Further Evidence of Sebaceous Differentiation Uniqueness: Holocrine Secretion of Sebocytes Is a Multistep, Cell-Specific Lysosomal DNase2-Mediated Mode of Programmed Cell Death

Christos C. Zouboulis

Holocrine secretion by sebocytes does not occur via increased cell volume, but rather from programmed DNA fragmentation and death, which differs from apoptosis. Moreover, it can be enhanced with increased rates of induced terminal sebocyte differentiation. Fischer et al. address the mode of holocrine sebocyte secretion, and they demonstrate that its mechanism differs from that of apoptosis, necroptosis, and cornification, being a multistep, cell-specific lysosomal DNase2-mediated mode of programmed cell death.

Lipogenesis is the major function of sebaceous glands (Zouboulis, 2004). After formation, sebaceous gland cells (sebocytes) mature gradually from undifferentiated to differentiated lipid-producing cells (Plewig and Christophers, 1974). The sebaceous lipids, a mixture containing squalene and wax esters, as well as cholesterol esters, triglycerides, and possibly free cholesterol (Nikkari, 1974), constitute the liquid phase of sebum, whose discharge represents the most obvious sebaceous gland activity. The solid phase of sebum is formed by cell debris, which occurs due to rupture of the sebaceous gland cell (sebocyte) membranes and cellular degradation (holocrine secretion). In turn, holocrine secretion has long been considered to result mechanically from an in vivo increase in sebocyte volume, by a factor of 100–150, due to significant intracellular lipid accumulation (Tosti, 1974).

However, terminally differentiated sebocytes are not only characterized by accumulations of lipid droplets in their cytoplasm and increased cell volume but also by nuclear degradation (Zouboulis et al., 1994). This latter finding disputed the mechanistic concept of holocrine sebocyte secretion. Indeed, Wróbel et al. (2003) detected cytoplasmic accumulation of lipid droplets, cell enlargement, and nuclear fragmentation in SZ95 sebocytes in vitro after treatment with arachidonic acid. Moreover, they observed increased externalization of nonapoptotic phosphatidylserine levels in SZ95 sebocytes, a dose-dependent reduction in bcl-2 mRNA and protein expression, enhanced DNA fragmentation, and increased caspase-3 levels after treatment with staurosporine, a potent inhibitor of phospholipid Ca2+-dependent protein kinase. However, staurosporine did not enhance sebaceous lipogenesis. On the other hand, 5α-dihydrotestosterone inhibited SZ95 sebocyte death without involving apoptotic pathways. The authors concluded that the pattern of natural sebocyte death, at least in culture, did not occur mechanically from increased cell volume but rather from programmed cellular DNA fragmentation and death, which differs from apoptosis and which can be enhanced by increased rates of induced terminal sebocyte differentiation.

In 2011, Kurihara et al. provided evidence that gene expression and production of ATP-binding cassette subfamily B member 1 is involved in lipid secretion in insulin-differentiated hamster sebocytes. They determined that sebaceous lipogenesis is associated with increased levels of intracellular ATP, and they confirmed its correlation with nonapoptotic phosphatidylserine exposure. On the other hand, Liman and Alan (2013) reported that specific proliferating cell nuclear antigen/Bax/survivin expression patterns could reflect specific cell differentiation states in the chicken uropygial gland, a specialized holocrine secretory gland, and that holocrine secretion in this gland is realized primarily by way of apoptosis, thus disputing the findings by Wróbel et al. (2003) and Kurihara et al. (2011). The currently known modifiers of sebocyte lipogenesis and programmed cell death are shown in Table 1.

