Tumor and Stem Cell Biology

Aberrant Activation of Fatty Acid Synthesis Suppresses Primary Cilium Formation and Distorts Tissue Development

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Abstract

Aberrant activation of fatty acid synthesis is a key feature of many advanced human cancers. Unlike in classical lipogenic tissues, this process has been implicated in membrane production required for rapid cell proliferation. Here, to gain further insight into the consequences of tumor-associated fatty acid synthesis, we have mimicked the lipogenic phenotype of cancer cells in Xenopus embryos by microinjection of RNA encoding the lipogenic transcription factor sterol regulatory element binding protein 1c (SREBP1c). Dramatic morphologic changes were observed that could be linked to alterations in Wnt and Hedgehog signaling, and ultimately to a distortion of the primary cilium. This is a sophisticated microtubular sensory organelle that is expressed on the surface of nearly every cell type and that is lost in many cancers. SREBP1c-induced loss of the primary cilium could be confirmed in mammalian Madin-Darby canine kidney (MDCK) cells and was mediated by changes in the supply of fatty acids. Conversely, inhibition of fatty acid synthesis in highly lipogenic human prostate cancer cells restored the formation of the primary cilium. Lipid-induced ciliary loss was associated with mislocalization of apical proteins, distortion of cell polarization, and aberrant epithelial tissue development as revealed in three-dimensional cultures of MDCK cells and in the developing mouse prostate. These data imply that tumor-associated lipogenesis, in addition to rendering cells more autonomous in terms of lipid supply, disturbs cilium formation and contributes to impaired environmental sensing, aberrant signaling, and distortion of polarized tissue architecture, which are all hallmarks of cancer.

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Introduction

Development and progression of cancer is often associated with a remarkable increase in de novo fatty acid synthesis in the tumor cells themselves (1–3). Activation of this pathway occurs as a result of common oncogenic and environmental insults and involves changes at all levels of lipogenic enzyme regulation (2, 3). The progressive activation of lipogenesis correlates with a poorer prognosis and shorter disease-free survival for multiple tumor types. Unlike in classical lipogenic tissues, activation of this pathway in cancer cells has been implicated in the biosynthesis of membranes, which are required for rapid cell proliferation, and it is thought to be part of a more general metabolic switch that renders cancer cells more autonomous in terms of their supply of building blocks available for cell growth (4). In support of this concept, chemical-mediated or small interfering RNA–mediated downregulation of pathway components of de novo lipogenesis blocks cell proliferation and restricts tumor growth (1, 3, 4). The observations that the lipogenic pathway is frequently activated early in cancer development and that its activation occurs irrespective of the proliferative status of the individual cancer cells suggest that the consequences of increased lipogenesis extend beyond its expected role as a provider of lipids for rapid cell proliferation. Activation of growth factor signaling and protection from cell death are just a few of the proposed new roles of this pathway (5, 6).

Here, to gain further insight into the implications of increased lipogenesis for cancer cell biology, we mimicked the lipogenic phenotype of cancer cells in the Xenopus laevis embryo model of tissue development and in several mammalian models of epithelial morphogenesis. This study reveals...
an unexpected link between cellular metabolism and environmental sensing. We propose that in addition to rendering cells more autonomous in terms of lipid supply, disturbed lipogenesis impairs the formation of the primary cilium and contributes to aberrant signaling and distortion of polarized tissue architecture, which are all hallmarks of malignant progression.

Materials and Methods

Plasmid constructs and in vitro transcription

The promoter-reporter constructs pTopFLASH and 8×Gli-BsI55/LucII (referred to as pGli-luc) were kindly provided by Dr. Hans Clevers (Hubrecht Institute, Utrecht, the Netherlands) and Dr. Hisato Kondoh (University of Tokushima, Tokushima, Japan), respectively. The pCMV6-XL4 plasmid encoding fatty acid synthase (FASN) was purchased from OriGene, and the pcDNA3.1-ACCFull-IVSclen30 plasmid encoding acetyl-CoA carboxylase was kindly provided by Dr. N. Dalla Venezia (Lyon, France). For expression of nSREBP1c in Xenopus embryos, the cDNA corresponding to amino acids 1 to 490 of the human sterol regulatory element binding protein 1c (SREBP1c) was subcloned into the pCS2+ vector (7). Control embryos were injected with synthetic RNA encoding β-galactosidase. Capped RNAs were synthesized by in vitro transcription with the use of SP6 RNA polymerase (Promega).

