Sirtuins in Skin and Skin Cancers

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Abstract

The sirtuins are a family of proteins that comprise class III of the histone deacetylases. These NAD⁺-dependent proteins have been found to be intricately involved in a variety of important and skin-relevant cellular functions and processes, including aging, UV damage response, oxidative stress, and wound repair. In addition, recent research is unraveling the role of sirtuins in a variety of skin diseases, including melanoma and nonmelanoma skin cancers. In this review, we provide a discussion on the potential roles and implications of different sirtuins in skin-specific cellular processes, which may have relevance to skin health and skin diseases. Based on the available literature, the sirtuins appear to be important targets in the management of a variety of skin diseases from cosmetic (e.g., skin aging) to fatal conditions (e.g., melanoma).

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Introduction

Sirtuins (SIRT) comprise 1 of 4 classes of histone deacetylases (HDACs; I–IV) that play important roles in a variety of cellular functions. This class III of HDACs is entirely dedicated to the SIRTs, based on their homology to the yeast protein SIR2 (silent information regulator 2), their conserved catalytic domain, and nicotinamide adenine dinucleotide (NAD⁺) dependence [1, 2]. Seven members of the sirtuin family (SIRT1–7) have been identified so far. Although their core domain is conserved, they differ in their N- and C-terminal domains (Fig. 1) [3]. SIRTs are evolutionarily conserved from prokaryotic through eukaryotic cells, and although they are classified as HDACs, the family is responsible for several types of posttranslational modifications in both histone and nonhistone proteins, which are important for a variety of cellular processes. Some of the nondeacetylase activities for each sirtuin are outlined in Figure 1. Additionally, despite structural similarities, each sirtuin has its own biological niche, performing unique functions via regulating critical mechanisms in the cell.

As an indication of the different roles they play biologically, the different sirtuin members have been found to be located in relatively discrete cellular compartments

Fig. 1. Sirtuin function and localization. A visual representation of the 7 mammalian sirtuins. Relative size and nondeacetylation activities are shown, as well as subcellular location. SIRT's 1 and 2 function in both the nucleus and cytoplasm, with SIRT1 showing more nuclear functions and SIRT2 more cytoplasmic ones. SIRT6 is found in the nucleus, and SIRT7 is associated with the nucleolus, while SIRT's 3–5 are primarily mitochondrial. Dark blue bands represent the conserved core catalytic domains while surrounding bands indicate the N- and C-terminal regions of each sirtuin. References for the nondeacetylation activities are as follows: (1) [87]; (2) [88]; (3) [89]; (4) [90]; (5) [91]; (6) [92]; (7) [93]; (8) [94]; (9) [95]; (10) [96].

(shown in Fig. 1). For example, SIRT's 1, 6, and 7 are located mostly in the nucleus, consistent with their roles in transcription facilitation and epigenetic regulation [4]. SIRT2 is found mainly in the cytoplasm, enabling it to interact with proteins involved in gluconeogenesis, the immune and inflammatory response, and microtubule stabilization [5, 6]. SIRT's 3, 4, and 5 are most commonly found within the mitochondria, which is in accordance with their importance in cellular metabolism (reviewed in Michan and Sinclair [7]). Interestingly, some of these sirtuins have been shown to be both nuclear and cytoplasmic, suggesting nuclear-cytoplasmic shuttling, and intracellular locations and roles which could be dependent on tissue and cell type (discussed in Haigis and Guarrente [8]).

While the roles of sirtuins have been explored extensively in other systems, their roles in the skin and skin cancers are less well defined (discussed in Serravallo et al. [9]). Here, we discuss the function of sirtuins in the skin as it pertains to aging, ultraviolet (UV) radiation damage, and oxidative stress, as well as several skin diseases, with an emphasis on skin cancers.

