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Dystroglycan is not required for maintenance of the luminal epithelial basement membrane or cell polarity in the mouse prostate

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Abstract

Background—Dystroglycan is a cell-surface receptor for extracellular matrix proteins including laminins and perlecan. Prior studies have shown its involvement in assembly and/or maintenance of basement membrane structures, cell polarity and tissue morphogenesis; and its expression is often reduced in prostate and other cancers. However, the role of dystroglycan in normal epithelial tissues such as the prostate is unclear.

Methods—To investigate this, we disrupted dystroglycan expression in the prostate via a conditional gene targeting strategy utilizing Cre recombinase expressed in luminal prostate epithelial cells.

Results—Contrary to expectations, deletion of dystroglycan in luminal epithelial cells resulted in no discernable phenotype as judged by histology, basement membrane ultrastructure, localization of dystroglycan ligands, cell polarity or regenerative capacity of the prostate following castration. Dystroglycan expression remains in keratin-5-positive basal cells located in the proximal ducts where dystroglycan expression is elevated in regenerating prostates.

Conclusions—Our results show that dystroglycan in luminal epithelial cells is not required for the maintenance of basement membranes, cell polarity or prostate regeneration. However, it is possible that persistent dystroglycan expression in the basal cell compartment may support these or other functions.

Keywords

dystroglycan; basement membrane; polarity; epithelium; prostate

INTRODUCTION

Dystroglycan (DG) was first identified as a component of the dystrophin-glycoprotein complex in skeletal muscle (1). It is composed of a highly glycosylated extracellular α subunit non-covalently linked to a transmembrane β subunit, which are post-translationally derived products of a single gene (2,3). α DG binds to several extracellular matrix proteins including various laminins and perlecan which are important for both the structural and

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signaling functions of basement membranes (3,4). The cytoplasmic domain of β DG is tethered to the actin cytoskeleton via linker proteins such as dystrophin and utrophin (5). This domain has also been shown to interact with various signaling molecules, though a clear signaling function for DG has yet to emerge, *e.g.* (6–8). In skeletal muscle, disruption of this linkage via mutations in laminin-211 or dystrophin or by inappropriate carbohydrate modifications on α DG which are necessary for extracellular matrix binding lead to various forms of muscular dystrophy indicating that this linkage is critical for the integrity of this tissue (9).

DG is expressed in many other non-muscle cells including epithelial, neural and adipose cells that all share the property of being in direct contact with basement membranes (10). DG expression has been observed in all epithelia examined to date including the prostate (10,11). In the normal human prostate, the expression of laminins (111/123, 211, 332, 511/521) and perlecan have been documented (12,13). Of these, all but laminin-332 have been demonstrated as a DG ligand (3,14,15). Constitutive disruption of DG in the mouse leads to embryonic lethality around E6.5 (16). Mutant embryos fail to develop Reichert's membrane, one of the first basement membrane structures formed in the rodent embryo. Moreover, embryonic stem cells lacking DG fail to bind laminin or perlecan on their surface and embryoid bodies fail to develop a sub-endodermal basement membrane (17–19). More recently, Weir *et al.* have demonstrated that DG is required for laminin assembly on the basal surfaces of mammary epithelial cells (20). Together these findings suggest that DG is important for the assembly of basement membranes.

Compared to skeletal muscle, far less is known about DG function in epithelia. A requirement for DG function in epithelial cells has been demonstrated in flies, worms and mammals. Function blocking antibody experiments have shown that DG is required for kidney, lung and salivary gland epithelial morphogenesis in mice (21,22). In *Caenorhabditis elegans* mutation of DG disrupts the development of the gonadal epithelium, where it may be involved in the maintenance, but not assembly, of its basement membrane (23). In addition to its aforementioned role in basement membrane assembly/maintenance, other studies have indicated a role for DG in epithelial polarity. Several studies indicate a requirement for DG in the polarity of the *Drosophila melanogaster* follicular epithelium as well as in cultured mammary epithelial cells (20,24,25). Complementary roles for basement membrane laminins and perlecan in the establishment and/or maintenance of epithelial polarity have also been defined (26–28). Thus, an emerging body of work indicates that DG, through its interactions with its ligands may be critically involved in epithelial polarity in certain contexts.

