Chapter 31
N-Acetylcysteine for Reduction of Oxidative Stress/ Damage and Prevention of Melanoma

Pamela B. Cassidy, Sancy A. Leachman, and Douglas Grossman

Key Points

- *N*-acetyl-L-cysteine is a potent antioxidant that is commercially available as an over-the-counter supplement that has demonstrated efficacy for several medical applications.
- The drug is FDA-approved for acetaminophen poisoning. Its metabolism and basis of its biological activity are well understood.
- *N*-acetyl-L-cysteine has been used in several experimental systems, ranging from skin cancer prevention to modification of psychological conditions.
- Here we review its biological basis and potential clinical applications, with emphasis on our work developing *N*-acetylcysteine as an agent for melanoma chemoprevention.

Keywords NAC • Oxidative stress • UV • Melanoma • Nevi

Abbreviations

8-OH-dG	8-Hydroxy-2′-deoxyguanine
Cys	Cysteine
GGTase	Gamma-glutamyl transferase
GR	Glutathione reductase
GSH	Glutathione
GSSG	Glutathione disulfide
GST	Glutathione *S*-transferase

P.B. Cassidy
Department of Medicinal Chemistry, Huntsman Cancer Institute, University of Utah,
2000 Circle of Hope, Salt Lake City, UT 84112, USA
e-mail: pamela.cassidy@hci.utah.edu

S.A. Leachman
Department of Dermatology, Huntsman Cancer Institute, University of Utah,
2000 Circle of Hope, Salt Lake City, UT 84112, USA

D. Grossman, M.D., Ph.D. (✉)
Department of Dermatology, University of Utah Health Sciences Center, Oncological Sciences
at the University of Utah, 30 North 1900 East, Salt Lake City, UT 84132, USA
e-mail: doug.grossman@hci.utah.edu

R.R. Watson and S. Zibadi (eds.), *Bioactive Dietary Factors and Plant Extracts in Dermatology*,
Nutrition and Health, DOI 10.1007/978-1-62703-167-7_31,

NAC	*N*-Acetylcysteine
NADPH	Nicotinamide adenine dinucleotide phosphate reduced form
NAPQI	*N*-Acetyl-*p*-quinonimine
Protein-SG	Protein-glutathione mixed disulfide
ROS	Reactive oxygen species
UV	Ultraviolet radiation
γ(gamma)-GCS	Gamma-glutamylcysteine synthase

N-Acetyl Cysteine Is a Prodrug That Supports the Biosynthesis of Glutathione

N-acetyl-L-cysteine (NAC) is a prodrug of L-cysteine, which is the rate-limiting amino acid required for the biosynthesis of glutathione (GSH) [1]. The tripeptide thiol GSH (γ(gamma)-glutamylcysteinylglycine) is the most abundant small-molecule antioxidant in the body with levels as high as 10 mM in some tissues [2]. NAC begins its transformation into GSH by rapid deacetylation in the liver, giving cysteine (Cys). Cys is imported into melanocytes (the cells from which melanomas arise) and other cells in either its reduced form by the transporter Slc1A4 [3] or in its oxidized form (cystine) by Slc7A11 [4]. GSH itself is present in the extracellular space, but cannot cross the cell membrane; Cys must first be salvaged by cleavage of the tripeptide by γ(gamma)-glutamyltranspeptidases (GGTases) [5]. Once inside the cell, the first peptide bond in GSH is formed between the γ(gamma)-carboxylate of glutamate and the amino group of Cys in a reaction catalyzed by γ(gamma)-glutamylcysteine synthase (γ(gamma)-GCS). Feedback inhibition of γ(gamma)-GCS results in the tight regulation of basal levels of GSH in most tissues. Glutathione synthase then catalyzes the formation of the peptide bond between the α-carboxylate of Cys and the amino group of glycine to give GSH (Fig. 31.1) [1].

The biological activity of NAC is generally attributed to its ability to reduce oxidative stress in tissues by relieving the depletion of GSH, which in turn arises from a variety of insults including ultraviolet radiation (UV). The vital cellular functions of GSH are as follows: (1) scavenging and metabolizing reactive oxygen species (ROS) and reactive nitrogen species, (2) detoxifying electrophiles arising from xenobiotics and/or their metabolites, as well as endogenous electrophilic species such as oxidized lipids, (3) providing a reservoir for Cys, and (4) modulating critical cellular processes such as DNA synthesis, microtubular-related processes and immune function (reviewed in reference 6). Here we focus on the first two processes which are important for the antioxidant activity

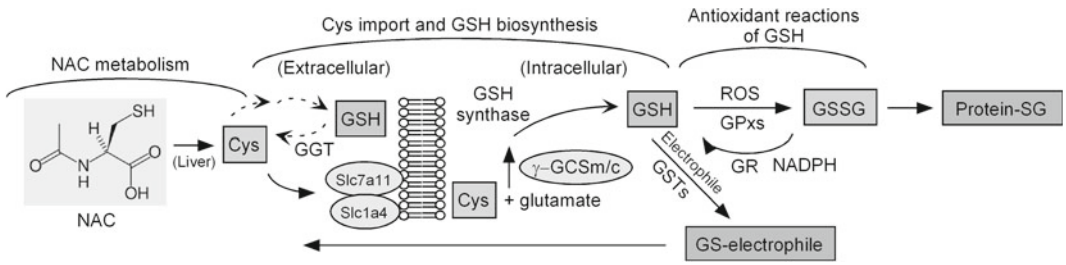

Fig. 31.1 The metabolism of NAC, the biosynthesis of GSH, and the antioxidant activities of GSH in the melanocyte are depicted schematically. *NAC metabolism*—NAC is deacetylated in the liver yielding Cys, which is imported cells by the transporters Slc1A4 and Slc7A11 (*Cys import*). Cys can also be salvaged by cleavage of GSH by GGTases. *GSH biosynthesis*—GSH is formed by the sequential actions of the γ(gamma)-GCS and GS. *Antioxidant reactions of GSH*—GSH is the reductant in the detoxification of hydrogen peroxide and peroxidized lipids in reactions catalyzed by GPxs, forming GSSG. Regeneration of GSH by reduction with NADPH is catalyzed by GR. Accumulated GSSG can oxidize protein thiols by formation of mixed protein-GSH disulfides (Protein-SG), thereby altering protein function. GSH is also consumed when electrophiles are detoxified by GSTs. Refer to list of abbreviations and text for further details

of GSH in the skin (Fig. 31.1). UV irradiation induces the formation of ROS [7], radicals, and oxidized lipids [8] in skin cells and tissues including human nevi [9]. Many toxic species are removed from the tissue in enzymatic reactions that consume GSH. GSH is the reductant in the detoxification of hydrogen peroxide and certain peroxidized lipids in reactions catalyzed by the glutathione peroxidases (GPxs) [10]. These reactions result in the formation the oxidized form of GSH, glutathione disulfide (GSSG). Regeneration of GSH by reduction with NADPH is catalyzed by glutathione reductase (GR) [1]. However, severe oxidative stress can overwhelm the cells' reductive capacity, and accumulated GSSG can oxidize critical protein thiols by formation of mixed protein-GSH disulfides, thereby altering protein function. This effect can be ameliorated by active export of GSSG, but the result is depletion of cellular GSH [11]. GSH levels are also decreased as a consequence of detoxification of electrophiles by the glutathione *S*-transferases (GSTs). These reactions involve the irreversible formation of covalent bonds between the thiol of GSH and the electrophile. While in almost all cases the resulting conjugate is much less toxic to the cell, it must be exported from the cell for processing and excretion; loss of GSH is the net result. The effects of the loss of this essential antioxidant on the cell can be catastrophic as illustrated below in our discussion of the molecular etiology of acetaminophen toxicity.

