thematic review

Thematic review series: Skin Lipids

Sebaceous gland lipids: friend or foe?

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Abstract Sebaceous glands are intriguing glands that are found throughout the human body except on the palms of the hands and soles of the feet. The true function of these glands has yet to be determined, but there are several theories, including antioxidant effects, antibacterial effects, and transport of pheromones. Sebaceous glands produce lipids that are involved in the pathogenesis of one of the most prevalent diseases of adolescence, acne. Although the majority of lipids produced by the sebaceous gland are also produced in other areas of the body, there are two that are characteristic of the sebaceous gland, wax esters and squalene. This review seeks to present an update on the physiology of the sebaceous glands, with particular emphasis on the production of sebaceous lipids.—Smith, K. R. and D. M. Thiboutot. **Sebaceous gland lipids: friend or foe? *J. Lipid Res.* 2008.** 49: **271–281.**

Supplementary key words wax esters • sebum • sebum functions • acne

Sebaceous glands are found all over the human body except on the palms of the hands and soles of the feet. The glands are numerous on the face and scalp and are sparse in areas such as the back. They can number as many as 400–900 glands/cm^2 on the face. Sebaceous glands are usually found in association with a hair follicle, which, together, is referred to as a pilosebaceous unit (**Fig. 1**). The sebaceous gland is located in association with the upper portion of the hair follicle, where it is not affected by the hair cycle. Sebaceous glands can be unilobular or multilobular. Although a majority of sebaceous glands are part of a pilosebaceous unit, some glands can be found without an associated hair follicle. Special nomenclature exists for such glands based on their location on the body. Fordyce spots are found on the lip and buccal mucosa. Meibomian glands and glands of the Zeiss are found on the eyelids, and Montgomery areolar tubercles are found in association with lactiferous ducts. Although the glands have several different names, they serve the same purpose: to secret sebum via holocrine rupture of individual sebocytes. There are several reviews that discuss the function and regulation of human sebaceous glands (1, 2).

DISEASES OF THE SEBACEOUS GLAND

Acne

Acne is the most common disorder involving the sebaceous gland. It is estimated that the prevalence of acne in adolescents reaches 100%. The pathogenesis of acne centers on the interplay of: *1*) sebum (lipid) production by the sebaceous gland; *2*) colonization of the hair follicle by *Propionibacterium acnes*; *3*) hyperkeratinization of the upper follicle; and *4*) release of inflammatory mediators into the skin (3). Acne cannot occur without sebum, which serves as a nutrient source for *P. acnes*. Few therapeutic agents, apart from 13-*cis*-retinoic acid (RA) and systemic antiandrogens (for use in women only), are effective inhibitors of sebum production. There is an unmet need in the treatment of acne for agents that safely reduce sebum production in both men and women.

Seborrhea

Seborrhea is more commonly known as oily skin. Areas that are commonly affected are those that contain a higher density of sebaceous glands, such as the face, ears, scalp, and upper part of the trunk. Seborrhea may predispose patients to the development of seborrheic dermatitis, a disorder with red, scaly patches of skin all over the body.

Sebaceoma

A sebaceoma is a benign tumor of the sebaceous gland. Sebaceomas can also be seen as part of hereditary neoplasm cancer syndromes such as Muir Torre, which is caused by a disruption in DNA mismatch repair.

Sebaceous carcinoma

Sebaceous carcinoma is the name given to a variety of malignant tumors that undergo aggressive sebaceous

Manuscript received 20 August 2007 and in revised form 31 October 2007.

Published, JLR Papers in Press, November 1, 2007.
DOI 10.1194/jlr.R700015-JLR200

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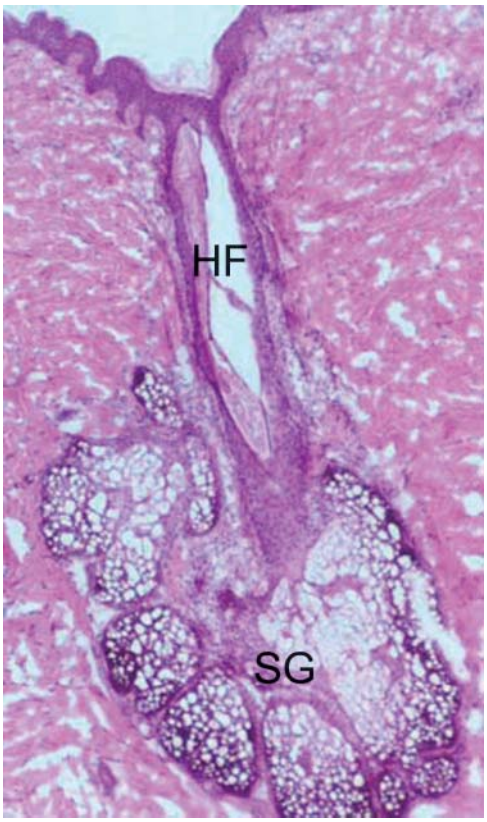

Fig. 1. Cross-section of a pilosebaceous unit. In the center of the figure is a hair follicle (HF) surrounded by a multilobular sebaceous gland (SG).

differentiation. These tumors are extremely rare and consist of two different types: ocular, which is more common, and extraocular. The mainstay of treatment is surgery, but radiation may also be used for patients with eyelid neoplasms.