Here we assess the expression and function of DG in the mouse prostatic epithelium. Using Cre-lox technology we generated a prostate-specific DG knockout mouse. We show that DG expressed in luminal epithelial cells is not essential for maintenance of the basement membrane composition or ultrastructure and its expression is not required for prostate cellular homeostasis. Furthermore, we show that DG is expressed in the castration-resistant basal cell population of the prostate and in the proximal ducts, currently implicated as a stem cell niche. DG protein expression increases in involuted prostates in association with a highly-folded basement membrane. Residual expression of DG in both castrate and intact mice is due to the lack of Cre expression in the basal cell compartment. Thus, contrary to expectations, we show that DG is not required for the maintenance of the basement membrane or the polarity associated with luminal epithelial cells in the prostate. Residual expression of DG basal/stem cell population of the prostate, may contribute to the lack of expected phenotypes in this organ.

MATERIALS AND METHODS

Animals

All procedures involving animals were performed according to The University of Iowa Animal Care and Use Committee policies. 129.B6 female $DG^{fl/fl}$ mice ((29); kind gift from Dr. Kevin Campbell) were crossed with male B6.D2 PB-Cre4 mice (NCI Mouse Models of Human Cancer Repository). F_1 $DG^{fl/wt}$; Cre⁺ males were bred to $DG^{fl/wt}$; Cre⁻ females. The F_2 $DG^{fl/fl}$; Cre⁺ males were bred to $DG^{fl/fl}$; Cre⁻ females. F_3 males were used for analysis. Herein WT refers to animals with a $DG^{fl/+}$; Cre⁺ or $DG^{fl/fl}$; Cre⁻ genotype and KO refers to animals with $DG^{fl/fl}$; Cre⁺ genotype. Female 129-Gt(ROSA)26^{Sor/J} mice ((30), The Jackson Laboratories) were also bred to male B6.D2 PB-Cre4 mice. Beta-galactosidase activity was assayed in the F_1 generation. For genotyping, tail DNA was extracted (REDExtract-N-Amp Tissue PCR Kit, Sigma) and PCR was performed. All animals were assessed at 6 months of age unless otherwise noted.

For castration/regeneration studies, mice at 6 to 9 months of age were anesthetized (100 mg/kg ketamine, 10 mg/kg xylazine) and castrated. Castrated mice were euthanized by CO₂ asphyxiation after 21 days. Remaining castrated mice were implanted with testosterone pellets (Innovative Research of America) at 15 mg/kg for 21 days. Testosterone-treated mice were euthanized after 21 days.

Antibodies

Anti- α DG (IIH6, gift from Dr. Kevin Campbell (2)), anti- β DG (8D5, Novocastra), CK5 (AF138, Covance), Ki-67 (NCL-Ki67p, Novocastra), p63 (clone 4A4, BD Pharmingen), perlecan (MAB1948, Chemicon), pan-laminin ((31); gift from Dr. Jeanne Snyder), laminin α 5 ((32), gift from Jeffrey Miner).

Histology and immunofluorescence analysis

Mouse urogenital organs were removed as a unit and fixed in 4% paraformaldehyde for 4 hours at 4°C and then transferred to 30% ethanol overnight. Prostates were dissected from the seminal vesicles and bladder. Prostates were processed through an ethanol to xylene gradient for paraffin embedding using a Ventana Automated Tissue Processor. 5 μ m sections were cut using a Leica RM2125 rotary microtome. Tissues were deparaffinized and hydrated through a xylene to ethanol gradient followed by citrate buffer or proteinase K antigen retrieval. After 1X phosphate buffered saline (PBS) wash, slides were blocked for 1 hour at room temperature (RT) in blocking solution (2% donkey serum, 1% BSA, 0.05% Tween-20, 0.1% Triton X-100 in 0.01M PBS). Slides were incubated with primary antibody in blocking solution overnight at RT. After two 1X PBS washes, slides were incubated with secondary antibody and nuclear stain 4',6-diamidino-2-phenylindole (DAPI) in blocking solution for 1 hour at RT. Slides were washed twice and coverslipped. Number of keratin 5 (K5)-positive cells per 100 DAPI-positive epithelial nuclei were counted for 3 separate areas of the ventral prostate per mouse. WT (n=3) and KO (n=2) mice were assessed at 6 months of age. Resulting data were averaged and analyzed by t-test. Sections were analyzed independently by two individuals, and similar results were obtained. Ki67 positive cells were quantified as described for K5-positive cells. WT (n=3) and KO (n=3) were assessed at six months of age. For hematoxylin and eosin staining, prostate sections were processed under standard conditions.