Fischer et al. (2017) address the mode of holocrine sebocyte secretion and demonstrate that its mechanism differs from apoptosis, necroptosis, and cornification. To determine the mechanism of DNA degradation during sebocyte cell death, they inactivated candidate DNA-degrading enzymes by specifically targeted specific DNase gene deletions in keratinocytes and sebocytes in genetically constructed mouse strains. Among the different enzymes studied in hair follicle-associated sebaceous glands and the preputial glands, epithelial cell-specific deletion of lysosomal DNase2 blocked DNA degradation. DNA breakdown during sebocyte differentiation coincided with the loss of lysosome-associated membrane protein 1, and it was accelerated by the abrogation of
autophagy, the central cellular program of lysosome-dependent catabolism. Suppression of DNA degradation by the deletion of DNase2 resulted in aberrantly increased concentrations of residual DNA and decreased amounts of the DNA metabolite, uric acid, in secreted lipids. These results suggest that programmed cell death, which terminally differentiated sebocytes undergo, is a lysosome-dependent mechanism regulated through cell-autonomous DNA degradation by lysosomal DNase2.

Interestingly, the histone 3 protein, which is recruited by sebocytes, becomes a component of nucleosomes and is then condensed into secreted microvesicles. The so-called sebosomes, containing lipid particles (Nagai et al., 2005), were found by Fischer et al. (2017) to disappear in terminally differentiated sebocytes in wild-type mice but not in DNase2-skin knockdown mice. These data indicate that DNase2-mediated DNA degradation is required for the proteolysis of histone 3 in terminally differentiated sebocytes. Together with the finding that DNA degradation results in the formation of relevant amounts of uric acid as a component of sebum, an antioxidant present on the skin surface (Thiele et al., 2001), the results of Fischer et al. (2017) illustrate that nuclear degradation during holocrine secretion of sebocytes is a multistep process. Histone 3 (Nagai et al., 2005) and 4 (Lee et al., 2009) proteins are essential for the fate of sebocyte lineage. Myc-induced chromatin modifications leading to methyltransfer changes in the abundant trimethylated histone 3 and 4 proteins of epidermal stem cells induce them to exit their niche and differentiate into seocytes, escorting them during sebocyte differentiation (Frye et al., 2007).

Taking into account the fact that sebocytes are differentiated epithelial cells originating from the same stem cells as follicular keratinocytes, it is increasingly astonishing to recognize the high level of cell lineage uniqueness that sebaceous differentiation can reach!

**CONFLICT OF INTEREST**

The author states no conflicts of interest.

### Table 1. Modifiers of sebocyte lipogenesis and programmed cell death

<table>
<thead>
<tr>
<th>Modifier</th>
<th>Lipogenesis</th>
<th>Programmed cell death</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arachidonic acid</td>
<td>↑↑ release</td>
<td>↑</td>
<td>Wröbel et al., 2003</td>
</tr>
<tr>
<td>Endocannabinoids</td>
<td>↑</td>
<td>↑</td>
<td>Dobrosi et al., 2008</td>
</tr>
<tr>
<td>Extracellular Ca²⁺ reduction</td>
<td>↑</td>
<td>↑</td>
<td>Zouboulis et al., 2016</td>
</tr>
<tr>
<td>Nicotine</td>
<td>↑</td>
<td>↑</td>
<td>Hu et al., 2014</td>
</tr>
<tr>
<td>nPKCδ and aPKCζ inhibitors</td>
<td>↑</td>
<td>↑</td>
<td>Géczy et al., 2012</td>
</tr>
<tr>
<td>Insulin/IGF-1</td>
<td>↑</td>
<td>—</td>
<td>Kurihara et al., 2011; Makrantonaki et al., 2008</td>
</tr>
<tr>
<td>Isotretinoin</td>
<td>—</td>
<td>↑</td>
<td>Nelson et al., 2011</td>
</tr>
<tr>
<td>Capsaicin (high concentration)</td>
<td>↑</td>
<td>↑</td>
<td>Tóth et al., 2009</td>
</tr>
<tr>
<td>Capsaicin (low concentration)</td>
<td>↓</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>R-verapamil</td>
<td>↓ TG</td>
<td>—</td>
<td>Kurihara et al., 2011</td>
</tr>
<tr>
<td>PPARδ, α, β activators</td>
<td>↓ release</td>
<td>↓</td>
<td>Schuster et al., 2011</td>
</tr>
<tr>
<td>Sox9 silencing</td>
<td>↓</td>
<td>↑</td>
<td>Shi et al., 2016</td>
</tr>
</tbody>
</table>

Abbreviations: IGF, insulin-like growth factor; PKC, protein kinase C; PPAR, peroxisome proliferator-activated receptor; TG, triacylglycerols.