NAC for Acetaminophen Overdose

Elimination of acetaminophen from the body is facilitated by sulfation and glucuronidation [12]. If acetaminophen levels rise so quickly as to overwhelm these pathways, the drug is metabolized by the mixed-function oxidase cytochrome p450 CYP2E1 to the toxic electrophilic species *N*-acetyl-*p*-quinonimine (NAPQI). NAPQI can be safely eliminated from cells after formation of a covalent adduct to GSH in a reaction catalyzed by GSTs. However, in cases of overdose or in patients where GSH levels are already compromised, GSH becomes severely depleted, leaving unconjugated NAPQI free to react with proteins in the liver. This can result in failure of the organ if left untreated [13]. NAC is approved by the FDA for treatment of acetaminophen toxicity. Administration of NAC protects the liver by resupplying the tissue with Cys, which supports the synthesis of GSH and ultimately allows the safe elimination of NAPQI. For treatment of acetaminophen toxicity a total of 300 mg/kg NAC is administered in a loading dose of 150 mg/kg in 200 mL diluent over 60 min, followed by 50 mg/kg over 4 h and 100 mg/kg over 16 h [14].

NAC Sources

Acetadote® brand of NAC, available by prescription in the United States, is supplied by Cumberland Pharmaceuticals as a solution (10 and 20%) for i.v. administration (Table 31.1). We chose to use the aqueous solution (acetylcysteine) formulated for oral ingestion because it is packaged under nitrogen

Table 31.1 Sources and formulations of NAC

Prescription drug	Dose/formulation	Supplier	Cost
Acetylcysteine	10 and 20% for inhalation or oral ingestion	American Regent, Inc.; Mayne Pharma, Inc.	------
Acetadote®	10 and 20% for i.v. injection	Cumberland Pharmaceuticals	------
Dietary Supplements			
N-Acetylcysteine	1,000 mg tablet	Source Natural	120 for $20.79
N-Acetylcysteine	600 mg capsule	Now Foods	250 for $21.73
N-Acetylcysteine	600 mg capsule	Swanson Premium	100 for $6.49

which stabilizes the drug by eliminating contact with oxygen. Dietary supplements containing NAC are widely available in health food stores and on the Internet. FDA regulations concerning the manufacture of these products are different than those for prescription drugs, but all domestic and foreign companies that manufacture dietary supplements for distribution in the United States, must comply with the Dietary Supplement Current Good Manufacturing Practices (cGMPS) for quality control (http://www.fda.gov/Food/DietarySupplements/default.htm). The greatest concern with these products is loss of potency via oxidation, which can be accelerated if the drug is not stored in a cool and dry location.

Toxicology and Adverse Events

Single i.v. doses of NAC at 1,000 mg/kg in mice, 2,445 mg/kg in rats, 1,500 mg/kg in guinea pigs, 1,200 mg/kg in rabbits and 500 mg/kg in dogs were lethal. There are no well-controlled studies of NAC in pregnant women and the drug is classified as "Pregnancy Category B" [14].

In a post-marketing study involving 4,709 adults who received i.v. injections for acetaminophen overdose, the following adverse reactions were reported: urticaria or facial flushing (6.1%), pruritus (4.3%), respiratory symptoms (1.9%), edema (1.6%), hypotension (0.1%), and anaphylaxis (0.1%). In our clinical trial we have safely administered NAC orally to a total of 72 patients, although in our studies, the single oral dose of 1,200 mg [9], was far lower than the i.v. doses given for acetaminophen poisoning (300 mg/kg or 21,000 mg for a 70 kg person). To our knowledge, no adverse events have been reported by others following oral ingestion of the drug.

Oxidative Stress and Melanoma

There is correlative evidence to suggest that one link between UV radiation and melanoma is generation of oxidative damage [15, 16]. In the *Xiphophorus* fish model, the UV action spectrum for melanin-dependent oxidant production is identical to that for melanoma induction [17], and oxidative dysregulation in human melanoma cell lines correlates with aggressive behavior [18]. Melanocytes isolated from melanoma patients display increased sensitivity to peroxidizing agents that correlates with endogenous antioxidant imbalance [19], and elevated ROS have been found in melanocytes from dysplastic nevi relative to normal skin from the same individuals [20]. Interestingly, melanoma is quite rare in albino individuals who lack melanin [21], and, while melanocytes may be protected by endogenous melanin which can directly absorb photons and quench UV-generated ROS [22]. At higher UV doses oxidized melanin actually participates in the generation of ROS [17], and we recently found that melanocytes maintain higher levels of oxidative stress than keratinocytes or fibroblasts isolated from the same individuals [23], possibly due to ROS generated during melanin biosynthesis [24].

Multiple oxidizing species capable of damaging cellular structures and DNA are induced in the skin by UV; these include hydrogen peroxide, hydroxyl radical, superoxide, nitric oxide and oxidized lipids [25]. If high levels of these species persist, mutations in DNA can result directly from reactions with DNA causing the formation of modified bases such as 8-hydroxyguanine (8-OHdG). These DNA modifications can lead to mutations if not repaired prior to DNA replication [26]. Studies of early human melanoma lesions show loss of heterozygosity for DNA repair genes [27], and next-generation sequencing of a melanoma genome revealed a significant rate of the G→T transversion, which is a signature mutation arising from unrepaired oxidative DNA damage [28]. We previously showed that oral delivery of the antioxidant NAC can protect mouse skin against UV-induced generation of ROS and 8-OHdG, and depletion of GSH [7]. In addition, we found that administration of NAC just prior

to, and immediately following, UV exposure significantly delayed the onset of UV-induced melanoma in our animal model (discussed in detail below) [7]. This is direct evidence for a role of oxidative stress in UV-induced melanoma, and provides the rationale for targeting oxidative stress for melanoma chemoprevention. In summary, the production of melanin itself may increase oxidative stress in melanocytes, and oxidation of DNA has the potential to produce cancer-causing mutations in these cells.

Antioxidant Response and the Melanocyte

More that 90% of melanomas are thought to be sporadic, but of those that are familial, 20–40% arise in persons with germ-line mutations in the *Cdkn2a* locus [29]. The penetrance of mutations *Cdkn2a* varies according to geographic location, with an incidence rate of melanoma in carriers of 58% in Europe, 76% in the United States, and 91% in Australia, by 80 years of age. A recent meta-analysis of genetic modifiers in 96 families with germ-line *Cdkn2a* mutations showed that risk for melanoma was increased 4.6-fold for carriers that had two mutations in another gene important to melanocyte biology, *MC1R*. This increased risk is reflected in the median age of onset in *Cdkn2a* mutation carriers which is decreased from 47 years of age for *MC1R* wild-type individuals, to 37 years in *MC1R* mutants ($P<0.0001$) [30]. The melanocortin-1 receptor gene (*MC1R*) codes for a seven-pass transmembrane protein expressed on the cell surface of melanocytes [31]. Loss-of-function (LOF) mutations in *MC1R* commonly result in the red-hair phenotype (red hair, light eye color and the inability to tan) in humans. Functional MC1R protects the skin from the mutagenic effects of UV by promoting pigment (melanin) synthesis, and by upregulating the expression of DNA repair and antioxidant genes in melanocytes [32, 33]. In fact, the expression of antioxidant genes that have antioxidant response elements (AREs) in their promoters (including those controlling GSH biosynthesis, Fig. 31.1) is downstream of MC1R [34, 35]. Binding of the MC1R ligand stimulates transcription of *Nrf2*, which in turn encodes a subunit of the transcription factor complex that binds to AREs and activates transcription of antioxidant genes [32]. We therefore believe that impairment of both pigmentation *and* antioxidant response contribute to the fourfold increased risk for melanoma observed in individuals (without *Cdkn2a* mutations) carrying two LOF *MC1R* alleles [36]. This is consistent with the synergistic effect manifested by the increased melanoma risk observed in individuals harboring mutations in both *Cdkn2a* and *MC1R*, and highlights the importance of oxidative stress as a target for therapeutic intervention in the process of melanomagenisis.