X-gal Histochemistry

Mouse urogenital organs were removed and prostates were dissected from the seminal vesicles and bladder. Prostates were fixed for 1 hour at 4°C in 4% paraformaldehyde followed by incubation in 30% sucrose overnight at 4°C. The tissue was then embedded in

Optimal Cutting Temperature embedding medium, frozen briefly in liquid nitrogen and stored at -20°C overnight. Tissues were cut using the Zeiss Micron Model HM505E at 10 μm . Slides were stored at -80°C . For staining, slides were washed one time in 1X PBS and incubated for 30 minutes at RT in rinse buffer (100 mM sodium phosphate pH 7.3, 2 mM MgCl_2 , 0.01% sodium deoxycholate, 0.02% NP-40). Following aspiration of the rinse buffer, slides were incubated in staining solution (rinse buffer plus 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide and 1 mg/ml X-gal) for 4 hours at 37°C . Slides were rinsed in PBS, counterstained with eosin, and coverslipped.

Transmission Electron Microscopy

Mouse prostates were fixed with 2% paraformaldehyde/2% glutaraldehyde in PBS, rinsed three times in 0.1 M sodium cacodylate, then postfixed with a solution of 1% OsO_4 and 1.25% potassium ferrocyanide in cacodylate buffer. After three rinses in cacodylate buffer, the samples were exposed to a 1% aqueous tannic acid solution for 20 minutes, dehydrated through a graded series of acetones, and embedded in Eponate-12 (Ted Pelling, Inc., Redding, CA). After embedment and polymerization, 110 nm sections were cut using a Leica UC6 Ultramicrotome, and these were stained with 5% uranyl acetate followed by Reynold's lead citrate. Sections were viewed on a JEOL JEM-1230 transmission electron microscope.

Protein Isolation and Western Blot

Prostates were removed from intact or castrated mice and lobes were individually dissected. Lobes from each mouse were combined and SDS lysis buffer (50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, 1% Triton X, dH_2O , complete protease inhibitors (Sigma)) were added. After incubation for 5 min on ice, prostate were homogenized for 5 seconds. Sample Buffer (2 M sucrose, 2 M Tris-HCl, 1:20 2-Mercaptoethanol and trace amounts of bromophenol blue) was added to the lysates in a 1:1 ratio. Samples were boiled for 5 minutes and stored at -80°C . Protein quantification was carried out using the BioRad Protein Assay following the manufacturer's instructions. Protein lysates (40 μg) were loaded on a 4–20% gradient, Tris-HCl, 10 well, 50 μl ReadyGel and electrophoresed for 1 h at 150 volts. Protein was then transferred onto a polyvinylidene fluoride membrane at 350 mA for 1 h on ice. Blots were blocked in blotto (50 mM Tris, 100 mM NaCl, 0.1% Tween 20 and 5% dried milk) 1 h at RT. Blots were incubated with a 1:500 dilution of I1H6, 1:200 dilution of 8D5 (NovoCastra) and 1:2000 dilution of beta-actin (Sigma) in blotto overnight at 4°C . Blots were washed 2 $\times$ 5 minutes in blotto at RT. Blots were then incubated with a 1:5,000 dilution of goat anti-mouse IgM peroxidase for αDG (Roche) or 1:20,000 donkey anti-mouse peroxidase for βDG and actin (Jackson ImmunoResearch Laboratories) for 1 h at RT. Blots were washed in 50 mM Tris, 100 mM NaCl, and 0.1% Tween 20 3 $\times$ 5 minutes. Blots were developed using SuperSignal West Pico Chemiluminescent Substrate (Pierce).

RESULTS

DG expression in the mouse prostate

DG expression has been examined in many tissues but has not been characterized in the mouse prostate. In order to determine the localization of DG in the mature mouse prostate we stained sections with antibodies to both α and β DG. DG was localized to the basolateral surfaces of epithelial cells in contact with the basement membrane (Fig. 1A,D). Importantly, these cells reacted with the glycosylation-sensitive antibody I1H6 (Fig. 1A) indicating that DG is post-translationally modified in prostate epithelial tissue as it is in others such as muscle and nerve. We observed similar DG staining in epithelial cells of all four lobes of the mouse prostate with the ventral lobes expressing the highest amounts of DG (data not shown).

