Expanding Our Understanding of Human Skin Aging

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Two very different studies expand our understanding of human skin aging. In the first study, Hüls et al. show an association between nitrogen dioxide levels in outdoor air and number of lentigines on the cheek. In the second study, Bowman and Birch-Machin show that mitochondrial complex II activity in human skin fibroblasts decreases with age.

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Two very different studies expand our understanding of human skin aging. In the first study, a "Letter to the Editor," Hüls et al. (2016) show an association between nitrogen dioxide (NO_2) levels measured in the environment and the geometric mean number of cheek lentigines in two separate populations.

Outdoor NO_2 levels associated with cheek lentigines

Environmental pollution in the form of poor air quality is a major worldwide health problem, and recognition of the significant effects of air pollution on the skin is growing. Cigarette smoking and ambient soot levels are known to promote clinically visible skin changes (Vierkotter et al., 2010). Hüls et al. (2016) report a new association between an environmental pollutant, NO_2, which is generated from combustion such as from vehicles, and visible cheek lentigines. A dose effect of NO_2 on the geometric mean number of cheek lentigines was observed: an increase of 10 $\mu g/m^3$ in NO_2 level was associated with 24–25% more cheek lentigines.

The strength of the Hüls et al. (2016) article is in the association between NO_2 levels and cheek lentigines in two separate populations, one in Germany and the other in China, both in individuals over 50 years old. There is evidence suggesting that environmental insults to the skin are more likely to manifest with clinically visible phenotypes in individuals with genetic susceptibility. Recently, wrinkle severity has been reported in elderly women to be associated with decreased lung function (specifically the ratio of forced expiratory volume to forced volume capacity), but only in individuals who carry specific matrix metalloproteinase promoter variants (Vierkotter et al., 2010). The Hüls et al. article did not genotype the participants in the two cohorts, but determining whether the increased lentigo phenotype is visible only in individuals carrying a specific genotype could be informative, especially if the genetic associations are the same across two populations. Furthermore, if this association between NO_2 and increased numbers of cheek lentigines could eventually be shown to serve as a marker for damage to other organ systems such as the lungs (as wrinkle formation has been reported to associate with decreased lung function), individuals could be screened for susceptibility to pollution so that they could minimize exposures.

Another important question the Hüls et al. (2016) study brings up is whether the increase in number of cheek lentigines is attributable to a systemic effect from chronic inflammation via the lungs or attributable to more regional effects on the skin. Hüls et al. did not detect a significant association between NO_2 levels and the number of lentigines on other sun-exposed areas such as the forehead, dorsal hand, or forearm. This suggests that a regional effect is likely needed—for instance, through NO_2 exposure to cheek skin. One possibility is that NO_2 (or its breakdown products, such as photolysis-mediated hydroxyl radicals [Trebs et al., 2009]), may penetrate cheek skin more effectively than other sun-exposed anatomic sites.

> Mitochondria and pollution: two studies expanding our understanding of human skin aging.

How NO_2 might contribute to lentigo formation

Although Hüls et al. (2016) did not show a mechanistic connection between NO_2 levels and cheek lentigines, it is known that NO_2 can undergo photolysis, with generation of reactive breakdown products that can potentially affect both the skin and lungs (Ayyagari et al., 2007). Mechanisms hypothesized to promote the so-called "environmentally-induced lentigo" include macromolecular damage and activation of aryl hydrocarbon receptors on skin cells (Nakamura et al., 2015), although the specific role of NO_2 remains to be determined.

Ultraviolet radiation has been shown to induce pigmentation via the aryl hydrocarbon receptor in mice (Jux et al., 2011), and it can also induce mitochondrial DNA damage (Berneburg et al., 2004). Clearly, ultraviolet radiation is the primary environmental insult driving lentigo formation, and Hüls et al. (2016) report lentigines in sun-exposed anatomic sites only. The additional role of NO_2 appears to be that of increasing the number of lentigines. Because geometric means were reported by Hüls et al., likely because of nonnormal distributions of lentigo counts, perhaps a subset of the populations studied are susceptible to increased numbers of lentigines. This

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possibility, although not explored in the Hüls et al. study, would include genetic predisposition (suggested by uncommon genodermatoses with increased lentigo formation such as Peutz-Jeghers syndrome, although not necessarily at play in the study by Hüls et al.), comorbidities, and/or exposure to other pollutants in addition to NO_2.

Because the increase in geometric mean numbers of cheek lentigines per unit of increase in NO_2 was fairly small, prospective studies are needed to confirm the findings reported by Hüls et al. (2016). Ideally, these studies would use more individualized measurements of environmental exposures including NO_2, ultraviolet radiation dosimetry, clinical histories, and genotype, although these types of studies would require significant resources over long periods of time.

Foreskin fibroblasts show decreased mitochondrial complex activity with increasing chronological age

A second study in this issue, by Bowman and Birch-Machin (2016), is an in vitro study that shows decreasing mitochondrial complex II activity in foreskin fibroblasts with increasing chronological age. Using foreskin (presumably a sun-protected anatomic location) from 27 individuals whose ages spanned eight decades, Bowman and Birch-Machin found that the rate of complex II activity per unit of mitochondria was decreased in cultured fibroblasts, but not in keratinocytes, with increasing chronological age. These observations were supported by parallel declines in complex II subunit transcript and protein levels. The results of this study are consistent with known decreases in mitochondrial complex II activity in numerous animal models, including the skin of naturally aged mice (Velarde et al., 2012), although in mice the effects were seen primarily in keratinocytes, not fibroblasts. In addition, decreased mitochondrial complex activity was associated with shortened lifespan in the nematode (Pfeiffer et al., 2011) and fruitfly (Tsuda et al., 2007), indicating the pro-aging effect of mitochondrial dysfunction.

