HHS Public Access

Author manuscript

Curr Pharmacol Rep. Author manuscript; available in PMC 2016 June 01.

Published in final edited form as:

Curr Pharmacol Rep. 2015 June 1; 1(3): 206–215. doi:10.1007/s40495-015-0027-9.

An Overview of Ultraviolet B Radiation-Induced Skin Cancer Chemoprevention by Silibinin

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Abstract

Skin cancer incidences are rising worldwide, and one of the major causative factors is excessive exposure to solar ultraviolet radiation (UVR). Annually, ~5 million skin cancer patients are treated in United States, mostly with nonmelanoma skin cancer (NMSC), which is also frequent in other Western countries. As sunscreens do not provide adequate protection against deleterious effects of UVR, additional and alternative chemoprevention strategies are urgently needed to reduce skin cancer burden. Over the last couple of decades, extensive research has been conducted to understand the molecular basis of skin carcinogenesis, and to identifying novel agents which could be useful in the chemoprevention of skin cancer. In this regard, several natural non-toxic compounds have shown promising efficacy in preventing skin carcinogenesis at initiation, promotion and progression stages, and are considered important in better management of skin cancer. Consistent with this, we and others have studied and established the notable efficacy of natural flavonolignan silibinin against UVB-induced skin carcinogenesis. Extensive pre-clinical animal and cell culture studies report strong anti-inflammatory, anti-oxidant, DNA damage repair, immune-modulatory and anti-proliferative properties of silibinin. Molecular studies have identified that silibinin targets pleotropic signaling pathways including mitogenic, cell cycle, apoptosis, autophagy, p53, NF-κB, etc. Overall, the skin cancer chemopreventive potential of silibinin is well supported by comprehensive mechanistic studies, suggesting its greater use against UV-induced cellular damages and photocarcinogenesis.

Keywords

Photocarcinogenesis; Chemoprevention; Silibinin; DNA repair; Mitogenic signaling; Apoptosis

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Compliance with Ethics Guidelines

Conflict of Interest Rahul Kumar and Gagan Deep declare that they have no conflict of interest.

Rajesh Agarwal declares a financial interest in Protechsure Scientific, Inc. which is developing skin products containing silibinin.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

1. Introduction

Skin is continuously exposed to sunlight [primarily ultraviolet radiation (UVR)] and other toxicants that eventually causes skin cancer, which is the most common malignancy worldwide [1-3]. Among all the factors, UVR is the most important etiological factor in skin cancer development, and accounts for about 50-90% of total reported skin cancer cases [4, 5]. In addition, increased popularity of outdoor activities and tanning devices as well as rapid depletion of the ozone layer have also contributed to enhanced UVR exposure [6]. Therefore, not surprising, incidences of skin cancer are continuously rising. Epidemiological analysis reveal that annually in the United States alone, ~5 million skin cancer patients are treated, and that between 40 and 50 percent of Americans are susceptible to develop skin cancer at least once by the age of 65. These statistics clearly suggest that skin cancer is a major health issue.

In general, skin cancers can be broadly classified into two groups: (A) cutaneous melanoma and (B) non melanoma skin cancer (NMSC), the latter is further classified into (i) basal cell carcinoma (BCC) and (ii) squamous cell carcinoma (SCC). Cutaneous melanoma is aggressive form of skin cancer and despite the fact that only 5-10% of total diagnosed skin cancer cases are melanoma, it causes approximately 75% of all skin cancer-related deaths because of its high metastatic potential. It is estimated that approximately 90% of melanomas are caused by exposure to UVR [2, 7]. Regarding NMSC, majority of the cases are BCC which is more common than all other human malignancies combined. Almost all BCC occurs on excessively UVR exposed body sites, especially the uncovered ones such as face, ear pinna, neck, scalp, shoulders and back [8]. Other than UVR, exposure to arsenic, radiation, chronic inflammatory conditions in skin, and burns, scars, infections complications or even tattoos are also the contributing factors for BCC. Similar to BCC, the primary cause for SCC is also the excessive exposure to solar UVR [9]. Both BCC and SCC could be easily treated when diagnosed early and are rarely fatal, but in a small percentage of cases, BCC and SCC have shown metastatic potential and cause death. Moreover, BCC and SCC development as well as their treatment/s could be painful and disfiguring.