SEBACEOUS GLAND DEVELOPMENT

Sebaceous gland development occurs during the 13th to 16th weeks of gestation from the most outward bulges on the developing hair follicles in the human fetus. The gland remains attached to the hair follicle by a duct that serves as the canal for sebum to flow to the hair follicle and ultimately to the skin surface. The bulge area of the hair follicle contains the epithelial progenitor cells, which repopulate the continually cycling hair follicle. These epithelial progenitor cells in skin give rise to the epidermis as well as the epithelial component of skin appendages, including hair follicles and associated sebaceous glands. Several pathways have been discovered to be involved in hair follicle and sebaceous cell development. **Figure 2** gives an overview of the pathways that are involved in sebaceous gland development.

At least three pathways have been found to be of importance to sebaceous gland development. These pathways are the Wnt signaling pathway, the c-*myc* signaling

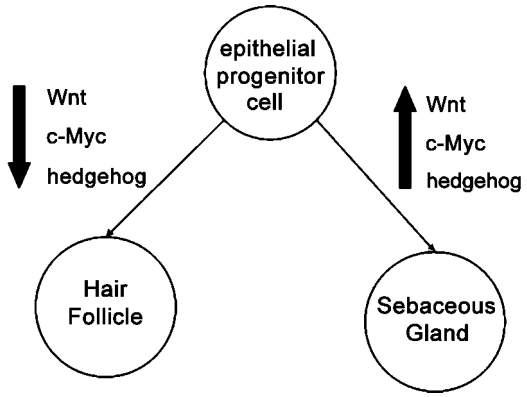

Fig. 2. Pathways that affect sebocyte differentiation.

pathway, and the hedgehog signaling pathway (Fig. 2). Wnt signaling occurs through the stabilization of β-catenin. Mice with mutations in β-catenin, which decrease Wnt signaling through a binding defect in β-catenin, have an increased number of sebaceous glands over normal mice (4). The next player in the sebaceous gland development pathway is c-*myc*. The *Myc* gene, which encodes the c-Myc protein, is a downstream target of the β-catenin/T-cell factor transcription factor. Transgenic mice overexpressing c-*myc* in the basal layer of skin have an increase in sebaceous gland size and number (5–7). In addition to the increase in sebaceous gland proliferation, there is a decrease in the number of hair follicles. The third pathway involved in sebaceous gland development is the hedgehog signaling pathway. Hedgehog protein family members mediate transcriptional effects through Gli proteins. Transgenic mice have been created for proteins along the hedgehog signaling cascade. The skin of mice with a gain-of-function mutation for hedgehog signaling was found to have an increase in the number of sebaceous glands associated with hair follicles as well as of ectopic sebaceous glands not associated with a hair follicle (8). It is also interesting that c-*myc* was found to be upregulated in the skin of mice with a gain of function of hedgehog signaling. These mice give evidence that these pathways are important for sebaceous gland development and that some cross-talk exists between these pathways.

The sebaceous gland is a holocrine gland, which indicates that the glandular secretion consists of cells from the gland itself. The sebaceous gland contains two kinds of cells (sebocytes): peripheral cells and central cells (**Fig. 3**). The peripheral cells are cubodial or flattened and are immature cells that contain no lipids. When the sebocytes progress to the center of the gland, they mature. As the cells differentiate, there is an increase in smooth endoplasmic reticulum, where the lipids are produced, and Golgi apparatus for packaging of the lipids. The central cells are bigger than the peripheral cells, and the majority of the increase in size is attributable to the increase in cytoplasmic lipids. The increase in lipid accumulation can be detected by Oil Red O staining. Ultimately, as the cells differentiate, they reach the center of the gland, where they disintegrate and release their contents into the follicle.

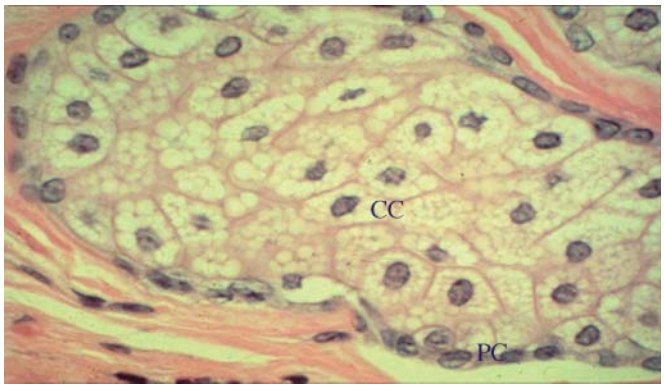

Fig. 3. Close-up image of a sebaceous gland. This is one lobule of a sebaceous gland, showing the flattened peripheral cells (PC) and the central cells (CC), which have a frothy appearance as a result of the accumulation of lipids.

The cell that is most like the sebocyte is the adipocyte. Data from adipocyte models have been used for extrapolation into sebocytes. Both cell types have a similar physiology, the accumulation of lipids, for two separate functions. For the sebocyte, the function is to release lipids that eventually make their way to the skin surface, as opposed to the energy-storing function of the adipocyte lipids. What makes the sebocyte and adipocyte similar is that they both accumulate lipids. Both cells have similar receptors and express enzymes important for lipid production [LDL, liver X receptor (LXR), diacylgylcerol acyltransferase (DGAT), and stearoyl-CoA desaturase 1 (SCD1)]. Treatment of both adipocytes and sebocytes with LXR agonists causes a decrease in lipogenesis. It would be very interesting to obtain more data directly comparing sebocytes and adipocytes.