Culture of Xenopus embryos and injection of RNA/DNA

In vitro–fertilized Xenopus eggs were cultured in 0.1X Marc’s Modified Ringers [0.1 M NaCl, 1.8 mmol/L KCl, 2 mmol/L CaCl2, 1 mmol/L MgCl2, 5 mmol/L HEPES (pH 7.6)]. Synthetic RNA and/or reporter plasmids were microinjected into one-cell to four-cell stage embryos while they were submerged in 1X Marc’s Modified Ringers with 6% Ficoll (Amersham). At least 1 hour after injections, embryos were transferred to 0.1X Marc’s Modified Ringers to develop. The embryos were staged according to the criteria proposed by Nieuwkoop and Faber (The stages of embryonic development; http://www-cbd.ups-tlse.fr/organismes/nieuwkoop/nieuwkoop.html).

Wnt/β-catenin and Hedgehog activity assays

One-cell stage embryos were coinjected with the appropriate promoter-reporter construct (100 pg) and nSREBP1c-encoding RNA (100 pg), as indicated. Embryos were lysed in Reporter Assay Lysis Buffer (Roche), and luciferase activity was measured.

Modulation of lipogenesis in cell lines

Madin-Darby canine kidney (MDCK) and LNCaP cells were obtained from the American Type Culture Collection in 2008 and 2009, and were authenticated by karyotyping in December 2009. Cells were grown in two dimensions on cover slips in DMEM/Ham’s F-12 or RPMI 1640 supplemented with 10% FCS (Invitrogen). To ectopically express nSREBP1c, MDCK cells were infected on day 1 and day 4 after seeding with 30 pfu/cell of an adenovirus encoding the first 403 amino acids of SREBP1c (kindly provided by Dr. Fabienne Foufelle, Universite’ Paris 6, Paris, France; ref. 8). For expression of lipogenic enzymes, MDCK cells were transiently transfected with the use of FuGENE HD Transfection Reagent (Roche). For three-dimensional cultures, MDCK cells were infected with 30 pfu/cell 1 day after plating. The next day, cells were trypsinized and seeded in Matrigel (BD Biosciences) at a concentration of 50,000 cells/50 μL of Matrigel. MDCK cells were treated with soraphen A (kindly provided by Drs. Klaus Gerth and Rolf Jansen; Helmholtz Zentrum für Infektionsforschung GmbH, Braunschweig, Germany; refs. 9, 10) or palmitic acid, as previously described (11, 12). LNCaP cells were reverse transfected with 50 mmol/L small interfering RNA targeting FASN (s5030; Ambion) with the use of Lipofectamine RNAiMAX (Invitrogen). Silencer Select Negative Control #1 small interfering RNA from Ambion was used as control. LNCaP cells were treated with 2.5 μmol/L 25-hydroxycholesterol as indicated (Sigma–Aldrich).

Modulation of lipogenesis in the mouse prostate

The left anterior prostate of 5-to-6-week-old male 129SveV mice was injected with 250 × 10^6 pfu of the nSREBP1c-encoding virus or control virus. One week after injection, animals were euthanized by cervical displacement. Anterior prostates were resected and sectioned for histologic and immunofluorescence analysis. Experiments were done according to international regulations and were approved by the local Ethics Committee for Animal Experimentation at Ghent University.

Confocal immunofluorescence microscopy

LNCaP and MDCK cultures were fixed in paraformaldehyde at day 6 or 7 post-seeding, respectively. Xenopus embryos and mouse prostates were fixed in parafomaldehyde and embedded in agarose VII (Sigma–Aldrich). Sections of 80 and 300 μm, respectively, were cut with a vibratome. Cells and sections were permeabilized, blocked, and incubated with primary antibodies and Alexa Fluor-conjugated secondary antibodies (Invitrogen). Primary antibodies used were: acetylated tubulin (Sigma–Aldrich), ZO-1 (Zymed Laboratories), gp114/carcinoembryonic antigen (CEA; Abcam), FASN (Cell Signaling Technology), and E-cadherin (Abcam). The nuclei were stained, and cells and sections were mounted (see Supplementary Methods). Images and Z-stack shots were acquired with the use of an Olympus Fluoview FV1000 confocal laser scanning microscope (Olympus).