Since excessive exposure to solar UVR is the main reason for skin cancer, photocarcinogenesis is an area of extensive research. UVR consists of UVA (320-400 nm), UVB (280-320 nm) and UVC (200-280 nm), where UVB and UVC are most effective in causing skin cancer; however, majority of UVC radiation is filtered by atmospheric ozone layer, and therefore, it has not much biological relevance to skin cancer [7, 10]. Majority of solar UVR reaching the earth surface is UVA (90-99%) and only 1-10% is comprised of UVB. Earlier studies with mouse skin models showed the complete skin carcinogenic potential of both UVA and UVB radiations [11, 12]. However, higher exposure doses are needed for UVA-caused photocarcinogenesis in mice when compared to UVB in terms of both dose and duration, and also has a longer tumor latency period, which is attributed to its weak tumor initiating potential [12]. Therefore, UVB exposure is the most clinically relevant form of UVR for the development of skin cancers. The skin tumor initiating potential of UVB has been attributed to its strong absorption by DNA in skin keratinocytes resulting in DNA lesions including cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts, which are major contributors to photocarcinogenesis [13, 14]. Furthermore,

UVR generates reactive oxygen species (ROS) in skin and induces oxidative DNA damage such as 8-hydroxy-2′-deoxyguanosine (8-OHdG) [15, 16]. UVB-generated ROS can also act as tumor promoter by activating cellular mitogenic signaling pathways promoting proliferation and inflammation [17, 18]. The importance of DNA damaging potential of UVB can be highlighted by the fact that almost 60% SCCs carry UVB signature mutations in tumor suppressor gene p53, such as CC→TT and C→T transitions [14, 19, 20]. UVB exposure also causes immunosuppression, photoaging and sunburn depending on the duration as well as levels of exposure [21]. UV-induced signature mutations (CC→TT and C→T transitions) in PTCH1 (in the mitogenic sonic hedgehog pathway) and p53 genes represent the most significant pathogenic events in BCC and found in almost 50% of sporadic BCC [22, 23]. Recent research showed that there are other genes also which are mutated in BCC such as STAT5B (signal transduction and activation of transcription), CRNKL-1 (crooked neck pre-MRNA splicing factor 1), and that telomerase reverse transcriptase (TERT) promoter also contributed towards development of this malignancy [24, 25].

For skin cancer prevention, efforts have been made to improve awareness about risk factors including minimizing sunlight exposure especially during mid-day hours, effective use of sunscreens, wearing protective clothing when outdoors and by avoiding the use of UV tanning devices. However, the current options and strategies have been largely ineffective as evident by continuous increase in skin cancer incidences, the related morbidity and medical care expenses. Moreover, few studies have even shown that sunscreen use enhances melanoma risk, further demanding novel and alternative measures to lower skin cancer risk [26]. Many epidemiological studies have shown that modification in lifestyle factors including dietary patterns could be helpful in the prevention of two-thirds of cancer cases [27-29]. As the complete skin carcinogenic potential of UVB radiation has been attributed to its potential to directly damage DNA and other cellular macromolecules as well as oxidative stress generation, nontoxic natural compounds that a) could reduce solar UVR-caused DNA damage, and/or b) possess anti-oxidant, anti-inflammatory and/or immune-modulatory effect could be useful against photocarcinogenesis [10, 13, 30-32]. In this regard, many non-toxic natural compounds including silibinin, green tea extract, black tea extract, epigallocatechin 3-gallate, etc. have shown their strong efficacy against photocarcinogenesis at initiation, promotion and complete carcinogenesis stages [13, 32-37]. The present review is focused on the effectiveness and associated mechanism(s) of **Silibinin** against UVB-induced skin carcinogenesis.

2. Silibinin and photocarcinogenesis prevention

Silibinin is the main bioactive flavonolignan present in milk thistle extract and is a dietary supplement for its hepatoprotective activity [38, 39]. The milk thistle plant (Silybum marianum L., Family Asteraceae) is currently cultivated in several countries for commercial production of milk thistle extract [13], mostly Silymarin that contains 40% (w/w) silibinin as the major anticancer agent [40]. Several studies have reported strong antioxidant property of silibinin and its capability to scavenge and neutralize free radicals and ROS [10, 13, 32, 41, 42]. Furthermore, several *in vivo* studies have shown that silibinin is free of potential

adverse effects in both animal and human studies, which is a desired component for cancer chemopreventive agents [13, 43-45].

