, and thus represent attractive research targets for this field. Because they present increased prevalence of basal/stem and luminal progenitor cells, Pb-PRL mouse prostates offer a useful resource and allow for the characterization of these populations in vivo and functional testing ex vivo/in vitro. Establishing the underlying mechanisms of basal/stem and luminal progenitor cell amplification in Pb-PRL mice are current research aims in our laboratory, involving possible paracrine factors secreted in response to prolactin/Stat5 signaling in luminal cells and/or microenvironmental stimuli from the dense stromal compartment of Pb-PRL prostates.

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conditions, such as inflammation, might lead to the reactivation of lineage plasticity observed during developmental stages [7].

It is logical to speculate that if basal in origin, the progression of prostate cancer should rely on the differentiation of the transformed basal cell into a luminal progenitor allowing for the formation of a luminal-like tumor. On the other hand, if the cell-of-origin of the tumor were luminal, then the presence of a transformed luminal progenitor would seem essential for tumor initiation. In both scenarios, luminal progenitors appear key to prostate tumorigenesis. Unfortunately, there is very little information in the literature about luminal progenitors in the prostate epithelium, let alone their role in prostate tumorigenesis. In human tumors, luminal progenitors have been proposed to promote prostate cancer progression [8]. In mice, M. Shen and C. Abate-Shen have described the presence of castration resistant Nkx3.1+ (CARN) cells, which are luminal cells that survive androgen-deprivation and can regenerate the prostate gland upon restoration of testosterone levels [9]. These data indicate that androgen-independent luminal progenitors may be present in the prostate in physiological conditions. In agreement, androgen-independent luminal cells have recently been reported to be present in human prostate tumors by the group of G. Risbridger [10].

For several years, our laboratory has studied the effects of local prolactin (PRL) expression in the pathophysiology of numerous PRL target tissues, including the prostate [11-14]. Prolactin is a protein hormone produced in the anterior pituitary and secreted into the circulation as an endocrine hormone of which its best known function is the promotion of lactation in the mammary gland [15]. However, extra-pituitary expression of PRL is now well established and autocrine roles of this hormone continue to be described in different tissues [16]. In the prostate, expression of PRL and activation of its main signaling molecule Stat5 (signal transducer and activator of transcription 5) have been correlated to prostate cancer progression and recurrence [17-21]. To understand the effects of PRL/Stat5 signaling in the prostate, we have used a PRL-transgenic mouse model where PRL expression is driven by a short fragment of the probasin (Pb) promoter, which is prostate-specific. As initially reported by Kindblom et al. [22], local PRL expression leads to prostate tumors exhibiting hyperplastic epithelial and stromal compartments and prostate intraepithelial neoplasia (PIN). Our initial studies demonstrated a clear amplification of the p63+ basal/stem cell compartment in the prostate epithelium of Pb-PRL mice, illustrated by the accumulation of clustered basal cells displaying abnormal morphology (round shape and positioned perpendicularly to the basement membrane) [23]. In a recent article published in The American Journal of Pathology, we have reported further evidence of this amplification using additional basal/stem cell markers, such as CK-5 and the FACS markers alpha6-integrin (CD49f) and stem cell antigen 1 (Sca-1) [24]. These FACS markers characterize a prostate cell subpopulation shown to contain basal cells displaying self-renewal properties, called “LSC” cells for Lin−Sca-1+CD49fhigh [25, 26]. Using immunohistological stainings, we have further shown that the epithelial regions containing clustered basal cells in our Pb-PRL mouse model very frequently displayed high Sca-1 staining and presence of double positive CK-5+CK-8+ cells (assumed to represent intermediate stages of differentiation of a basal cell into a luminal cell) [24]. We have interpreted these findings as signs of differentiation of the amplified basal cells into the luminal lineage. Given that the Pb promoter is androgen-regulated, and hence supposed to be activated only after the onset of puberty, the expression of PRL in Pb-PRL mice is expected to begin once prostate development has been accomplished. Thus, it would seem that PRL could induce basal-to-luminal differentiation in non-developmental stages of prostate physiology. However, further evidence is necessary to verify this conclusion and to explore the possible underlying mechanisms.