LIPIDS OF THE SEBACEOUS GLAND

Human sebum contains cholesterol, cholesteryl esters, squalene, fatty acids, diglycerides and triglycerides, and wax esters. **Table 1** gives a comparison of the epidermal lipids and sebum. Human sebum is unique compared with the sebum of other animals (**Table 2**). The reasons for the uniqueness of human sebum are not known but can be hypothesized to be attributable to differences in the functions of sebum among species. In humans, the function of sebum is not really known, but several theories are discussed below. In animals, sebum content may be more specialized based on each animal's specific needs, environment, waterproofing (for animals that spend a majority of time in the water), etc. Although the content of sebum may not be the same, new insights about the function of human sebum may be determined from studying sebum in other animals.

TABLE 1. Components of sebum and epidermal lipids

Lipids	Sebum Weight	Epidermal Surface Lipid Weight
	%	
Triglycerides, diglycerides, and free fatty acids	57	65
Wax esters	26	NA
Squalene	12	NA
Cholesterol	2	20

NA, not applicable.

TABLE 2. Components of sebum from different animals

Lipids	Mouse (96)	Rat (97)	Rabbit (98)	Rat Preputial (97)
		% weight		
Triglycerides, diglycerides, and free fatty acids	9	9	NA	62
Wax esters	5	25	4	
Squalene	NA	0.5	NA	1.5
Cholesterol	13	5	4	2

NA, not applicable.

Cholesterol makes up ~2% of sebaceous gland lipids. Cholesterol is not unique to the sebaceous gland, is found throughout the body, and is a component of cellular membranes. All of the carbons used in cholesterol biosynthesis are derived from acetate. Squalene is the linear intermediate in cholesterol biosynthesis, and in other tissues it is quickly converted to lanosterol and finally to cholesterol. Squalene is produced by the fusion of two molecules of farnesyl pyrophosphate through the action of squalene synthase. Squalene is not only found in sebum: levels of squalene are increased in the serum of postmenopausal woman with coronary artery disease (9). Squalene accounts for 12% of the lipid composition of sebum and is not found in the internal organs or among the other epidermal surface lipids. It is very interesting that the squalene produced in sebaceous cells is not converted to cholesterol.

There are several possibilities for why there is a buildup of squalene in the sebaceous gland with little conversion of squalene to lanosterol and cholesterol. The first possibility is that there is an overexpression of, or an increase in the activity of, the squalene-producing enzyme, squalene synthase, inside the cell. Although squalene synthase levels have never been measured in sebocytes, several studies have determined squalene synthase mRNA levels in response to an anaerobic environment, which is found inside sebaceous glands. The yeast squalene synthase gene ERG9 in *Saccharomyces cerevisiae* has decreased expression in anaerobic conditions (10). Sterol-response element binding proteins 1a and 2 (SREBP1a and SREBP2) have been shown to increase the transcription of human squalene synthase in the livers of transgeneic mice overexpressing SREBPs (11, 12). SREBP1 has been shown to be increased in SEB-1 cultured human sebocytes in response to insulin and insulin-like growth factor (IGF) (13). These results suggest that the levels of squalene synthase are affected by environmental conditions present in the sebaceous gland and in the transcription factors found in the sebaceous gland.

Another possibility for why squalene is so abundant in the sebaceous gland is that there is a decrease in the levels and/or activity of the enzymes that process squalene into cholesterol. Squalene must be converted to squalene 2,3-epoxide by the enzyme squalene oxidocyclase to proceed to becoming cholesterol. For squalene oxidocyclase to catalyze the reaction, molecular oxygen is needed, and because the sebaceous gland has an anaerobic environment, this may become the rate-limiting step for the conversion of squalene to cholesterol. The appearance of squalene as a major component of sebum may be a result of the unique environment of the sebaceous gland.

Wax esters, like squalene, are unique to sebum and not produced anywhere else in the body. They account for ~25% of sebaceous gland lipids, and their production is important in the survival of the sebaceous gland. This can be seen in mice that lack these lipids. In the acyl-CoA:DGAT1 knockout mouse, there is sebaceous gland atrophy and hair loss (14). DGAT is an important enzyme in the synthesis of triglycerides and has two forms, DGAT1 and DGAT2, which differ in sequence and localization (15). DGAT1 is involved in the synthesis of wax esters, unlike DGAT2 (16), and is expressed in most tissues, including the skin and the sebaceous gland (14, 15). The abnormalities seen in the DGAT1 knockout mouse are not present until after puberty, which is when the sebaceous gland becomes most active. When analyzing the fur lipids of the DGAT1 deficient mouse, little to no wax esters are found. An interesting twist to this study is that in DGAT1-deficient mice, when bred on an obese mouse background with a deficiency in leptin, the abnormalities in the sebaceous gland and fur return to normal. This suggests that leptin has an effect on the production of wax esters in the sebaceous gland when DGAT is absent (14). It also may be possible that the absence of DGAT leads to the buildup of precursors that act as signaling molecules. In the leptin-deficient mouse, these signaling molecules either do not build up or do not effectively transmit the signal that leads to sebaceous gland involution. This is an area in which more research needs to be done to determine the mechanism involved in sebaceous gland dysfunction in the DGAT-deficient mouse.

Although the other lipids produced in the sebaceous gland can be found in other areas of the body, some of these lipids have features that are unique to the sebum. Sebaceous gland fatty acids, for example, are branched-chain fatty acids that are uncommon in other organs (17, 18). To synthesize the branched fatty acids, it is thought that branched intermediates are used to extend the fatty acid chain.