How the Use of NAC Overcomes Pitfalls in Traditional Chemoprevention?

There are many obstacles associated with conventional chemoprevention approaches for cancer, which usually involve chronic drug administration. Besides maintaining and monitoring patient adherence over time, unintended toxicities may be associated with chronic ingestion of any agent, and it is generally not possible to assess clinical benefit until the end of the trial (i.e., did the intervention group develop less cancer?). Another consideration is that the chemopreventive agent may not be administered in conjunction with the specific oncogenic stimulus, which for many cancers is unknown. For melanoma, the long latency time and low (annual) risk of tumor development are such that large numbers of patients would need to be treated and monitored for many years to determine whether a given preventive agent is effective. Finally, a combination of these factors may yield unanticipated adverse (or paradoxical) results as observed in various prevention trials of antioxidants [37]. For example, patients who took β-carotene and retinol for 2 years exhibited an increased risk of lung cancer [38], and mixed antioxidant supplementation over 7 years was associated with increased risk of skin cancer

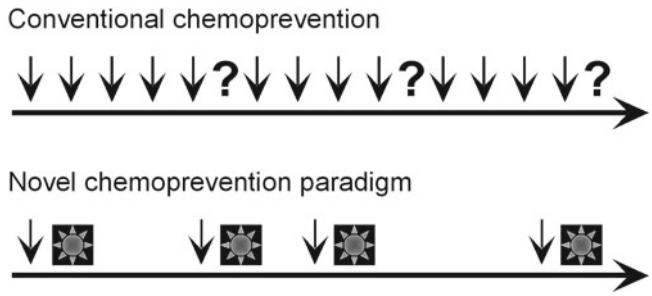

Fig. 31.2 Chemoprevention paradigms. (**a**) Conventional chemoprevention is characterized by chronic administration of an agent (*arrows*), usually not in conjunction with specific oncogenic stimulus (often unknown, *?*). (**b**) Our proposed novel use of NAC involves episodic administration (*arrows*) in anticipation of known oncogenic stimulus (UV exposure,). From the authors' previously published work [7]

in French women [39]. Here we propose a novel chemoprevention strategy for melanoma that bypasses most of these obstacles associated with conventional cancer chemoprevention. Patients could take NAC as a prophylactic "sunburn pill" in anticipation of sun exposure to protect their skin against UV-induced oxidative stress/damage. In sharp contrast to conventional chemoprevention protocols, in which an agent is administered chronically and (usually) independent of mutagenic insult that may be unknown or poorly defined (Fig. 31.2, conventional chemoprevention), this scenario would involve episodic drug administration, in conjunction with exposure to a presumed mutagenic insult (UV radiation), targeting a presumed mutation-initiating pathway (oxidative stress/damage) (Fig. 31.2, novel chemoprevention paradigm). Additional advantages of this approach over conventional strategies include avoidance of potential toxicities that could be associated with chronic ingestion of any agent, and the *presumed benefit* that would be afforded by reduction in potentially carcinogenic oxidative damage in the skin over the course of many UV exposures.

NAC Prevents UV-Induced Skin Cancers in Mouse Models

D'Agostini et al. [40] have shown that NAC is able to significantly modulate the formation of UV-induced skin tumors in the SKH-1 hairless mouse. In this study, mice were exposed daily to light from halogen quartz bulbs with emission covering a broad spectrum of visible light as well as UVA and UVB. NAC administration was initiated 3 days before the beginning of irradiation and throughout the remainder of the study in the drinking water at a dose calculated to deliver 1,000 mg/kg bodyweight daily. NAC affected both the tumor latency and multiplicity in this model. The earliest light-related skin lesions were detectable in animals treated with UV alone after 300 days and this was delayed until 390 days in irradiated mice treated with NAC. After 480 days the UV-treated mice had significantly more tumors compared to those treated with UV plus NAC (5.76 ± 1.06 versus 2.14 ± 0.57, $P < 0.001$). The nature of the light-induced skin lesions ranged from pre-neoplastic lesions such as epidermal hyperplasia, to benign tumors such as papillomas, evolving towards keratoacanthoma-like tumors, appendage/basal tumors, carcinomas in situ and squamocellular carcinomas. Of the animals treated with UV alone, 21% had squamocellular carcinomas, but none of the animals treated with NAC as well as UV developed these advanced tumors.

Our examination of the effects of NAC on melanoma began with a study of melanocytes in culture [7]. We found that hydrogen peroxide produced in UV-irradiated (960 J/m [2]) melan-a mouse melanocytes was reduced to the levels of untreated controls with the addition of 5 mM NAC to the culture

medium. We also found that free nonprotein thiols (principally GSH) were reduced by approximately 20% within 48 h after UV irradiation at the same dose. Addition of NAC (10 mM) to the medium of the irradiated cells boosted the level of free thiols to 130% of that in unirradiated controls. When we measured UV-induced DNA damage in melanocytes, we found that UV increased the number of cells staining positive for the oxidized base 8-OH-dG to fivefold more than that observed in control cells, and that 5 mM NAC reduced the number of positive cells to the same as that in unirradiated cells. However, immunochemical analysis of DNA isolated 48 h after treatment from UV-irradiated cells showed that NAC had little if any effect on UV-induced cyclobutane dimer formation. Thus, we concluded that the principle effect of NAC is on UV-induced oxidative stress (as measured by thiol depletion), and that oxidative damage to DNA was also relieved, but with no apparent effect on the formation/repair of direct photoproducts.

We next studied the effects of NAC in vivo using the HGF/survivin mouse model of melanoma [41]. The expression of the HGF transgene causes melanocytes in the skin of these mice to localize to the dermal/epidermal junction, in contrast to wild-type mice where the majority of melanocytes are found at the base of the hair follicle where they are protected from the mutagenic effects of UV [42]. HGF mice develop cutaneous melanomas when subjected to a single neonatal dose of UV radiation. Expression of the survivin transgene in the melanocytes of HGF mice decreases the latency of melanoma development and increases metastases both in the lymph nodes and in the lungs [41]. In our NAC study, the fact that tumor formation requires that the animals be irradiated shortly after birth, dictated that in order to deliver NAC orally, the drug must be supplied to the dams, then trans-placentally to their offspring and/or via the milk of nursing females. In order to show the efficacy of this delivery method, we first examined GSH levels and 8-OH-dG in the skin of neonatal HGF mice born to females given water containing NAC (equivalent to approximately 1.9 g/kg/day 2–3 days before until 2 weeks after delivery). Compared to the UV-irradiated skin of animals receiving no NAC from their mothers, the UV-irradiated skin of NAC treated animals contained significantly higher levels of free thiols and less positive staining for 8-OH-dG (Figure 3 in reference 7). Convinced that our delivery method was effective, we then compared the effects of NAC on UV-induced tumor formation. The time for 50% of animals to form tumors of at least 1 mm in diameter was increased from 13.8 weeks in control animals to 20.6 weeks in NAC-treated animals ($P = 0.0003$, Fig. 31.3). In addition, tumors were significantly smaller in treated animals at early time points, but this difference disappeared by the end of the study at 32 weeks. Tumors were collected for histological examination and metastases were determined by necropsy. There were no significant differences between cytologic atypia in tumors or in rates of metastasis to the lymph nodes and lungs. Thus, the predominant effect of orally delivered NAC before (and briefly following) UV irradiation was a delay in tumor formation.

