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Transgelin: An androgen-dependent protein identified in the seminal vesicles of three Saharan rodents

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ABSTRACT

During the breeding season, a major androgen-dependent protein with an apparent molecular weight of 21 kDa was isolated and purified from the seminal vesicles of three Saharan rodents (MLVSP21 from *Meriones libycus*, MSVSP21 from *Meriones shawi*, and MCVSP21 from *Meriones crassus*). The 21-kDa protein was isolated and purified from soluble seminal vesicle proteins of homogenate by one-dimensional polyacrylamide gel electrophoresis (SDS-PAGE). Using polyclonal antibodies directed against $POSVP_{21}$ (*Psammomys obesus* seminal vesicles protein of 21 kDa), a major androgen-dependent secretory protein from sand rat seminal vesicles, identified previously as transgelin, we showed an immunological homology with $POSVP_{21}$ by immunoblotting. These three major androgen-dependent proteins with a same apparent molecular weight of 21 kDa designated as $MLVSP_{21}$ (*Meriones libycus* seminal vesicles protein of 21 kDa), $MSVSP_{21}$ (*Meriones shawi* seminal vesicles protein of 21 kDa), and $MCVSP_{21}$ (*Meriones crassus* seminal vesicles protein of 21 kDa) were localized by immunohistochemistry and identified by applying a proteomic approach. Our results indicated that the isolated proteins $MLSVP_{21}$, $MSSVP_{21}$, and $MCSVP_{21}$ seem to correspond to the same protein: the transgelin. So that transgelin can be used as a specific marker of these rodent physiological reproduction mechanisms.

1. Introduction

Several proteins secreted by the seminal vesicles [1–5], prostate[6–8], epididymis [9–11], and vas deferent [12] of adult males have been studied extensively as markers of testosterone action. It has been shown in several species of mammals that the majority of the secreted components from seminal vesicles are proteins that are rapidly synthesized in the presence of androgens [13].

In fact, androgens play an important role in the development, growth, and maintenance of differentiated functions of seminal vesicles [14,15], and the expression of some of these proteins is dependent on the presence of testosterone [16].

In addition, in order to be regulated by steroid sex hormones, seminal vesicle and prostate are dependent on reciprocal stromal/epithelial interactions mediated by several molecules such as growth factors [17,18].

In a series of investigations, the rodent has been proved to be a good experimental model for the study, and some of the protein components of rat and mouse seminal vesicle secretions have been identified in several species: for example, the guinea pig [19,20] and the mouse [21,22].

In the mouse, the seminal vesicles secrete a similar group of androgen-regulated proteins [4,22,23], and the mechanism by which testosterone regulates the expression of these proteins has been investigated. It has been shown

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that androgens acted mainly on gene transcription of a secretory protein of 99 amino acids (MSVSP99) and that this transcriptional regulation was exerted by steroid-receptor complexes interacting with enhancer sequences adjacent to the gene [24].

In the rat seminal vesicles, the physiological roles of the SVS proteins are still not yet well-defined; however, it has been proposed that the lower molecular weight SVS IV-VI proteins serve as proteinase inhibitors in the vaginal and uterine tract and help maintain a suitable environment for sperm motility and fertilization [25]. The SVSI-II proteins appear to be components of the rat copulatory plug, with the SVS II protein being the major component [26]. Clotting of the SVS II protein with itself and other SVS proteins is catalyzed by a transglutaminase that produces extensive covalent cross-linking between glutamine and lysine and the formation of γ-glutamyl-ε-lysine [27].

Finding the structure and function of these proteins has been attempted in order to understand their roles in seminal vesicle physiology and their effects on gamete activity.

We previously reported that in sand rat *Psammomys obesus* seminal vesicles, one major secretory androgen-dependent protein designated as $POSVP_{21}$ (i.e., *P. obesus* seminal vesicles protein of 21 kDa) was abundantly synthesized when the androgen level increased. It accounts for over 22% of soluble proteins from homogenate of seminal vesicles during the breeding season. During the nonbreeding season, very large part of this protein was greatly reduced. Gernigon et al. have reported that the expression of this protein is depended on the presence of testosterone [28].