Most unexpectedly, our FACS analyses of Pb-PRL and wild-type mouse prostates have led us to the finding of a new luminal subpopulation, which are Sca-1+ cells, seemingly representing luminal progenitors (mature luminal epithelial cells do not express Sca-1) [24]. In FACS profiles, this population displays Lin−Sca-1+CD49fmed staining and to the best of our knowledge had not been described in any of the mouse models studied so far. We have named these cells "LSC-med", in comparison to the previously described and above-mentioned LSC (basal/stem) population, which we now refer to as "LSC-high". As observed for basal/stem cells, the LSC-med population also was amplified in Pb-PRL prostates compared to wild-type controls (representing about 10% of Lin− cells versus 2%, respectively). We have characterized LSC-med cells and shown that they expressed the luminal cytokeratin CK-8 but not the basal cytokeratin CK-5. The capacity of LSC-med cells to generate spheres was tested in 3D culture conditions in a sphere assay. This functional test has been used for many organs to evaluate stem-like properties in cell populations [27]. Compared to LSC-high cells, LSC-med cells generated very few spheres but of larger sizes, suggesting a lower but certain degree of stemness. Most importantly, LSC-med cells were able to differentiate into Sca-1− (mature) luminal cells when stimulated with dihydrotestosterone in colony culture. In prostate tissue slides of Pb-PRL mice, Sca-1+ luminal cells were positive for the proliferation marker Ki-67 at a significantly higher frequency than basal cells and Sca-1−...
luminal cells [24]. These data have led us to conclude that the development of prostate tumors induced by PRL might depend on the amplification/appearance of these rapidly proliferating luminal progenitors. It is currently not clear whether LSC-med cells and CARN cells represent the same luminal progenitor population. Unfortunately there are no expression markers that allow sorting of CARN cells by FACS to permit their characterization. Our ongoing research aims to establish whether LSC-med cells are resistant to castration, and whether they express the transcription factor Nkx3.1.

Given the amplification of LSC-med cells, Pb-PRL mouse prostates offer a valuable source of these luminal progenitors to allow for their characterization in terms of tumor initiation and progression (the prevalence of LSC-med cells in wild-type prostates being too low to obtain a sufficient amount of material for functional assays, see above). On this subject, our current research aims include i) to establish whether LSC-med can be cells-of-origin of prostate cancer; ii) given that Sca-1 has no known human homolog [28], to determine the expression of other prostate markers allowing for the search for LSC-med cells in normal prostate tissue and/or prostate tumors; iii) as mentioned above, test whether LSC-med cells are resistant to castration and if they can regenerate the prostate epithelium upon androgen restoration.

From a different but complementary perspective, our interests rely on paracrine interactions that may underlie the amplification of the basal/stem cell compartment in response to PRL stimulation and Stat5 signaling. Indeed, it was evident from our analysis of Pb-PRL prostates that activation of Stat5 was absent from the basal layer of the prostate epithelium [23, 24], indicating that these cells might not be direct targets of PRL stimulation. Given that only the luminal layer (including LSC-med cells) displays Stat5 activation in Pb-PRL mice, it remains to be established whether LSC-med cells originate through basal-to-luminal differentiation from the amplified basal cells, or if active Stat5 in mature luminal cells could induce the expression of Sca-1 in these cells and thus promote their becoming LSC-med (Figure 1). We are unaware of any examples in the literature of in vivo de-differentiation of luminal cells into less mature cells exhibiting features of luminal progenitors. In contrast, evidence for basal-to-luminal differentiation has been reported in conditions of inflammation [27] or PTEN deletion [16]. We thus tend to favor the hypothesis that LSC-med cells originate through an induction of basal-to-luminal differentiation of basal cells in response to PRL stimulation. This has led us to propose the existence of paracrine mechanisms (yet to be elucidated) through which the activation of Stat5 in the luminal layer (or other non-epithelial compartment) will lead to the secretion of (an) unknown paracrine factor(s) able to stimulate basal/stem cell amplification and differentiation (Figure 2A). Some of our current research thus focuses on unveiling these possible paracrine mechanisms. We have not yet established clearly whether Stat5 is activated in the stroma of Pb-PRL prostates, and thus this complex compartment (consisting of smooth muscle fibers, fibroblasts, and endothelial, immune, and neural cells [29]) remains a possible source of paracrine messengers that might participate in basal/stem cell amplification in response to PRL (Figure 2A). Of note, Pb-PRL prostates display mild inflammation [22], which we have started to characterize [30]. In addition, since mounting evidence shows that activated fibroblasts secrete important cancer-promoting signals [29, 31, 32], these cells remain an attractive and plausible origin for paracrine factors participating in the observed Pb-PRL epithelial phenotype.
It is interesting to note that the activation of Stat5 and the expression of stem-like characteristics appear to follow an opposite trend in the epithelium of Pb-PRL prostates, as exemplified in Figure 2B, where stem-like properties are mostly present in the basal compartment and decreasing towards LSC-med luminal progenitors, whereas Stat5 is clearly present in luminal cells, and even intermediate cells, but never in the basal cell layer. A possible link between Stat5 activation and pro-differentiation signals (signifying the absence of stem-like properties) has been reported for breast epithelial cells/tumors [33, 34]. Whether this is also true for prostate epithelial cells, and what the implications might be remains to be determined.

In summary, our recent findings have demonstrated the existence of Sca-1+ luminal cells that we have named LSC-med cells and that we propose to represent luminal progenitors [24]. Further research on these cells' functional properties and on their role in prostate tumorigenesis is warranted. In addition, paracrine interactions among epithelial and/or stromal cell types will help establish the mechanisms of amplification of basal/stem cells and LSC-med cells in response to local PRL stimulation.

Conflicting interests

The authors declare that they have no conflicting interests.
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