SEM microscopy

Two-dimensional MDCK cultures were fixed 7 days after seeding with 2.5% glutaraldehyde in 0.1 mol/L Na-cacodylate buffer (pH 7.3; 1 h at room temperature). After post-fixation in 2% osmiumtetroxide in H2O (1 h at 4°C) and washing in H2O, cells were dehydrated and incubated in hexamethyldisilazane (2 × 15 min at room temperature). Cells were dried.
mounted on SEM stubs, coated with platinum, and scanned with the use of a JEOL JSM7401F scanning electron microscopy (SEM) device.

**Statistical analysis**

Statistical evaluation of results was done with the use of the Student’s unpaired t-test available on SigmaPlot 8.0 software.

**Results**

**Ectopic expression of nSREBP1c in Xenopus embryos induces a complex developmental phenotype**

To gain further insight into the implications of increased lipogenesis for cancer biology, we mimicked the lipogenic phenotype of cancer cells in the *X. laevis* embryo as an experimental model of tissue development. To activate the lipogenic pathway we injected *Xenopus* embryos at the one-cell to four-cell stages with RNA encoding the mature nuclear form of SREBP1c, further referred to as nSREBP1c. SREBP1c is a transcription factor that is frequently activated in human cancer cells and that substantially contributes to the activation of lipogenesis in tumors (2, 3). The activation of the lipogenic pathway was confirmed at the level of FASN mRNA expression and lipogenic activity (Supplementary Figs. S1 and S2). At the end of the gastrula stage, gastrulation defects, such as incomplete blastopore closure, were observed in >40% of the embryos (Figs. 1A, stage 13, blue arrow; and 1B, stage 26). From stage 18 onwards, nSREBP1c-injected embryos exhibited signs of abnormal anterior development, as evident by the absence of the cement gland (Fig. 1A, stage 18, red arrow). At later stages, a high percentage of embryos (36%) displayed severe malformations of head structures (Figs. 1A, stage 32; and 1B, stage 26). A fraction of the embryos had a shortened body axis (Fig. 1A, stage 32). Most embryos with gastrulation or severe anterior defects died before stage 45 (Fig. 1B, stage 45). Embryos with a milder anterior phenotype survived, had a smaller head than the wild-type controls, and failed to form and/or laterally position the eyes (Figs. 1A, stage 47, yellow arrow; and 1B, stage 45).

**Expression of nSREBP1c modulates Wnt and Hedgehog signaling in Xenopus embryos**

The complex phenotype induced as a result of nSREBP1c injection was reminiscent of disturbances in at least two developmental signaling pathways. The absence of anterior structures is typically associated with overactivation or insufficient inhibition of early zygotic Wnt/β-catenin signaling in the anterior region of the embryo (13). In addition, the positioning of the eyes near the central midline was strikingly similar to the phenotype of animals treated with Hedgehog pathway inhibitors, including cyclopamine (14). Thus, these phenotypic similarities suggested that nSREBP1c expression exerted differential effects on at least two key developmental signaling pathways: (a) activation of canonical Wnt/β-catenin signaling and (b) inhibition of Hedgehog signaling. To test this hypothesis, *Xenopus* embryos were coinjected with RNA encoding nSREBP1c and luciferase reporter constructs (pTopFLASH and pGli-Luc) that allowed the monitoring of the activity of the canonical Wnt and Hedgehog pathways, respectively. Consistent with the phenotypic malformations, ectopic expression of nSREBP1c in *Xenopus* embryos resulted in a 3-fold to 4-fold increase in Wnt reporter activity and a 2-fold to 3-fold decrease in Hedgehog reporter activity (Fig. 2).

