Loss of the ssecks/gravin/akap12 Gene Results in Prostatic Hyperplasia

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Abstract

SSeCKS/Gravin/AKAP12 (SSeCKS) is a kinase scaffolding protein that encodes metastasis-suppressor activity through the suppression of Src-mediated oncogenic signaling and vascular endothelial growth factor expression. SSeCKS expression is down-regulated in Src- and Ras-transformed fibroblasts, in human cancer cell lines and in several types of human cancer, including prostate. Normal human and mouse prostates express abundant SSeCKS in secretory epithelial cells and, to a lesser extent, in the surrounding mesenchyme. Here, we show that the loss of SSeCKS results in prostatic hyperplasia in the anterior and ventral lobes as well as increased levels of apoptosis throughout the prostate. Dysplastic foci were observed less frequently but were associated with the loss of E-cadherin staining and the loss of high molecular weight cytokeratin-positive basal epithelial cells. SSeCKS-null prostate tissues expressed significantly higher relative levels of AKTpos473 compared with wild-type controls, suggesting that SSeCKS attenuates phosphatidylinositol-3-OH kinase signaling. The data suggest that SSeCKS-null mice have increased susceptibility for oncogenic transformation in the prostate. [Cancer Res 2008;68(13):5096–103]

Introduction

Src-suppressed-C Kinase Substrate (SSeCKS), encoded by the rodent orthologue of human Gravin/AKAP12, is a scaffolding protein for protein kinase (PK) A and PKC (1) originally identified as a suppressor of v-Src oncogenic signaling and transformation (2, 3). Fluorescence in situ hybridization analysis revealed that SSeCKS maps to 6q24-25.2, a deletion hotspot in advanced human prostate, breast and ovarian cancer, implicating a role for the loss of SSeCKS expression in prostate cancer (CaP) progression (4, 5).

SSeCKS/Gravin/AKAP12 (SSeCKS) is a potential metastasis inhibitor of CaP (6). SSeCKS expression is abundant in untransformed prostate cell lines and in normal human and rodent prostate luminal epithelial cells but down-regulated in CaP cell lines and in high-grade cancers in humans (4, 5). The tetracycline-regulated reexpression of SSeCKS in Mat-LyLu CaP cells had little effect on the growth of primary s.c. tumors or on the colonization of metastatic sites by tumor cells, yet it severely inhibited the growth of macroscopic lung metastases (5). The SSeCKS metastasis-suppressing activity likely involves its ability to down-regulate proangiogenic factors such as vascular endothelial growth factor (VEGF) because the forced reexpression of VEGF isoforms 165 or 121 partially rescues the ability to SSeCKS reexpressing Mat-LyLu cells to form macroscopic lung metastases (4).

In this study, we have generated ssecks knockout (KO) mice by disrupting the major exon encoding the three SSeCKS protein isoforms: α, β, and γ. KO mice survive to adulthood with no major physiologic abnormalities except a consistent phenotype of decreased fertility for both males and females. Significantly, males exhibited evidence of prostatic hyperplasia—especially in the anterior lobe—starting as early as 2 months, with dysplastic foci appearing at month 9. These data strongly suggest that loss of SSeCKS/Gravin/AKAP12 produces a cancer-prone condition in the prostate.

Materials and Methods

Construction of targeting plasmid. The ssecks (mouse akap12) gene was isolated from BAC 27244 (strain 129SvJ) contained within the pBACII vector (Incyte Genomics). The BAC DNA was cut with Sal I and the fragments spliced into pCR-XL vector (Invitrogen). Two contiguous Sal I fragments representing upstream 10.3-kb and downstream 3.0-kb SSeCKS genomic fragments (pCR-XL-10.3 and pCR-XL-3.0, respectively) covering all of exons 2 to 3 (Fig. 1A) were identified by Southern blotting and DNA sequencing (data not shown). A 6.5-kb fragment containing the 5’ end of exon 2 plus upstream intronic sequences was amplified from pCR-XL-10.3 by PCR using the following primers: sc4F, 5′-CGCTGGAAAGGTTTCTGTTTCTCTTTCTG-3′ (Xho I site underlined) and sc4R, 5′-GGCGTACCTCTTCTGCTACATCTTCTGACCTCTCTTCG-3′ (Sal I); this fragment was then cut with Xho I and Sal I and spliced into the Xho I site in pTCT5.2 (gift of Tom Luftkin, Genome Institute of Singapore) upstream of the LacZ and neomycin6 cassettes, to produce pTCT5.2/6.5. A 3-kb Xba I/Spe I fragment containing the 3’ end of exon 2, all of exon 3, and downstream intronic sequences was spliced from pCR-XL-3.0 into the Xba I site of pTCT5.2/6.5 to produce pTCT5.2-ssecks KO DNA (destroying the 5′-Xba I site).