Another aspect that distinguishes sebaceous lipids from other human lipids is the pattern of unsaturation seen in sebaceous lipids. The "normal" mammalian pathway of desaturation involves inserting a double bond between the ninth and tenth carbons of stearic acid (18:0) to form oleic acid (18:1Δ9). A Δ6 double bond can be added only after the Δ9 double bond is in place. The Δ6 desaturase enzyme (fatty acid desaturase-2) converts linoleate and α-linoleate into long-chain polyunsaturated fatty acids. Within human skin, Δ6 desaturase mRNA and protein expression is restricted to differentiating sebocytes located in the suprabasal layers of the sebaceous gland (19). This enzyme catalyzes a "sebaceous-type" reaction of converting palmitate into the monounsaturated fatty acid sapienate, a 16 carbon fatty acid with a single *cis* double bond at the sixth carbon from the carboxyl end. Sapienic acid is the most abundant fatty acid in human sebum and is not present in the sebum of other hair-bearing animals. Elongation of the chain by two carbons and insertion of another double bond between the fifth and sixth carbons yields sebaleic acid (18:2Δ5,8), a fatty acid thought to be unique to human sebum. This work identifies Δ6 desaturase as the major fatty acid desaturase in human sebaceous glands and suggests that the environment of the sebaceous gland permits catalysis of the sebaceous-type reaction and restricts the catalysis of the polyunsaturated fatty acid-type reaction (19). The unsaturated fatty acids play a prominent role in the sebaceous gland.

The importance of these unsaturated fatty acids in the sebaceous gland can be seen in the SCD1-deficient mouse. SCD catalyzes the Δ^9-*cis* desaturation of methylene-interrupted fatty acyl-CoA substrates and is the rate-limiting factor in those reactions. The preferred substrates for SCD are palmitoyl- and steraoyl-CoA. Expression of SCD1 has been found in the liver, eyelid, white adipose tissue, and skin of the mouse. SCD1 knockout mice exhibit a narrow eye fissure, thinner hair coat than wild-type mice, and atrophy of the sebaceous glands. The remnant of the sebaceous gland no longer has its foamy appearance. The skin of these mice was also found to have lower levels of wax esters and monounsaturated fatty acids. The asebia mouse has an extensive natural deletion in the SCD1 gene and shows scant to absent hair, in combination with fibrous tissue replacement of hair follicles and hypoplastic to absent sebaceous glands (20). This mouse is used as a model for alopecia and suggests that the sebaceous gland may be involved with hair development. This hypothesis is supported by the fact that sebaceous glands are scant in certain forms of alopecias (21). This mouse is very useful for determining the interaction of sebaceous glands with hair follicles.

The majority of the body receives its lipids through the uptake of circulating lipids. Sebaceous glands express at least two different receptors involved in the uptake of circulating lipid, FATP4 and LDL receptor. FATP4 is a fatty acid transporter that has been shown to be expressed in sebaceous glands (22). It has also been shown that sebaceous glands and the human sebocyte cell line SEB-1 express the LDL receptor (22, 23). The uptake of circulating lipids is also suggested by the observation that upon beginning a fast, the incorporation of free fatty acids into sebum is reduced by 20% (24, 25). Also of note is that transgenic mice overexpressing apolipoprotein C-I have sebaceous gland atrophy (26). All of these results indicate that the uptake of circulating lipids is an important step in the production of sebaceous lipids.

Most studies to determine the uptake of lipids into the sebaceous gland have used radiolabeled lipids. In one

study, to determine the fate of circulating lipids, punch biopsies of skin were incubated with radiolabeled palmitic acid, oleic acid, and monounsaturated and polyunsaturated lipids (27). Acetate was incorporated into all of the cellular and secreted lipids, and palmitate was incorporated into all of the fatty acid-containing lipids. Palmitate was elongated to oleic acid and was incorporated into polar lipids, then triglycerides, but not into other lipids. Linoleic acid was the only fatty acid that appeared to be subjected to β-oxidation. The ability of sebaceous cells to synthesize wax esters correlated with the β-oxidation activity. Thus, the oxidation of linoleic acid is specific to sebaceous cells and correlates with their function and differentiation. These results provide evidence that the sebaceous gland selectively used fatty acids. Palmitic acid is the preferred fatty acid for incorporation into wax esters, and linoleic acid undergoes β-oxidation (27). The sebaceous gland provides an interesting model to study lipid production that is different from that found in other areas of the body.

SEBACEOUS GLAND FUNCTION

Although the mechanisms by which sebaceous glands produce and release their lipid products are fairly well understood, little is known of the putative function of sebum. There are, however, several theories. Sebum may represent a delivery system for antioxidants, antimicrobial lipids, and pheromones (28, 29). One school of thought is that sebum functions to deliver antioxidants to the surface of the skin in the form of vitamin E (28). Vitamin E is a known antioxidant and its primary form is α-tocopherol. There is a correlation between the characteristic sebaceous lipid squalene and α-tocopherol levels on the surface of the skin. Increased levels of α-tocopherol are found on the face, where there is a greater population of sebaceous glands, compared with the upper arm. It is believed that α-tocopherol is the main antioxidant on the skin (30). The antioxidant function of sebum may be important, because it is hypothesized that the buildup of reactive oxygen species on the skin surface could cause a breakdown of the skin barrier and some of the signs of aging. The delivery of vitamin E through sebum could serve an important function in preventing aging and maintaining a healthy skin barrier.

