ORIGINAL ARTICLE

Prostate anatomy in motheaten viable (meY) mice with mutations in the protein tyrosine phosphatase SHP-1


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KEYWORDS
Prostate; Anatomy; Differentiation; SHP-1; Motheaten viable mouse

Abstract
Objective: To study prostate and seminal vesicle anatomy in viable motheaten (meY) mice with mutations in the PTPN6 gene leading to a severe reduction in the activity of protein tyrosine phosphatase SHP-1. Homozygous meY mice exhibit multiple anomalies that include immunodeficiencies, increased proliferation of macrophage, neutrophil, and erythrocyte progenitors, decreased bone density and sterility.

Materials and methods: We analyzed macro- and microscopic anatomy of the seminal vesicle and prostate macro- and microscopic anatomy of 5 meY/meY and 8 wt/wt adult 7-week-old mice. Computerized morphometric analysis was performed to measure the relative changes appearing in the epithelial volume of the different prostatic lobes.

Results: All mice studied revealed normal genital organs (penis, testis, epididymis, vas deferens) and bladder. The seminal vesicle was absent in all meY/meY individuals analyzed, being normal and very noticeable in wt/wt mice. The different glands that compose the prostatic complex (anterior, ventral and dorso-lateral prostate) were atrophied in meY/meY mice: anterior prostate 0.4 times, ventral 0.19 times, dorsal 0.35 times and lateral 0.28 times those of the respective regions in wt/wt mice. Microscopically, meY/meY mice revealed scarce and large prostatic ducts, acini severely atrophic with empty lumen and scarce loose epithelial component forming tufts and infoldings, and hyperplastic changes in fibromuscular stroma.

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Conclusions: The prostate of me"/me" mice exhibits signs of aberrant differentiation and the resulting phenotype may be related to the loss of function of SHP-1. Prostatic anomalies in these mice affect, together with defects in sperm maturation, their sterility. These data suggest that SHP-1 plays an important role in prostate epithelial morphogenesis.

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Anatomia de la próstata en ratones motheaten viable (me") con mutaciones en el gen de la proteína tirosina fosfatasa SHP-1

Resumen

Objetivo: Estudiar la anatomía de la próstata y la vesícula seminal en ratones motheaten viable (me") con mutaciones en el gen PTPN6 que conlleva una severa reducción en la actividad de la proteína tirosina fosfatasa SHP-1. Los ratones me" homocigotos muestran múltiples anomalías que incluyen inmunodeficiencias, aumento en la proliferación de macrófagos, neutrófilos y progenitores de eritrocitos, disminución de la densidad ósea y esterilidad.

Material y método: Se analizó la anatomía macro y microscópica de la vesícula seminal y de la próstata, tanto a nivel macro como microscópico, de 5 ratones me"/me" (homocigotos me") y 8 ratones wt/wt (tipo salvaje) adultos de 7 semanas. Se ha realizado análisis morfológico computarizado para medir cambios relativos en el volumen epitelial de los diferentes lóbulos prostáticos.

Resultados: Todos los ratones estudiados mostraron órganos genitales (pene, testículos, epididímos, deferentes) y vejiga normales. La vesícula seminal se encontraba ausente en todos los ejemplares me"/me" analizados, siendo normal y muy llamativa en ratones wt/wt. Las diferentes glándulas que componen el complejo prostático (próstata anterior, ventral y dorsolateral) se encontraron atroficas en ratones me"/me": próstata anterior 0.4 veces, ventral 0.19 veces, dorsal 0.35 veces y lateral 0.28 veces el tamaño de las respectivas regiones en ratones wt/wt. A nivel microscópico los ratones me"/me" mostraron ductos prostáticos mayores y escasos, acinos severamente atroficos con luces vacías y escaso y suelo componente epitelial formando penachos y pliegues, y cambios hiperplásicos en el estroma fibro muscular.

Conclusiones: La próstata de ratones me"/me" muestra signos de diferenciación aberrante y el fenotipo resultante puede estar relacionado con la pérdida de función SHP-1. Las anomalías prostáticas en estos ratones influyen, junto con los defectos de la maduración espermática, en su esterilidad. Estos datos sugieren que SHP-1 desempeña un importante papel en la morfogénesis epitelial prostática.