Effects of NAC on UV-Irradiated Human Skin Tissues

Investigators at the University of Michigan studied the effects of topical NAC on the UV-induced signaling that leads to photoaging of human skin [43]. They found that in untreated skin, topical application of a 20% aqueous solution of NAC under occlusion, virtually eliminated the oxidized form of GSH (GSSG), and increased reduced GSH levels by 50%. They also showed that UV stimulates ERK/MAP kinases and promotes the accumulation of the protein factor cJun. The transcription of *cJun* is increased by oxidative stress [44], whereupon the resulting protein forms a heterodimeric complex with the constitutively expressed cFos [45]. This complex is known as the transcription factor AP-1. The accumulation of cJun is related to photoaging by virtue of the fact that the transcription of the matrix metalloproteinase (MMP) collagenase, an AP-1 target gene, is induced by UV. In their working model for the pathophysiology of photoaging, the Michigan group hypothesizes that damage to the extracellular matrix by UV-induced MMPs is imperfectly repaired after UV exposure, resulting in an

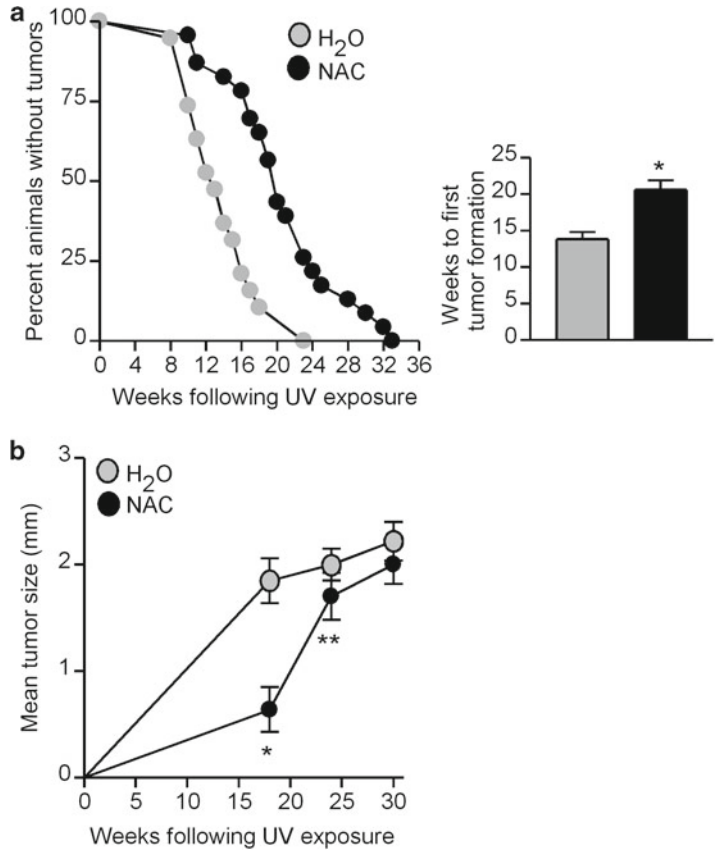

Fig. 31.3 Oral NAC delays onset of UV-induced melanoma. (**a**) Percent of animals without tumors. Pregnant female mice were provided with either water containing NAC ($n=19$) or water alone ($n=21$). Neonates from these females were irradiated with UV (3,900 J/m^2) 2 days after birth. Animals were monitored for 40 weeks for tumor formation. NAC-treated animals required significantly long to develop tumors ($P=0.0003$) (**b**) Tumor size. Tumors in NAC-treated animals were smaller at early timepoints but were similar in size by 30 weeks after irradiation. *$P<0.001$, **$P=0.14$. From the authors' previously published work [7]

invisible solar scar. With repeated intermittent UV exposures, the solar scars accumulate, eventually resulting in visible skin wrinkling or photoaging. When NAC was applied 24 h prior to irradiation of skin at two times the minimal erythemal dose (2 MED), the UV-induced accumulation of cJun and induction of collagenase transcription, was relieved. This is supportive of the prediction that by decreasing UV-induced MMP expression, NAC will prevent photoaging in human skin.

Oral NAC Protects Melanocytic Nevi Against UV-Induced Oxidative Stress

Melanocytes make up less than 10% of the cells in the human epidermis. Nevi (clonal neoplasms of melanocytes) have a significantly higher percentage of melanocytes and we therefore considered them an excellent model system for studying UV-induced oxidative stress in this cell type. In order to avoid any potential risks of exposing patients to UV radiation, we developed an ex vivo system for evaluating UV-induced oxidative stress in nevi. After removal from the patient, nevi were divided into roughly equal fragments and one was irradiated while the other was left as untreated control. The tissue was placed in a cell culture incubator and we found it to be viable for up to 72 h. Following treatment with UV at 400–4,000 J/m^2, we measured ROS levels that were significantly elevated 48 h after treatment in nevi treated with the highest dose. GSH levels in nevi treated with 4,000 J/m^2 UV were depressed by 25–30% at both 24 and 48 h after irradiation.