Several other functions have been suggested for sebum, including antibacterial effects and the delivery of pheromones. This hypothesis of antibacterial function was derived from the observation that fatty acids of sebum may exhibit self-sterilizing properties. Studies performed with fractions of sebaceous lipids suggest that sebum can affect the viability of *Streptococcus* but not *Staphylococcus* or *Escherichia coli* (29). The components of sebum that are hypothesized to have the greatest antibacterial effect are oleic and palmitoleic acids. Administration of palmitoleate in wild-type C57BL/6 and mutant *flake* mice, which have an increase in spontaneous skin infections, causes a decrease in the size of bacterial lesions (31). The mechanism by which oleic and palmitoleic acids are thought to inhibit fatty acid synthesis in bacteria is through the inhibition of FabI. FabI catalyzes the final and rate-limiting step of the chain elongation process in bacteria. Unsaturated fatty acids have been found to be inhibitors of FabI. The fatty acids tested, including oleic acid and palmitoleic acid, had the ability to inhibit *S. aureus* FabI. These fatty acids were effective against *S. aureus* as well as *S. pyogenes*, although the mean inhibitory concentration was lower for *S. pyogenes* (32). The lipids were not effective against *E. coli* or *Pseudomonas aeruginosa*. These antibacterial properties could explain one of the possible functions of sebum in human. It has also been proposed that sebum functions as a transporter of pheromones, but to date, there are no data to support this hypothesis.

Another possible function of the sebaceous gland is hydration of the stratum corneum (SC). There is a marked decrease in SC hydration in the asebia SCD1 knockout mouse compared with wild-type and heterozygote mice. Supplementation of these mice with sebum-like lipids did not restore normal hydration (33). Notably, the supplemented lipids did not contain wax diesters, because they are not commercially available. Supplementation with glycerol, however, did increase hydration of the SC in these mice. Glycerol is a by-product of triglyceride breakdown; therefore, triglycerides were supplemented on the skin to determine whether they alone could improve hydration. Triglycerides alone could not improve hydration. Glycerol produced by the breakdown of triglycerides by sebaceous gland-associated lipase is needed to maintain hydration of the skin (33). These results suggest that the production of glycerol in the pilosebaceous follicle is important for SC hydration. It has also been recognized that the sebaceous gland may have a function in waterproofing in mice. Mice with a DGAT1 deficiency retained more water on their fur compared with normal mice after 3 min of immersion (14). Although these studies all suggest that sebum plays several important roles, the exact function of the sebaceous gland in humans is still a relative mystery.

The finding that mice with sebocyte dysfunction also tend to have fur/hair abnormalities leads to yet another possible function of sebum as an important member of the hair follicle. The asebia mouse, in addition to a disruption in sebocyte function, also has a disruption of hair. The mouse has been used as a model of cicatricial alopecias (34). Also, other mice with abnormalities in sebaceous gland function have dysfunction of the hair (14, 20). It is possible that there is a disruption in the whole pilosebaceous unit in these knockout mice, but because deficiencies in the enzymes primarily affect the sebaceous gland, it is more likely that the sebaceous gland is important for a proper-functioning hair follicle. This is a very interesting topic of study and may lead to better treatments for conditions such as alopecia.

REGULATION OF SEBACEOUS GLAND FUNCTION

Many compounds have been shown to regulate sebaceous gland function, and several of these compounds are

discussed below. The measure for determining sebaceous gland activity is sebum output. Squalene and wax esters are the most reliable measures of sebum production, because they are unique to sebum and will not be affected by lipids from other skin cells. For most of the agents discussed below, their effects on sebum production are known, but the exact mechanism by which sebum production is altered has yet to be elucidated.

Androgens

Clinical and experimental evidence indicates that androgens affect sebaceous gland function. The majority of circulating androgens are produced by the gonads and the adrenal gland, but they can also be produced locally within the sebaceous gland from dehydroepiandrosterone sulfate, an adrenal precursor hormone. Androgen receptors are expressed in the basal layer of the sebaceous gland and in the outer root sheath keratinocytes of the hair follicle (35, 36). When free testosterone enters the cell, it is quickly reduced to 5α-dihydrosterone (DHT) by the 5α-reductase enzyme. The activity of 5α-reductase is increased in the sebaceous gland in proportion to the size of the gland (37). DHT is ~5–10 times more potent than testosterone in its interaction with the androgen receptor. Upon binding to its receptor protein, DHT is translocated to the nucleus and initiates the transcription of androgen-responsive genes. It has been shown in a hamster ear model that DHT increases sebaceous gland size by increasing sebocyte proliferation and the rate of total lipid synthesis. DHT increases the mRNA of proteins involved in fatty acid, triglyceride, squalene, and cholesterol synthesis. This effect is mediated by the SREBPs. By inhibiting SREBP's effect with 25-hydroxycholesterol, there was a 50% decrease in the lipid synthesis increase by DHT alone (38). Androgens exert their effect on sebaceous glands by increasing the proliferation of sebocytes and increasing lipid production through SREBPs.

