

NAD in Skin: Therapeutic Approaches for Niacin

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Abstract: The maintenance and regulation of cellular NAD(P)(H) content and its influence on cell function involves many metabolic pathways, some of which remain poorly understood. Niacin deficiency in humans, which leads to low NAD status, causes sun sensitivity in skin, indicative of deficiencies in responding to UV damage. Animal models of niacin deficiency demonstrate genomic instability and increased cancer development in sensitive tissues including skin. Cell culture models of niacin deficiency have allowed the identification of NAD-dependent signaling events critical in early skin carcinogenesis. Niacin restriction in immortalized keratinocytes leads to an increased expression and activity of NADPH oxidase resulting in an accumulation of ROS, providing a potential survival mechanism as has been shown to occur in cancer cells. Niacin deficient keratinocytes are more sensitive to photodamage, as both poly(ADP-ribose) polymerases and Sirtuins are inhibited by the unavailability of their substrate, NAD⁺, leading to unrepaired DNA damage upon photodamage and a subsequent increase in cell death. Furthermore, the identification of the nicotinic acid receptor in human skin keratinocytes provides a further link to niacin's role as a potential skin cancer prevention agent and suggests the nicotinic acid receptor as a potential target for skin cancer prevention agents. The new roles for niacin as a modulator of differentiation and photo-immune suppression and niacin status as a critical resistance factor for UV damaged skin cells are reviewed here.

Key Words: Skin, NAD, niacin, PARP, Sirtuin, nicotinic acid receptor, actinic keratosis, skin cancer.

INTRODUCTION

Non-melanoma skin cancers (NMSC), which include basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), are keratinocyte-derived tumors. Although many environmental and genetic factors contribute to the development of skin cancers, the most important etiological factor in NMSCs is chronic exposure to UV radiation from sunlight [1, 2]. Primary prevention of NMSC has focused on education about the harmful effects of UV radiation present in the sunlight. However, even though the use of sunscreens has grown widely, this has not resulted in a decrease of skin cancer incidence [3]. Some studies have even indicated that sunscreen use is associated with an increase in the incidence of malignant melanoma due to increased exposure time [4, 5]. Approaches that can reverse early signs of skin cancer (actinic keratosis) or prevent progression to malignancy are urgently needed and may be much more effective than sunscreens alone due to increased motivation by subjects at increased risk. The recent discoveries that niacin can modulate skin differentiation and photo-immune-suppression have stimulated a new interest in its mechanism(s) of action in skin.

2. NIACIN

Niacin, also known as vitamin B₃ or vitamin PP, is a water-soluble vitamin that occurs in two chemical forms:

nicotinic acid and nicotinamide (also known as niacinamide). Niacin can be obtained directly from the diet or synthesized from dietary tryptophan in the liver and possibly kidney in some species. However, the synthesis of niacin from tryptophan is extremely inefficient, where the dogma suggests that approximately 60 mg of dietary tryptophan produces 1 mg of niacin in the presence of vitamins B₂ and B₆ (Fig. 1). Furthermore, metabolic studies suggest this is highly unlikely in humans under moderate niacin deficiency [6] and *in vitro* studies have shown that not all human cells contain the enzymes required for the utilization of tryptophan for the formation of pyridine nucleotides [7]. Of interest to this review is the fact that human skin cells appear to rely strictly on salvage pathways for synthesis of pyridine nucleotides [8].

2.1. Niacin Functions

Both nicotinic acid and nicotinamide are dietary precursors for the synthesis of important coenzymes involved in hydride ion transfer: nicotinamide adenine dinucleotide [NAD(H)] and nicotinamide adenine dinucleotide phosphate [NADP(H)] (Fig. 1). NAD and NADP serve as coenzymes for hundreds of enzymes [9]. The roles of NAD as a coenzyme in oxidative metabolism, specifically in the citric acid cycle, fatty acid oxidation, and glycolysis are well understood. The transfer of electrons from NADH formed in these processes to oxygen is the major source of adenosine triphosphate (ATP) in aerobic metabolism and also is essential in the less efficient production of ATP in glycolysis. NAD also serves as a substrate for nicotinamide adenine dinucleotide phosphate [NADP(H)] biosynthesis. This coenzyme serves an analogous role for transfer of electrons in biosynthetic reactions, functioning as hydride ion donor for

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reductive biosyntheses including fatty acid synthesis, cholesterol synthesis, and hydroxylations (Fig. 1).

Besides its role as a coenzyme, NAD^+ was first shown in 1963 to serve as a substrate for covalent protein modifications [10]. Since then, considerable progress has been made towards understanding the multiple roles of NAD^+ as a substrate for mono-ADP-ribosylation, poly(ADP-ribosyl)ation, and NAD-dependent protein deacetylation (Fig. 1). Furthermore, besides protein modification, NAD^+ may be also used for the synthesis of signaling molecules, including ADP-ribose (ADPR) [11-13], cyclic ADP-ribose (cADPR) [14, 15], nicotinic acid adenine dinucleotide phosphate (NAADP) [16, 17], and 2'-phospho-cyclic ADP-ribose (P-cADPR) [18, 19], all important in Ca_2^+ signaling (Fig. 1).