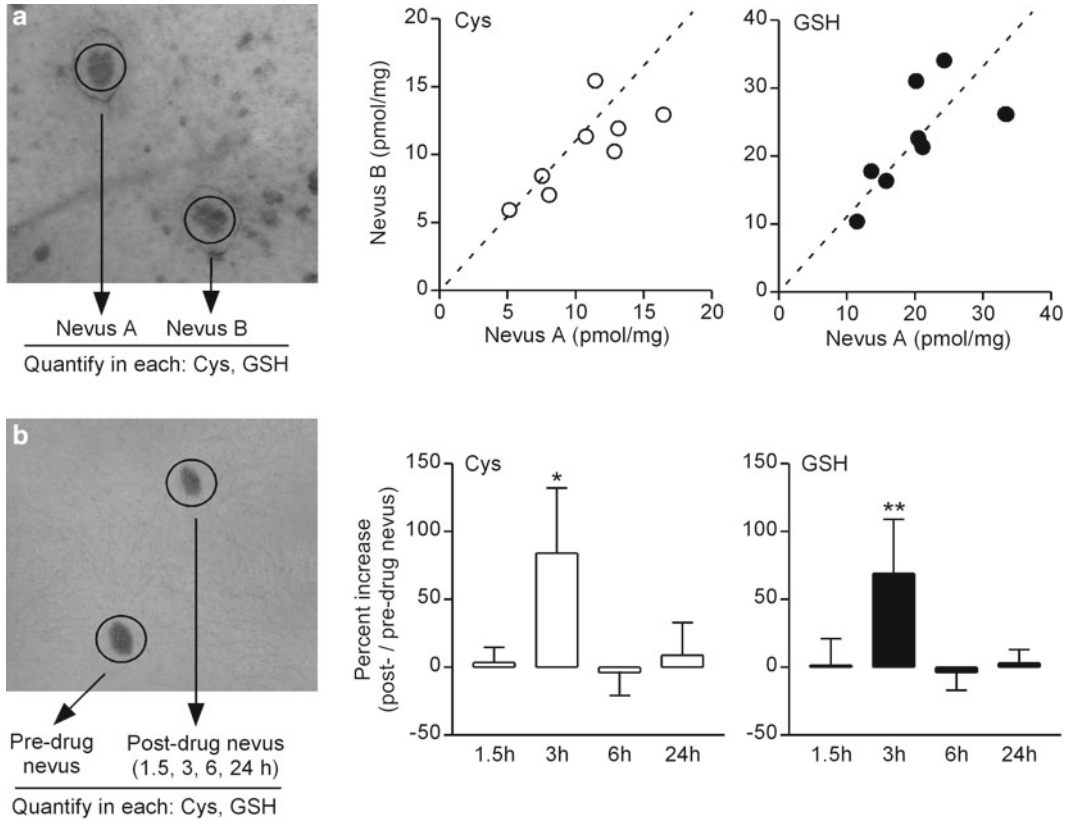

Fig. 31.4 Internevus correlation of oxidative biomarkers, and NAC-mediated oxidative modulation in nevi. (**a**) Two similar-appearing nevi were removed from individual patients ($n=8$), and Cys and GSH content was determined (*left*). For each patient, values for Cys (*left plot*, *open circles*) and GSH (*right plot*, *closed circles*) are expressed as a single data point reflecting each pair of nevi (nevus A, abscissa; nevus B, ordinate). *Dotted lines*, theoretical correlation where data points should fall for pairs of nevi with identical values. For Cys measurements, correlation coefficient (r) is 0.77 (95% confidence interval, 0.14–0.95; $P=0.03$). For GSH measurements, correlation coefficient (r) is 0.69 (95% confidence interval, −0.03 to 0.94; $P=0.06$). (**b**) Two similar-appearing nevi were removed from individual patients immediately before, and either 1.5, 3, 6, or 24 h ($n=5$–6 at each time point) following ingestion of 1,200 mg NAC (*left*). Cys and GSH content (normalized to nevus weight) were determined for each nevus, and data expressed as percent increase in postdrug versus predrug nevus (*right*). $*P=0.047$; $**P=0.016$ (Wilcoxon signed-rank tests). From the authors' previously published work [9]

Next we examined the safety and tolerability of oral NAC. We found that 600 mg NAC, administered in a single dose of a 20% solution diluted into tomato juice to mask the salty taste, was well tolerated by the first two patients. Therefore, the remaining patients (70) were given a 1,200 mg dose with the idea that the larger dose would have an increased chance to protect against oxidative stress. All patients were surveyed by telephone 24 h after drug ingestion in order to assess any side effects such as nausea or itching. In all cases the drug was well tolerated, confirming the safety of a single oral high dose of NAC.

We then conducted a pilot study of the delivery of NAC to nevi after oral administration. We felt that it was important, in determining parameters for suitable drug delivery to nevi, to use a "predrug" nevus as a reference to control for interpatient variability. However, we realized that this strategy would not work if there was significant variability in nevi from the same patient (Fig. 31.4a). In order to assess inter-nevus variability, we determined Cys and GSH levels in eight sets of two nevi of similar appearance, each harvested from the same patient. We found that while levels of these thiols varied

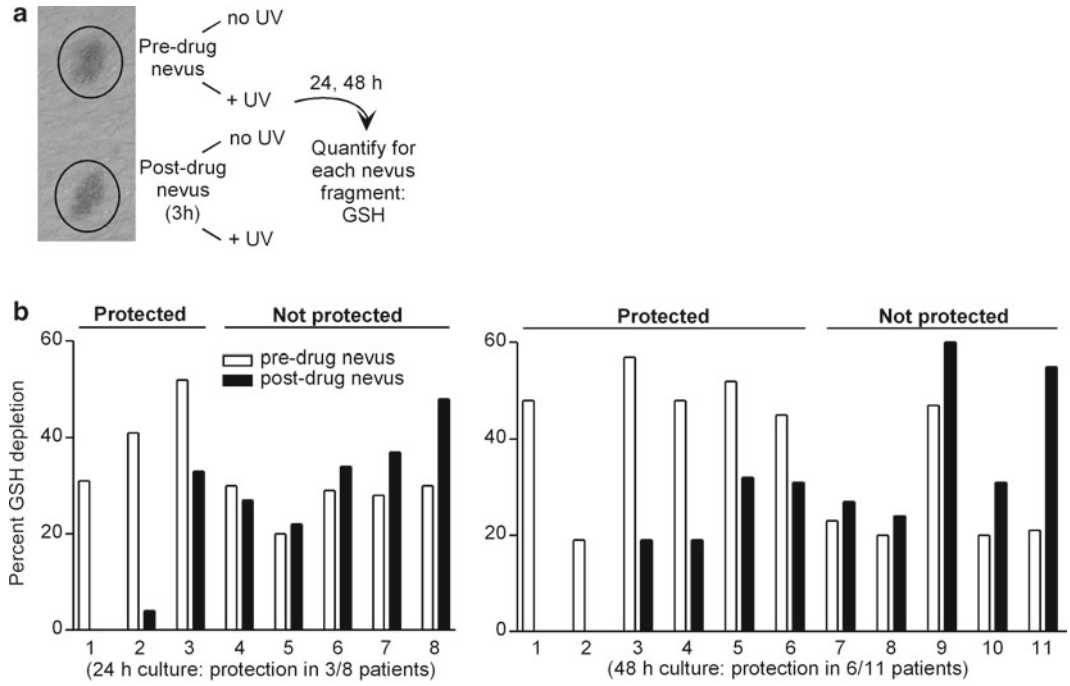

Fig. 31.5 NAC-mediated protection against UV-induced oxidative stress. (**a**) Two similar-appearing nevi were removed from 19 patients immediately before and 3 h following ingestion of 1,200 mg NAC. Fragments of each nevus were either untreated or UV irradiated (4,000 J/m2), then cultured for either 24 h (8 patients) or 48 h (11 patients). GSH content (normalized to nevus weight) was determined for each nevus fragment. (**b**) Data expressed as percent UV-induced GSH depletion (UV treated versus untreated) in fragments of predrug nevi (*open columns*) and postdrug nevi (*filled columns*) from each patient. For nevi cultured 24 h, there was protection (i.e., less GSH depletion) in three of eight patients (*left*); for nevi cultured 48 h, there was protection in 6 of 11 patients (*right*). From the authors' previously published work [9]

considerably between patients, there were good correlations between levels measured in nevi harvested from the same patient. In order to characterize the pharmacokinetics of Cys delivery to nevi, we removed a nevus just before drug ingestion, then either 1.5, 3, 6, or 24 h later (Fig. 31.4b). Using an HPLC-based assay to detect thiols in the nevus tissues, we found a significant elevation of both Cys and GSH at 3 h, which returned to baseline after 6 h.