There have been several clinical studies examining the role of androgens in the stimulation of sebum production. Exogenous administration of testosterone and dehydroepiandrosterone increases sebaceous gland size and sebum production (39). Other studies have shown that the development of acne in the prepubertal period of development has been associated with increased serum levels of dehydroepiandrosterone sulfate (40, 41). Subjects who lack the androgen receptor and are androgen-insensitive have no sebum production (42). Also, excess production of androgens by tumors (ovarian or adrenal) is often associated with the development of acne. Production of lipids in the sebaceous gland occurs mostly in the smooth endoplasmic reticulum, whose size is increased with testosterone. Despite the fact than an increase in androgens is associated with increased sebaceous gland size and sebum production, there are no data to indicate that the sebaceous gland is involved in locally increasing the concentration of androgens. An increase was found in the activity of the androgen-metabolizing enzymes found on the face, chest, and back compared with the sebaceous glands in non-acne-prone areas when normalized for gland size (43). Determining what causes this increase in androgens that increases sebum production is important to understanding sebaceous gland pathophysiology.

Estrogens

Although it is known that estrogens suppress sebum production, little is known about the mechanisms by which this occurs. Their effect on sebum production is greater when given systemically as opposed to topically (44), and estrogen-containing hormonal birth control is used in women as a treatment for acne. The dose of estrogen required to suppress sebum production is greater than that required to suppress ovulation (45). The most potent estrogen is estradiol, which is produced from testosterone by the action of the enzyme aromatase. Aromatase is active in the ovary, adipose tissue, and other peripheral tissues. Estradiol can be converted to the less potent estrogen, estrone, by the action of the 17β-hydroxysteroid dehydrogenase enzyme. Both aromatase and 17β- hydroxysteroid dehydrogenase are present in the skin (46, 47). There are currently several hypotheses that suggest a mechanism for the suppression of sebum production by estrogens. These include the notions that estrogens directly antagonize androgen activity, estrogens inhibit the production of androgens by gonandal tissue through a negative feedback loop, and estrogens regulate genes involved in lipid production. Rats given testosterone and estrogen simultaneously have a high rate of mitosis but a reduction in gland size and sebum secretion (48, 49). Based on these results, it is thought that estrogens work principally to decrease intracellular lipid production.

Retinoids

This class of vitamin A-derivative pharmacological agents still continues to be used to treat acne. Isotretinoin (13-*cis*-RA) is the most potent pharmacological inhibitor of sebum secretion. Histological changes in sebaceous gland size can be seen after 8 weeks of treatment. The sebaceous glands have a reduced size and the sebocytes appear undifferentiated with decreased lipid accumulation. The mechanisms behind the effects of 13-*cis*-RA are not yet known. It has been determined, however, that 13-*cis*-RA causes cell cycle arrest and apoptosis in the immortalized human sebocyte cell line SEB-1 (50). Isotretinoin is not known to interact with any currently identified retinoid receptors, and it is thought that it may act as a prodrug that delivers all-*trans*-RA and 9-*cis*-RA to the cell. Isotretinoin has been shown to preferentially metabolize into all-*trans*-RA in the immortalized human sebocyte cell line SZ95 (51). Treatment with 9-*cis*-RA and all-*trans*-RA shows a decrease in sebosuppressive effects compared with 13-*cis*-RA (52). Despite the potent activity of 13-*cis*-RA, it is a teratogen; therefore, it is important to continue to search for an alternative non-teratogenic compound to inhibit sebum production.

LXR

Another receptor recently found to be expressed in sebocytes is the LXR. LXRs act as cholesterol sensors and

have been shown to regulate genes involved in the efflux of cholesterol and phospholipids out of the cell upon binding to oxysterols. This receptor has been reported to form heterodimers with the retinoid X receptor. These proteins have been shown to be important to keratinocyte differentiation and epidermal permeability barrier homeostasis (53, 54). These receptors were recently detected in cultured sebocytes and sebaceous glands. Positive staining was apparent in the sebaceous gland within the nucleus. The receptors are also expressed in the SZ95 sebocyte cell line. LXR agonists have been shown to inhibit sebocyte proliferation in vitro and to promote lipogenesis in this cell line (55). The localization of LXRs in sebaceous glands is a very recent result and is a new field of study for how they function in sebocyte physiology.

Peroxisome proliferator-activated receptors

With the discovery of the peroxisome proliferator-activated receptors (PPARs), recent advances have been made in understanding the regulation of lipid metabolism. PPARs are orphan nuclear receptors that exert their action by forming heterodimers with retinoid X receptors and binding to specific response elements on DNA consisting of direct repeats of AGGTCA spaced by one nucleotide (DR-1 sites) (56). There are three subtypes of PPARs (α, β, and γ1–γ3) that differ in their tissue distribution and respective roles in mediating lipid metabolism (57).

PPARs mediate epidermal growth, differentiation, and lipid metabolism. PPARα ligands increase the formation of cornified envelopes and the expression of differentiation proteins in fetal epidermis, normal human keratinocytes, and raft cultures (58–61). In addition, increases in mRNA for a variety of lipogenic enzymes were noted after treatment of keratinocytes with PPARα agonists. The PPAR-regulated genes, fatty acid transport protein, acyl-CoA synthase, and CD36 may mediate lipid uptake into keratinocytes (62, 63). The expression of CD36 in human keratinocytes is induced by treatment with ligands of each PPAR subtype. Administration of the formerly available PPARγ agonist troglitazone to patients improved psoriasis and inhibited keratinocyte proliferation, suggesting that this class of drugs may be beneficial in treating diseases of the skin (64).

