Review of: "Single-cell atlas of epithelial and stromal cell heterogeneity by lobe and strain in the mouse prostate"

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Potential competing interests: The author(s) declared that no potential competing interests exist.

Comments on the manuscript:

“Single-cell atlas of epithelial and stromal cell heterogeneity by lobe and strain in the mouse prostate”

Introduction to the review

Already Galen (129-209 or 131-216) carried out experiments in animals by destroying this or that part of the brain, demonstrating its central role in the coordination of organs. Among the fields requiring this type of method, physiology has often had recourse to amphibians or mammals. At the end of the 19th century, to understand the causes of embryonic development, the first models were often marine animals such as sea urchins or ascidians, the choice of which responded not only to scientific criteria but also to practical criteria linked to the artificial reproduction of species whose external fertilization could be carried out under microscopic control. Amphibians, common in ponds and whose tadpoles can be taken without difficulty, have also often been used, as have birds, in particular *Gallus domesticus*, whose eggs are easy to obtain in barns. These models have made it possible to understand the mechanisms regulating development. Another model, *Drosophila melanogaster*, first used to understand mutations and then the laws of population genetics, was introduced by Morgan in the 1910s.

Animal models have been or are also used to examine the effects of pharmaceutical or toxic products, cosmetics or to examine the effects of a particular pathology, which concerns the article presented here.

The use of animal models is now part of the methodological arsenal of biomedical research laboratories. I would dare to say that its use has become classic. During research requiring an animal model, the first question that arises is the choice of the model, which must be the right animal of the right sex and at the right stage (embryonic, juvenile, adult) depending on the question to be studied. Over the decades, these models have multiplied, in order to respond more and more precisely to the questions asked. Alongside first wild and then farmed animals, the use of the animal model has taken on a new dimension with transgenic individuals since the 1980s. Directly inherited from spontaneously mutant strains and powerful molecular biology methods, they allow to directly address the effects of one or more genes, either by suppressing this or these, or on the contrary by causing their overexpression.

To choose the right model, it is necessary to know perfectly the microscopic tissue anatomy in order to appreciate the modifications related to pathological and physiological factors. For this, histological techniques have provided increasingly

efficient staining and morphometry methods. Today the anatomical, histological, cytological structure is no longer enough to characterize a tissue, it is necessary to examine the distribution of genes in the different cells to have a detailed view of the phenomena. For this, it is necessary to highlight common genes but also characteristic genes and the choice of the gene whose expression is sought arises and it is not so simple.
The work presented here follows this logic. Reading this rich publication led me to appreciate this work which could be a model of its kind.

Review

The manuscript is divided into an introduction, the results themselves divided into five paragraphs and conclusions. The materials and methods are specified at the end of the publication. There are 60 bibliographical references. Results are expressed on five figures.

Introduction

In the introduction, the others recall that animal models are important for conducting research in biology and particularly in the biomedical field. Today there are many animal models and for any research, the choice must be made on the best animal model suitable for research, which requires above all to know the model well. As said above, the work presented here falls within this logic and concerns two strains of mice particularly dedicated to the study of prostate cancer.
First, it is important to give an anatomical description of the organ concerned, here the prostate, with a mapping of the cells in the different lobes. Today, the development of molecular biology and gene databases makes it possible to establish an extremely fine mapping of cells by highlighting the genes or their expression products at the level of the latter.
However, animal models possess anatomical differences with human beings. The authors thus describe the structure of the mouse prostate which, like most rodents. These differences have been observed in mice and other common rodents used as models, but also in animals living in the wild in different areas, such as the desert lizard *Gerbillus tarabuli* (https://pubmed.ncbi.nlm.nih.gov/28162243/), and consists of four lobes instead of three in humans.
I can only completely agree with the authors who insist on the importance of animal models in the study of prostate cancer - and I will add cancers and other pathologies in general, provided however that choosing the right model, which has been done for prostate cancer (see ref 1 of the manuscript: https://pubmed.ncbi.nlm.nih.gov/23610450/)
The models themselves have differences. In the manuscript, the authors describe them as follows in the two models chosen: the strains Hi-MYC and Lo-MYC mouse models whose potential lesions have been precisely localized. These important differences are at the level of the various parameters of prostate cancer in the different mouse models chosen (progression of the cancer, duration of survival, etc.) This is how they analyzed in depth the FVB/ N.J. (http://www.slate.com/articles/health_and_science/the_mouse_trap/2011/11/black_6_lab_mice_and_the_history_of_biomedical_research.html)and the C57BL/6J (http://www.slate.com/articles/health_and_science/the_mouse_trap/2011/11/black_6_lab_mice_and_the_history_of_biomedical_research.html; https://www.jax.org/strain/001800) line(https://www.gempharmatech.us/en/humanization-mouse-models/?gclid=EAIaIQobChMItOqcsK3A9gIVjal3Ch0PswbmEAAYASAAEgLdS_D_BwE#?

utm_term=c57bl%2F6&utm_campaign=Gem-Europe&utm_source=adwords&utm_medium=ppc&hsa_acc=5410931798&hsa_cam=15425842040&hsa_grp=130703098255&hsa_ad=565415088139&hsa_src=g&hsa_tgt=kwd-916967341172&hsa_kw=c57bl%2F6&hsa_mt=p&hsa_net=adwords&hsa_ver=3, and others…) commonly used for biomedical research.

Results and discussion

This part is divided into five paragraphs and a conclusion.

