What’s Eating the Epidermis? In Vivo Autophagy Manipulation via Subcutaneous MicroRNA Delivery

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Autophagy, discovered as a starvation-induced cellular recycling pathway, routes protein aggregates, damaged organelles, and pathogens to lysosomes and also supports normal tissue homeostasis. Although prior studies linked autophagy to epidermal differentiation, infection, and carcinogenesis, Wang et al. report upstream regulation of autophagy by microRNAs. Subcutaneous delivery of microRNA mimics and antagonists modulated autophagy in vivo, suggesting a novel potential therapeutic strategy in dermatology.


The 2016 Nobel Prize in Physiology or Medicine was awarded to Yoshinori Ohsumi “for his discoveries of mechanisms of autophagy,” reflecting a resurgence of enthusiasm in many fields of biology that have refocused on this ancient cellular recycling pathway, conserved from yeast to humans. Cutting-edge research on autophagy has elucidated its critical involvement in processes ranging from aging and neurodegeneration to infectious disease and carcinogenesis (Choi et al., 2013). Investigative dermatology has also experienced an uptick in published autophagy research, with many recent studies reporting diverse roles for autophagy in keratinocytes, which depend on this degradative route for protein turnover of cellular structures that must undergo major remodeling during specialized processes of differentiation. The wholesale clearing of organelles that occurs in the maturing erythrocyte is a particularly striking example (Schweers et al., 2007). In the epidermis, keratinocytes undergo a similar process of culling all their organelles and nuclei. Akinduro et al. (2016) reported that autophagy occurs in a constitutive manner in the epidermis and contributes to normal nuclear breakdown (nucleophagy) during keratinocyte cornification. They also showed that the nuclear retention in cornified layers typically seen in psoriasis (called parakeratosis) correlated with impaired autophagy.

Ultimately, autophagy delivers substrates to the lysosome, where an acidic pH and specialized enzymes permit cargo degradation, but the pathway is somewhat complex (Figure 1). An ordered assembly of cytoplasmic and membrane-associated autophagy-related proteins (Atgs) is required to identify and physically sequester cytoplasmic debris, protein aggregates, and dysfunctional organelles into autophagosomes, which can be visualized by electron microscopy as round vacuolar structures delimited by a double membrane (Mizushima et al., 2010). Forming autophagosomes can also be distinguished by light microscopy using the canonical marker LC3 (or a related Atg8 family member), a ubiquitin-like protein that is lipidated and inserted into an isolation membrane that surrounds the intended cargo to form a phagophore. Closure of the phagophore double membrane generates the mature autophagosome, which eventually fuses with lysosomes through the activity of the SNARE protein complex. A recent genetic mouse model confirmed that mutation of SNAP29, a component of the autophagosomal SNARE complex, causes the ichthyosis phenotype seen in the neurocutaneous disorder called CEDN1 syndrome (Schiller et al., 2016) and confirms that an impaired ability to complete the autophagic degradation

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Clinical Implications

- Autophagy is a critical lysosomal degradative pathway in keratinocytes.
- MicroRNAs serve as upstream regulators of autophagy in the epidermis.
- Epidermal autophagy can be manipulated in vivo via subcutaneous microRNA injection.

process can result in defective epidermal cornification.

Using transgenic mice expressing fluorescent LC3 (GFP-LC3), Wang et al. (2018) show accumulation of GFP-LC3—positive puncta (representing mature autophagosomes) by fluorescence microscopy of epidermal tissue cross sections after depletion of miR-107. The endogenous miR was markedly down-regulated after subcutaneous injection of an antagomiR (a small RNA sequence that binds to and antagonizes endogenous miR having a complementary sequence). Additionally, treatment of cultured human epidermal keratinocytes with the miR-107 antagomiR correlated with an increased number of visible vacuoles seen by phase-contrast microscopy. To further probe the connection between miR-107 and autophagy, Wang et al. administered a synthetic mimic of miR-107 subcutaneously. Increasing miR-107 levels reduced the accumulation of GFP-LC3—positive puncta in the epidermis, suggestive of more rapid turnover of autophagosomes via fusion with lysosomes, where the GFP fluorescence is quenched and the delivered protein substrates are broken down by proteases. Mechanistically, Wang et al. show that experimental up-regulation or down-regulation of miR-107 was correlated with opposing effects on the phosphorylation (de-activation) status of dynamin. This GTPase is known to regulate the end stages of autophagy, namely regeneration of the cellular pool of acidified lysosomes that are available to fuse with additional autophagosomes.