The Skin and the Impact of Sirtuins on Chronological Aging

As the largest organ in the body, the skin performs many different and vital functions. Through its complex structure, it acts to both protect the body from damage as well as to provide a surface for external interactions. The functions of the skin range from simple barrier activities

to complex endocrine signaling and biomolecule synthesis activities [10]. Damage to the skin can disrupt or alter its ability to fulfill these crucial roles and can come in many forms, including environmental stresses and physical injuries, and can even be exacerbated by psychosocial stress [10, 11]. Each of these stressors can have different effects on the skin, and some may not show any visible effects for a long time, such as damage from UV exposure and oxidative stress. To complicate matters, some potentially damaging agents like UV radiation may actually be beneficial or even essential to a healthy body when used in moderation (discussed in Holick [12]). To maintain the necessary balance of protection and selective penetration, the skin must tightly regulate its activities via a complex network of cells, nerves, chemicals, and lipids that are structured in a way to best accomplish its varied roles.

Keratinocytes, melanocytes, Langerhans cells, and Merkel cells make up the majority of the cells in the skin. The importance of each of these cells, along with the structure, function, and key molecules present in the skin, are discussed in depth and explained in an article on the integumentary system by McLafferty et al. [10]. Briefly, the skin consists of a series of layers, ranging from the external epidermis to the dermis. The outermost layer of the skin, the epidermis, consists mainly of keratinocytes and is responsible for the bulk of its barrier functions (reviewed in Boer et al. [13]). Underneath the epidermis is the dermis. This layer contains the structural and nutritive components necessary to support a healthy epidermis, as well as the various vessels, glands, and nerves that make the skin so complex [10]. The close proximity of the

skin to the environment facilitates aging, cellular, and tissue damage, wounds, and diseases, all of which appear to be linked to the sirtuins.

From the time of their discovery, the sirtuins have been implicated in the process of aging. Preceding their identification in mammals, lifespan extension via caloric restriction was widely studied as one of the primary functions of the yeast homolog protein Sir2 (reviewed in Sinclair [14]). It was initially thought that this would translate to mammals, and although many reports have found similar results, several other studies have found no effects of sirtuins on lifespan [15–20]. There was more consistent success when using small molecule activators, but initial successes, as with some of the initial yeast and fly studies [21], were likely due to off-target effects [22, 23]. Thus, the final verdict on the ability of sirtuins to directly affect aging remains an ongoing quest (reviewed in Kanfi et al. [19] and Dang [24]). However, beyond direct effects, sirtuins have been found to interact with other major longevity factors, including AMP-activated protein kinase, phosphatidylinositol-3-OH kinase, insulin-like growth factor 1, and mechanistic target of rapamycin (reviewed in Thandavarayan et al. [25]). Indeed, the process of aging itself is still not clearly understood and includes aspects of lifespan, cellular changes (such as apoptosis, protein modifications, and increased senescence), and cosmetic changes. Prevalent theories for these changes include decreased telomere length from repeated cell division, exhaustion of a limited neuronal or stem cell population, accumulation of cellular toxins over time, or repeated DNA damage from environmental stresses [26–29]. Because of findings supporting their role in longevity, there is abundant evidence regarding the link between sirtuins and aging throughout the body (reviewed in Watroba and Szukiewicz [30]).

In the skin, the physical characteristics associated with aging are visually apparent. Aged skin becomes thin and shows an increase in wrinkles, as well as a decrease in hydration and skin elasticity [31, 32]. Interestingly, researchers have found that several sirtuins have a changed expression profile depending on the age of the person or cell line being studied. For example, a 2014 study found that SIRT1 levels decreased with age in dermal fibroblasts isolated from female donors who ranged from 20 to 67 years old [33]. Another study found that both SIRT1 and SIRT6 levels decreased in human dermal fibroblasts with higher passage numbers, and that these levels were associated with aging biomarkers found in the same cells [34]. Since fibroblasts are involved in the production of the extracellular matrix in the skin, these studies suggest that

decreasing SIRT levels in aged fibroblasts may have an effect on the chronological aging processes. To take this a step further, Sharma et al. [35] found that there was a greater resistance in human dermal fibroblasts from older human subjects to reprogramming using classical Yamanaka factors, as well as higher SIRT6 levels in younger cells. However, they found that adding SIRT6 while reprogramming allowed the aged cells to have an increased reprogramming efficiency. These findings taken together suggest that both SIRT1 and SIRT6 are important molecules in skin cell aging, and warrant further exploration, especially because skin health is considered a factor representing overall “good health.”