**Expression of nSREBP1c impairs primary cilium formation in Xenopus embryos and in mammalian cells**

Our data that nSREBP1c abrogated Hedgehog signaling and activated Wnt/β-catenin signaling were highly reminiscent of the effects induced by disruption of the primary cilium in previously published studies (15–21). The primary cilium is a sophisticated microtubular sensory organelle that is expressed on the surface of nearly every cell (22–24). It functions as an antenna that senses external cues and translates them into changes in intracellular signaling pathways, including Wnt and Hedgehog signaling, which coordinately regulate organ development and tissue homeostasis. Hence, we investigated whether expression of SREBP1c in *Xenopus* embryos affected the formation of the primary cilium. The floor plate of the neural tube is known to express primary cilia that can easily be visualized (21). We injected one-cell stage *Xenopus* embryos in the animal pole region with RNA encoding nSREBP1c or with control RNA. Immunofluorescence staining of acetylated tubulin, which is a component of the cilary axoneme, revealed several large cilia in the lumen of the neural tube of control embryos at stage 26, whereas the nSREBP1c-expressing embryos had only a few or no cilia present (Fig. 3; Supplementary Movies S1 and S2). To provide further evidence for a direct causal link and to explore whether similar effects occur in mammalian cells, we studied the effects of expressing nSREBP1c on primary cilium formation in the MDCK cell line, one of the prime mammalian models to investigate primary ciliogenesis. To activate the lipogenic pathway, MDCK cells were infected with an adenovirus encoding nSREBP1c or an empty control virus. Increased lipogenic activity was confirmed by western blotting for FASN expression and assessment of lipogenic activity (Supplementary Figs. S3 and S4). The control cultures readily displayed primary cilia on ~70% of the cells 7 days post-seeding. Infection with the nSREBP1c adenovirus decreased the percentage of ciliated cells to 22% and induced a marked enlargement of the cells (see below; Fig. 4A). Trypan blue exclusion assays showed that overexpression of nSREBP1c did not induce an increase in the number of dead cells (Supplementary Fig. S5), indicating that the effect of SREBP1c on cilium formation is not caused by nonspecific effects due to cell stress or cytotoxicity. Taken together, these data showed that tumor-associated SREBP1c expression impaired primary cilium formation in *Xenopus* embryos and mammalian cells.

**Modulation of primary cilium formation by nSREBP1c involves changes in fatty acid supply**

To confirm that the effects of SREBP1c on primary cilium formation involved changes in the fatty acid supply, we did four complementary experiments. In the first experiment, we infected MDCK cells with the nSREBP1c-encoding adenovirus and then blocked fatty acid synthesis by treating the cells
with soraphen A (9, 10), a potent inhibitor of fatty acid synthesis (25). Treatment with soraphen A largely reversed the effect of nSREBP1c on primary ciliation; there was outgrowth of primary cilia from the cells and a reduction in the cell surface area (Fig. 4A). In the second experiment, we transfected MDCK cells with expression constructs encoding FASN and acetyl-CoA carboxylase, which are the two downstream targets of nSREBP1c that catalyze fatty acid synthesis. Expression of FASN- and acetyl-CoA carboxylase significantly reduced the percentage of ciliated cells from 79% for control cells to 27% for FASN- and acetyl-CoA carboxylase-transfected cells (Fig. 4A). In the third experiment, we attempted to mimic the effect of nSREBP1c expression on ciliation by treating nontransfected cells with palmitic acid, which is the primary end product of fatty acid synthesis. Increasing doses of palmitic acid resulted in a dose-dependent decrease in ciliation, similar to the effects observed with nSREBP1c expression (Fig. 4A; Supplementary Fig. S6). These effects were not caused by general lipid-mediated cytotoxicity and cell death because the treatment did not significantly increase the number of dead cells (Supplementary Fig. S7). Loss of the primary cilium upon nSREBP1c expression or palmitic acid treatment was confirmed by scanning electron microscopy (Fig. 4B). Finally, we explored whether cancer cells that display high rates of lipogenesis and lack a primary cilium,
such as LNCaP prostate cancer cells, would reexpress the cilium after blocking fatty acid synthesis. As shown in Fig. 4C, only 5% of LNCaP cells in control conditions displayed a cilium. After small interfering RNA–mediated knockdown of FASN (Supplementary Fig. S8), acetylated tubulin-positive cilia were discernable in 15% to 20% of the cells. Similarly, blockage of the SREBP1c-activating cleavage with 25-hydroxycholesterol, which decreased the lipogenic activity by ∼40%, increased the percentage of ciliated cells 5-fold (Supplementary Fig. S9).