The recent emergence of the importance of PPARs as mediators of adipogenesis and lipid metabolism in other tissues raises the question of whether these receptors regulate lipid production in human sebaceous glands (65). Chimeric mice have been generated that lack expression of PPARγ in the skin (66). Studies in these mice demonstrate that a functional PPARγ receptor is required for the development of adipose tissue and sebaceous glands. In rat preputial cells, mRNAs for PPARδ and PPARγ1 have been identified using RNase protection assays (65). Rat preputial cells serve as a model for human sebocytes (67). Ligands of PPARα such as WY-14643 and a PPARγ ligand, the thiazolidinedione BRL-49653, induce the accumulation of lipid droplets in rat preputial sebocytes but not rat keratinocytes. PPARs are expressed in human skin and sebaceous glands, where they may play a role in mediating sebum production. Using RT-PCR and immunohistochemistry, the expression of each of the PPAR subtypes was noted in human sebocytes and human skin (68, 69). RT-PCR showed expression of PPARα, -δ, -γ1, and -γ2 in SZ95 immortalized sebocytes (68). In SEB-1 immortalized sebocytes, agonists of PPARs (α, δ, γ, and pan-agonist) have been shown to increase lipid production. This family of receptors is a possible target for the suppression of sebaceous gland function (70).

IGF and growth hormone

Growth hormone is secreted by the pituitary gland and acts on the liver and peripheral tissues to stimulate the production of IGFs. There are two forms of IGF, IGF-1 and IGF-2, with IGF-1 being the most abundant. It has been hypothesized that growth hormone may be involved in the development of acne (71). Acne is most prevalent in adolescents during a time when growth hormone is maximally secreted and serum levels of IGF-1 are highest. In addition, IGF-1 can be produced locally within the skin, where it can interact with receptors on the sebaceous gland to stimulate its growth. Furthermore, conditions of growth hormone excess, such as acromegaly, are associated with seborrhea and the development of acne. In some tissues, the actions of IGF-1 can be mediated by androgens, and this may be true of the sebaceous gland as well. In cultures of rat preputial cells, growth hormone increased lipid droplets in the presence of insulin. Insulin can also act at the IGF-1 receptor, although with a 2-fold decreased affinity. Treatment of SEB-1 immortalized sebocytes with high doses of insulin (100 μM) and physiological doses of IGF-1 (20 ng/ml) increased lipid production as assessed by lipogenesis. Treatment of SEB-1 cells with insulin and IGF-1 also increased the mature forms of SREBP-1 (13). This suggests that stimulation of the IGF-1 receptor is important for lipid production in sebaceous glands.

The mechanisms used by IGF to increase lipid production and the expression of mature SREBP-1 have been under investigation. Inhibition of the mitogen-associated kinase pathway has been shown to have no effect on lipid production or SREBP-1 expression in the presence of IGF. On the other hand, inhibition of the phosphoinositide kinase-3 pathways decreased lipid production and SREBP expression in sebocytes treated with IGF (23). Although the phosphatidylinositol 3-kinase pathways have been shown to be important for increased lipid production and SREBP expression in response to IGF, more research is needed to determine other pathways that affect the expression of SREBPs in sebocytes.

Growth hormone was more potent than IGF-1 and IGF-2 in increasing lipid droplets. Dihydrotestosterone plus insulin induced lipid-forming colonies. These data suggest that growth hormone stimulates sebocyte differentiation beyond that found with IGF or insulin; yet it had no effect on growth. Increases in growth hormone and IGF-1 production may contribute in complementary ways to the increase in sebum production during puberty and in patients with acromegaly (72).

Evidence exists for the role of epidermal growth factor, IGF-1, and keratinocyte growth factor in modulating sebaceous gland growth. Sebocytes express receptors for growth factors such as epidermal growth factor and IGF-1 (73). The growth of sebocytes is enhanced by supplementation of cell culture medium with epidermal growth factor and insulin. Treatment of experimental animals with keratinocyte growth factor stimulates the growth of hair and sebaceous glands (74, 75). In cultures of hamster sebocytes, epidermal growth factor, transforming growth factor α, and basic fibroblast growth factor each augmented the growth of hamster auricular sebocytes, whereas each of these agents suppressed the intracellular accumulation of triglycerides (76).

Melanocortins

Melanocortins include melanocyte-stimulating hormone and adrenocorticotrophic hormone. They have a role in regulating feeding behaviors, body weight, pigmentation, and immune function. Melanocortin signaling inside the cells is generated through the formation of cAMP. It has also been determined that cholera toxin generates cAMP and can be used to differentiate sebocytes (77). Melanocortins have been shown to increase sebum production in rodents, and mice that lack the melanocortin-5 receptor (MC5R) have reduced sebum production (78, 79). MC5R was detected in normal human skin and cultured keratinocytes but not in melanocytes or fibroblasts (80). MC5R has also been identified in human sebaceous glands (81, 82). It is suggested that MC5R is a marker of sebocyte differentiation. This theory comes from the observation that MC5R is expressed in the central part of the gland and not the periphery, where the immature cells reside. Also, MC5R expression is seen in cultured sebocytes that have been treated with cholera toxin to induce differentiation (82). This distribution is different from the expression of MC1R, which is expressed in both undifferentiated and differentiated sebocytes (82). There are no known mutations of MC5R involved with disorders of the sebaceous gland, such as acne, and sebaceous gland neoplasms. All of the receptor variants respond similarly to stimulation with α-melanocyte-stimulating hormone, which suggests that there is no causative role of MC5R in sebaceous gland dysfunction.