2.2. Niacin and Skin

The first indication of the beneficial effects of niacin to human skin was the observation that it could reverse the dermatitis associated with pellagra. Pellagra (from "pelle agra" meaning rough skin) is caused by severe niacin deficiency and was first described by Casal in 1735 as "mal de la rosa", with symptoms of dermatitis, diarrhea, dementia and death [20]. Dermatitis occurred especially in skin areas exposed to sunlight, and was characterized by skin thickening, scaling and hyperkeratinization [21]. The observation that the dermatitis observed in pellagra patients, along with other symptoms of pellagra, could be reversed by oral niacin supplementation lead to establishing niacin deficiency as the major etiological component of pellagra [22].

A deficiency of either niacin or tryptophan can result in pellagra in a nutritionally compromised person. However, pellagra-like dermatitis (secondary pellagra) can occur even when adequate quantities of niacin are present in the diet, but other diseases or conditions interfere with its intake, absorption and/or processing, such as prolonged diarrhea, anorexia nervosa, chronic alcoholism, chronic colitis, severe ulcerative colitis, regional ileitis, hepatic cirrhosis, carcinoid tumor, Hartnup's syndrome, and tuberculosis of the gastrointestinal tract [23-27]. An excess of dietary leucine can interfere with niacin utilization and result in pellagra [28]. In patients with carcinoid syndrome, tumor cells convert tryptophan into serotonin, depressing endogenous niacin production. Isoniazid, an antituberculosis medication, is an analog of niacin and may also suppress endogenous niacin production and produce pellagra [24]. In Hartnup's disease dietary amino acids including tryptophan are poorly absorbed [29]. Symptoms of pellagra are also observed in long-term administration of 5-fluorouracil, as this drug inhibits the conversion of tryptophan to nicotinic acid [29]. Other drugs, including pyrazinamide, 6-mercaptopurine, hydantoins, ethionamide, phenobarbital, azathioprine, and chloramphenicol, may also cause pellagra by interfering with the tryptophan-niacin pathway. In HIV disease one of the many dysfunction occurring is a severe plasma tryptophan and niacin deficiency, sometimes leading to a pellagra-like state [30, 31].

3. NAD AND POLY(ADP-RIBOSE) POLYMERASE

In addition to its role as a co-factor in redox reactions and as a regulator of the redox state (NAD^+/NADH), NAD func-

tions as a substrate for numerous classes of ADP-ribosyl transferases (Fig. 1), enzymes involved in cellular processes including transcription, calcium homeostasis, DNA repair, cell death, neoplastic transformation, etc [32, 33]. Considerable evidence now indicates that the relative NAD content of cells can influence cellular responses to genomic damage by multiple mechanisms. For example, NAD^+ is a substrate for the poly(ADP-ribose) polymerase (PARP) family of enzymes, which are important in DNA damage responses, including repair, maintenance of genomic stability, signaling following stress responses that influences apoptosis, telomere function, transcription regulation, and numerous other cellular functions [34-37]. Interestingly, PARP-1 functions both as a structural component of chromatin and a modulator of chromatin structure through its intrinsic enzymatic activity, promoting the formation of compact, transcriptionally repressed chromatin structures [38].

The beneficial effects of niacin have been attributed to the involvement of PARP-1 as a target for cancer prevention based on studies from many laboratories, including our own, that have demonstrated the involvement of PARP-1 in the maintenance of genomic integrity following genotoxic stress [34, 39]. PARP-1 functions in the synthesis of chromatin-associated ADP-ribose polymers (PAR) that function in cellular recovery from DNA damage and maintenance of genomic stability. The activation of PARP-1 by DNA strand breaks leads to complex signaling pathways that can enhance cell survival, result in cell death by apoptosis, or cause energy loss that leads to necrosis. In cases where the amount of damage is relatively small, PARP-1 activation enhances cellular recovery by interaction with other proteins such as p53 and the nuclear proteasome to stimulate both DNA repair and histone degradation such that the cell can fully recover from the genotoxic stress. When the damage is relatively higher, PARP-1 plays a key role in effecting cell death by apoptosis through its transcriptional activation role involving the NF- κ B pathway and by preventing ATP depletion and DNA repair through PARP-1 cleavage [39]. Finally, when the damage is very high, PARP-1 overactivation can lead to cellular necrosis through depletion of first NAD and then ATP with a resulting loss of all energy dependent functions. A more recent study has suggested that a drop in cellular NAD levels itself can trigger the mitochondria to initiate cellular apoptosis [40]. Inhibitors of PAR metabolism alter the repair of DNA strand breaks [41, 42] and increase the cytotoxic effects of DNA alkylating agents [41-44]. These inhibitors also increase the frequency of malignant transformation by these agents [45, 46]. Blocking ADP-ribose polymer metabolism using a molecular genetic approach that involved overexpression of the DNA binding domain of PARP-1 also has shown inhibition of DNA repair and increased cytotoxicity [47]. Furthermore, mice with homozygous disruption of the PARP-1 gene show increased sensitivity to cytotoxic agents and increased susceptibility to chemical carcinogens [48].