Activation of the lipogenic pathway distorts cell polarization and protein sorting

The expression of the primary cilium at the apical surface of epithelial cells is often linked to cell polarization. In several experimental systems, cilium formation is considered the terminal stage of cell polarization, and conversely, disruption of the primary cilium affects cell polarization (26, 27). To assess whether the distortion of the primary cilium in response to activation of lipogenesis is accompanied by changes in epithelial cell polarization, MDCK cells were plated on a solid substrate and infected with the nSREBP1c-encoding adenovirus or empty control virus. The cells were allowed to polarize for 7 days and were examined for changes in the cellular distribution of the tight junction marker ZO-1, the apical proteins gp114/CEA and gp135/podocalyxin, and the lateral marker E-cadherin. nSREBP1c expression induced a dramatic morphologic change in MDCK cells. In contrast to control cells, which had a tall columnar epithelial phenotype, nSREBP1c-transduced cells displayed a more flattened morphology, which caused the cells to seem larger (Fig. 5). The formation of ZO-1–containing tight junctions was unaffected by nSREBP1c expression. The lateral marker E-cadherin was also unaffected. In contrast, the apical proteins gp114/CEA and gp135/podocalyxin were mislocalized. Under control conditions, gp114/CEA was predominantly localized to the apical surface, whereas it was enriched

Figure 2. nSREBP1c expression modulates Wnt and Hedgehog signaling in Xenopus embryos. A, luciferase reporter assay for canonical Wnt/β-catenin signaling activity. Xenopus embryos were injected at the one-cell stage with pTopFLASH together with 100 pg nSREBP1c RNA or control RNA. The luciferase activity was measured at stage 11.5. Error bars, SE; **, P < 0.005 (NControl = 4 pools of 3 embryos; NnSREBP1c = 4 pools of 3 embryos). B, luciferase reporter assay for Hedgehog signaling activity. Xenopus embryos were injected at the one-cell stage with pGli-Luc together with 100 pg nSREBP1c RNA or control RNA. The luciferase activity was measured at stage 19. Error bars, SE; ***, P < 0.0005 (NControl = 21 embryos; NnSREBP1c = 23 embryos).