In order to explore the function of DG in the mouse prostate, we employed a conditional gene targeting strategy as we have previously used (29,33). To generate a null DG mutant prostate epithelium, we utilized a transgenic mouse line with prostate epithelial-specific expression of Cre recombinase (PB-Cre4) (34). Cre expression is controlled by the modified rat probasin promoter *ARR2PB* resulting in specific, but variable, expression in the luminal epithelial cells of all prostatic lobes. PB-Cre4 mice were bred to mice bearing the floxed DG allele to generate a prostate epithelium-specific DG knockout mouse. Loss of both α and β DG from the epithelium was detectable by 3 months of age (Fig. 1B,E) in all lobes of the prostate (data not shown). This experiment also established the specificity of the prostate epithelial staining with antibodies directed to both α and β DG. In addition, muscle surrounding the urethra expressed DG indicating prostate-specific loss (Fig. 1B,E insets). Interestingly, the DG KO animals maintained small patches of DG-positive cells (arrow in Fig. 1B). Incomplete Cre-mediated recombination in the PB-Cre4 strain has been described previously and may reflect variable transgene expression in different prostate lobes and/or the presence of basal cells in which the androgen-dependent *ARR2PB* promoter is not expected to be active (34).

Normal morphology, ultrastructure, cellular composition and function of DG knockout prostates

In order to assess the consequences of a loss of DG expression in the mouse prostate, we evaluated the histology, ultrastructure and cellular composition of DG knockout prostates compared to littermate controls. Histologic assessment of all four lobes of the prostate in DG WT (n= 8) and KO (n= 8) mice at 12 months of age showed no abnormalities in gland morphology (Fig. 2A–C and D–F) respectively. This includes both the epithelial and stromal compartments, the latter remaining as a thin band of cells surrounding the glands. Additionally, DG knockout prostates were indistinguishable from control littermates at both 6 and 18 months (data not shown). Moreover, although the preceding mice were on a mixed B6.129 background, we have also evaluated prostate-specific knockouts in an extensively backcrossed B6 background at 5 months of age (n=2) and also observed no histological differences (data not shown).

We have previously shown that DG is involved in the establishment and/or maintenance of basement membranes in some tissues (18,33). We therefore examined the basement membranes of DG knockout prostates at the level of ultrastructure and immunohistochemistry. Transmission electron microscopy was used for ultrastructure analysis. The basement membrane was present on the basal side of the epithelium in both WT (n=2) and KO (n=2) animals. No apparent changes in continuity, morphology or distance from the epithelium were observed (Fig. 2 G,J). Moreover, on the apical surface of the cells microvilli and intact tight and adherens junctions were observed (Fig. 2 H,I,K,L). These data indicate that DG is not required for polarity of luminal epithelial cells. The basement membrane is composed primarily of laminins, collagen IV, perlecan and nidogen/entactin. DG binds to both laminins and perlecan through conserved G-domains in these molecules (4). Therefore, we assessed the expression and localization of perlecan and laminin by immunofluorescence. Perlecan and laminin were present in the basement membrane of the prostate glands and staining intensity and distribution was unaltered by the status of DG (Fig. 3 A,D and B,E) respectively. Although no changes were evident using a pan-laminin antibody, changes in the expression of particular laminin isoforms in the DG knockout prostates was possible. The expression of laminin isoforms has not yet been extensively evaluated in the mouse prostate basement membrane. We therefore assessed expression of laminin-511/521 by probing with antibodies specific for the $\alpha 5$ chain of laminin. Laminin-511/521 staining was similar between DG KO prostates and WT

littermates (Fig 3. C,F). We did not detect expression of the laminin $\alpha 1$ chain in adult mouse prostate epithelial basement membranes (data not shown).

The prostate is composed of two primary cell populations; basal and luminal cells. Unlike human prostatic epithelium, mouse basal cells do not form a continuous layer juxtaposed to the basement membrane and are instead spread discontinuously throughout the epithelium except in the proximal ducts where basal cells are concentrated. Consequently, mouse luminal epithelial cells are in direct contact with the basement membrane. Utilizing basal cell specific marker K5, we assessed the role of DG in maintaining cellular composition of the prostate (Fig 3. G,I). There was no significant difference in the percentage of K5-positive cells in the epithelial compartment between WT (3.7 ± 2.4) and KO (4.3 ± 2.3) mice at six months of age ($p=0.603$, t-test). Epithelial cell/extracellular matrix interactions are known to affect cell proliferation and apoptosis. We therefore examined proliferation by Ki-67 staining (Fig 3. H,J). There was no significant difference in proliferation in the epithelium between WT (0.58 ± 0.90) and KO (0.25 ± 0.49) mice at six months of age ($p=0.408$, t-test).