Among other members of the PARP family, PARP-2 is activated by DNA strand breaks, similarly to PARP-1. In fact, in the absence of PARP-1, PARP-2 is required for repair of single strand breaks, maintenance of genomic stability and embryonic viability [49, 50]. PARP-3 associates with the daughter centriole within centrosomes and interferes with

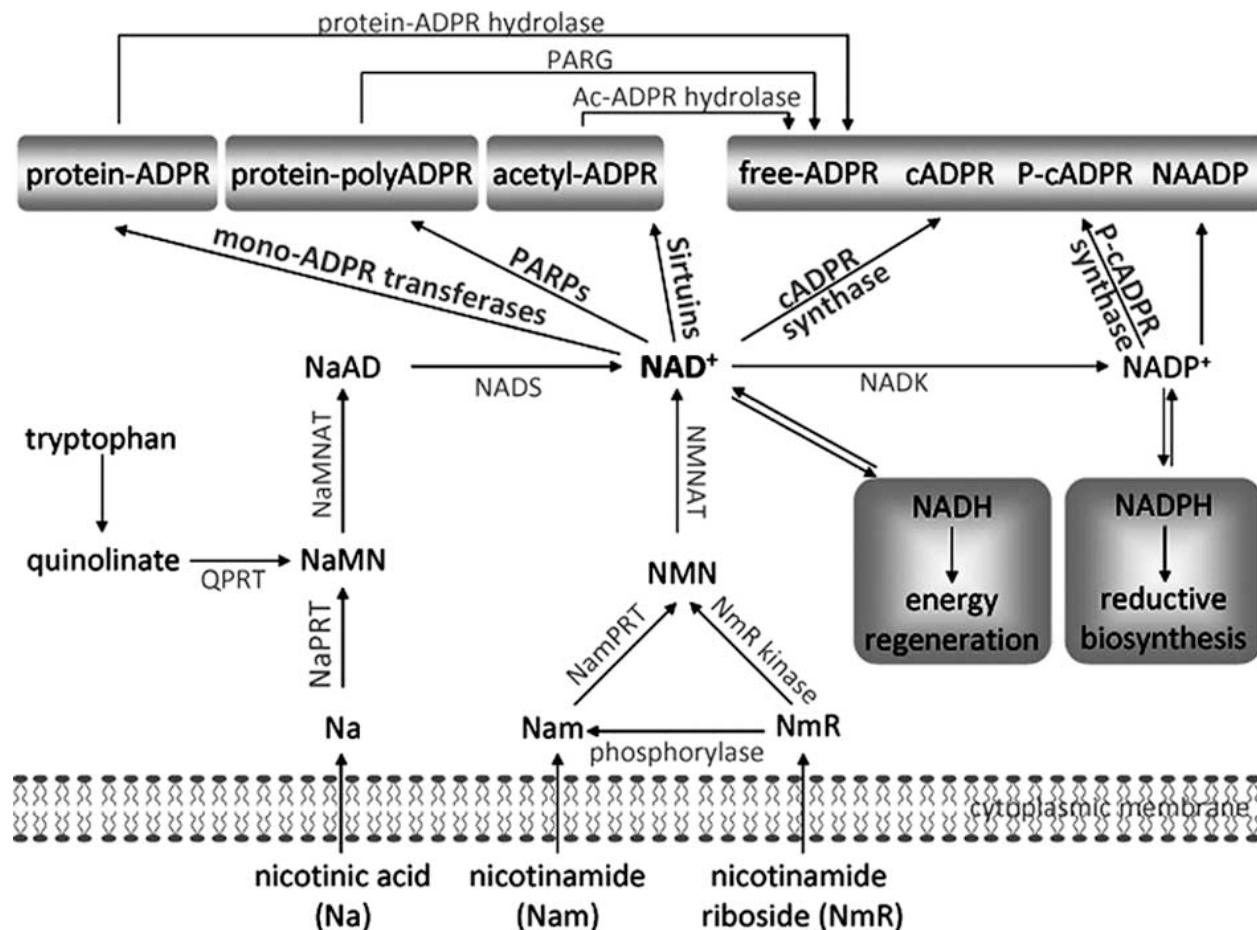


Fig. (1). Mammalian NAD metabolic pathways. Nicotinic acid (Na), nicotinamide (Nam), and nicotinamide riboside (NmR) serve as precursors for the synthesis of nicotinamide adenine dinucleotide (NAD^+). NAD^+ can be used in various cellular processes both as a coenzyme in metabolic pathways, as a substrate for covalent protein modifications, and for generation of signaling nucleotides. Nicotinamide phosphoribosyltransferase (NamPRT), nicotinamide mononucleotide adenylyl transferase (NMNAT), nicotinic acid mononucleotide adenylyl transferase (NaMNAT), nicotinic acid phosphoribosyltransferase (NaPRT), NAD synthetase (NADS), NAD kinase (NADK), quinolinic acid transferase (QPRT), ADP ribose (ADPR), cyclic ADP ribose (cADPR), nicotinic acid adenine dinucleotide phosphate (NAADP), 2'-phospho-cyclic ADP-ribose (P-cADPR), poly-ADP ribose polymerase (PARP), poly-ADP ribose glycohydrolase (PARG).