The most preferred and frequently used animal model to study UVR-caused skin cancer is SKH-1 hairless mouse skin which parallels both histologically and pathological features of human NMSCs [46]. Furthermore, the multi-stages of photocarcinogenesis in this mouse model have been well defined and characterized. Following UVB exposure, exposed mouse skin showed epidermal hyperplasia, and further UVB irradiation leads to the development of benign papilloma which further progresses to SCC [13]. These characteristics in this mouse model closely followed the development of NMSC in humans [47]. Therefore, this model has been used extensively to conduct mechanistic as well as preventive studies related to photocarcinogenesis [46]. Our studies in this model have clearly shown that silibinin is a strong chemopreventive agent against photocarcinogenesis, and produces its activity irrespective of application on the dorsal side of mouse skin either before or after UVR exposure or given through diet. Mechanistic and biomarker analyses showed that silibinin activity against photocarcinogenesis was by inhibiting cell proliferation, mitogenic signaling and induction of apoptosis [43, 44, 48-50]. Furthermore, it was reported that dietary feeding of silibinin at 1% dose (w/w) for two weeks prior to a single UVB exposure strongly inhibits CPD formation, cell proliferation, sunburn cells, but increases p53 and its downstream molecule p21/Cip1 in irradiated SKH-1 hairless mouse skin [43]. The observed prevention by silibinin following dietary feeding is of utmost importance in chemoprevention of skin cancer. In bioavailability studies, we found that following oral dosing of silibinin (50 mg/kg body weight) to mice, it peaks at 1 hour post-administration in skin [51]. In the same study, silibinin was also detected in liver, lung, stomach, pancreas and prostate, indicating the appreciable bioavailability of this phytochemical [51]. Recent studies by others and us have also established the anti-carcinogenic potential of silibinin against several other neoplasms such as liver, lung, colon and prostate, when administered orally, confirming its broad-spectrum anti-cancer effects [10, 45, 52-56]. Overall, these findings make silibinin a potent and promising chemopreventive agent against UVB-induced skin cancer with minimal toxicity and remarkable efficacy.

3. Molecular mechanism(s) of skin cancer prevention by silibinin

Due to strong preventive effects of silibinin against photocarcinogenesis, efforts have been made to understand the molecular mechanism/s underlying its chemopreventive efficacy. It is evident from the published studies by us and others that silibinin targets several molecular pathways in mouse skin which are described in detail below.

3.1 Silibinin inhibits UVB-caused DNA damage

As mentioned earlier, UVB is absorbed by epidermal DNA causing various photoproducts formation including CPDs, 6-4 photoproducts, DNA-DNA/protein crosslinks, cytosine photohydrates, DNA strand breaks, etc. [13, 57]. Among these, formation of CPDs is quite frequent and important, and therefore, it is the well-studied form of UVB-caused skin epidermal DNA damage. Due to the importance of genomic integrity, there are various DNA repair mechanisms in normal cells that continuously monitor and repair majority of the DNA damage efficiently, but those DNA repair mechanisms could be ineffective in the case of

severe damage to DNA which results in introduction of mutations in key genes following replication of damaged DNA (due to faulty repair) [58]. The most common mutations are CC→TT and C→T transitions which often are considered as UVB signature mutations [14, 19]. The cells harboring these mutations are referred as initiated cells, which can lie dormant for a long period of time. Our earlier studies with SKH-1 hairless mice and JB6 epidermal cells have shown strong inhibition of UVB-caused CPDs formation [43, 48, 59, 60] together with an accelerated removal of CPDs in JB6 cells by silibinin [59]. Dietary silibinin also showed similar inhibitory results towards UVB-caused CPDs formation in mouse skin. These results strongly correlate with silibinin-mediated decrease in UVB-induced skin cancer incidence and a strong reduction in number of tumors in SKH-1 hairless mouse skin as summarized above.

Exposure of UVR to murine and or human epidermal cells causes a reduction in the levels of antioxidant enzymes and generates ROS, which leads to oxidative modification in DNA, proteins and lipids [61]. The ROS-mediated DNA strand breaks, base modifications and cross-links could lead to errors during replication causing genomic instability that is considered an important prerequisite for cancer development [62, 63]. Under oxidative stress conditions, lipids get oxidized and generate lipid peroxidation products, such as reactive aldehydes, which could react with DNA and form lesions, and hence, initiate cell transformation [64]. The strong protective efficacy of silibinin against ROS-induced cellular damages has been attributed to its strong antioxidant potential. In addition, silibinin has been shown to increase glutathione-S-transferase (GST) and quinine reductase (QR) activities in the skin which are involved in the removal of cellular reactive moieties [51]. Thus, it has been suggested that silibinin could also prevent DNA damage via decreasing oxidative stress.