### REFERENCES


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Treatment of Low-Risk Basal Cell Carcinoma

Nicole W.J. Kelleners-Smeets1,2, Klara Mosterd1,2 and Patty J. Nelemans3

With the continuously rising incidence and changing populations of patients with basal cell carcinoma, evidence about the different treatment modalities is mandatory. Randomized clinical trials, such as the surgery versus imiquimod for nodular superficial basal cell carcinoma trial, can provide this evidence. Patients can then be informed about all aspects of alternative treatment options so that conscious, shared decisions can be made.


Basal cell carcinoma (BCC) is the most common skin cancer, and its incidence continues to rise. Surgical excision (SE) is still the most commonly used treatment for BCC, although there are numerous other therapeutic options. Optimal treatment depends on patient and tumor characteristics such as size, location, histological subtype, and previous treatment. A Cochrane review reveals that there is little good quality research on comparative effectiveness of treatments for BCC and that there is a need for head-to-head comparisons of treatments, with long-term follow-up (Bath-Hextall et al., 2007). Trials that included noninvasive treatments were performed mainly by industry, and they were often placebo controlled. However, it seems more relevant to compare new treatments to the “gold standard,” which is surgery. The Surgery versus Imiquimod for Nodular Superficial basal cell carcinoma (SINS) trial of Williams et al. (2017) is therefore an important study, as it compares a commonly used noninvasive treatment, imiquimod cream, with SE. This randomized clinical trial shows that SE remains the most effective treatment for a primary low-risk superficial or nodular BCC. The percentage of lesions with treatment success after treatment with imiquimod cream is reported to be 82.5% after a follow-up period of 5 years. However, for some reason, the authors did not use a time-to-event analysis, such as Kaplan Meier analysis, to account for the censored observations in 118 of the 501 originally randomized patients, who were lost to follow-up. Therefore, actual treatment success may be somewhat lower, because the percentage, 82.5%, was based on the 383 patients for whom data on outcome was available after 5 years. Most treatment failures occurred within the first year after treatment. This is in line with the findings of Roozeboom et al. (2016), who recently reported 3-year follow-up data of a randomized clinical trial comparing imiquimod cream with 5-fluorouracil cream and photodynamic therapy. In this study, recurrences after photodynamic therapy continued to occur up to 3 years after treatment. Findings from both trials suggest that imiquimod cream might still represent a clinically useful alternative to SE. The fact that almost no recurrences appeared after the first years of follow-up refutes suggestions of a possible progressive rise in BCC recurrences after 3 years of follow-up and suggestions that recurrences in the imiquimod group were difficult to identify. Furthermore, a concern that recurrences had transformed from superficial to morpoeic forms is rebutted (Williams et al., 2017).

Randomized controlled trials are of great importance in gaining evidence for making a conscious shared decision by physicians and patients.

Currently, no treatment competes with the efficacy of SE for BCC. However, imiquimod cream is probably the best alternative, noninvasive treatment. The trial of Williams et al. (2017) was designed as a noninferiority trial. This means that up-front, a lower efficacy is accepted because the investigators

1Department of Dermatology, Maastricht University Medical Centre, Maastricht, The Netherlands; 2GROW, School for Oncology and Developmental Biology, Maastricht University Medical Centre, Maastricht, The Netherlands; and 3Department of Epidemiology, CAPHRI, School for Public Health and Primary Care, Maastricht University, Maastricht, The Netherlands

Correspondence: Nicole W.J. Kelleners-Smeets, Department of Dermatology, Maastricht University Medical Centre, P Debyelaan 25, PO Box 5800, 6202 AZ Maastricht, The Netherlands. E-mail: n.kelleners.smeets@mumc.nl

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