G₁/S cell cycle progression [51]. PARP-4 acts as a catalytic component of vault particles which are thought to function in intracellular transport and are involved in multidrug resistance in human tumors [52]. PARP-5 (also referred as tankyrase-1) and PARP-6 (tankyrase-2) regulate telomere homeostasis by diminishing the ability of the negative regulator of telomere length, TRF-1, to bind telomeric DNA and therefore, promoting telomere elongation [53, 54]. It becomes evident that the PARP family plays crucial roles in regulating key signaling pathways and therefore, maintaining optimal NAD levels is critical.

4. PARPS IN RESPONSE TO PHOTODAMAGE

Extensive data can be found on the consequences of NAD deficiency and its effect on PARP inhibition following DNA damage induced by chemical agents [55-57] and various studies have shown PARP activation in response to UV irradiation [57-59]. With respect to responses to UV light, PARP activation has been shown to participate in carcinogenesis, DNA repair, and cell death. PARP inhibition during UVB-induced skin carcinogenesis in mice, acts as a cocarcinogen by accelerating the onset of tumors and increas-

ing the severity of cancers [60]. PARP activation has also been linked to UVB-induced apoptosis in cultured cells and in mouse skin [57, 61]. Numerous studies have attempted to demonstrate that inhibition of PARP following UV damage could enhance the cytotoxic effects of UV, analogous to what has been shown with alkylating agent damage. However, no link has been demonstrated between PARP inhibition and cytotoxicity upon UV irradiation. In fact, a previous study suggests that PARP does not participate directly in the repair of UV-induced pyrimidine dimers [62]. Thus, the role of PARP activity during recovery from UV damage is not well defined.

In a model of niacin deprivation, we have shown that keratinocytes are severely sensitized to photodamage, shown as an increase in both DNA damage and cell death (unpublished data). PARP inhibition through substrate deprivation not only can explain the increased cell death due to unrepaired DNA damage, but also can explain the increase in DNA strand breaks in two ways. First, unavailability of NAD^+ leads to a catalytically inactive PARP that can have a dominant negative influence on DNA repair, leading to unrepaired DNA breaks produced by endonucleases in the

DNA repair process. Second, PARP-1 functions both as a structural component of chromatin and a modulator of chromatin structure through its intrinsic enzymatic activity, promoting the formation of compact, transcriptionally repressed chromatin structures [38]. Therefore, PARP inhibition could lead to a more relaxed chromatin, which would become more prone to DNA damage.

5. NAD AND SIRTUINS

In addition to NAD⁺ serving as a substrate for ADP-ribosyltransferases, it is a substrate for NAD⁺-dependent protein deacetylases (Sirtuins) (Fig. 1). Sirtuins (SIRT) are members of a family of NAD⁺-dependent enzymes that remove acetyl groups from acetylated lysine residues in proteins and in doing so, regulate the biological function of their targets [63, 64]. SIRT belongs to the Silent information regulator 2 (Sir2) family of histone deacetylases (HDACs), which play a central role in epigenetic gene silencing, DNA repair and recombination, cell-cycle regulation, microtubule organization, and in the regulation of aging [65-68].

All SIRT share a conserved catalytic domain, which is reported to function as a NAD⁺-dependent protein deacetylase and, in some cases, as a putative mono-ADP-ribosyltransferase [63, 69-71]. During the deacetylation reaction, SIRT consume one NAD⁺ molecule to generate acetyl-ADP-ribose and nicotinamide [70]. During mono-ADP-ribosylation, SIRT consume one NAD⁺ molecule to generate the ADP-ribosylated product and nicotinamide [63, 72, 73]. A conserved histidine residue in the catalytic core of the SIRT is important for the deacetylase and/or mono-ADP-ribosyltransferase activities [63]. However, acetyl-ADPR can be metabolized to ADPR, which is highly reactive in glycating lysine residues; thus, distinguishing these two reactions is technically challenging. Additionally, in *in vitro* studies employing NAD⁺, numerous enzymes can generate ADPR magnifying the challenge. Thus, further studies are needed to distinguish whether or when these enzymes are catalyzing a deacetylase reaction or an ADP-ribosylation reaction.