Genomic stability is essential for cell survival, and in response to DNA damage, either the progression of cell cycle is halted for an adequate time to repair DNA damage when the damage is mild or reparable, or induce apoptotic death when the DNA damage is severe or irreparable. These DNA damage-related cellular responses are multifaceted and involve a complex signaling network in which p53 plays a crucial role [58]. Studies have shown that following DNA damage, p53 is activated rapidly, and this activation is predominantly through post-translational modifications and interactions with other cellular co-factors [58, 65]. Following UVB exposure, p53 is phosphorylated at specific serine sites resulting in its stabilization and accumulation. Upon stabilization, p53 translocates to the nucleus and subsequently transactivates specific target genes involved in the pathways of DNA damage repair, cell cycle progression and/or apoptosis-related signaling [66, 67]. Due to its importance, not surprisingly, the DNA binding domain of p53 is found to be a hot spot of mutations during the cancer development as observed in several malignancies including skin cancer [9, 68]. In fact, 50-60% NMSC cases in humans harbor UVB signature mutations in p53 gene, which is comparable to murine skin tumors [9, 69]. In this regard, we and others have reported that UVB exposure to mouse skin and cultured epidermal cells caused a moderate to extensive apoptosis based on the UVB dose along with an accumulation of p53 protein [13, 44, 48, 60, 70-73]. Importantly, silibinin further increased p53 level in SKH-1 hairless mouse skin epidermis, irrespective of its application on the dorsal side of mouse skin either before or after UVR exposure or given through diet. [44, 48]. Silibinin also

increased p53 level in UVB exposure caused skin tumors [44]. In these studies, p21/Cip1, a downstream target of p53, was also found to be upregulated which contributed towards cell cycle arrest by inhibiting cyclin-dependent kinase (CDK)-cyclin complex [48]. Similarly, in cell culture experiments with epidermal JB6 and HaCaT cells, we reported that silibinin treatment further increases UVB-induced phosphorylation at serine 15 site which causes stabilization of p53 and is related to apoptosis [70, 71]. However, at lower UVB dose, silibinin inhibited apoptotic cell death in HaCaT cells [71]. Silibinin treatment also facilitated DNA damage repair by activating a p53 downstream target, namely GADD45α (growth arrest and DNA damage-inducible protein alpha), in both JB6 cells and SKH-1 hairless mouse skin [60]. Furthermore, another study by us showed that silibinin conferred protection against DNA damage and apoptosis in epidermal cells both in cell culture and murine skin by modulating IL-12 level indicating immune-modulatory potential of silibinin [59]. Overall, these findings clearly suggested that silibinin prevents UVB-induced DNA damage as well as potentiates the DNA repair machinery, as major mechanisms of its efficacy against photocarcinogenesis during tumor initiation stage.

3.2 Dual efficacy of silibinin towards UVB-induced pro- and anti-apoptotic machineries in mouse skin

Excessive exposure to UVB radiation could cause severe DNA damage which leads to removal of damaged cells via apoptotic death pathway by modulation of various pro- and anti-apoptotic proteins, dependent or -independent of p53 involvement manner [74, 75]. Induction of apoptosis in DNA damaged cells is of high importance, as its failure not only predisposes cells harboring DNA damage to the carcinogenesis, but also induces resistance to therapeutic interventions. Though apoptotic cell death following stress condition is widely regarded as a protective mechanism, excessive apoptosis of epidermal cells (sunburn) may compromise skin layer which acts as the first barrier for humans against adverse effects of various environmental toxins [76]. As discussed in section 3.1, in the case of mild damage to DNA, silibinin protects normal epidermal cells from apoptosis; while following severe damage, silibinin further potentiates apoptotic death, suggesting its dual action against UVB-induced photodamage. In our previous short term study, silibinin treatment reduced both apoptotic and sunburn cell populations in SKH-1 hairless mouse skin after UVB exposure at 180 mJ/cm^2 dose, either once or 5 days [43]. However, in long term study of 25 weeks, silibinin treatment further increased the apoptosis induced by UVB in both skin tumors and chronically UVB-exposed uninvolved mouse skin [44]. In the same study, it was also found that silibinin treatment caused activation of caspase 3 with a concomitant decrease in survivin levels in UVB-induced skin tumors and these pathways might be important contributors in silibinin-induced apoptotic cell death [44]. These results suggest that following UVB exposure, depending on the level of DNA damage, silibinin acts as DNA damage sensor and induces or inhibits apoptotic cell death.