One function of the prostate is to secrete and store prostatic fluid which is a component of semen. We therefore assessed function of the prostate by fertility. We assessed the number of pups per litter for 5 litters sired by each of 3 WT and KO males (15 litters each genotype). Loss of DG from the prostate does not affect fertility as there was no significant difference in number of pups per litter between WT (6.1 ± 1.6) and KO (6.5 ± 2.6) mice ($p=0.677$, t-test).

Normal regeneration of DG knockout prostates

The prostate is able to undergo multiple cycles of involution following androgen ablation (castration) and regeneration with reintroduction of androgens. Epithelial cell/extracellular matrix interactions are essential for cell proliferation and differentiation necessary for regeneration. We therefore determined how loss of DG from the prostate epithelium would affect this process. Prostate-specific DG KO and WT littermate mice were castrated between 6 and 9 months of age. Twenty one days after castration, prostates were regenerated by reintroduction of testosterone. Prostates were assessed after 21 days of testosterone treatment. As expected, castration resulted in a significant decrease in individual gland size and overall tissue size (Fig. 4 A,B). Following restoration of androgens, DG knockout prostates were able to regenerate comparably to WT littermate controls as assessed by histology (Fig. 4 C,D). Expression and localization of laminin and perlecan were unchanged compared to WT animals (data not shown).

DG expression in the castrate-resistant basal cell population

Involution of the prostate after androgen ablation results in apoptosis of luminal cells with survival of a basal cell population resistant to the effects of castration. Both α and β DG expression was significantly enhanced in WT mice after castration compared with intact controls (Fig 5 A,C,G). The castrated prostate takes on a pleated appearance due to the delayed degradation of the basement membrane as the glands undergo apoptosis (35). Therefore increased DG expression may be associated with the excess basement membrane. Interestingly, DG expression in the KO mice was similar to WT mice indicating DG is expressed in the androgen-independent basal cell population and that this expression is maintained in PB4-Cre expressing mice (Fig. 5 A,D). DG expression was lost after regeneration of the prostate although some patches of cells with expression were evident, similar to intact control animals (Fig. 5 B,E and C,F).

Due to the ability of the prostate gland to undergo repetitive cycles of involution and regeneration, an androgen-independent stem cell population must exist to regenerate the glands (36). Furthermore, this population exists as a part of the larger androgen-independent basal cell population and is thought to reside in a stem cell niche located in the proximal prostatic ducts; although recent evidence indicates the existence of a distinct luminal stem cell population (37–39). We therefore examined the expression of DG in the proximal ducts (Fig. 6A,B). DG was expressed in the basal cell population of the proximal ducts, identified by anatomic location K5 and p63 staining, in both WT and KO mice (Fig. 6. C–F) suggesting DG may be expressed in this prostate stem cell population. To confirm the lack of expression of Cre from the *ARR2PB* promoter in this compartment, we crossed PB-Cre4 mice to the 129-Gt(ROSA)26^{Sor/J} reporter strain (30). As expected, this showed β -galactosidase staining in the distal prostate (Fig. 6G) but not in the proximal ducts penetrating the periurethral muscle (Fig. 6H)

DISCUSSION

DG has been most extensively studied in skeletal muscle although it is expressed in many other tissues including epithelia. As mentioned above, accumulating data indicates that DG is involved in the development of epithelial tissues including formation of basement membranes and the establishment of cell polarity. On the other hand, disruption of DG in the mouse epiblast using Mox2-Cre (which creates an embryo constitutively lacking DG); resulted in mice that survived to birth, though they died shortly thereafter (40). Although epithelial tissues were not extensively examined in these mice, this phenotype suggests that DG might have a more limited role in epithelial development. We therefore sought to determine the role of DG in the prostatic epithelium using a prostate-specific knockout mouse. Utilizing the PB-Cre4 transgenic mouse we deleted DG in the prostate from mice carrying a floxed DG allele. Given the prior work in this area, we were surprised to find that loss of DG expression did not lead to any observed phenotype. Specifically we evaluated histology, ultrastructure, expression of DG ligands, cellular composition, proliferative status, regenerative capacity, and fertility in mice in cohorts of mice up to 18 months of age. These data afford several conclusions regarding the role of DG in the prostatic epithelium.