The mechanism by which mitochondrial dysfunction promotes aging is thought to be via oxidative stress. Oxidative stress is known to lead to epidermal skin thinning in SOD-deficient mice (Velarde et al., 2012). More recently, oxidative stress has been observed to increase in human dermal fibroblasts if complex II function is abrogated (Anderson et al., 2014). Oxidative stress has been reported to lead to mitochondrial DNA damage in human dermal fibroblasts (Quan et al., 2015), potentially leading to a vicious cycle of increased oxidative stress (Rinnerthaler et al., 2015).

The main limitation of the Bowman and Birch-Machin (2016) study is the lack of clinical histories of the 27 participants. For instance, there may be confounding effects on the skin fibroblasts from older individuals with significant smoking histories or who may have lived in areas with air pollution for long periods of time, both of which may promote oxidative stress. Future studies will need to address these potential confounders.

Another question arising from Bowman and Birch-Machin's (2016) study is how mitochondrial complex II activity is decreased although complex IV activity is not. Loss of autophagy with age can lead to accumulation of dysfunctional mitochondria and increased levels of oxidative stress (Green et al., 2011; Lopez-Armada et al., 2013). It could be hypothesized that with age, abnormal mitophagy could result in the sparing of mitochondria with complex II dysfunction. This hypothesis remains to be explored.

Potential connection between mitochondrial dysfunction and lentigo formation

Mitochondrial complex II mutations in humans lead to variable clinical phenotypes (Rivner et al., 1989). Although cutaneous lentigines are not reported as one of the clinical signs, pigmentary alterations in the retina have been observed (Rivner et al., 1989). The Bowman and Birch-Machin (2016) study describes decreased mitochondrial complex II activity with increasing chronological age, and future studies of sun-exposed skin sites, with clear clinical histories, will likely clarify

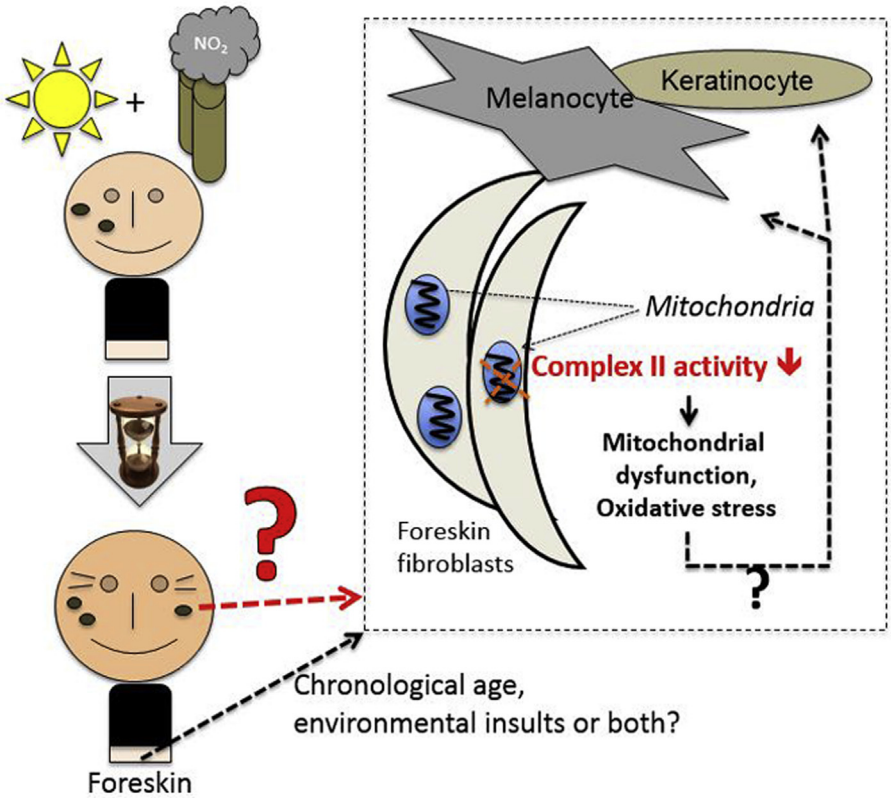

Figure 1. Is there a link between nitrogen dioxide, increased cheek lentigines, and oxidative stress? Multiple pathways are likely to control human skin aging, and future studies are needed to see whether pollution related phenotypes may be related to cellular processes such as mitochondrial dysfunction.

whether this phenomenon is exacerbated by external insults. Interestingly, the aryl hydrocarbon receptor has been reported to play a role in protecting the skin from carcinogen-mediated oxidative stress (Melchini et al., 2011), although some studies indicate that the aryl hydrocarbon receptor may also promote inflammation (Stockinger et al., 2014). Although it is known that ultrafine particulate pollutants induce oxidative stress and mitochondrial damage (Li et al., 2003), NO_2 has not been shown specifically to induce oxidative stress in vivo. If NO_2 is capable of increasing oxidative stress in vivo, pathways such as the aryl hydrocarbon receptor pathway may respond in a manner that protects the skin from environmentally induced oxidative stress, and this response may include lentigo formation. This possibility remains to be examined (Figure 1).

Airborne environmental pollution is a growing worldwide health problem, and understanding how various components of pollutants, particularly as they may interact with ultraviolet radiation to cause cellular damage, is of critical importance in therapeutic efforts to maintain healthy skin into advanced age and to repair or reverse cutaneous damage.

CONFLICT OF INTEREST

The author states no conflict of interest.

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