Generation of mutant mice. J1 embryonic stem (ES) cells were electroporated with pTCT5.2-ssecks KO DNA linearized in the vector backbone, and then selected for colony formation on neo6 feeder fibroblasts in medium containing G418 (400 μg/mL) by the RPCI Gene Targeting and Transgenic Resource Facility (Aimee Stablerowski, Director). Two ES cells clones, #31 and 240, were identified with a targeted ssecks gene by Southern blotting using lacZ- and exon 3–specific probes, and by long-run PCR using sc24R (the complement of sc24F) (Fig. 1, below) plus primers for genomic sequences upstream of those in pCR-XL-10.3, and with Xba I BF (the complement of Xba I BR, below) plus sc19B, 5′-TCATCCCGCCCTGCACTGTGA-3′ (Fig. 1A). The PCR primers used to detect the wild-type (WT) allele were sc24R, 5′-CCGTGTACTACTAAAGGAGTGTAGTACC-3′ and sc19B, 5′-CCTGGTAGTCGTCGGAGTTCGGAG-3′.

Histology of SSeCKS-KO Mice. The tissues from 129 × C57BL/6 mice were fixed and stained by the RPCI Cell Analysis Resource Facility (Jennifer Black, Director) as follows: fixation of tissues in 10% formalin for 4 h at 4°C (http://cancerres.aacrjournals.org/).

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Note: Supplementary data for this article are available at Cancer Research Online.
(EMD Chemicals) in PBS solution, embedding in paraffin by standard methods. Blocks were sectioned (5 μm) and stained with H&E (Sigma). For prostate lobe isolation, the urogenital tract containing the bladder, seminal vesicles, urethra, and ampullary gland was excised, weighed, and then individual prostatic lobes were isolated using a dissection microscope. Total prostate weight (all four lobes) was determined.

**Immunohistochemistry.** Sections were deparaffinized with xylene and rehydrated with graded ethanol solutions. After antigen retrieval by boiling in 10 mmol/L citrate buffer (pH 6.0) for 20 min, the sections were treated with 3% peroxide in methanol for 15 min at room temperature to quench endogenous peroxidase. The sections were incubated with rabbit (Rb) polyclonal anti-Ki67 antibody (pAb; 1:1,000 dilution; Novocastra), mouse (M) anti-E-cadherin monoclonal antibody (mAb; 1:2,500 dilution; Becton Dickinson), Rb anti-AKT<sup>873</sup>P mAb (1:100 dilution; 736E11; Cell Signaling), anti–high molecular weight cytokeratin M-mAb (1:50 dilution; MAb-34;1E12; DAKO), or anti–SSeCKS Rb-pAb (1:2,000, ref. 7) overnight at room temperature and then incubated with horseradish peroxidase–conjugated swine anti-Rb (1:100; DAKO) or Rb anti-mouse (1:200; DAKO) IgG Ab, followed by incubation with 3,3-diaminobenzidine (DAB) chromogen (Biogenex Laboratories). The slides were counterstained with either Mayer’s hematoxylin, dehydrated, cleared, and coverslipped. Terminal deoxynucleotidyl transferase biotin-dUTP Nick End Labeling (TUNEL) staining was carried out using ApopTag Plus Peroxidase in situ Apoptosis kit (Chemicon) according to the manufacturer’s instruction.