Several *in vitro* cell culture studies have also reported a series of variable apoptotic effects of silibinin following UVB exposure which were dependent on the severity of DNA damage. Silibinin, at physiologically achievable doses, strongly induced apoptosis in A431 cells [72]. Importantly, silibinin inhibited apoptotic death in HaCaT cells (human immortalized keratinocytes) following UVB irradiation at lower doses (5 and 30 mJ/cm^2),

but enhanced apoptotic cell death following UVB exposure at a higher dose of 120 mJ/cm^2 [71]. Molecular analyses revealed that silibinin treatment results in protection against UVB-induced activation of caspase 9 and PARP cleavage compared to UVB alone group, irrespective of its treatment before or immediately after UVB exposure [71]. Similar silibinin treatments were also found to alter the Bcl2 family of proteins (both pro- and anti-apoptotic proteins) as well as the interaction and localization of Bcl2, Bax, Bak, Bad, Bcl-xL and cytochrome c [71]. Furthermore, survivin level was restored following silibinin treatment post-UVB irradiation. Recently, it was reported that silibinin pre-treatment resulted in inhibition of UVB-caused apoptosis in HaCaT cells by down regulation of FADD (Fas-associating protein with death domain) and inhibition of procaspase-8 cleavage [77]. In addition, silibinin inhibited the UVB-induced apoptosis in A431 cells by promoting autophagy [78]. Further studies revealed that UVB activates IGFR1-PI3K-Akt signaling axis in A431 cells inhibiting autophagy, and silibinin down-regulates this signaling axis and promotes autophagy, and thus opposed the pro-apoptotic effects of UVB [78, 79]. The induction of autophagy by silibinin arrested the cell growth transiently and promoted the repair of damaged DNA, and finally rescued the UVB-exposed cells from apoptosis. Overall, above summarized findings convincingly suggest the dual effectiveness of silibinin in inhibiting or inducing UVB-caused apoptosis in both animal and cell culture models.

Unlimited replicative or proliferative potential is considered as the essential characteristics of cancer cells including skin cancer. In this regard, the increased protein levels or interactions of cyclins and CDKs, together with a decrease in CDK inhibitors (CDKIs) expression, have been shown to play a casual role in UVB-caused skin carcinogenesis [80, 81]. Cell cycle analysis showed that majority of epidermal cells such as JB6 and HaCaT are in G1 phase which is further prolonged following UVB-induced DNA damage [60, 71], via an increase in p21/Cip1 protein that could be dependent or -independent of p53 involvement [71]. p21/Cip1 is one of the first cell cycle regulatory protein molecules upregulated in cells following DNA damage by various stress conditions including UVB, to halt the progression of cell cycle and thus proliferation of the cells harboring damaged DNA[82]. It has been observed that following chronic exposure to UVR, the protein levels of the cell cycle regulatory molecules are altered in the SKH-1 hairless mouse skin and silibinin was found to reduce UVB-induced expression of CDKs 2 and 4, cyclins B1, A, E and D, Cdc2, Cdc25c and to up regulate the expression of CDKIs (p21/Cip1 and p27/Kip1) [44, 83]. Our cell culture findings also showed silibinin's inhibitory activity on cell cycle progression following UVB-exposure by regulating CDKs and CDKIs [71, 84]. Overall these studies suggest silibinin's effects on cell cycle regulators which might be an important mechanism towards its anti-proliferative action.

3.3 Silibinin inhibits UVB-caused inflammation in mouse skin

Chronic inflammation is a critical component and prerequisite for tumor progression in skin carcinogenesis [6, 85, 86]. Skin protects from both injury and infection; whereas immune cells play a critical role in both wound healing and tissue repair, the inflammatory reactions initiated by these cells are essential in various skin pathological conditions as well as skin cancer. These immune cells secrete various growth factors, cytokines, prostaglandins and chemotactic polypeptides, and thus lead to the condition of chronic inflammation in skin

which further contributes towards UV-induced skin carcinogenesis [3, 85, 87]. Clinical manifestations of UVB-induced skin inflammation are erythema, edema and hyperplastic epithelial responses induced by infiltration and accumulation of inflammatory cells. Recent research suggests that UVB positively regulates autophagy in dermal inflammatory cells and inhibits their apoptotic death; however, it negatively regulates autophagy in epidermal cells and promotes their apoptosis. The apoptosis of epidermal cells results in secretion of inflammatory cytokines, mediating pro-inflammation reaction among dermal inflammatory cells, and induces chemotaxis and recruitment of neutrophils and mast cells, leading to skin inflammation [88].