1. Delineation of mouse prostate cell types reveals significant lobe and strain-specific differences in epithelial compartments

The first work consisted in dissecting the lobes of the two selected strains and describing with precision the histology of each of the four lobes. Histological characteristics were thus highlighted.

To characterize all the components (genes characteristic of intermediate filaments, fibroblasts, endothelial and immune cells, smooth muscle and pericytes), genetic markers were used from a library of single-cell RNA sequencing (scRNA-seq) prepared for each strain and each prostatic lobe. These different genes are expressed differently depending on the strains. Similarly, the authors demonstrated that the transcription profiles of epithelial cells were distinct from stromal cells (which seems obvious, but still had to be demonstrated).

2. Distinguishing features of mouse prostate lobes in luminal epithelial compartments that 154 are conserved between strains

Differences between cell types and lobes are observed at the same time in each of the mouse lines. The authors show that there are subtle differences in the expression of several genes encoding proteins involved in several physiological mechanisms, such as *AY761184*, whose protein is involved in the antimicrobial defense system, or*C1s2* and *Mt3* reported in two genes. expression signatures based on Gleasn score in human prostate cancer.

3. Rare luminal epithelial populations

In addition to cells characteristic of each of the lobes, there are rare cells expressing characteristic genes: endocrine cells, luminal cells and progenitor-like cells. Here too, particularly subtle differences were demonstrated in these cells present in the two strains of mice studied.

4. Stromal cell populations are primarily conserved across lobe and strain

Stromal cells (smooth muscle, pericytes and fibroblasts) and immune cells grouped by type are observed in the lobes of both strains. Two distinct populations of fibroblasts, subglandular fibroblasts and interstitial fibroblasts appear on examination, and even closer examination of non-epithelial cell groups reveals yet other subtypes. A distribution of immune cells including macrophages, T cells, and mast cells, across the different lobes has been demonstrated. Here too, there are subtle differences between the lobes for each of the two lines of mice.

5. Two fibroblast populations with distinct molecular features and localization in mouse 275 prostate

Two types of fibroblasts characterized by their genetic profiles have been highlighted, “prostatic” fibroblasts which could be the equivalent of the interstitial fibroblasts of the human prostate and “ductal” fibroblasts which would be the equivalent of glandular fibroblasts. Although the urethra is not the subject of our research, "urethral" fibroblasts are certainly very rare or even absent, but this is a hypothesis.

Conclusions

At the end of this study on the prostate of two mouse strains used as models, hitherto uncharacterized cell types were discovered, molecular markers characteristic of several cell types were identified, which allows a very fine *in situ* examination of the prostate tissues. Powerful scRNA-seq analysis of normal mouse prostates revealed lobe- and strain-specific differences at the epithelial cell level, which may be related to morphological differences in prostate lobes in mice. That basal and luminal epithelial cells are characteristic of the mouse strain used does not appear to have been reported in previous studies. These very marked differences in gene transcription that are observed between basal and luminal epithelial cells in the two strains may warrant strain-specific clustering.

The observation of these differences observed in the mouse prostate suggests that there are also interindividual differences in the luminal and basal cells of the human prostate, which may be linked to a pathological risk. The histological differences that exist between the lobes of the prostate result both from the differences observed at the level of the luminal cells and from the composition of the stroma.

Stromal cell types and their distribution are conserved across both mouse strain and prostatic lobe. In the wild type, no difference in the composition of immune cell types is observed between the two strains of mice studied.

Materials and Methods

The materials and methods are described in detail. Several powerful methods were used to determine with the greatest possible precision the nature of the different cells and their distribution in the prostate lobes of the two models.

I will, however, make two remarks. The first concerns the maintenance of mice in rearing until they reach 6 months (**Mouse models and prostate dissectio**n): what were the rearing conditions? are indeed important and can have repercussions on the health of the animals Have there been examinations of their state of health?

the second concerns **Single-cell RNA-sequencing and data pre-processing**. “Libraries for scRNA-seq were prepared using the 10x Genomics Chromium Single Cell 3’ Library and Gel bead Kit V2 (CG00052_RevF) (https://assets.ctfassets.net/an68im79xiti/RT8DYoZzhDJRBMrJCmVxl/6a0ed8015d89bf9602128a4c9f8962c8/CG00052_SingleCell3_ReagentKitv2UserGuide_RevF.pdf?) according to the manufacturer’s protocol for each dissected prostate lobe.": as this technique is the basis of a large part of the work and is therefore fundamental for this research, it would seem useful to me that the protocol be recalled here with any adaptations to the material studied.

Figures

The many figures are essential. They illustrate the text, showing the results obtained. Even if these figures are particularly dense, they are useful for the follow-up of this particularly coherent study.

Figure 1B: A few explanations of the different structures found in the different parts of the prostate would seem useful to me, especially for the reader less familiar with this organ.

Conclusions of review

In conclusion, I would say that this well-conducted study provides essential elements for a detailed knowledge of the anatomy of the prostate of two mouse models used in biomedical research. This work is welcome because, in the current state of knowledge, it has become urgent to know more and more precisely the animal models that are used. The current development of the possibilities offered by molecular biology makes it possible to map at the finest level, i.e. at the level of the presence and expression of genes, the components of the organs studied, here the prostate. This work shows the differences that may exist between the prostates of two strains of mice used as models and their differences with human prostates which, even if the anatomy is different, have equivalent structures.

This useful and well-done work is well worth publishing after some suggested improvements.