**Southern hybridization analysis.**Liver DNA was extracted by overnight digestion at 35°C in 100 mmol/L NaCl, 10 Tris (pH 8.0), 25 mmol/L EDTA, 0.5% SDS containing 100 μg/mL Proteinase K (Calbiochem, EMD Biosciences), followed by incubation at 37°C for 1 h with RNase A (100 μg/mL; Sigma), phenol/chloroform separation, and ethanol precipitation. Thirty micrograms of the genomic DNA were digested with XbaI and blotted as described previously (7). A probe downstream of SSeCKS exon 3 and outside the targeting vector was prepared by PCR reaction using BAC 27244 DNA template plus primer (3,10). The NH2-terminal 103 amino acid (a.a.) of SSeCKS is expressed in many tissues and cell types as two major isoforms, α and β (8, 9), each of which is encoded by spliced transcripts controlled by an independent promoter (3, 10). The NH2-terminal 103 amino acid (a.a.) of α-SSeCKS and 8 a.a. of β-SSeCKS are encoded by small exons (1A1 and 1A2 for α-SSeCKS) and 8 a.a. of β-SSeCKS are encoded by small exons (1A1 and 1A2 for α, 1B for β) that then splice to a common exon 2 (3) that encodes the remaining ca. 1,500 a.a. (representing 94% of the α isoform or 99.5% of the β isoform). Exon 2 also encodes the entire testes-specific γ-SSeCKS isoform, whose unspliced transcript originates from flanking intronic sequences (11) knocked out the α transcript by inserting a gene-trap vector into exon 1A1 in mouse ES cells, with the resulting Tl15 mice showed no physiologic deficiencies. Given that most tissues and cell types coexpress both

Results

**Establishment of ssecks/gravin/akap12 KO mice.** SSeCKS/Gravin/AKAP12 (SSeCKS) is expressed in many tissues and cell types as two major isoforms, α and β (8, 9), each of which is encoded by spliced transcripts controlled by an independent promoter (3, 10). The NH2-terminal 103 amino acid (a.a.) of α-SSeCKS and 8 a.a. of β-SSeCKS are encoded by small exons (1A1 and 1A2 for α, 1B for β) that then splice to a common exon 2 (3) that encodes the remaining ca. 1,500 a.a. (representing 94% of the α isoform or 99.5% of the β isoform). Exon 2 also encodes the entire testes-specific γ-SSeCKS isoform, whose unspliced transcript originates from flanking intronic sequences (11) knocked out the α transcript by inserting a gene-trap vector into exon 1A1 in mouse ES cells, with the resulting Tl15 mice showed no physiologic deficiencies. Given that most tissues and cell types coexpress both

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**Figure 1.** Targeted disruption of the ssecks/gravin/akap12 gene. A, a partial map of the ssecks locus and diagrams showing the gene-targeting strategy. **Filled boxes,** exons 2 and 3 (Ex2 and Ex3, respectively). An upstream 6.5-Kb fragment was amplified with primers sc4F/R, spliced upstream of the lacZ/neoR cassette in pTCT5.2, followed by the insertion of the downstream XbaI/SpeI 3.0-Kb ssecks fragment, as described in Materials and Methods. ES clones with the targeting vector inserted into an ssecks genomic locus were confirmed by Southern blotting on EcoRI cut genomic DNA using lacZ and 3′-exon 2 probes (hatched boxes), as well as long-run PCR analysis using primers NeoF and sc19R (data not shown). B, PCR analysis of mouse tail DNA showing ssecks WT or targeted (mt) alleles in WT (+/+), heterozygous (+/−) and homozygous (−/−) mice. C, Southern blot analysis of XbaI-cut genomic DNA from WT (+/+), heterozygotes (+/−), or KO (−/−) mice probed with a 480-bp fragment mapping downstream of exon 3 and outside the targeting vector (A). Whereas the 3-Kb XbaI fragment is diagnostic of the WT allele, the 15-Kb XbaI fragment is diagnostic of the recombinant KO allele (A).

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α and β isoforms and given that these isoforms are virtually identical, it is likely that β-SSeCKS provides compensatory functions in the TL15 mice.