By using modern genetic tools, mammalian model systems, and microscopic approaches, basic science studies that are focused on the ancient autophagy pathway have the potential to suggest novel therapeutic approaches using compounds that function as autophagy modulators, many of which are already in clinical use. These include some that are regularly prescribed by dermatologists (e.g., hydroxychloroquine, rapamycin/sirolimus) (Levine et al., 2015). However, Wang et al. (2018) suggest that miR may be a new target in the quest to manipulate autophagy. Relative to small-molecule chemical inhibitors or activators, synthetic miR mimicking or antagonizing endogenous miR would potentially offer greater specificity given their unique nucleotide sequence. Supporting the viability of miR as a therapeutic target in the skin, Wang et al. showed robust manipulation of the level of miR-107 in vivo by subcutaneous delivery of a synthetic miR or an antagomiR. This represents an important technical advance for the field of investigative dermatology. Cutaneous injection is routinely performed by dermatologists to locally deliver medications for recalcitrant plaques of psoriasis (e.g., corticosteroids), viral warts (e.g., bleomycin or cidofovir), or certain types of squamous cell carcinomas (e.g., methotrexate), diseases that have already been linked to autophagy. In light of the findings presented by Wang et al. showing effective delivery of synthetic miR or antagomiR that allow local and sequence-specific manipulation of endogenous keratinocyte miR, one could foresee therapeutic efforts to favorably alter autophagic flux via miR-107 or, more broadly, to similarly target other endogenous miR that have been associated with skin diseases.

CONFLICT OF INTEREST

The author states no conflict of interest.

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REFERENCES


Figure 1. Autophagy allows cells to recycle cellular debris, protein aggregates, and injured organelles. To sequester cargo intended for degradation, cells begin by forming an isolation membrane. Immature LC3 resides in the cytoplasm, but upon up-regulation of autophagy, pro-LC3 is cleaved and then lipidated (activated) by Atg family members 3, 4, and 7. Mature LC3 is subsequently incorporated into the elongating isolation membrane via the activity of Atg 5, 12, and 16L to surround the cargo, forming a phagophore. Closure of the double membrane generates a mature autophagosome, which can subsequently fuse with lysosomes via the activity of SNARE protein complexes, thus forming an autolysosome capable of cargo degradation.
Tanning Addiction in Adolescents: Directions for Measurement and Intervention Development

Mary K. Tripp

Little is known about tanning addiction in adolescents. Miller et al. found that 7.0% of 11th grade students met addiction criteria. After adjusting for all other comorbidities, the odds of addiction were two times greater for students who reported problem use of marijuana or obsessive-compulsive disorder symptoms. The likelihood of addiction increased with problem substance use and psychological symptoms.

Miller et al. (2018) make a significant contribution to our understanding of tanning addiction and related comorbidities in adolescents (Miller et al., 2018). There is evidence for biological addiction to UV exposure, potentially mediated by the UV-induced release of β-endorphin, which is an endogenous opioid (Fell et al., 2014). However, it is not clear to what extent physical dependence on UV exposure may explain a pattern of frequent or repetitive tanning behavior. Very little is known about tanning addiction in adolescents; virtually all research on tanning addiction and related comorbidities has been adapted from widely used tools designed to screen for problem substance use and psychological symptoms for their associations with tanning addiction.

Prevalence of Tanning Addiction in Adolescents

Miller et al. (2018) found that 7.0% of the 11th grade students in their study met the criteria for tanning addiction as assessed by a modified version of the Cut down, Annoyed, Guilty, Eye-opener (CAGE) instrument, referred to as mCAGE (Table 1). The indoor or outdoor tanning behavior of students was not assessed in this study, so the proportion of tanners who were classified as addicted is not known. According to the 2015 Youth Risk Behavior Survey (YRBS), 9.0% of 11th grade students in the United States reported using an indoor tanning device at least once during the past year (Kann et al., 2016). In light of this statistic, the prevalence of tanning addiction in the current study is striking, because it may suggest that a large proportion of 11th grade students who report any indoor tanning behavior may be characterized as addicted to tanning. However, differences in sample designs and characteristics between the Miller et al. study and YRBS limit direct comparisons of prevalence estimates. The prevalence of indoor tanning behavior in Los Angeles, California, where this study was conducted, may differ from national estimates. Less is known about the outdoor tanning practices of adolescents, and it is not clear if tanners in this study were mostly engaged in indoor or outdoor tanning behaviors.

There has been much work in recent years to develop and psychometrically evaluate measures that are valid and reliable to assess tanning addiction, but currently there is no recognized criterion standard. Several measures have been used across studies, and there is a lack of consensus on which measure, or combination of measures, may be most useful. Some tanning addiction instruments have been adapted from widely used tools designed to screen for substance dependence. For example, the CAGE has been used to assess problem drinking (Ewing, 1984) and has been modified (i.e., mCAGE) to assess tanning addiction (Warthan et al., 2005). The mCAGE has a total of four items and, given its brevity, was selected for use in the Miller et al. (2018) study. Indeed, an advantage of the mCAGE is its potential for use as a brief screening tool, which would facilitate the triage of tanners to treatment strategies for tanning addiction. However, evidence is lacking for the use of mCAGE in assessing tanning addiction in adolescents. Adolescents are an understudied group in the measurement literature on tanning addiction; thus, the study by Miller et al. provides a valuable opportunity to gain...