Mammalian SIRT have diverse cellular locations, target multiple substrates, and affect a wide range of cellular functions. SIRT1 is localized to the nucleus and is the most extensively studied member of the SIRT family. It has been shown to play an important role in the regulation of stress induced cell fate in mammalian cells and has therefore been described as a guardian against cellular oxidative stress and DNA damage [74]. SIRT1 promotes cell survival by inhibiting apoptosis or cellular senescence induced by stresses including DNA damage and oxidative stress [75-77]. SIRT2 is a cytoplasmic protein that co-localizes both with chromatin during mitosis and microtubules in the cytoplasm [78]. SIRT2 functions in the control of mitotic exit in the cell cycle where increased SIRT2 activity severely delays cell cycle progression through mitosis [79]. SIRT2 also acts as a mitotic checkpoint protein that prevents chromosomal instability and the formation of hyperploid cells in early metaphase [80]. SIRT3 is a mitochondrial protein [81, 82] with deacetylase activity linked to adaptive thermogenesis [83] and longevity [84, 85]. However, some authors believe that SIRT3 is a nuclear protein that translocates to the mitochondria upon cellular stress [86]. SIRT4 also is a mitochondrial pro-

tein and contains the conserved SIRT domain, but it apparently does not possess *in vitro* deacetylase activity [73]. SIRT4 has been reported as a modulator of glutamate dehydrogenase (GDH), a mitochondrial enzyme that converts glutamate to α -ketoglutarate [73]. GDH controls amino acid-stimulated insulin secretion by regulation of glutamine and glutamate oxidative metabolism [73]. SIRT5 is the least characterized member of the SIRT family and was only recently reported to deacetylate cytochrome c [87]. SIRT5 has been described as a mitochondrial protein [88] with weak deacetylase [89] and no ADP-ribosyltransferase activity [73]. SIRT6 is a nuclear protein associated with heterochromatin that promotes resistance to DNA damage and suppresses genomic instability in mouse cells, in association with a role in base excision repair (BER) [88, 90]. SIRT6 deficient mice display multiple pathologies that resemble human aging including lymphopenia, loss of subcutaneous fat, decreased bone density, hypoglycemia, decreased insulin-like growth factor 1 (IGF-1) levels, and premature death [90]. SIRT6 is suggested to possess both ADP-ribosyl-transferase [72] and deacetylase activity [90]. Finally, SIRT7 is a widely expressed protein localized to the nucleolus that associates with condensed chromosomes during mitosis [88, 91]. SIRT7 expression is abundant in tissues with high proliferation rates and low in non-proliferating tissues [91]. SIRT7 has been suggested to play a role as a positive regulator of RNA polymerase I transcription and may be required for cell viability in mammals [91]. SIRT7 interacts with RNA polymerase I and histones, and positively regulates the transcription of active rRNA genes (rDNA). Depletion of SIRT7 has been shown to stop cell proliferation and trigger apoptosis [91]. Like the other members of the SIRT family, the NAD binding core domain is conserved in SIRT7, but no deacetylase or ADP-ribosyltransferase activity has been demonstrated to date [91]. It has been suggested that SIRT7 regulates rDNA transcription in a NAD⁺ dependent manner, such that the NAD⁺/NADH ratio might modulate SIRT7 to link the cellular energy status with rRNA synthesis and ribosome production [92].

6. SIRTUINS IN SKIN

Work from our lab examined the expression of SIRT in human skin cells and found that the seven SIRT family members are expressed in fibroblasts and keratinocytes. It also shows that SIRT may play a role in the differentiation process of keratinocytes, as the gene expression of SIRT1 and SIRT7 is decreased and SIRT4 and SIRT5 are increased during this process. Supporting our observation, a recent report has shown that SIRT1 promotes differentiation of normal keratinocytes [93]. We've also found that SIRT expression is altered both in pre-malignant (actinic keratosis) and malignant (SCC) skin disease. In some tissues it has been shown that tumor cells require SIRT overexpression [94]. In skin malignancies (SCC) this is true for all SIRTs (unpublished data). We have also seen that in response to photodamage, the expression of several SIRTs is altered in keratinocytes. Furthermore, we showed that SIRT's responses to photodamage differ between normal (NHEK) and immortalized keratinocytes (HaCaT), which may be indicative of alterations associated with early skin carcinogenesis.

7. NIACIN DEFICIENCY INCREASES ROS IN HUMAN KERATINOCYTES

Removal of niacin from the cell culture medium allows the control of intracellular NAD and the investigation of NAD dependent responses following DNA damage stress. Using this approach, it has been shown that HaCaT keratinocytes, unlike many non-immortalized human cells, become severely NAD depleted while maintaining their ability to divide indefinitely under these conditions [8]. These results confirm that normal human keratinocytes require the pre-formed vitamin to synthesize NAD utilizing the salvage pathway, as the synthesis of NAD from tryptophan through the *de novo* pathway seems to be absent. Interestingly, the long-term survival mechanism in niacin deficient HaCaTs seems to involve the accumulation of reactive oxygen species (ROS) and increased DNA damage, possibly caused by the increased expression and activity of NADPH oxidases [8].

It has been hypothesized that since NAD falls to extremely low levels under niacin deprivation in immortalized cells, the utilization of glucose by glycolysis is not functional and that cells require an alternative carbon source for energy [8]. In the case of HaCaTs, we show evidence that niacin deficiency leads to utilization of glutamine as the primary carbon source. The utilization of glutamine for energy is linked to the generation of NADPH, which is recycled by NADPH oxidases (NOX), with the subsequent generation of ROS by NOX [8]. Thus, adequate NAD modulates survival pathways that normally limit ROS dependent signaling that may be activated as a survival mechanism during early skin carcinogenesis.