The Role of Sirtuins in UV Radiation and Photoaging

Premature development of the physiological changes observed in chronological skin aging, as well as uneven pigmentation, a deepening of wrinkles, and a rough texture, can occur as the result of the process known as photoaging [31, 32]. Photoaging is strongly correlated with sun exposure, with both UVA (320–400 nm) and UVB (290–320 nm) radiation contributing to its progression [36]. Most UVB is absorbed in the epidermis, where it causes sunburn and damages cellular DNA through the formation of cyclobutane pyrimidine dimers and pyrimidine (6–4) pyrimidone photoproducts [37, 38]. DNA damage that is not adequately repaired can lead to increased cellular senescence, apoptosis, and carcinogenesis [37]. UVA penetrates more deeply into the dermal layer, damaging DNA, proteins, and lipids indirectly through the generation of reactive oxygen species (ROS), as well as breaking down collagen through the activation of the tissue-remodeling matrix metalloproteinases (MMPs) [38]. Sirtuins play a role in both UVA- and UVB-mediated events, suggesting that they could be key participants in photoaging.

SIRT1 has been shown to play a role in photoaging, especially through the inhibition of MMPs and subsequent collagen degradation. The SIRT1 activators resveratrol and metformin have been shown to inhibit MMP-9 and prevent collagen degradation when applied to human fibroblast cells or mouse skin prior to UV radiation exposure [39]. Resveratrol has similarly been shown to inhibit MMP-1 expression, whereas SIRT1 knockdown increases MMP-1 and -3 levels [40]. This protective role of SIRT1 in UV-induced photoaging has also been found in clinical samples, as SIRT1 and MMP-1 expression has been shown to increase in response to UVA radiation in

both human skin *in vivo*, as well as human fibroblasts *in vitro* [41, 42]. Together, this suggests that SIRT1 activators have therapeutic potential in photoaging prevention.

The case for SIRT1 involvement in UVB-mediated DNA damage has been demonstrated in several *in vitro* experiments using human fibroblasts. UVB radiation has been shown to decrease SIRT1 protein levels in these cells [43]. Interestingly, the natural compound juglone (5-hydroxy-1,4-naphthalenedione) which is found in several plants, has been shown to restore SIRT1 to normal levels after UVB treatment, suggesting that SIRT1 might play a role in preventing UVB-induced carcinogenesis [43]. Overexpressing SIRT1 in human fibroblasts reinforces this possibility, as it results in protection from UVB-induced cellular senescence and oxidative stress, presumably through the suppression of p53 acetylation [44]. However, *in vivo* studies show that the SIRT1 story is more complex, and the level of SIRT1 expression is critical for its role in UVB protection. Contrary to the *in vitro* findings in fibroblasts, keratinocyte-specific homozygous SIRT1 deletion suppresses skin cancer development in mice via p53 activation and UVB-induced apoptosis, whereas heterozygous SIRT1 deletion promotes UVB-induced skin carcinogenesis [45]. Thus, as has been seen in many of the cancer studies to date, the role of SIRT1 as a tumor promoter or suppressor in UVB-induced cancer initiation is unclear and might vary with cell/tissue type or protein levels.

Research on the role of the remaining sirtuins in UV damage response is limited. Lang et al. [46] have shown that SIRT4 levels increase in fibroblasts exposed to UVB radiation *in vitro*, and this correlates with an increase in cellular senescence. This finding was corroborated *in vivo* by an observed elevation in SIRT4 levels in naturally photoaged human skin samples [46]. Benavente et al. [47] have shown that solar simulated light (containing both UVA and UVB) induces upregulation of both SIRT1 and 4 mRNAs, which appear to play roles in resistance to photodamage. SIRT6 has also been shown to increase in human keratinocytes in response to UVB exposure, and silencing its expression results in increased UVB-induced apoptosis in these cells [48]. This suggests that SIRT1, 4 and 6 play protective roles in the UVB damage response.