To produce a mouse deficient in all SSeCKS isoforms, the 4.8-Kb SSeCKS exon 2 was targeted for deletion in mouse ES cells using a vector containing a lacZ-Neo cassette flanked by ssecks sequences upstream of exon 2 and downstream of exon 3, which encodes the 3'-untranslated region of all SSeCKS isoform mRNAs (Fig. 1A). Two ES clones, #31 and #240, were identified by PCR- and Southern blot–based assays for the incorporation of the targeting vector into the endogenous ssecks locus (data not shown). These ES cells were then inserted into strain 129SvJ blastocysts to produce chimeric mice. The production of germine-stable ssecks+/− and −/− mice was confirmed by short-run PCR analysis of tail DNA using oligonucleotide primers sets specific for either the WT or mutated alleles (Fig. 1B), or by long run PCR using primers flanking both exons 2 and 3 (data not shown). Figure 1C shows that the ssecks−/− mouse DNA loses a 3 Kbp XbaI fragment, which converts into a 15 Kbp fragment, when hybridizing to a probe mapping downstream of Exon 3 and outside the targeting vector. Western blotting of organ lysates normally enriched for SSeCKS, such as the testes, bladder, lung, brain, heart, ovary, and prostate, showed an absence of detectable SSeCKS isoforms in ssecks−/− mice (Supplementary Fig. S2).

After founder mice with germ-line incorporation were produced, matings between heterozygous mice produced offspring with normal Mendelian distribution for WT, heterozygous, and null progeny (Table 1). Matings between heterozygotes or between ssecks−/− mice produced normal litter sizes of between 8 and 10 pups. However, both male and female ssecks+/− mice displayed delayed fertility (Supplementary Table S1). Specifically, ssecks−/− males took an average of 2 weeks to impregnate WT females, and similarly, ssecks−/− females took 1 to 2 weeks to be impregnated by WT males, compared with 1 to 2 days required for impregnation using WT mating pairs. Similar fertility delays occurred in mating pairs of ssecks−/− mice. Significantly, 10% to 25% of ssecks−/− mice (depending on sex and on ES line) were fully infertile as deemed by an inability of males to impregnate 4 WT females or females to be impregnated by fertile WT males over a month-long mating period. Interestingly, ssecks−/− females nested and suckled pups normally. Given the similar defects in males and females, and given that the testes, ovary and brain are normally enriched for SSeCKS expression (8), it is unclear whether the fertility delays are due to developmental deficits in reproductive organs or in responses to endocrine-mediated mating signals.

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Table 1. Offspring from matings of SSeCKS heterozygotes

spontaneous death in otherwise healthy-appearing ssecks+/− and ssecks−/− mice as young as 4 months, representing 3% to 4% of the +/− or KO colonies (Supplementary Table S1). Post mortem analysis indicated cardiomegaly in all these cases, although sacrificed age-matched +/− or −/− mice (randomly picked) showed no such defect. A small number of KO mice (especially line #240) displayed a limb-grasping phenomena, a marker of neuromuscular deficit,4 possibly correlating with anomalous brain histologies such as a defective Purkinje cell layer (Supplementary Fig. S1, compare C and D). Other anomalous histologic findings in the SSeCKS−/− mice were liver granulomas (Supplementary Fig. S1A and B), increased cell size and vascularization in the lung (Supplementary Fig. S1E and F), and an increased red pulp layer surrounding germinal centers in the spleen (Supplementary Fig. SIG–HI).

We noted that starting at roughly age 4 months, ssecks−/− mice displayed macroscopically and palpably enlarged urogenital growths. Post mortem analysis indicated that the KO mice had >2-fold increases in urogenital weights compared with age-matched male WT mice (Supplementary Fig. S3), correlating with similar increases in prostate weights in KO mice starting as young as 2 months (Fig. 2B). Analysis of formalin-fixed prostatic lobes after staining with H&E (Fig. 3) indicated hypercellularity in the KO anterior and ventral lobes compared with the same lobes in WT mice (Fig. 2A), plus foci of hyperplasias and, to a lesser extent, dysplasias. One hundred percent of the KO mice displayed hypercellularity in the anterior lobe compared with age-matched WT controls (Supplementary Table S2), although it should be stressed that prostates from WT mice older than 9 months exhibited typical age-related hyperplasia compared with younger (5- to 8-month-old) WT counterparts (12).