Earlier studies support the concept that DG is required for the assembly and/or maintenance of some basement membrane structures in various tissues (16,18,33). Because the PB-Cre4 transgene is not extensively expressed during early urogenital development and early branching morphogenesis, we cannot assess DG function during this period when basement membranes are first assembled in the prostate (34). However, our data indicate that DG is not required for the subsequent maintenance of the basement membrane associated with luminal epithelial cells up to 18 months of age. We found no differences in the ultrastructure or molecular composition using pan-laminin, laminin $\alpha 5$ and perlecan antibodies, representing known DG ligands. Residual expression of DG is observed in what are most likely to be K5-positive basal cells, in which the *ARR2PB* promoter is not active. Because this population comprises <5% of the cells in the distal epithelium of the prostate it would seem unlikely to account for the maintenance of the entire basement membrane structure. However, basal cells extend processes over a large area which could play a role in maintenance of the basement membrane (41,42). Thus, our data indicate that DG in luminal epithelial cells is not required for the maintenance of the basement membrane in the prostate. Perhaps other ECM receptors are involved in this process, or, once assembled, the intrinsic self-assembly properties may act to maintain basement membrane structure (43). DG may still be required for the initial assembly or maintenance of basement membranes in some tissues; such as Reichert's membrane or the pial basement membrane (16,33). Prior studies showing that DG is required for laminin assembly in cultured mammary epithelial cells involves *de novo* assembly (20). However, a requirement for DG in basement

membrane assembly or maintenance is likely to be tissue-specific. The basement membrane between the visceral endoderm and epiblast and in analogous structures in embryoid body preparations that recapitulate the development of the epiblast, as well as the basement membrane in skeletal muscle, persists in the absence of DG function (16,29,44). This may reflect a role for DG in the assembly or maintenance of certain types of basement membranes as both Reichert's membrane and the pial basement membrane are relatively thick and/or are not formed between continuous cell layers unlike that between the visceral endoderm and epiblast.

Another unexpected finding is that DG is not required for the maintenance of epithelial polarity in mouse luminal prostatic epithelial cells. Luminal epithelial cells in the DG knockout prostate showed polarized features such as apical microvilli and tight junctions. Prior studies in mammary epithelial cells have indicated a role for DG in polarity (20). In this case, the role for DG in polarity may be secondary to its role in laminin assembly as the latter may provide polarizing cues. In *Drosophila melanogaster* it has become evident that the role of DG in epithelial polarity is context-dependent as the role of DG in polarity is evident only under conditions of energetic stress (24,25). It will be interesting to determine if a similar role for DG in maintaining polarity under energetic stress exists for mammalian epithelial cells.

Here we also found that DG is expressed in the basal cell compartment of the prostate epithelium, including those in the proximal ducts which are thought to contain prostate stem cells (37,38). Since the *ARR₂PB* promoter is not active in these cells, DG is not disrupted in this compartment. This likely explains why prostate regeneration following castration is normal in mice with prostate-specific DG knockout. Androgen ablation (castration) results in loss of androgen dependent luminal cells from the prostatic ducts by apoptosis while the androgen independent basal cells survive. Other approaches will be required to test the role of DG in this compartment. Earlier studies showing a role for DG in muscle regeneration suggest that DG may play a role in stem cell function in other tissues (29). Following castration, prostatic ducts involute, resulting in a folded basement membrane surrounding the atrophied glands. Interestingly, we found that both α and β DG were elevated in the atrophied glands in WT animals. This suggests that membrane fragments may be associated with the residual basement membrane. This residual basement membrane may be involved in the regeneration of the prostate and suggests that *de novo* basement membrane assembly may not be required for prostate regeneration. It will be interesting to determine whether DG associated with this residual basement membrane plays a role in maintaining this structure or in subsequent regeneration.