8. NIACIN AS A CHEMOPREVENTION AGENT

In vitro as well as animal studies show that modulation of NAD⁺ by nutritional control results in altered cellular processes and sensitivity to DNA damage. Cells cultured under conditions of nutritional depletion of NAD are more sensitive to the cytotoxic effects of DNA-damaging agents [35, 95]. This sensitivity is thought to be due to reduced availability of NAD, resulting in impaired PARP-1 function and DNA repair mechanisms. In human cells, niacin deficiency has been shown to alter the expression of p53, induce inherent genomic instability, and reduce survival following exposure to solar simulated light [96, 97]. Conversely, increased NAD⁺ or more active NAD biosynthesis allows cells to recover more efficiently after DNA damage [96].

Animal studies have shown that UV exposure during niacin deficiency leads to sensitized skin [98], while niacin supplementation protects mouse skin from UV irradiation [99]. Other studies employing niacin deficiency have shown changes in NAD⁺ and poly(ADP-ribose) metabolism altering p53 expression, increasing genomic instability, impairing cellular responses to DNA damage, and increasing cancer incidence [100-104].

In human subjects, pilot data have demonstrated that the NAD content of skin varies widely and inversely correlates with malignant phenotype [96, 105]. NAD content of normal skin in subjects diagnosed with squamous cell carcinoma is significantly lower than that of individuals showing pre-

cancerous lesions [96]. Preliminary data also suggest that niacin supplementation may have protective effects against DNA damaging agents [99, 106-108]. In addition, another factor associated with the development of skin cancer is the UV radiation-induced immunosuppression [109, 110]. Several studies have shown that topical and oral delivery of nicotinic acid and nicotinamide can prevent the immunosuppression and skin tumor induction by UV irradiation [99, 111, 112]. Thus, niacin supplementation may limit skin damage and consequently skin cancer by targeting multiple mechanisms including reduction of DNA damage and optimizing responses to DNA damage. These findings along with the evidence that the NAD content of human tissues, including skin, is deficient in a significant portion of the population, raise the question as to whether niacin status (NAD content) is an important protective factor for genotoxic stresses resulting directly and indirectly from exposure to sunlight.

9. NICOTINIC ACID RECEPTOR EXPRESSION IN HUMAN KERATINOCYTES: A NEW ROLE IN SKIN?

The use of niacin as a chemopreventive agent for the treatment of early signs of skin cancer involves assessment of which chemical form of the vitamin is most beneficial. Both nicotinic acid and nicotinamide serve as precursors of the coenzymes NAD and NADP. However, some *in vitro* evidence indicates that high concentrations of nicotinamide, but not nicotinic acid, have inhibitory effects on NAD-dependent protein deacetylases (SIRT6) and poly(ADP-ribose) polymerases (PARPs) [113, 114]. Furthermore, nicotinic acid appears to be a preferred candidate for therapeutic use since it has additional biological effects unrelated to those as a vitamin [115]. In recent years, nicotinic acid was found to bind two very similar orphan G-protein coupled receptors, HM74A (also referred to as GPR109A or PUMA-G in mice) and HM74 (GPR109B) [116]. This discovery has provided the possibility of gaining further insight into the therapeutic mechanism(s) of action for nicotinic acid.

These two receptors are encoded by separate genes in tandem organization where the HM74 gene encodes a protein with a carboxyterminal extension of 24 amino acids as compared to HM74A [117]. HM74A has recently been identified as an important pharmacological target for the treatment of dyslipidemia as it binds nicotinic acid with high affinity [118-121]. HM74A is expressed in adipose tissue where, upon binding of its ligand, it has been shown to inhibit the activity of adenylyl cyclases, resulting in lowered cAMP levels. As a consequence, the hydrolysis of triglycerides and the release of free fatty acids into the bloodstream are reduced. This leads to the positive impact of elevating high-density lipoprotein (HDL) and reducing low-density lipoprotein (LDL) cholesterol observed upon nicotinic acid intake. For this reason, nicotinic acid is the most effective drug in use for modulating hyper-lipidemia, increasing HDL cholesterol levels, and preventing coronary heart disease [122].

Amongst various tissues, HM74A is also expressed in skin macrophages and Langerhans cells where it has been reported to mediate an intense flushing and burning sensation of the skin in response to nicotinic acid, the common

side effect of high-dose niacin intake [123]. In order to circumvent this problem our lab has developed the esterified niacin derivative, lauryl nicotinate, for transdermal delivery of the drug through the skin, providing controlled rates of delivery that alleviate the flushing response during therapy to modulate serum cholesterol levels. During mechanistic studies of lauryl nicotinate, we discovered that niacin causes the release of leptin and downstream signaling of leptin has profound effects on epidermal renewal, wound healing and hair follicle biology in skin (Fig. 2) [106, 124]. These findings led to an investigation of niacin derivatives that preferentially target delivery of niacin to skin cells and the functions of niacin in skin biology. Of interest, we have found that myristyl nicotinate stimulates skin differentiation, consistent with a role in cancer prevention (Fig. 3) [106]. Our unpublished studies also show that HM74 and HM74A receptors are expressed in human skin, mainly in the stratum basale and stratum spinosum in the epidermis, so it is possible that the beneficial effects to skin [106, 124] observed by topical application of niacin are mediated by signaling through the nicotinic acid receptor, at least in part (Fig. 3).