Having determined the optimal timing for both drug delivery and UV-induced GSH depletion, we set about conducting a test of the effects of NAC administration on UV-induced oxidative stress in human nevi. The experimental design is shown in Fig. 31.5a. Patients with two suitable nevi were recruited from our high risk melanoma clinic. Before ingestion of the drug one nevus was removed, and 3 h after NAC administration, the second nevus was removed. Immediately after harvest, each nevus was divided in half and one portion was UV-irradiated. After 24–48 h, GSH levels were measured in each of the four nevus fragments from every patient. Our results showed that in 9 of the 19 participants treated in this study, UV-induced GSH depletion was relieved (Fig. 31.5b).

Our current research is aimed at determining why some of the patients' nevi were not protected by NAC. In new analyses of the data, we noted that tissues from patients responsive to NAC-mediated chemoprotection exhibited significantly higher levels of UV-induced GSH depletion in control (predrug) nevi than did nevi from patients that were not protected (Fig. 31.6a, Cassidy and Grossman, unpublished analysis). This may represent a threshold effect, whereby UV-induced production of oxidizing species in some patients may not have been sufficient to activate Nrf2-dependent antioxidant responses, which include transcriptional activation of Cys transporters and the GSH biosynthetic

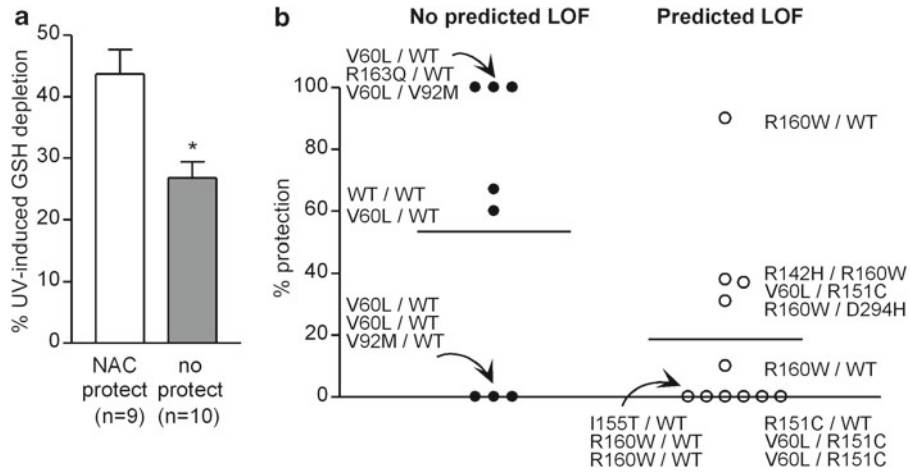

Fig. 31.6 Preliminary analysis of NAC-mediated protection and effect of *MC1R* mutation. (**a**) Magnitude of UV-induced GSH depletion in untreated nevi is greater in patients with nevi protected by NAC than those not protected. $*P = 0.003$ (2-tailed *t* test). (**b**) GSH levels were determined and percent protection is shown. Results are grouped based on indicated *MC1R* genotype (*left*, *filled circles*, no predicted loss of function (LOF); *right*, *open circles*, predicted LOF). *Solid line* represents mean percent protection

pathway [46]. This is a likely significant factor since our trial design included administration of an identical dose of UV to nevi from every participant, regardless of their skin type. Our future trial design will incorporate measurements of MED in normal skin and delivery of a "biologically equivalent" dose of UV as determined by this objective measurement of UV sensitivity. Using this design, persons with high MED get a higher UV dose than those with low MED with the intent to elicit an equivalent amount of oxidative stress in the nevi of each patient. This new strategy should result in a higher percentage of patients with significant GSH depletion in pre-drug nevus, which should then facilitate increased biosynthesis of GSH in the post-drug nevus and protection of the tissue against oxidative stress.

We also considered genetic factors that might make the antioxidant response of some nevi less robust than others. To explore this concept, we retrospectively sequenced *MC1R* (which as discussed above is known to affect oxidative stress in melanocytes) in the patients from our completed study. Interestingly, we found that while five of eight patients with wild-type *MC1R* or non-LOF polymorphisms experienced protection from UV-induced depletion of GSH, 7 of 11 patients with one or more LOF *MC1R* mutations were *not* protected (Fig. 31.6b, Cassidy and Grossman, unpublished). Given the dependence of Nrf2-mediated antioxidant gene transcription in melanocytes on MC1R activation [34], it is plausible that loss of MC1R function in these patients in the context of GSH depletion by UV, may compromise the downstream antioxidant response, leading to an inability to upregulate GSH synthesis. Thus, *MC1R* genotype and sensitivity of nevi to UV-induced oxidative stress may be important biomarkers of chemoprevention efficacy in our system, and incorporating these factors into our trial design are the focus of our continued efforts to develop personalized melanoma chemoprevention strategies.

Other Potential Applications

There are numerous potential therapeutic applications for NAC in humans, in addition to those relating to skin cancer detailed above. Most of these additional applications exploit the antioxidant activity of NAC, which may be beneficial in ameliorating the effects of acute or chronic inflammation and

oxidative damage in various organs. In patients with pulmonary fibrosis, NAC (600 mg three times daily) improved lung function in patients already taking immunosuppressive drugs [47]. In patients undergoing cardiac angioplasty, combined i.v. and oral dosing of NAC before and after the procedure prevented contrast dye-induced nephropathy [48]. Aberrant ROS production also may play a role in the autoimmune disease systemic lupus erythematosis (SLE), and NAC can decrease auto-antibody production in SLE-prone mice [49]. Oral NAC (600 mg three times daily for 2 weeks) improved endothelial dysfunction in SLE patients [50], and a clinical trial to test whether oral NAC can decrease auto-antibody production in these patients is underway (ClinicalTrials.gov, NCT00775476). Finally, in cystic fibrosis, NAC scavenges myeloperoxidase activity in patient sputum [51] and stimulates chloride efflux from airway epithelial cells [52], and a recent phase II trial in patients receiving either 700 mg or 2,800 mg orally showed that extracellular GSH increased in sputum although there was no alteration in clinical or inflammatory parameters [53]. In patients with history of preterm labor and bacterial vaginosis, oral NAC (600 mg daily) significantly increased gestational age at delivery compared to placebo [54].

NAC attenuates decline in muscle Na+/K+-pump activity [55], and oral NAC (1,800 mg) reduces respiratory muscle fatigue during heavy exercise [56]. NAC reduces keratinocyte proliferation, and there is a case report of a patient with lamellar ichthyosis improving following 5 weeks of topical application of NAC [57]. NAC penetrates the blood–brain barrier, and given the known role of its metabolite Cys as a modulator of the glutamatergic system and potentiator of dopamine release, NAC may potentially influence reward-reinforcement pathways in the brain [58]. Thus, NAC may be therapeutically useful in psychiatric disorders allegedly related to oxidative stress (e.g., schizophrenia, bipolar disorder) as well as psychiatric syndromes characterized by impulsive/compulsive symptoms (e.g., trichotillomania, pathological nail biting, gambling, drug addiction) [59]. In adult patients with trichotillomania (compulsive hair pulling), 1,200 mg oral NAC daily was found to reduce hair pulling in over half the patients [60]. One study in marijuana users found that 2,400 mg NAC per day decreased drug use [61], and another in cocaine addicts found that 2,400 mg NAC reduced cravings for cocaine [62]. On the other hand, NAC at doses up to 2,400 mg per day does not appear to be useful for tobacco cessation [63]. A placebo-controlled trial found that 1,000 mg per day NAC plus existing medication over 6 months improved global function and akathisia in schizophrenia patients [64]. A 6-month trial of 2,000 mg NAC per day in patients with bipolar disorder showed reduction in depressive symptoms [65]. Finally, NAC has been tested against placebo in Alzheimer's disease following 3 and 6 months of treatment, and comparison of interval change favored NAC treatment on nearly every outcome measure, although significant differences were obtained only for a subset of cognitive tasks [66].