More recently, this protein was purified and characterized. Its site of synthesis was determined and its peptidic sequence was analyzed. $POSVP_{21}$ was identified, such as transgelin [29,30].

The present study sought to expand our investigations to other Saharan rodents, such as Libyan jird *Meriones libycus*, *Meriones shawi*, and desert jird *Meriones crassus*. They are also characterized by a seasonal reproduction cycle.

Transgelin is a 22-kDa protein identified as SM22, WS3-10, or mouse p27. The DNA and protein sequences of SM22 protein are highly conserved across species, and homologues have been found in invertebrate species, as distant as *Caenorhabtidis elegans* (unc-87) [31] and *Drosophila melanogaster* (mp20) [32]. Transgelin, also named SM22α, is a cytoskeleton-associated protein that is expressed abundantly in smooth muscle tissues of normal adult vertebrates [33]. Its expression is one of the earliest markers of smooth muscle differentiation during embryogenesis [34].

Several investigations suggested that SM22α might play a significant role in differentiation or cellular senescence by stabilizing the cytoskeleton through actin binding. [35].

Recent studies reported that the transgelin identified in smooth muscles and blood vessels was also found in the fibroblasts and in some epithelia such as the intestinal epithelium, the epithelium of the mammary channel [36], the glomerular epithelium [37], and the prostate epithelium [38].

In this work, we explored the possible expression of transgelin in the seminal vesicles of three Saharan species: *Meriones libycus*, *Meriones shawi*, and *Meriones crassus*.

2. Materials and methods

2.1. Animals

The sand rat *P. obesus* is a diurnal herbivorous rodent that lives around wadis in the Sahara desert. The Libyan jird *Meriones libycus* and the desert jird *Meriones crassus* are nocturnal herbivorous and granivore Saharan rodents belonging to the Gerbillidae family. They live in a superficial burrow arranged under the largest bushes. So, they benefy of shade procured by plants [39].

The adult animals were trapped in the wild in the regions of Béni Abbès (30°07′N, 2°10′W) and M'sila (35°N, 4°E) (during the breeding season) and euthanized 24 to 48 hours later to study the proteins secreted by the seminal vesicles and other secretory organs of the male genital tract. Dissected tissues were fixed or stored at −80 °C for RNA or protein extraction.

2.2. Extraction of proteins from adult sand rat seminal vesicles

In normal adult males, proteins obtained either from secretions or from homogenized tissues were used. Frozen tissue samples were homogenized for 30 seconds at 4 °C in 2 or 4 mL buffer A, in a glass-glass handheld homogenizer (Braun, Melsungen, Germany). After centrifugation at 12,000 × *g* for 10 min at 4 °C, the supernatant fluid was retained and used as "homogenate."

The concentration of soluble proteins was determined by the micro-method procedure of Bradford assay [40].

2.3. Protein gel electrophoresis

One-dimensional (1D) electrophoresis was performed under denaturing conditions [41] and carried out on slab gels (140 mm × 120 mm × 1.5 mm) using a Biorad model 220; sodium dodecyl sulfate protein samples (100 μg) were applied to 15% resolving gels with 4.5% stacking gel and run at 20 mA at room temperature until the tracking dye (bromophenol blue) reached the bottom of the gels. The gels were then stained with 0.25% (wt/vol) Coomassie Brilliant Blue in an aqueous solution containing 50% (vol/vol) methanol and 10% (vol/vol) acetic acid for 45 minutes at room temperature and destained in a solution without dye. The apparent molecular weights of the proteins were calculated using the mobility of standard proteins as a reference.

2.4. Immunoblotting

Western blotting of polyacrylamide gels onto nitrocellulose sheets was carried out according to Towbin et al. [42]. After 1D electrophoresis, proteins were electroblotted onto a polyvinylidene difluoride membrane (Immobilon-Psq, Millipore, Bedford, MA, USA) using a