Several observations have shown that topical application of a nicotinic acid derivative has beneficial effects on skin barrier integrity by increasing the stratum corneum and moderately increasing the thickness of the epidermis, reducing transepidermal water loss and enhancing specific markers of differentiation [106, 124]. It also has been proposed that nicotinic acid enhances wound healing by stimulation of

leptin secretion. The presence of the nicotinic acid receptor in the same area where these effects are taking place points towards the possibility that signaling through this receptor is responsible for the enhanced differentiation and leptin secretion being observed. Whether leptin is derived from adipocytes in the subcutaneous layers, from keratinocytes, or both, remains to be determined. Further research will be necessary to define the exact mechanisms mediating the effects of topical niacin and whether they are mediated *via* the niacin receptor and/or leptin. This is particularly exciting after finding that the receptors are expressed differentially in skin with preferential expression in human epidermal keratinocytes. The nicotinic acid receptor thus represents a potential target for developing skin cancer prevention agents as well as therapeutics to benefit other dermatological diseases (Fig. 2).

10. CONCLUSIONS

Niacin deficiency in humans causes sun sensitivity, indicative of DNA repair abnormalities [98]. In fact, animal models of niacin deficiency demonstrate genomic instability [100] and increased cancer development in sensitive tissues including skin [125]. However, the maintenance and regulation of cellular NAD(P)(H) content and its influence on cell function are poorly understood. The development of a model of niacin modulation in human skin cells not only has confirmed that human keratinocytes require the preformed vitamin, niacin, to synthesize NAD utilizing the salvage pathway, as the synthesis of NAD from tryptophan through the