Disruption of epithelial cell/extracellular matrix interactions is a hallmark of cancer due to the disruption in regulation of cell proliferation, apoptosis, survival and differentiation. Loss of DG function may be associated with the progression of epithelial cancers including prostate cancer (11,45). Overexpression of DG in breast cancer cells inhibits cell proliferation and decreases tumorigenesis (46). These data suggest loss of DG from the epithelium may result in disruption of tissue homeostasis. However, in the normal prostate, tissue architecture remained unchanged by histological assessment of DG KO mice from 3–18 months of age compared with WT animals. We also showed that number and localization of K5-positive basal cells as well as proliferation as assessed by Ki-67 staining remained the same. These data suggest DG does not have a direct role in regulation of proliferation, survival and differentiation of adult mouse prostate luminal epithelial cells. Thus, loss of DG function in luminal cells is insufficient to initiate neoplastic progression in the mouse prostate. However, it remains possible that loss of DG function contributes to tumor progression when combined with other molecular or cellular alterations.

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Fig. 1. Dystroglycan localization and expression in the ventral lobe of a prostate specific knockout mouse

DG is expressed on the basal surface of the epithelium (yellow, arrows) in the prostate gland as shown by α -DG (IIIH6) and β -DG (8D5)-specific antibodies (A,D). DG expression is lost in DG KO mice (B,E) compared with WT (A,D) although patchy areas of expression remain (arrowheads) (B,E). Tissues stained with secondary antibodies only were used as a negative control (C,F). Muscle surrounding the bladder neck was used as a positive control (inset boxes). Nuclei were stained with DAPI (blue). Scale bar 50 μ m. Autofluorescence of luminal secretions (*).

Fig. 2. Histology and ultrastructure of the prostate

Hematoxylin and eosin stain of the ventral (A,D); lateral (B,E) and dorsal (C,F) prostatic lobes in DG WT (n=8) and KO (n=8) mice were similar at six months of age. Scale bar (A–F); 50 μ m. Transmission electron microscopy of the basement membrane (arrows) of the lateral lobe of DG WT (G) and KO (J) mice. Epithelial microvilli and tight junctions (arrows) of the lateral lobe of DG WT (H,I) and KO (K,L) mice, respectively. Two WT and two KO mice were analyzed. Epithelial cell (*). Scale bars (H,I,K,L) 1.0 μ m (G,J) 0.4 μ m.

Fig. 3. Basement membrane and cellular composition of the ventral lobe of the prostate
 Extracellular matrix proteins and DG binding partners perlecan and laminin (green) showed a similar localization and staining intensity in DG WT (A,B) and KO mice (D,E) respectively. Likewise, laminins 511/521 (red) were also unchanged (C,F). Basal cell number (G,I) as assessed by anti-K5 staining (red) and proliferation rate (H,J) as assessed by Ki-67 staining (green) were similar. Nuclei were stained with DAPI (blue). 3 WT and 3 KO animals were assessed for each protein. Scale bar 50 μ m.

Fig. 4. Involution and regeneration of the lateral and ventral lobes of the mouse prostate
DG WT (A) and KO (B) mice were castrated at 6 to 9 months of age. Testosterone was introduced 21 days post castration and regenerated prostates were analyzed 21 days after reintroduction of testosterone. WT (C) and KO (D) prostates regenerated normally. Scale bar 150 μ m.

Fig. 5. DG expression following involution and regeneration of the mouse prostate

DG expression is enriched after involution in both WT and KO mice (A,D) and is reduced after restoration of androgens allowed for regeneration in KO mice. Immunofluorescence staining for DG (orange) was performed on the ventral lobe of castrated WT (A) and KO (D) mice, regenerated ventral lobe (B,E) and normal ventral prostate (C,F) respectively. Nuclei were stained with DAPI (blue). Muscle surrounding the bladder neck was used as a positive control (inset boxes) 6 mice of each genotype were assessed. Scale bar 50 μm. (G) αDG (11H6) and βDG (8D5) protein expression was enriched in protein lysates of castrated (C1, C2) prostates compared with intact (I) prostates as shown by western blot.

Fig. 6. Expression of DG in the basal cell population of the prostate proximal ducts
 Immunofluorescence staining for DG (red) expression in the proximal ducts of WT and KO mice (A,B). Basal cells are indicated by P63, red (C,D,) and Keratin 5, green (E,F) expression. Nuclei are stained with DAPI (blue). 3 mice of each genotype were assessed. Scale bar 50 μ m. X-gal staining (blue) detects Cre recombinase activity in the distal ducts (G) but not the proximal ducts (H) of the mouse prostate ventral lobe. Scale bar 50 μ m.