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Introduction

Phosphorylation is one of the main changes that proteins undergo after being synthesized. It has been estimated that between 30 and 50% of the proteins in eukaryotic organisms can be phosphorylated. This change is directly involved in the control of different cellular processes such as: migration, proliferation, apoptosis, differentiation, metabolism, immunity, learning, and memory. Therefore, it is not surprising that changes in protein phosphorylation patterns have been associated with cancer, diabetes, and inflammatory and neurodegenerative illnesses. The phosphorylation level of a protein is the result of the activity of 2 types of enzymes: protein kinases in charge of phosphorylation and protein phosphatases responsible for dephosphorylation.

Within the phosphatase group is the SHP-1 protein, codified by the PTPN6 gene, which is mainly expressed in hematopoietic and epithelial cells. The existence of 2 strains of mice with mutations affecting this phosphatase gene has enabled us to know its regulatory role. These 2 strains are called motheaten (me") and motheaten viable (me"). Their genetic analysis has revealed that those me mice lacked SHP-1, whereas the me" ones expressed a deficient form of the enzyme with an activity of 20%. The homozygous mice (me/me or me"/me") showed hematopoietic abnormalities which considerably shortened their lives (2 to 3 weeks for the former ones and 9 to 12 for the others). These mice suffered from extramedullary hematopoiesis, splenomegaly, dermatitis, and hemorrhagic pneumonitis. Alterations of the lymphoid ontogeny were observed, which led to chronic inflammations and a picture of systemic autoimmune disease evidenced by hypergammaglobulinemia, the presence of autoantibodies and tissue damage caused by the presence of circulating immune complexes. Natural killer (NK) lymphocytes and cells of the erythroid line were also altered. The analysis of all these alterations, obviously due to the absence or defect in SHP-1, has demonstrated that this phosphatase is a negative regulator of multiple signals in hematopoietic cells, including those activated by interleukins, growth factors, adhesion and immunoreceptors and it has enabled us to classify SHP-1 as a potential tumor suppressor gene. Loss or reduction of SHP-1 has also been observed in various kinds of lymphomas. 
and leukaemias. All of the above has led to consider SHP-1 as a tumor suppressor gene.

SHP-1 is also expressed in epithelial cells which are present in the ovary, exocrine pancreas, breast, thyroid, and the prostate. Although the studies conducted on these cell types are less abundant, emerging data show that SHP-1 is also going to be a key element in the regulation of processes activated by growth factors, neuropeptides, and cytokines. Our research group was the first to demonstrate the presence of this enzyme in the ventral prostate of a rat, in the human prostate tumor cell lines PC3 and LNPCA, and in the human prostate. We have also proven that in PC3 cells the somatostatin neuropeptide inhibits cell proliferation by increasing SHP-1 activity and its expression.

The studies performed with motheaten and motheaten viable mice were mainly confined to cells of the hematopoietic line. Therefore, the aim of this work was to conduct a histological analysis of the genitourinary system of male me" mice.

**Materials and methods**

The me" (C57BL/6J-Ptpn6<sup>me</sup>) mutant heterozygous mice were provided by The Jackson Laboratory (Bar Harbor, Maine, USA). The me"/<sup>me</sup> mutant homozygous mice as well as the +/- wild ones used as controls were obtained by mating of the me"/+ heterozygotes. All of the mice were reared in the animal facility at the University of Alcalá (Alcalá de Henares, Madrid) following the regulatory protocols for animal care and husbandry of the Community of Madrid. Eight wild mice (wt/wt) and 5 adult homozygous mice me"/me" were slaughtered at 7 weeks of age in order to study the gross and microscopic anatomy of the prostatic and seminal complex. Genotypes at the PTPN6 locus were determined by PCR to verify the wt/wt and me"/me" phenotypes. The primers used were: 5′-GTT ATT GAA CAA GGA CCA AGG-3′ (common), 5′-GAG GTG GAG AAA GGC CGG GT-3′ (wild) and 5′-GAG GTG GAG AAA GGC CGG GA-3′ (me"). These primers, as well as the protocol suggested, were those proposed by The Jackson Laboratory.