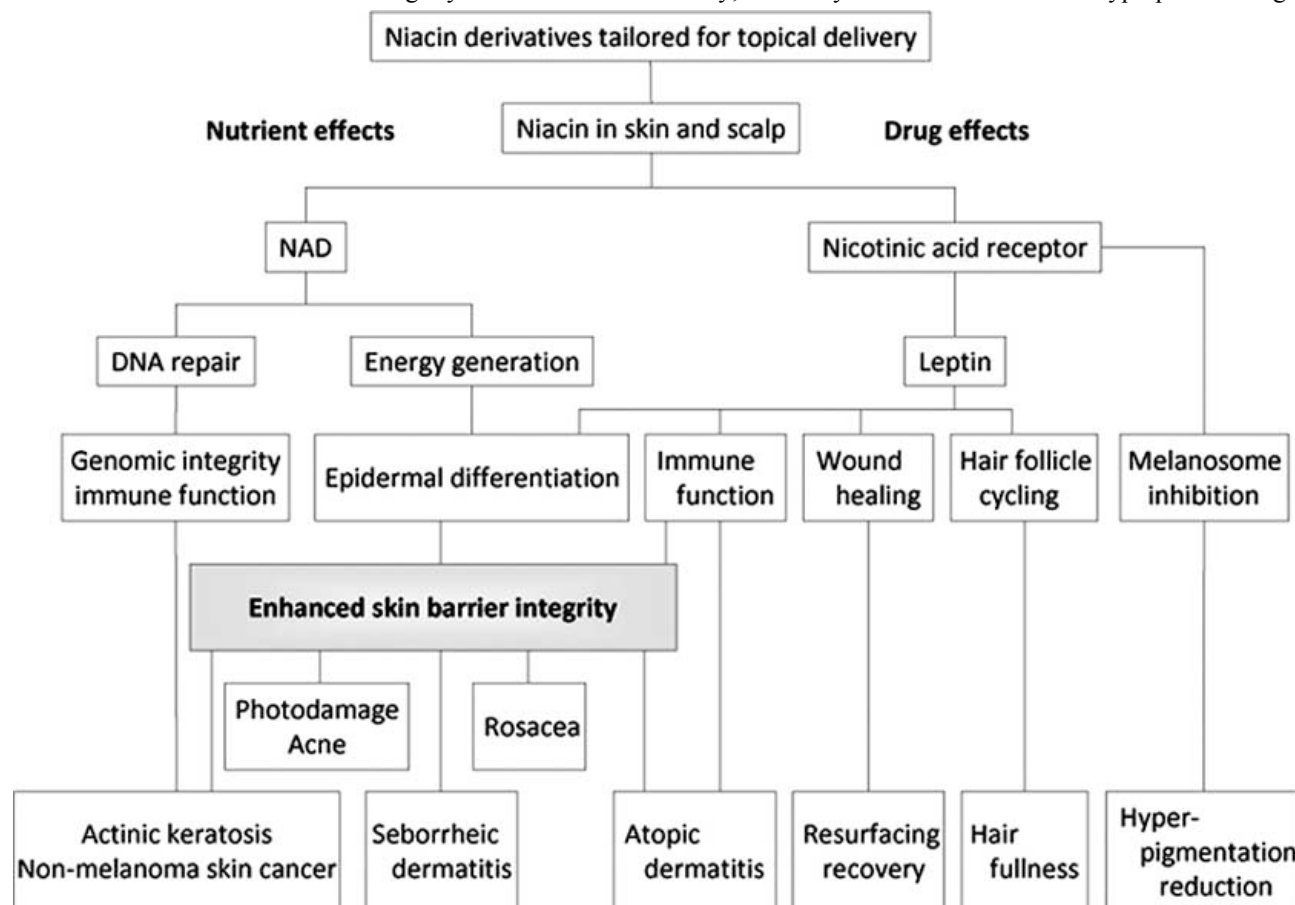


Fig. (2). Nutrient and drug effects of niacin in skin. Topically delivered niacin can exert its beneficial effects on skin both as a nutrient, through its maintenance of NAD levels, and as a drug, signaling through the nicotinic acid receptor.

de novo pathway seems to be absent, but provides a tool to identify NAD-dependent signaling events that may be critical in early skin carcinogenesis [8].

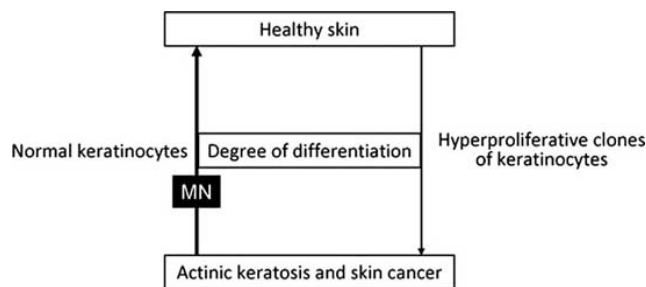


Fig. (3). Enhancing differentiation prevents progression of hyperproliferative cells toward actinic keratosis. Agents that induce skin differentiation prevent the progression of hyperproliferative cells to pre-cancerous (actinic keratosis) and cancerous (skin cancer) states. Niacin derivatives tailored for topical delivery, e.g. myristyl nicotinate (MN), stimulate skin differentiation, potentially serving as skin cancer prevention agents.

Niacin deficient skin is more sensitive to photodamage, possibly because PARPs and SIRT activities are restricted by the unavailability of their substrate, NAD^+ , leading to unrepaired DNA damage upon UV exposure and subsequent cell death. PARP inhibition could lead to a more relaxed chromatin, which would become more prone to DNA damage. NAD^+ is also consumed by SIRT activity to deacetylate histones, among other targets. Histone deacetylation leads to a more compact chromatin structure and gene silencing [126]. Taken together, niacin deficiency could lead to a more open DNA structure, with more active gene expression and greater sensitivity to damage and translocation events [98]. Therefore, both SIRT and PARPs are critical following DNA damage and maintaining an optimal cellular NAD level is crucial to assure a proper DNA repair after photodamage.

Various studies have provided links to niacin's role as a potential skin cancer prevention agent and we have suggested the nicotinic acid receptor as a potential target for skin cancer prevention drugs. Finding that keratinocytes express the nicotinic acid receptors supports the idea that niacin plays an important role in preventing skin disease as signaling through this receptor may be responsible for the enhanced skin differentiation and wound healing observed upon niacin treatment [106, 124].

Niacin deficiency drives keratinocytes during early carcinogenesis to proliferate and survive under conditions that promote genomic instability and DNA damage, thus driving the carcinogenesis process in skin [8]. Therefore, niacin has potential as a chemopreventive agent used for treatment of early events in skin cancer (e.g. actinic keratoses) as it would sustain proper DNA damage repair and maintenance of genomic stability, as well as drive skin differentiation to create a proper skin barrier and function.

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ABBREVIATIONS

ADP	= Adenine dinucleotide phosphate
ADPR	= ADP ribose
ATP	= Adenosine triphosphate
BCC	= Basal cell carcinoma
cADPR	= Cyclic ADPR
GDH	= Glutamate dehydrogenase
HDAC	= Histone deacetylase
HDL	= High-density lipoproteins
LDL	= Low-density lipoproteins
NAD(H)	= Nicotinamide adenine dinucleotide (reduced)
NADP(H)	= Nicotinamide adenine dinucleotide phosphate (reduced)
NAADP	= Nicotinic acid adenine dinucleotide phosphate
NMSC	= Non-melanoma skin cancer
PAR	= ADP-ribose polymers
PARP	= Poly-ADP ribose polymerase
P-cADPR	= 2'-Phospho-cADPR
ROS	= Reactive oxygen species
SIRT	= Sirtuin
SCC	= Squamous cell carcinoma

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