Several studies have shown the important roles of arachidonic acid, prostaglandins, cyclooxygenases (COX), lipoxygenase, tumor necrosis factor-α (TNF α), inducible nitric oxide synthase (iNOS), and several transcription factors including AP-1, NF-κB and STAT3 in inflammation [85]. The arachidonic acid pathway is at the core of inflammatory response. In this pathway, COX enzymes are responsible for the formation of prostaglandins (PGE2, PGF2α, and PGD2), prostacyclin and thromboxane, while lipoxygenase generates 5-HPETE which is converted to leukotrienes. COX2 is over-expressed in several cancers and considered an attractive drug target [89]. We reported previously a strong increase in the protein levels of COX2 levels in both the skin and skin tumors in mice following sustained exposure to a dose of UVB that was physiologically relevant to human exposure [50]. UV-caused increase in COX2 expression is known to induce PGE2 that is the major COX product involved in photocarcinogenesis [90]. Animal studies have clearly shown the roles of PGE2 receptors EP1, EP2 and EP4 in UVB-induced skin carcinogenesis [91]. Not surprisingly, a population-based case-control analysis in United Kingdom provides evidence that patients predisposed to NMSC might benefit from chemoprevention with Nonsteroidal anti-inflammatory drugs (NSAIDs) [92]. PGE2 has also been shown to promote inflammation, immunosuppression, tumor cell proliferation, anti-apoptosis and tumor invasion, and thus, contributing significantly towards the development of skin cancer [85, 86, 93]. Silibinin has also been shown to strongly inhibit UVB-induced COX2 and iNOS levels in skin as well as skin tumors in SKH-1 hairless mice, irrespective of its topical application before or after UVB exposure or given in diet [50]. Furthermore, several transcription factors (AP-1, NF-κB, and STAT3) are known to regulate inflammatory cytokines, and UVB exposure has been reported to strongly activate these transcription factors. Consistent with its effects on COX2 and iNOS, similar silibinin treatments also inhibited UVB-caused NF-κB and STAT3 activation in both skin and skin tumors [50]; silibinin also strongly inhibited the activation of transcription factor AP-1 [84]. Together, these findings clearly suggested that silibinin inhibits inflammatory response in mouse skin following UVB exposure by inhibiting the expression and activity of COX2, and the level of pro-inflammatory cytokines through inhibition of AP-1, NF-κB and STAT3 transcription factors, indicating a strong anti-inflammatory action of silibinin, which might be contributing towards its photocarcinogenesis preventive potential.

3.4 Silibinin inhibits UVB-caused activation of mitogenic and survival signaling in mouse skin

One of the hallmarks of tumor promotion and malignant transformation is the activation of mitogen activated protein kinases (MAPKs) by both chemical tumor promoters and UVB [93, 94]. The UVB-caused activation of mitogenic signaling is mediated via extracellular regulated kinase 1/2 (ERK1/2), p38 kinase and c-jun-NH_2-kinase 1/2 (JNK1/2) MAPKs [94]. These serine/threonine kinases upon activation phosphorylate and subsequently activate various downstream target molecules, and regulate various cellular events including proliferation and apoptosis [95, 96]. While ERK1/2 activation is involved in cell proliferation and survival, p38 and JNK1/2 exert both anti- and pro-apoptotic functions dependent on stimuli and cellular context [97]. Furthermore, ERK1/2 activation also plays a critical role in UV and other tumor promoters caused cell transformation and tumor promotion [85, 98]. Akt also acts as a critical cell survival and anti-apoptotic signaling molecule and plays an important role in both promotion and progression stages of carcinogenesis [99]. The UVB-mediated activation of both MAPKs and Akt leads to the activation of AP-1 and NF-κB; and these two transcription factors regulate the expression of an array of genes required for growth and proliferation including inflammatory cytokines, and further help in maintaining chronic inflammatory conditions, to promote photocarcinogenesis [85, 100].