The animals were slaughtered using light anesthesia with ether. Anatomical dissection was performed following the recommendations of the *Bar Harbor Pathology Workshop*, the extraction of the genitourinary block and its gross dissection being carried out with the help of a dissecting microscope. The extracted genitourinary block contained the prostate lobules, the seminal vesicles (S), the ampullary glands (A), the proximal vas deferens (D), the bladder (B), and the proximal urethra (UR). The individual dissection of the mouse’s different portions or prostate glands enabled us to separate: the anterior prostate or coagulant gland (CG), ventral prostate (VP), dorsal prostate (DP), and the lateral prostate (LP) (Fig. 1). Each glandular portion of each animal was independently labeled. A histological evaluation of the genital structures was carried out in each individual, including the vas deferens, epididymis, and the testis. The status of spermatogenesis in the tubular component and Leydig cell density in the testicular interstitium were qualitatively assessed.

Figure 1  Anatomical schema showing the relationship between the accessory reproductive glands of the mouse: seminal vesicle (S), coagulant gland or anterior prostate (CG), ventral prostate (VP), dorsal prostate (DP), lateral prostate (LP), deferens (D), ampulla (A), bladder neck (BN) and urethra (U). The dorsal and lateral prostates form a single unit.

Samples were fixed in buffered formalin. The material was dehydrated in alcohols with an increasing concentration and stained with the conventional hematoxylin-eosin technique in 4-mm serial cuts. It was microscopically assessed and a computed morphometric analysis was performed using an optical microscope connected to a computerized image capture system (Leica Qwin 3.0). 10 measurements of each prostate area were taken in each individual. Data were submitted to variance analysis using the Statistical Analysis System program, 2004 (SAS). Differences between the averages were submitted to the Tukey test using the General Linear Models procedure (GLM).

**Results**

At the macroscopic level, all the mice analyzed showed normal urinary bladder and genital organs (penis, testicles, epididymis, and vas deferens) at the time of dissection. However, the studied homozygous mice me"/me" lacked seminal vesicles (S), unlike the wild-type mice wt/wt which did show these organs on both sides of the bladder (Fig. 2). The me"/me" mice also lacked the ampulla (A) or ampullary gland.

The histological analysis of the different prostate glands was then performed, firstly describing that of the wild mice +/- . The scattering lobes which make up the prostate gland complex are separated by a thin capsule covered with
mesothelium which can only be seen at a microscopic level. They consist of a lattice of ducts in the shape of branches, surrounded by a thin fibromuscular tunic. The portions or prostate glands showed histological differences. The dorsal prostate is covered by a simple columnar epithelium, sometimes slightly stratified, with glandular lights containing homogeneous eosiophilic secretions. The lateral prostate is quite similar to the posterior one, although it has a somehow flatter epithelium and larger luminal space. The coagulant or anterior prostate is intimately attached to the seminal glands in its concave portion. Histologically, it shows a more papillary pattern with an epithelium ranging from cubic to columnar and profuse glandular secretion. The ventral prostate shows flatter luminal borders and profuse serous secretion.

All the prostate lobes and, in a more significant way, the ventral gland were atrophic in all the me+/me Cre mice (Fig. 3). At the anatomical level, the most notorious finding was the reduction in prostate size, which was non-proportional to the reduction in the body weight of the me+/me Cre mice. The morphometric calculation evidenced that the anterior prostate was 0.4 times, the ventral prostate 0.19 times, the dorsal prostate 0.35 time, and the lateral one 0.28 times the size of the respective regions in C57BL/6J wt/wt mice (Fig. 4 and Table 1). From a histological point of view, the prostatic ducts were scarce and larger, and the acini were severely atrophic with empty lumen and scarce loose epithelial component forming tufts and infoldings. The fibromuscular stroma showed evident hyperplastic changes in all prostate regions. At a testicular level, epididymal atrophy, reduced spermatogenesis, and a smaller number of Leydig cells in the testicular interstitium were also confirmed.

Discussion

The mice used in this study showed that the SHP-1 tyrosine phosphatase plays a significant role in the morphogenesis of the genitourinary system.

me Cre mice, along with motheaten mice, are normally used as models of autoimmune and inflammatory diseases and have enabled us to decipher the involvement of this phosphatase in these diseases. Other alterations detected in these mice were the following: decreased thymus size, increased spleen size due to greater eritropoyesis and mielopoyesis, glomerulonephritis, an increasing number of pulmonary mast cells associated with allergic asthma, greater tolerance to glucose and sensitivity to insulin, retinal degeneration, osteopenia, and increasing bone resorption indexes. SHP-1 is also important when controlling the glial activation of both a normal and damaged nervous system. This regulatory role of astrocytes and microglia has also been evidenced with the me Cre model. However, the morphology of the genitourinary system of these mice had not been analyzed so far. Our results showed that the absence of SHP-1 alters the anatomy and histology of the accessory sexual glands of the me Cre mice; the prostate showed signs of aberrant differentiation with marked atrophy of the prostate epithelial component,