Connections between Oxidative Stress and Sirtuins in the Skin

The relationship between aging and UV exposure in the skin is closely intertwined with oxidative stress, as thoroughly reviewed by Kammeyer and Luiten [37] and

Rinnerthaler et al. [49]. Briefly, ROS are generated in UVA-exposed skin through the excitation of photosensitizers, which then transfer energy to molecular oxygen to produce superoxide anions, hydroxyl radicals, or singlet oxygen. These ROS have the capability to cause significant cellular damage, but also have a functional role in molecular signaling pathways. Endogenous controls for cellular damage include the conversion of ROS into less reactive species such as when superoxide dismutase reacts with superoxide anions to form hydrogen peroxide (H_2O_2). Although H_2O_2 is less reactive than other ROS, it still has the capability to cause oxidative stress through subsequent conversion to more harmful compounds, and due to its increased stability, it is frequently used to induce oxidative stress experimentally. Studies have shown that H_2O_2 -induced oxidative stress correlates with a decrease in SIRT1 levels in keratinocytes [50]. Treatment with the SIRT1 activator resveratrol has been shown to prevent H_2O_2 -induced cell death, decrease proliferation, and suppress senescence, whereas the SIRT1 inhibitors sirtinol and nicotinamide enhance H_2O_2 -induced cell death [50, 51]. Keratinocytes can also be protected from H_2O_2 damage and autophagy via melatonin treatment, an effect that is reversed through SIRT1 siRNA or sirtinol treatment [52]. Together, these data suggest that SIRT1 is an active player in the prevention of H_2O_2 -induced cell damage, although the mechanism is complex. Mechanistically, JNK signaling has been implicated upstream of SIRT1, and p53 has been shown to function downstream in H_2O_2 -induced keratinocyte death [50, 53]. Studies have also suggested a coordinating role between SIRT1 and AMP-activated protein kinase in the downstream activation of FOXO3 that affects H_2O_2 -induced cellular senescence and proliferation, as well as interactions between SIRT1 and FOXO3a in UVB-induced oxidative stress resistance [44, 51]. Interestingly, SIRT2 has also been shown to target FOXO3a in mouse fibroblasts, thereby regulating manganese superoxide dismutase, decreasing H_2O_2 -induced ROS, and promoting cell death [54]. Thus, multiple signaling pathways and multiple sirtuins seem to be involved in the cellular response to oxidative stress in the skin.

SIRT3 has been shown to play a role in skin maintenance through oxidative stress-induced keratinocyte differentiation, a process that is crucial for skin regeneration and maintenance, and is important in skin disease. Bause et al. [55] have shown that the process is strongly linked to SIRT3 expression, as its knockdown induces differentiation through increased ROS, and increases mitochondrial superoxide generation in response to H_2O_2 treat-

ment. This role for SIRT3 in oxidative stress regulation might extend to stress induced by environmental stressors, as a recent study showed that ozone exposure results in decreased SIRT3 levels, correlating with increased cellular H_2O_2 , reduced superoxide dismutase 2, and increased DNA damage [56]. Thus, SIRT3 may be involved in the management of oxidative stress in more than one process that is critical for normal skin maintenance.

Role of Sirtuins in Wound Healing and Other Skin Diseases

In addition to their roles in aging, UV damage, and oxidative stress, the sirtuins affect skin health in a number of different disease backgrounds. In 2013, Serravallo et al. [9] wrote a very thorough, detailed review on the topic, covering inflammatory, autoimmune, and hyperproliferative skin diseases, as well as cutaneous infections, inherited diseases, and cancer. Since then, a number of studies have expanded on the role of sirtuins in these areas.

Wound healing in the skin is impaired by knockdown or accelerated by activation of several different sirtuins. Activation of SIRT1, 2, and 3 through treatment with MC2562 or SIRT1 activation via resveratrol accelerates wound repair in a mouse model through increased keratinocyte proliferation [57]. In addition, SIRT1 knockdown at the wound site via shRNA results in dense, disordered collagen fibers during healing similar to those seen in hypertrophic scar formation, whereas collagen fibers similar to those seen in normal wound healing are observed after resveratrol treatment at the site [58]. SIRT6 knockdown in a diabetic mouse background further exacerbates the impaired wound healing associated with the db/db phenotype [25]. SIRT7^{-/-} mice also show impaired wound healing in an otherwise wild-type background [59]. Thus, it is likely that further exploration of sirtuins could lead to new treatments for disease-induced impairment of wound healing, or aid in the minimization of scar formation.