Human and mouse prostates show abundant SSeCKS staining in the luminal epithelial cells and, to a lesser extent, in the surrounding stroma (Supplementary Fig. S4). SSeCKS staining is lost in human lesions with a Gleason sum of ≥6 (5), and the loss of SSeCKS staining correlates with strong statistical significance with CaP adenocarcinoma [as opposed to prostatic intraepithelial neoplasia (PIN)], high Gleason sum, and poor clinical outcome.5

To determine whether the loss of SSeCKS correlated with changes in both epithelial cell proliferation and apoptosis rates, prostatic lobes from at least 10 KO and WT mice were stained for the S-phase marker, Ki67 (Supplementary Fig. S5), as well as for the apoptosis marker, TUNEL. All KO lobes exhibited at least 2-fold higher levels of proliferating cells, with the anterior lobe showing an almost 4-fold increase (Fig. 2C), as determined by visual analysis by 2 independent laboratory personnel. Using an automated, digitized scanning system (Ariol) of larger numbers of lobe samples

4 http://www.informatics.jax.org/searches/Phat.cgi?id=MP:0001513
(Supplementary Fig. S5I and J), we identified 8- to 10-fold increases in Ki67-positive cells in KO versus WT anterior lobes (data not shown). The slightly higher values determined by the Ariol analysis were probably due to a lower threshold for what is considered Ki67-positive staining. This automated analysis also calculated an average cellularity per duct of 417.1429 for WT-AP lobes versus 661.7143 for KO-AP lobes (SE, 0.09927; \( P < 0.01 \)), or an increase of 1.6-fold for KO lobes—the same as the manual counting data in Fig. 2A. The dorsal and lateral KO lobes displayed similar 2-fold increases in apoptosis compared with the same WT lobes (Fig. 2D), correlating with the lack of increase in relative hypercellularity in these lobes (Fig. 2A). In contrast, the KO anterior and ventral lobes displayed higher increases in proliferation than in apoptosis, correlating with the relative hypercellularity of these lobes to their WT controls. Taken together, these data indicate that SSeCKS plays a general suppressive role for both proliferation and apoptosis in the prostate but a greater role for proliferation control in the anterior and ventral lobes.

The anterior lobe, also known as the coagulating gland in the mouse, has secretory functions and is enriched for the developmental marker, NKX3.1, whose conditional knockout results in PIN (13). Interestingly, prostatic hypercellularity resulting from the loss of NKX3.1 is thought to be mediated through a hyperactivation of AKT (13, 14). Indeed, the anterior lobe of normal mice expresses the lowest level of all the lobes of the AKT antagonist PTEN (15), such that combining the \( \text{Nkx3.1}^{-/-} \) cells with a \( \text{Pten}^{+/--} \) background (total PTEN loss leads to embryonic lethality; ref. 16) greatly accelerates neoplastic progression in the prostate, especially in the anterior lobe (17). A similar acceleration in prostatic onco genesis in the anterior lobe was detected in \( \text{p18tarck}^{-/-} \text{Pten}^{+/--} \) mice (18). Given these background data and our finding of anterior lobe hyperplasia in the SSeCKS-/- mice, we addressed whether the loss of SSeCKS induced AKT activation. Thus, prostate lobes from KO or WT mice were stained for phospho-Ser473-AKT or with normal rabbit IgG as a negative control. Figure 4D shows that the KO anterior lobes had 2- to 3-fold more AKT phosphorylation than WT controls, and this increase is also reflected in Western blots of prostate lysates (Fig. 4B). Given the recent finding that SSeCKS controls Src activation via a direct SH3-mediated binding (19), these results suggest that SSeCKS normally attenuates Src-dependent AKT activation.