NAC has an excellent record of safety and efficacy in treating a wide range of conditions in which oxidative stress plays a critical role, and many more clinical trials are currently in progress (ClinicalTrials.gov). In this chapter, we have presented data from promising clinical and animal studies illustrating the potential utility of NAC in protecting against the consequences of UV-induced oxidative stress in the skin including photoaging as well as skin cancers. We believe that the insights that we and others have gained into molecular mechanisms of UV-induced oxidative stress and the protective effects of NAC, provide robust intermediate biomarkers of efficacy, and that larger scale phase III trials using photodamage and cancer incidence as endpoints could be justified in the near future.

References

1. Wu G, Fang YZ, Yang S, Lupton JR, Turner ND. Glutathione metabolism and its implications for health. J Nutr. 2004;134(3):489–92.
2. Meister A, Anderson ME. Glutathione. Annu Rev Biochem. 1983;52:711–60.
3. Hoek KS, Schlegel NC, Eichhoff OM, et al. Novel MITF targets identified using a two-step DNA microarray strategy. Pigment Cell Melanoma Res. 2008;21(6):665–76.

4. Chintala S, Li W, Lamoreux ML, et al. Slc7a11 gene controls production of pheomelanin pigment and proliferation of cultured cells. Proc Natl Acad Sci U S A. 2005;102(31):10964–9.
5. Zhang H, Forman HJ. Redox regulation of gamma-glutamyl transpeptidase. Am J Respir Cell Mol Biol. 2009;41(5):509–15.
6. Lu SC. Regulation of glutathione synthesis. Mol Aspects Med. 2009;30(1–2):42–59.
7. Cotter MA, Thomas J, Cassidy P, et al. N-acetylcysteine protects melanocytes against oxidative stress/damage and delays onset of ultraviolet-induced melanoma in mice. Clin Cancer Res. 2007;13(19):5952–8.
8. Haywood R, Andrady C, Kassouf N, Sheppard N. Intensity-dependent direct solar radiation- and UVA-induced radical damage to human skin and DNA, lipids and proteins. Photochem Photobiol. 2011;87(1):117–30.
9. Goodson AG, Cotter MA, Cassidy P, et al. Use of oral N-acetylcysteine for protection of melanocytic nevi against UV-induced oxidative stress: towards a novel paradigm for melanoma chemoprevention. Clin Cancer Res. 2009;15(23):7434–40.
10. Brigelius-Flohe R, Wingler K, Muller C. Estimation of individual types of glutathione peroxidases. Methods Enzymol. 2002;347:101–12.
11. Lu SC. Regulation of hepatic glutathione synthesis: current concepts and controversies. FASEB J. 1999;13(10): 1169–83.
12. Kane RE, Li AP, Kaminski DR. Sulfation and glucuronidation of acetaminophen by human hepatocytes cultured on Matrigel and type 1 collagen reproduces conjugation in vivo. Drug Metab Dispos. 1995;23(3):303–7.
13. Ferner RE, Dear JW, Bateman DN. Management of paracetamol poisoning. BMJ. 2011;342:d2218.
14. Schaefer JD. Acetadote. Physician's Desk Reference. 2011;65:986–989.
15. Meyskens Jr FL, Farmer P, Fruehauf JP. Redox regulation in human melanocytes and melanoma. Pigment Cell Res. 2001;14(3):148–54.
16. Wittgen HG, van Kempen LC. Reactive oxygen species in melanoma and its therapeutic implications. Melanoma Res. 2007;17(6):400–9.
17. Wood SR, Berwick M, Ley RD, Walter RB, Setlow RB, Timmins GS. UV causation of melanoma in xiphophorus is dominated by melanin photosensitized oxidant production. Proc Natl Acad Sci U S A. 2006;103(11):4111–5.
18. de Souza GA, Godoy LM, Teixeira VR, et al. Proteomic and SAGE profiling of murine melanoma progression indicates the reduction of proteins responsible for ROS degradation. Proteomics. 2006;6(5):1460–70.
19. Grammatico P, Maresca V, Roccella F, et al. Increased sensitivity to peroxidizing agents is correlated with an imbalance of antioxidants in normal melanocytes from melanoma patients. Exp Dermatol. 1998;7(4):205–12.
20. Pavel S, van Nieuwpoort F, van der Meulen H, et al. Disturbed melanin synthesis and chronic oxidative stress in dysplastic naevi. Eur J Cancer. 2004;40(9):1423–30.
21. Streutker CJ, McCready D, Jimbow K, From L. Malignant melanoma in a patient with oculocutaneous albinism. J Cutan Med Surg. 2000;4(3):149–52.
22. Riley PA. Melanin. Int J Biochem Cell Biol. 1997;29(11):1235–9.
23. Jenkins NC, Liu T, Cassidy P, et al. The p16(INK4A) tumor suppressor regulates cellular oxidative stress. Oncogene. 2011;30(3):265–74.
24. Urabe K, Aroca P, Tsukamoto K, et al. The inherent cytotoxicity of melanin precursors: a revision. Biochim Biophys Acta. 1994;1221(3):272–8.
25. Farmer PJ, Gidanian S, Shahandeh B, Di Bilio AJ, Tohidian N, Meyskens Jr FL. Melanin as a target for melanoma chemotherapy: pro-oxidant effect of oxygen and metals on melanoma viability. Pigment Cell Res. 2003;16(3):273–9.
26. Bruner SD, Norman DP, Verdine GL. Structural basis for recognition and repair of the endogenous mutagen 8-oxoguanine in DNA. Nature. 2000;403(6772):859–66.
27. Pashaei S, Li L, Zhang H, et al. Concordant loss of heterozygosity of DNA repair gene, hOGG1, in melanoma in situ and atypical melanocytic hyperplasia. J Cutan Pathol. 2008;35(6):525–31.
28. Pleasance ED, Cheetham RK, Stephens PJ, et al. A comprehensive catalogue of somatic mutations from a human cancer genome. Nature. 2010;463(7278):191–6.
29. Pho L, Grossman D, Leachman SA. Melanoma genetics: a review of genetic factors and clinical phenotypes in familial melanoma. Curr Opin Oncol. 2006;18(2):173–9.
30. Fargnoli MC, Gandini S, Peris K, Maisonneuve P, Raimondi S. MC1R variants increase melanoma risk in families with CDKN2A mutations: a meta-analysis. Eur J Cancer. 2010;46(8):1413–20.
31. Abdel-Malek ZA, Kadekaro AL, Swope VB. Stepping up melanocytes to the challenge of UV exposure. Pigment Cell Melanoma Res. 2010;23(2):171–86.
32. Kadekaro AL, Leachman S, Kavanagh RJ, et al. Melanocortin 1 receptor genotype: an important determinant of the damage response of melanocytes to ultraviolet radiation. FASEB J. 2010;24(10):3850–60.
33. Song X, Mosby N, Yang J, Xu A, Abdel-Malek Z, Kadekaro AL. alpha-MSH activates immediate defense responses to UV-induced oxidative stress in human melanocytes. Pigment Cell Melanoma Res. 2009;22(6): 809–18.