In addition to its role in wound healing, research has recently linked SIRT1 to several different skin diseases. Its activation via resveratrol has been shown to improve psoriasis in humans [60] and an induced psoriasis-like inflammation in mice [61]. SIRT1 is downregulated in systemic sclerosis and likely plays a role in the regulation of fibroblast activation in the disease via TGF- β signaling [62, 63]. SIRT1 also appears to play a protective role in vitiligo [64] and aids in maintaining skin barrier integrity

[65]. These recent findings underscore the importance of sirtuins in skin diseases and provide new avenues of treatment for these common ailments. Finally, advances regarding the role of SIRT1, as well as several other sirtuins, have been made in both melanoma and nonmelanoma skin cancers (NMSCs) as discussed in the following section.

Influences of Sirtuins in Skin Cancer

Skin cancer is one of the major health problems in the world, as 3.5 million cases of NMSC are estimated to be diagnosed in 2016 in the USA alone, along with more than 76,000 cases of melanoma [66]. Melanoma develops solely from melanocytes while NMSCs arise from other cells in the skin. NMSCs such as basal cell and cutaneous squamous cell carcinomas (BCCs and SCCs, respectively) are unlikely to metastasize and can generally be removed by a dermatologist, whereas melanoma can be more dangerous because it can rapidly metastasize if not diagnosed and removed in its early stages. To date, surgical and pharmacological treatment of melanoma has not been sufficiently effective. Currently, the most widely used melanoma treatments are immunotherapy and BRAF/MAPK pathway inhibitors [67, 68]. While immunotherapies are quite encouraging, they are successful in only a subset of melanoma patients and are associated with the induction of autoimmune and proinflammatory side effects [68]. BRAF/MAPK pathway inhibitors fail to control melanomas in the long term, with most patients acquiring resistance after approximately 12 months of treatment and subsequent cancer recurrence (reviewed in Welsh et al. [69]). Melanoma also develops resistance to other commonly used antineoplastic agents, such as doxorubicin, that are extremely successful in other cancers. Thus, the development of novel therapeutics for melanoma treatment is critical, and sirtuins are among the proteins being investigated as potential targets in both melanoma and NMSCs.

Melanoma

Targeting sirtuins as therapeutics for melanoma treatment is complicated by the fact that sirtuins have been found to work as both tumor suppressors and promoters depending on several factors, including cell and tissue type [for reviews, see 70–73]. Research on sirtuins in melanoma is still in an early enough stage that their function as tumor suppressors or promoters cannot be defined with certainty, but studies to date support the latter. The

first 3 studies on sirtuin function in melanoma were published concurrently in 2014. Kunimoto et al. [74] showed that SIRT1 is involved in lamellipodium extension and Akt-dependent melanoma cell migration. This could indicate potential for SIRT1 inhibition in the development of therapeutics to limit melanoma metastasis. Data from the remaining 2 studies showed SIRT1 overexpression and increased activity in melanoma cells and tissues [75, 76]. Inhibition via siRNA or tenovin-1 decreased proliferation and clonogenic survival, and increased G₀/G₁ cell cycle arrest and senescence-like properties, suggesting a role for SIRT1 as an oncogene. Mechanistically, MITF was shown to be an upstream regulator of SIRT1, and p53 and p21 were shown to be activated downstream upon SIRT1 inhibition. However, the network of proteins involved is complex, and a further study found that there may be a link between SIRT1 inhibition and the BUB family of cell cycle-regulating proteins [77]. This suggests that SIRT1 acts through several pathways in its promotion of cancer growth and more research into this network is needed.