SEBACEOUS GLAND MODEL SYSTEMS

It is very important to have a model system for sebaceous glands because of the difficulty in obtaining large enough numbers of cells in primary culture, attributable to fact that they rupture as they mature. In an ideal model: *1*) the entire gland should be used; *2*) the gland should be morphologically similar to that of human, with a sebaceous follicle, infundibulum, lobules, and a piliary unit; *3*) the model should be androgen-sensitive; and *4*) the model should be economical of both material and time (83). There are multiple sebaceous gland models that have been developed, and as with any model, there are advantages and disadvantages with each system.

Rat/mouse preputial model

The preputial gland in rodents is used for territorial marking. These glands are holocrine glands that mature in a manner similar to sebaceous glands and have been shown to be androgen-responsive. Preputial glands have been used as an androgen-responsive model since the 1950s. The primary limitation of this gland as a sebaceous model is that the composition of lipid produced by the preputial gland differs significantly from the lipid composition of human sebum (84) (Table 2), and it is not associated with a hair follicle (83). In 1979, it was shown that cells isolated from the mouse preputial gland tumor could be grown in monolayer (85), and this work was extended in 1989, when Rosenfield (86) showed that rat preputial glands can be disbursed into a single cell suspension and grown on a layer of 3T3 fibroblasts. These cells grow in monolayer and express K4, a keratin found in sebaceous cells (67). Overall, the preputial gland/cells grown in monolayer are viable models, with limitations that must be considered when interpreting data. Most importantly, the lipid composition and differentiation process are different from those for sebocytes.

Hamster ear/flank model

Like the rat and mouse preputial gland, the flank organ (costovertebral gland) of the hamster is also used by the animal for territorial marking. These glands are similar to human sebaceous glands in that they have an infundibulum, a sebaceous duct, multiple lobules, and a piliary unit that enters from below the gland (83). A benefit of this model is that hair can be shaved from the hamster and topical application of a drug can be made to one flank organ, whereas the other flank can serve as a control. Like the human sebaceous gland, the flank organ is responsive to androgens (87).

Another model used is the hamster ear model. The skin on the inside of the hamster ear contains a dense layer of sebaceous glands. These glands have similar morphology to the human sebaceous gland, similar turnover time, and are androgen-responsive (88). Like the flank organ model, the hamster ear sebocytes can be used for topical application of drugs; furthermore, they may be a better model than the flank organ because their size is similar to that of human sebaceous glands (83).

To date, no animal model has been found predictive in assessing the effects of antiacne drugs in humans (89). Because acne is an exclusively human disease and sebaceous gland activity and differentiation are species-specific, many have attempted to create a model using human sebocytes (84).

Growth of human sebocytes in monolayer

There have been several reports describing various ways to grow primary sebocytes. Most are variations on one of two techniques (90, 91). This work preceded the advent of growing sebaceous glands in organ culture. Most importantly, cells in primary culture exhibit an incomplete

differentiation (92). To circumvent the difficulties in collecting sufficient human skin, two cell lines have been created by SV40 immortalization of primary sebocytes: the SEB-1 cell line (93) and the SZ95 cell line (94).

Both cell lines *1*) have been passaged for several years, *2*) are androgen-responsive, *3*) produce lipid, including triglycerides, squalene, and wax esters, *4*) possess markers characteristic of sebocytes, and *5*) have proliferation inhibited by 13-*cis*-RA.

Although much more convenient and practical for large-scale studies, immortalized sebocytes do not fully differentiate, as shown by the decreased amount of squalene and wax esters produced compared with sebum.

Growth of human sebaceous cellnad glands in primary culture

It has been demonstrated that excised human sebaceous glands can be grown for up to 7 days in organ culture (95). Human chest skin from cardiac surgery is sheared and maintained on polycarbonate filters. In this environment, sebocytes differentiate as they would in vivo. Importantly, sebaceous glands maintained in organ culture respond to steroids and 13-*cis*-RA as do sebaceous glands in vivo. The primary drawbacks for this model include difficulty in obtaining skin, difficulty in preparing the glands for culture, limitations of the size of experiments that can be performed, and the fact that experiments are limited to treatments of up to 7 days from excision. Clearly, there are benefits and disadvantages to each model system. It is important to be aware of the shortcomings of each model when interpreting data, and more work is needed to provide a sebaceous model that more accurately reflects the intact human sebaceous gland.

FUTURE DIRECTIONS

Soon after isotretinoin became available in the 1980s, there was a decline in research into alternative mechanisms for controlling sebum production. Concern over the serious side effects associated with isotretinoin, however, creates a tremendous need for alternative effective approaches to suppressing sebum production. The molecular mechanisms by which retinoids, androgens, and other factors alter sebum production remain obscure, but it seems likely that the genes encoding lipogenic enzymes would be candidate targets for the regulatory influence of hormones and retinoids. There are two available human sebocyte cell lines (SEB-1 and SZ95), and use of these cell systems, in combination with the availability of various pharmacological agonists and antagonists of putative receptors, enzymes, and other proteins, can provide insight into the regulatory mechanisms controlling sebum production. The development of mouse models expressing larger and ectopic sebaceous glands may be useful for testing topical treatments for acne. Ideally, these studies will lead to the identification of alternative therapeutic target sites in the treatment of acne and possibly other diseases affecting the sebaceous gland.

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