Two hallmarks of transition from prostatic hyperplasia to early neoplasia is the loss of E-cadherin cell-cell staining and the loss of basal epithelial cells that scaffold the secretory ducts (20, 21). Therefore, we stained anterior lobes of WT and KO prostates for E-cadherin and for high molecular weight cytokeratins—a marker of the basal cell phenotype (22). Whereas WT anterior lobes showed strong cell-cell staining of E-cadherin, KO anterior lobes from mice as young as 4 months showed dysplastic foci with loss of all E-cadherin staining (Fig. 5A). Similarly, KO anterior lobes had dysplastic foci that lacked staining for the many basal epithelial cells found in WT samples (Fig. 5B). Taken with the evidence of

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**Figure 2.** SSeCKS-null mice exhibit increased prostate weight, cellularity, cell proliferation, and apoptotic index. A, quantification of hypercellularity of anterior (AP), dorsal (DP), lateral (LP), and ventral (VP) prostatic lobes in WT (white columns) versus KO mice (black columns). H&E-stained paraffin sections of individually dissected lobes from at least six age-matched WT or KO mice were analyzed for cellularity: the number of cells per duct were counted in three equally sized ducts for each lobe. Columns, mean; bars, SE; * \( P < 0.01 \); ** \( P < 0.005 \); B, the average total weight of prostates from WT versus KO mice. Prostates from at least six mice per age group were dissected, and the prostate weights (normalized to total body weight) were determined. Columns, mean; bars, SE; * \( P < 0.01 \); ** \( P < 0.005 \); C, the average level of apoptosis was determined on the same tissue samples as in panel C using immunohistochemical staining for TUNEL as described in Materials and Methods. Columns, mean; bars, SE; * \( P < 0.005 \).
increased cellularity from Fig. 2, these data strongly suggest that the loss of SSeCKS predisposes the mice to the development of early prostatic neoplastic events.

Discussion

The molecular events involved in the initiation and progression of CaP are just being elucidated. There is increasing evidence that the loss of function such as PTEN, NKX3.1, or Rb predispose prostate epithelial cells to a p53-dependent program of both increased cell proliferation and senescence in an attempt to prevent the initiation of cancer (23). Later events, such as the loss of p53 or the oncogenic activation of Ras alleles, most likely facilitate a break through this barrier, resulting in the onset of frank neoplasia. Thus, at least 70% of primary CaP show evidence of the loss of one PTEN allele (24), with more malignant cases showing evidence of severe PTEN losses (25); at least 80% of early CaP show a loss of heterozygosity in 8p21, correlating with the loss of NKX3.1 (26). In contrast, p53 mutation is rare in primary CaP yet present in a third to 42% of CaP cases with high stage/grade and/or evidence of metastasis (reviewed in ref. 27).

In this current study, we show that the loss of the ssecks/gravin/akap12 gene results in hyperplasia in all prostatic lobes compared with age-matched WT controls. Interestingly, statistically significant hypercellularity is apparent only in the anterior lobe, probably due to concomitant increases in apoptosis rates in the ventral, dorsal, and lateral lobes. Our laboratory as well as others showed that SSeCKS controls cell cycle progression in normal cells,

Figure 3. Hyperplastic changes in the prostates of SSeCKS-null mice. H&E-stained sections of anterior, dorsal, lateral, and ventral prostate lobes derived from 15-mo-old WT or KO mice. Scale bar, 50 μm.
oncogenically transformed cells induces apoptosis, possibly through the induction of p27 (31, 32). In contrast, SSeCKS reexpression in v-Src–transformed fibroblasts, which does not induce p27 expression (28), protects cells from anoikis.8