34. Kokot A, Metze D, Mouchet N, et al. Alpha-melanocyte-stimulating hormone counteracts the suppressive effect of UVB on Nrf2 and Nrf-dependent gene expression in human skin. Endocrinology. 2009;150(7):3197–206.
35. Herraiz C, Journe F, Abdel-Malek Z, Ghanem G, Jimenez-Cervantes C, Garcia-Borron JC. Signaling from the human melanocortin 1 receptor to ERK1 and ERK2 mitogen-activated protein kinases involves transactivation of cKIT. Mol Endocrinol. 2011;25(1):138–56.
36. Kennedy C, ter Huurne J, Berkhout M, et al. Melanocortin 1 receptor (MC1R) gene variants are associated with an increased risk for cutaneous melanoma which is largely independent of skin type and hair color. J Invest Dermatol. 2001;117(2):294–300.
37. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. JAMA. 2007; 297(8):842–57.
38. Omenn GS, Goodman GE, Thornquist MD, et al. Risk factors for lung cancer and for intervention effects in CARET, the beta-carotene and retinol efficacy trial. J Natl Cancer Inst. 1996;88(21):1550–9.
39. Hercberg S, Ezzedine K, Guinot C, et al. Antioxidant supplementation increases the risk of skin cancers in women but not in men. J Nutr. 2007;137(9):2098–105.
40. D'Agostini F, Balansky RM, Camoirano A, De Flora S. Modulation of light-induced skin tumors by N-acetylcysteine and/or ascorbic acid in hairless mice. Carcinogenesis. 2005;26(3):657–64.
41. Thomas J, Liu T, Cotter MA, et al. Melanocyte expression of survivin promotes development and metastasis of UV-induced melanoma in HGF-transgenic mice. Cancer Res. 2007;67(11):5172–8.
42. Noonan FP, Dudek J, Merlino G, De Fabo EC. Animal models of melanoma: an HGF/SF transgenic mouse model may facilitate experimental access to UV initiating events. Pigment Cell Res. 2003;16(1):16–25.
43. Kang S, Chung JH, Lee JH, et al. Topical N-acetyl cysteine and genistein prevent ultraviolet-light-induced signaling that leads to photoaging in human skin in vivo. J Invest Dermatol. 2003;120(5):835–41.
44. Kaur P, Kaur G, Bansal MP. Upregulation of AP1 by tertiary butyl hydroperoxide induced oxidative stress and subsequent effect on spermatogenesis in mice testis. Mol Cell Biochem. 2008;308(1–2):177–81.
45. Fisher GJ, Wang ZQ, Datta SC, Varani J, Kang S, Voorhees JJ. Pathophysiology of premature skin aging induced by ultraviolet light. N Eng J Med. 1997;337(20):1419–28.
46. Marrot L, Jones C, Perez P, Meunier JR. The significance of Nrf2 pathway in (photo)-oxidative stress response in melanocytes and keratinocytes of the human epidermis. Pigment Cell Melanoma Res. 2008;21(1):79–88.
47. Wuyts WA, Vanaudenaerde BM, Dupont LJ, Van Raemdonck DE, Demedts MG, Verleden GM. N-acetylcysteine inhibits interleukin-17-induced interleukin-8 production from human airway smooth muscle cells: a possible role for anti-oxidative treatment in chronic lung rejection? J Heart Lung Transplant. 2004;23(1):122–7.
48. Trivedi H, Daram S, Szabo A, Bartorelli AL, Marenzi G. High-dose N-acetylcysteine for the prevention of contrast-induced nephropathy. Am J Med. 2009;122(9):874 e9–15.
49. Suwannaroj S, Lagoo A, Keisler D, McMurray RW. Antioxidants suppress mortality in the female NZB x NZW F1 mouse model of systemic lupus erythematosus (SLE). Lupus. 2001;10(4):258–65.
50. Kudaravalli J. Improvement in endothelial dysfunction in patients with systemic lupus erythematosus with N-acetylcysteine and atorvastatin. Indian J Pharmacol. 2011;43(3):311–5.
51. Vasu VT, de Cruz SJ, Houghton JS, et al. Evaluation of thiol-based antioxidant therapeutics in cystic fibrosis sputum: focus on myeloperoxidase. Free Radic Res. 2011;45(2):165–76.
52. Varelogianni G, Oliynyk I, Roomans GM, Johannesson M. The effect of N-acetylcysteine on chloride efflux from airway epithelial cells. Cell Biol Int. 2010;34(3):245–52.
53. Dauletbaev N, Fischer P, Aulbach B, et al. A phase II study on safety and efficacy of high-dose N-acetylcysteine in patients with cystic fibrosis. Eur J Med Res. 2009;14(8):352–8.
54. Shahin AY, Hassanin IM, Ismail AM, Kruessel JS, Hirchenhain J. Effect of oral N-acetyl cysteine on recurrent preterm labor following treatment for bacterial vaginosis. Int J Gynaecol Obstet. 2009;104(1):44–8.
55. Murphy KT, Medved I, Brown MJ, Cameron-Smith D, McKenna MJ. Antioxidant treatment with N-acetylcysteine regulates mammalian skeletal muscle Na+-K+-ATPase alpha gene expression during repeated contractions. Exp Physiol. 2008;93(12):1239–48.
56. Kelly MK, Wicker RJ, Barstow TJ, Harms CA. Effects of N-acetylcysteine on respiratory muscle fatigue during heavy exercise. Respir Physiol Neurobiol. 2009;165(1):67–72.
57. Redondo P, Bauza A. Topical N-acetylcysteine for lamellar ichthyosis. Lancet. 1999;354(9193):1880.
58. Dean OM, van den Buuse M, Berk M, Copolov DL, Mavros C, Bush AI. N-acetyl cysteine restores brain glutathione loss in combined 2-cyclohexene-1-one and d-amphetamine-treated rats: relevance to schizophrenia and bipolar disorder. Neurosci Lett. 2011;499(3):149–53.
59. Sansone RA, Sansone LA. Getting a knack for NAC: N-Acetyl-Cysteine. Innov Clin Neurosci. 2011;8(1):10–4.
60. Odlaug BL, Grant JE. N-acetyl cysteine in the treatment of grooming disorders. J Clin Psychopharmacol. 2007;27(2):227–9.
61. Gray KM, Watson NL, Carpenter MJ, Larowe SD. N-acetylcysteine (NAC) in young marijuana users: an open-label pilot study. Am J Addict. 2010;19(2):187–9.

62. Mardikian PN, LaRowe SD, Hedden S, Kalivas PW, Malcolm RJ. An open-label trial of N-acetylcysteine for the treatment of cocaine dependence: a pilot study. Prog Neuropsychopharmacol Biol Psychiatry. 2007;31(2): 389–94.
63. Knackstedt LA, LaRowe S, Mardikian P, et al. The role of cystine-glutamate exchange in nicotine dependence in rats and humans. Biol Psychiatry. 2009;65(10):841–5.
64. Berk M, Copolov D, Dean O, et al. N-acetyl cysteine as a glutathione precursor for schizophrenia—a double-blind, randomized, placebo-controlled trial. Biol Psychiatry. 2008;64(5):361–8.
65. Berk M, Copolov DL, Dean O, et al. N-acetyl cysteine for depressive symptoms in bipolar disorder—a double-blind randomized placebo-controlled trial. Biol Psychiatry. 2008;64(6):468–75.
66. Adair JC, Knoefel JE, Morgan N. Controlled trial of N-acetylcysteine for patients with probable Alzheimer's disease. Neurology. 2001;57(8):1515–7.