Our *in vitro* studies have shown that silibinin causes the inhibition of growth and death in A431 cells by significantly inhibiting the activation of ERK1/2 [72]. Conversely, in this study, silibinin activated p38 and JNK1/2 MAPKs which was associated with enhanced apoptotic cell death [72]. Similarly, silibinin also modulated these MAPKs in UVB-irradiated JB6 cells [84]. Our *in vivo* studies clearly suggested a dual role of MAPKs in silibinin efficacy against photocarcinogenesis. When exposure was done acutely at a single UVB dose (180 mJ/cm^2), ERK1/2, p38 and JNK1/2 MAPKs and Akt were activated in the skin of SKH-1 hairless mice, which was inhibited significantly by silibinin irrespective of its topical application before or immediately after UVB irradiation or fed in diet [49]. Similar inhibitory effects of silibinin on MAPKs and Akt were observed in a chronic UVB exposure protocol when SKH-1 hairless mice were exposed with UVB dose of 180 mJ/cm^2/day for 5 days [49, 83]. Conversely, in UVB-induced skin tumors in SKH-1 hairless mice, similar silibinin treatment further increased the UVB-induced activation of ERK1/2, p38 and JNK1/2 MAPKs in tumor samples at 25 weeks; however, the Akt expression was strongly decreased by silibinin [83]. These results clearly showed that silibinin inhibited various oncogenic signaling pathways required for tumor progression; however, it remains to be ascertained whether silibinin is also effective against chronic UVB-induced sustained genetic abnormalities at progression stage of skin carcinogenesis [101, 102] (Figure 1). Overall, these findings suggest that the modulation of mitogenic signaling with silibinin is an important mechanism of its efficacy against UVB-induced skin cancer.

3.5 Pharmacokinetics and safety of silibinin

The oral bioavailability of silibinin is poor due to its poor solubility in water; therefore, silibinin complexes have been developed for improved absorption. The commercially available silibinin complexes are IdB 1016 (silipide) and Silybin-phytosome (siliphos),

which are formulation of silibinin with phosphatidylcholine, and have shown improved bioavailability compared to silibinin [103-105]. Barzaghi *et al.* determined the plasma silibinin levels in nine healthy volunteers following administration of single oral dose of silipide (equivalent to 360 mg silibinin) and found that free silibinin concentration in plasma reached a peak of 298 ± 96 ng/ml achieved at 1.6 ± 1 h [103]. In another study by the same group, nine healthy volunteers received silipide (equivalent to 120 mg silibinin) twice daily for 8 consecutive days. The terminal half-life of silipide in plasma on day 1 was 2.6 ± 1 h with no change on day 8. Urinary profile revealed that less than 3% of the administered dose was excreted via urine, with a significant proportion of the dose probably being excreted in the bile [103]. In a similar study in humans, twelve healthy volunteers received a single oral dose of silipide (equivalent to 80 mg silibinin) and the free silibinin concentration reached a peak of 141 ± 32 ng/ml in plasma which was achieved at 2.4 h [104].

Due to the promising efficacy of silibinin against various malignancies in pre-clinical animal models, silibinin was also tested in several clinical trials. In one such trial in 2006, patients with confirmed colorectal adenocarcinoma received daily dose of silibinin (in the form of silipide) at 360, 720 or 1440 mg, and after seven days blood and colorectal and liver tissues were collected [106]. Further analysis showed that silibinin levels were between 0.3–4.0 μmol/L in blood, between 28-141 nmol/gm in colorectal tissue and between 1.0-2.5 nmol/gm in hepatic tissue [106]. These doses of silibinin were not associated with any adverse effect and termed as safe [106]. In another phase I/II clinical trial of silibinin in prostate cancer patients, authors concluded that silibinin doses up to 13 gm per day were well tolerated in patients without much side effects [107, 108]. Plasma analysis showed that the half-life of silibinin in plasma was short and ranged between 1.79 – 4.99 h, which was consistent with earlier studies with healthy volunteers, with the peak levels close to 100 μM [108]. These studies clearly suggested that there is minimal safety concerns related to silibinin consumption in humans, and bioavailability at physiologically pertinent concentrations could be attained by using formulation with phosphatidylcholine. Though, so far, no such trial has been conducted in skin cancer patients, the non-toxic nature of silibinin and its strong efficacy against UVB-induced skin carcinogenesis in pre-clinical animal models strongly supports its evaluation in skin cancer patients.