Currently, limited information is available regarding the role of other sirtuins in melanoma. SIRT2 has shown potential as a regulator of several cancer progression genes in a study by Karwaciak et al. [78] and may be a good candidate for dual therapy when combined with doxorubicin as it decreases resistance to the antineoplastic agent and reduces the dosage needed for effect. Interestingly, it has recently been shown that loss of SIRT2 led to drug resistance in melanoma through BRAF and MEK inhibitors [79]. It was shown that loss of SIRT2 led to resistance of the BRAF inhibitor vemurafenib in A375 melanoma cells. In addition, inhibition of SIRT2 in melanoma was found to decrease colony formation ability. This suggests that SIRT2 may be a key contributor in the process of drug resistance in melanoma, as well as a regulator of cell growth. Taken together, these studies suggest a role for SIRT2 in melanoma progression and drug resistance.

The role of SIRT3 in melanoma has only been assessed in a recently published study from our laboratory, where we found that SIRT3 is overexpressed in both human melanoma cell lines and clinical tissue samples. Knockdown of SIRT3 in highly expressing melanoma lines via shRNA decreased cell growth and migration, colony formation, and induced senescence in vitro, as well as reduced tumor growth in a melanoma xenograft mouse model [80]. These antiproliferative changes were accompanied by a G₁ phase cell cycle arrest, as well as a decreased expression of several cyclins and cyclin-dependent kinases. Interestingly, forced overexpression of

SIRT3 in a melanoma line that had lower endogenous levels of SIRT3 had the opposite impact, leading to increased proliferation. Taken together, these data suggest that SIRT3 plays an oncogenic role in melanoma. This makes it likely that targeting SIRT3, as well as the other sirtuins, may lead to potential melanoma treatments.

Squamous Cell Carcinoma

While most studies on sirtuins in skin cancer focus on melanoma, recent research has also investigated their role in skin cancer's less deadly forms. Cutaneous SCCs are generally less aggressive than melanoma but can become metastatic if left untreated [81]. Thus, new therapeutic targets are needed to counter these neoplasms. Recent research suggests that sirtuins could prove useful in this respect, as several studies support oncogenic sirtuin function in SCC. All 7 sirtuins have been shown to be overexpressed at the mRNA level in SCC tissue samples, as well as several sirtuins in an SCC cell line (A431; SIRTs 1 and 3) and actinic keratosis (SIRTs 2, 3, 5–7) [47]. At the protein level, overexpression of SIRT6 has also been observed, and skin-specific deletion of SIRT6 in mice inhibits skin tumorigenesis via a mechanism involving COX-2 suppression [48]. However, not all evidence supports oncogenic sirtuin function in SCC. Contrary to the findings of SIRT2 overexpression at the mRNA level, a separate study has shown that SIRT2 protein is downregulated in SCC and, further, that SIRT2 knockdown by siRNA increases the risk of cancer [82]. Thus, the role of the sirtuins in SCC of the skin is unclear, and further elucidation of the mechanisms involved is necessary to assess their potential as novel therapeutics in the treatment of SCC.

Basal Cell Carcinoma

Although it is the most common skin cancer, BCC is rarely fatal. In most cases, BCCs are successfully treated with surgery or radiation therapy. However, some patients cannot undergo these treatments for various reasons, including location and complexity of the tumor [83]. Without proper treatment, BCCs may become more aggressive, invading surrounding tissues and even metastasizing [84, 85]. Therefore, novel molecular targets are needed to treat complex cases of BCC, and a recent study suggests sirtuins as potential candidates. The study shows expression of all 7 sirtuins in BCC patient samples, with SIRTs 2 and 3 showing downregulation relative to patient-matched normal tissue [86]. This finding suggests that SIRTs 2 and 3 may play a role in BCC pathogenesis, and further investigation could lead to their use as therapeutic targets or prognostic markers.

Conclusion

Since Sir2 was discovered in yeast, an abundance of research has shown a very important role of this class of HDACs in a variety of physiological functions and disease conditions. In the skin, several sirtuins have been found to play important roles in aging as well as in UV damage and oxidative stress responses. Additionally, recent research has implicated sirtuins in many skin conditions, including psoriasis and skin malignancies. Although the study of skin-based roles of sirtuins is relatively new, exploring this family of proteins may further lead to the development of novel therapeutics in skin disorders, as well as in both melanoma and NMSC management.

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