Given the important regulatory role for SSeCKS in cell cycle control, given that its expression is significant throughout embryogenesis in the mouse (8, 33), frog (34), and fish (35), and given that vertebrates have only one member of the SSeCKS/Gravin/AKAP12 gene family (3), it was surprising that the loss of both ssecks alleles did not result in embryonic lethality. Indeed, the relatively normal development of ssecks/−/− mice is consistent with the loss of other G1–S regulatory proteins such as p53 (36), pRb (37), p21 (38), and p27 (39) and implies the existence of at least one compensatory function for SSeCKS. Alternatively, SSeCKS may not be required for gross development but may encode functions contributing to supraphysiologic development such as synapse formation involving memory and learning capabilities.7 Consistent with the loss of other examples of G1–S regulators, ssecks/−/− and mice do not develop spontaneous neoplasias within their first 18 months, yet they do develop hyperplasias in some tissues/cell types known to normally express high levels of SSeCKS such as the prostate. Relating to other tissues with normally high SSeCKS protein levels, KO mice showed limited cardiomegaly (under 5% of cases), a high level of lung hypertrophy and increased vascularization, and some physiologic brain abnormalities such as defective Purkinje cell layers.

Consistent with the signaling pathways controlled by PTEN and Nkx3.1 in suppressing prostatic hyperplasia, SSeCKS seems to attenuate AKT signaling. There is mounting evidence that the activation of the AKT pathway is a major determinant in CaP initiation and progression in humans (40), and moreover, the loss of PTEN, a direct antagonist of AKT activation, is a marker for late-stage malignancy in CaP such as metastatic potential (40, 41). Although it is not yet clear how SSeCKS might control PI3K/AKT activation, it is known to inhibit the ability of activated Src to mediate downstream oncogenic signaling and cytoskeletal pathways (2, 42), possibly through the recently described direct interaction between the Src SH3 domain and an NH2-terminal PxxP motif in SSeCKS (19).

Our data indicate that the prostatic hyperplasia detected in the ssecks/−/− mice displays focal hallmarks of PIN-like lesions as well as dysplastic lesions. Specifically, ssecks/−/− male mice, but not age-matched WT male mice, showed increased incidence with age of focal losses of total cellular and cell-cell E-cadherin staining, a marker of luminal epithelia, as well as the loss of basal epithelial cells, as assessed by staining for high molecular weight cytokeratins. The incidence and penetrance of these effects parallels that detected in the prostatic hyperplasias of Pten+/− and Nkx3.1/−/− mice (14, 43). Moreover, the focal and heterologous evolution of the prostatic lesions in the ssecks/−/− mice are consistent with the pattern of prostatic hyperplasias exhibited in mice deficient in all members of the pRb family (Rb, p107, and p130), a model of PIN marked by severe PTEN deficiency (44). Interestingly, PTEN, Nkx3.1, and Rb functions are lost in human CaP due to either mutational inactivation or haploinsufficiency (45, 46), and indeed, the SSeCKS locus on human chromosome 6q24-25.2 is a known hotspot for deletion in prostate cancer malignancy (reviewed in ref. 3). It has been postulated that the inappropriate proliferation of cells due to the loss of these suppressor functions sensitizes cells to

7 Ted Abel, Univ. of Pennsylvania; personal communication.
8 S. Akakaura, P. Nochajski and I.H. Gelman, manuscript in preparation.
die as by premature senescence as a defense mechanism to prevent further oncogenic progression (23). Interestingly, ssecks−/− mouse embryo fibroblasts exhibit premature senescence. Taken together, these data are consistent with a role for SSeCKS in suppressing malignant CaP progression in the presence of other pro-oncogenic signals such as activated Ras. This implies that the loss of SSeCKS in the absence of oncogenic signaling renders prostatic luminal epithelial cells prone to either senescence or cancer, depending on how quickly pro-oncogenic mutations might arise. Given the convergent effects by SSeCKS, PTEN, and NKX3.1 on AKT signaling in the prostate, it will be interesting to determine whether combining the SSeCKS-null background with Pten+/− or Nkx3.1−/− mice will result in accelerated CaP formation and aggressiveness, as has been shown in mice combined for Pten+/− and Nkx3.1−/− (17).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Figure 5. Loss of cell-cell junctions and basal epithelial cells in SSeCKS-null AP lobes. Immunohistochemical analysis of E-cadherin (A) or high molecular weight cytokeratins (B) in the AP paraffin sections from 3-mo-old WT or KO mice. The portion of the inset boxed region in the middle of A is magnified at right. Scale bars, 50 μm.