4. Conclusion

The studies elaborated above clearly show that non-toxic silibinin possesses potent efficacy against all stages of UVB-induced skin carcinogenesis through its various attributes including anti-oxidant, DNA damage repair, immunomodulation, anti-inflammatory and anti-proliferative actions (Figure 1). Importantly, the chemopreventive potential of silibinin against photocarcinogenesis is strongly supported by comprehensive mechanistic rationale; therefore, its greater use is recommended against UV-induced cellular damages and photocarcinogenesis. In future, silibinin efficacy should also be tested in therapeutic settings where skin tumors have already progressed and it will be interesting to examine whether silibinin could inhibit UVB-induced sustained genetic abnormalities resulting in oncogenes activation and tumor suppressor genes inactivation (Figure 1). Considering the fact that skin cancer incidences are increasing worldwide and sunscreens offer only a partial protection,

silibinin offers a unique and novel chemopreventive option to better manage and reduce skin cancer burden.

Acknowledgment

Original studies with silibinin efficacy as skin cancer chemopreventive agent are supported by NCI R01 grant CA140368.

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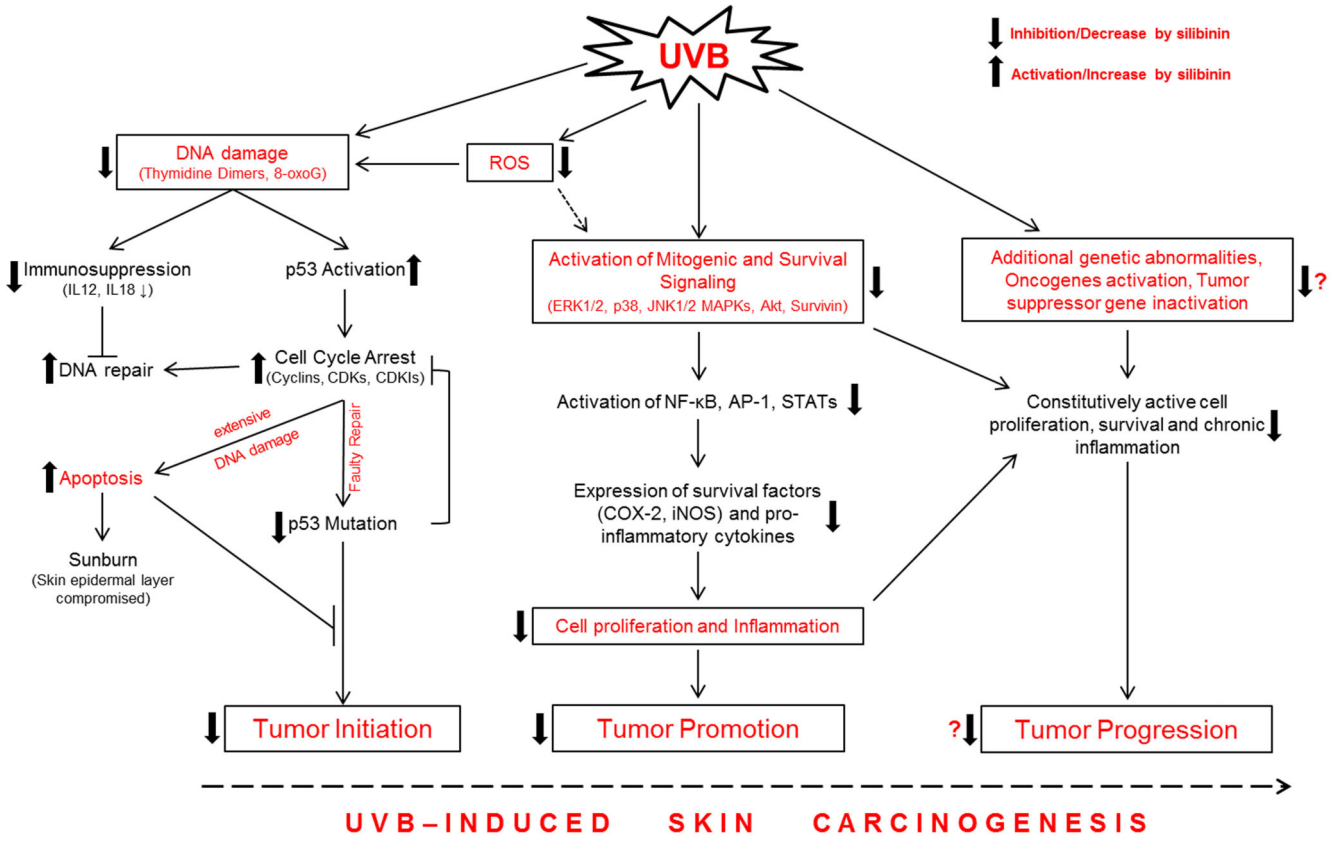

Figure 1.
Effect of silibinin on UVB-induced skin damage and carcinogenesis