Figure 3. Expression of nSREBP1c impairs primary cilium formation in Xenopus embryos. A, immunofluorescence microscopic visualization of primary cilia on the apical surface of floor plate cells in the neural tube of control and nSREBP1c-injected Xenopus embryos. Sections of embryos at the tailbud stage (stage 26) were stained with an antibody against acetylated tubulin (green) and 4',6-diamidino-2-phenylindole (DAPI; blue). Bar, 10 μm; dashed lines, border of the lumen of the neural tube; arrows, primary cilia. B, graphic representation of the quantification of primary cilia in sections of the neural tube (NControl = 35; NnSREBP1c = 37).
Figure 4. Expression of nSREBP1c impairs primary cilium formation in mammalian cells through modulation of fatty acid supply. A, effects of nSREBP1c expression and modulation of fatty acid supply on ciliogenesis in MDCK cells. MDCK cells were grown on cover slips; infected with an adenovirus encoding nSREBP1c or a control virus; transiently transfected with expression plasmids encoding acetyl-CoA carboxylase (ACC), FASN, and DsRed or empty control constructs; treated with 10 nmol/L soraphen A; or exposed to 100 μmol/L of exogenous palmitic acid, as indicated. At day 7 post-seeding, cells were stained with an antibody against acetylated tubulin (red or green). Nuclei were stained with DAPI (blue). Bar, 20 μm. Right, graphic representation of the percentage of ciliated cells; error bars, SE; ***, P < 0.005 (top: Ncontrol = 1068 and NnSREBP1c = 258; soraphen experiment: Ncontrol = 167 andNsoraphen A = 255; FASN/ACC experiment: Ncontrol = 202 and NFASN/ACC = 84; palmitic acid experiment: Ncontrol = 67, Npalmitic acid = 53). B, scanning electron microscopy of apical membranes of MDCK cells infected with nSREBP1c-encoding adenovirus or exposed to 100 μmol/L palmitic acid. Bar, 10 μm. C, effect of small interfering RNA (siRNA)-mediated knockdown of FASN on primary cilium formation in LNCaP cells. LNCaP cells were transfected with 50 nmol/L siRNA targeting FASN (siFASN) or with a control siRNA (Control). At day 6 post-seeding, cells were stained for acetylated tubulin (red). Nuclei were stained with DAPI (blue). Bar, 20 μm. Right, graphic representation of the percentage of ciliated cells. Error bars, SE; **, P < 0.05 (Ncontrol = 190; NsiFASN = 128).
Figure 5. Activation of the lipogenic pathway distorts cell polarization and protein sorting in epithelial cells. MDCK cells were grown on cover slips and infected with an adenovirus encoding nSREBP1c or a control virus (control). Where indicated, nSREBP1c-transduced MDCK cells were treated with soraphen A, or control cells were exposed to exogenous palmitic acid. At day 7 post-seeding, cells were stained for acetylated tubulin (cilia; red), the tight junction marker ZO-1 (green), the apical proteins gp114/CEA (green) and gp135/podocalyxin (green), or the lateral marker E-cadherin (red). Nuclei were stained with DAPI (blue). Top views as well as cross-sectional Z-stack projections are shown. Bar, 20 μm. Yellow arrowheads, enrichment of the apical protein gp114/CEA on the basolateral surface.
at the lateral surface in nSREBP1c-transduced cells. The apical marker gp135/podocalyxin partially disappeared in nSREBP1c-transduced cells, which is a phenomenon that has been linked to mislocalization of the protein (26, 28). Interestingly, these changes were reversed when cells were treated with soraphen A and were also induced in nontransfected cells exposed to exogenous palmitic acid.

**Activation of the lipogenic pathway distorts epithelial cyst formation and impairs ciliogenesis and normal gland formation during prostate development in mice**

The MDCK cell line, when grown as three-dimensional cultures in Matrigel, forms cysts, which is related to the process of epithelial polarization. This model is thus a unique *in vitro* system to study the mechanisms underlying epithelial morphogenesis (29–31). To assess whether nSREBP1c also affected this process, MDCK cells were infected with control or nSREBP1c-encoding virus and embedded in Matrigel. Numerous cysts were observed in the control cultures 7 days later. More than 95% of the cysts contained one single lumen surrounded by an average of nine cells at the maximal section and manifested large cilia that protruded into the central lumen (Fig. 6A). nSREBP1c-transduced cells formed fewer cysts and exhibited a polarization defect. Cysts that did grow out were overall much larger with ≥15 cells at the maximal section. In terms of the luminal content of cysts, 15% had a single lumen, 50% had >1 lumen, and 35% had a collapsed lumen. Moreover, only 25% of the cysts had primary cilia, and most of these cilia were deformed. Thus, these data suggest that enhanced lipogenesis perturbs normal

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**Figure 6.** Activation of the lipogenic pathway distorts epithelial cyst formation and tissue architecture in three-dimensional cultures of MDCK cells and in the developing mouse prostate. A, effect of nSREBP1c on epithelial cyst formation by MDCK cells. nSREBP1c-transduced or control MDCK cells were grown in Matrigel for 7 days, and fixed and stained for acetylated tubulin (red), and with Hoechst 33258 to visualize nuclei (blue). Bar, 10 μm. The nSREBP1c-infected cells formed larger cysts (S1), had >1 lumen (S2) or a collapsed lumen (S3), and had no or deformed cilia (S1–S3). Graph, overview of the different cyst morphologies observed under both conditions (N<sub>Control</sub> = 20; N<sub>nSREBP1c</sub> = 20). B, effect of nSREBP1c on ciliogenesis and tissue architecture in the pubertal mouse prostate. Mouse prostates were infected with an adenovirus encoding nSREBP1c or a control virus. Seven days post-intraprostatic adenoviral injection, prostates were stained for FASN expression (B1 and B1'), with H&E (B2 and B2'), or for acetylated tubulin (red; B3 and B3'). Nuclei were stained with DAPI (blue). Arrows, primary cilia; images in B3 and B3', Z-stack sections. Bar, 50 μm.
cyst formation, reflecting distorted epithelial tubule formation, which is a key feature of many epithelial cancers.