![Image](image_url)

**Figure 2** Gross anatomy of the prostate, vesicle, testicles, and vas deferens in C57BL/6J mice of the wild type and me Cre homozygotes. Absence of seminal vesicles and a significant decrease of the vesicoprostatic complex can be observed in me Cre mice, although the vas deferens, testicles, and penis are normal.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Morphometric calculation of the relative surface ratio between the different prostate glands.</th>
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<tbody>
<tr>
<td></td>
<td>Anterior</td>
</tr>
<tr>
<td>C57BL/6J wt/wt mice</td>
<td>10.829 ± 1.791</td>
</tr>
<tr>
<td>me Cre/me Cre α mice</td>
<td>4.321 ± 0.845</td>
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<tr>
<td>Relative ratio (%)</td>
<td>39.90</td>
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* Values expressed as mean ± SD on magnification of fixed microscope.
of the prostate which derives from the urogenital sinus. It adds secretions to semen and contributes to fertility. There is no equivalent in human beings. Its epithelium is columnar and simple, and it is surrounded by a muscular stroma which is more dense than the rest of the prostatic ducts. We also observed atrophy of the epididymis and a decrease in spermatogenesis and in the number of Leydig cells.

Previous data in the literature had already proven that the testicles of these mice showed a decreased number of Leydig cells, spermatogenesis stopped at the phase of secondary spermatocytes, and testosterone levels were lower than in the controls; therefore, no mature sperm was produced and these mice were sterile. In order to try and re-establish spermatogenesis and recover the fertility of these mice, they were treated with testosterone pellets; although we managed to complete spermatogenesis, these mice continued to be sterile probably due to the alterations of the accessory glands described in this study. It is known that the growth and development of the prostate and the seminal vesicles takes place from the end of the fetal phase to the adult phase and requires the presence of androgens. Given that testosterone was administered to these mice at 3 weeks of age, it might have been too late to recover the prostate and the seminal vesicles.

At present, it is increasingly apparent that the prostate function does not only depend on androgens; growth factors, cytokines, and interactions between the epithelium and the stroma also control the development and the normal functioning of the prostate and other accessory glands through mechanisms which require proteins in tyrosine phosphorylation, so the absence of SHP-1 tyrosine phosphatase can affect all these processes and cause the alterations observed in mev mice.

Nowadays we know that SHP-1 is present in the human prostate and its levels decrease in more metastatic

Figure 3 Completely cut histological section of the prostate and seminal complex. Absence of seminal vesicles and a relatively decreased size of the prostatic structures, with normal urinary bladder, are confirmed in mev mice.

which more severely affects the ventral gland, and absence of seminal vesicles and ampullary gland. This gland is a diverticular derivative of the proximal vas deferens of a wolffian origin which contrasts with the origin of the rest

Figure 4 Histological changes between the different regions of the prostate gland in C57BL/6J mice of the wild type and mev homozygotes. Decreased glandular secretion, epithelial atrophy, which is greater in the ventral prostate, and fibromuscular hyperplasia of the stroma.
high-grade tumors\textsuperscript{14,15,27}; that the presence of SHP-1 is required for proper functioning of the cell cycle; that it regulates the action of cytokines\textsuperscript{18} and cell survival routes such as PI3K/Akt; and that this phosphatase is located in the nucleus of prostate cells, so that it is possible for it to regulate gene expression.\textsuperscript{29} All these mechanisms controlled by SHP-1 may be directly and indirectly responsible for the anatomical and histological alterations found in the development of the accessory sexual glands of me\textsuperscript{r} mice.

In view of all these findings, we can conclude that SHP-1 plays an important role, not only in the hematopoiesis process and in neurogliogenesis, but also in prostate epithelial morphogenesis. These findings suggest that SHP-1 in human beings could have an influence on the development and proper functioning of the prostate, and alterations in its activity and/or its levels could be the starting point of various pathological processes such as prostate cancer.

\textbf{Conflict of interest}

The authors declare that they have no conflict of interest.

\textbf{Funding}

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