To further confirm that lipogenesis affects epithelial tissue formation in mammalian models, we ectopically expressed nSREBP1c in the pubertal mouse prostate, which is an excellent \textit{in vivo} model of epithelial tissue development. Anterior prostates of 5- to 6-week-old mice were injected with either an empty control virus or an adenovirus encoding nSREBP1c to activate the lipogenic pathway during prostate development. As shown in Fig. 6B, nSREBP1c locally increased lipogenesis, as shown by enhanced FASN expression. This was accompanied by appearance of scanty ciliated and poorly polarized epithelial cells, and a distortion of gland formation.

**Discussion**

The results from our study support an unexpected novel role for tumor-associated lipogenesis in cancer cell biology. In addition to its well-established involvement in the supply of building blocks for rapid cell proliferation, we provide evidence that activation of fatty acid synthesis, as observed in many advanced cancers, distorts cell polarization and suppresses the formation of the primary cilium. These conclusions are derived from findings on ectopic expression of SREBP1c in \textit{Xenopus} embryos and are substantiated in mammalian models. The involvement of fatty acids is supported by the observations that these effects can be mimicked by overexpression of lipogenic enzymes or by exogenous palmitic acid and can be reversed by inhibition of fatty acid synthesis. Our findings that enhanced fatty acid supply causes a dramatic flattening of the cells that is accompanied by mislocalization of apical proteins, together with recent reports that interference with apical protein transport impairs cell polarization and blocks ciliogenesis (27), suggest that perturbed apical protein transport and subsequent impairment of cell polarization may underlie defective ciliogenesis induced by activated lipogenesis. Conversely, as both phenomena are intricately linked (19, 26), defective ciliogenesis may subsequently prevent proper cell polarization, which reinforces the phenotype. The significance of these findings is supported by the mounting evidence that defective cell polarization and loss of the primary cilium often accompany cancer progression (24, 32–35). Because the primary cilium functions as a cellular antenna that concentrates and coordinates the activity of several signaling pathways, its loss may render cells less dependent on external cues. As a consequence, this loss may contribute to the constitutive activation of cancer-associated signaling pathways, such as Wnt signaling, which may be further activated by fatty acid synthase–mediated palmitoylation of Wnt ligands and by mutations in signaling components (5). Furthermore, as ciliogenesis utilizes the same structural elements that are necessary for mitotic spindle formation, suppression of ciliogenesis by activation of fatty acid synthesis may release a direct physical restraint on cell cycle (36). Accordingly, SREBP1c is stabilized by phosphorylation during cell cycle progression and is activated in cancer cells as part of the growth factor response (37, 38). Consistent with the loss of the primary cilium, increased lipogenesis was accompanied by distorted epithelial morphogenesis both in the MDCK and the mouse models. Together with the recent observation that ectopic prostatic expression of fatty acid synthase in transgenic animals resulted in prostatic intraepithelial neoplasia (6), our findings support a key role for tumor-associated lipogenesis in cancer progression and reinforce the importance of targeting this metabolic pathway for therapeutic intervention. Our observations that the effects on primary cilium formation can be mimicked by exogenous fatty acids may also reveal a novel interesting link between diet and cancer. Moreover, in view of the emerging evidence that loss of the primary cilium is involved in the etiology of other lipid-related diseases, including obesity and diabetes (39), our findings that pathologic SREBP1c expression and exogenous fatty acids suppress cilium formation may open an exciting new area of research that extends far beyond our original observations in the field of cancer biology.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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