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Mechanisms and preclinical efficacy of silibinin in preventing skin cancer

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Abstract

Eukaryotic cellular machineries including the genome face continuous challenge from environmental deleterious agents, as well as from the by products of their own metabolism. Our skin is the most important barrier. It protects us from xenobiotic and genotoxic agents including ultraviolet (UV) solar radiation and potential carcinogens, which are notorious for causing skin cancer. There is a rise in non-melanoma skin cancer (NMSC), which is diagnosed in more than a million people every year in the United States alone, and is also prevalent in the other Western countries. In addition to sunscreens, chemoprevention of skin cancer by natural non-toxic compounds is suggested as an effective strategy to prevent the incidence of skin cancer. Our extensive animal studies on silibinin, a non-toxic bioactive component in milk thistle, suggest that it has a strong potential to prevent skin cancer incidence, promotion and progression in response to chemical carcinogens and tumour promoters as well as UV radiation. Our data suggest that silibinin has multiple targets in the cell, and can be protective against the harmful effects of cytotoxic agents such as reactive oxygen species and inflammation. Further, silibinin modulates mitogenic and survival signalling, p53, Cip1/p21 and other cell cycle regulatory molecules to prevent UVB-induced skin carcinogenesis. Our ongoing studies also suggest the positive effect of silibinin on the repair of UVB-induced DNA damage in mouse skin. Overall, the protective efficacy of silibinin against skin cancer is supported by sound mechanistic rationale in animal and cell culture studies, and suggests its potential use for humans.

Keywords: Silibinin; Photocarcinogenesis; p53; DNA repair; Mitogenic and survival signaling; Apoptosis; Cell cycle

1. Introduction

Transformation of normal cells into the malignant state is determined by a number of factors that influence genetic as well as epigenetic molecular events. Mutagenic agents primarily cause oncogenic mutations including those in tumour suppressor genes that lead to tumour initiation. This is followed by non-mutagenic changes by endogenous and/or exogenous tumour promoters that increase the growth and proliferation of initiated cells. At later stages, additional changes at genetic as well as cellular level accelerate these processes during tumour progression. Mouse skin has been used as a standard paradigm to study all three stages – initiation, promotion and progression of carcinogenesis. Mouse models are also used to establish inflammatory responses to various xenobiotic agents including ultraviolet (UV) radiations and chemical carcinogens [1]. Many cell lines, representing initiation, promotion and progression of skin carcinogenesis have also been developed to study the detailed biological mechanisms *in vitro* and to identify critical targets for the prevention and better therapeutic outcomes [1–3].

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Although, awareness of the risk of skin cancer is increasing, numbers of cases of non-melanoma skin cancer (NMSC) as well as melanoma are expected to rise possibly due to increased popularity of outdoor activities such as sun-tanning; migration of fair-skinned individuals to more sun-intensive regions; and depletion in atmospheric ozone [4,5]. UV solar radiation consists of UVA (320–400 nm), UVB (280–320 nm) and UVC (200–280 nm). About 1–10% of solar radiation reaching the earth surface is comprised of UVB; the remainder is UVA. UVB is known to initiate tumour development. It is also a complete carcinogenic agent for the growth and development of NMSC, which is diagnosed in more than a million people each year in the United States of America alone [5,6]. UVB radiation exerts its tumour initiating effects primarily through the formation of cyclobutane pyrimidine dimers and 6–4 photoproducts [7,8] in DNA, in addition to generation of reactive oxygen species leading to the damage of DNA and other molecular targets [9]. UVB is also known to cause sunburn, photoaging and immunosuppression depending on the levels of exposure [10–14]. UV radiation is also causally linked with melanoma development, which is influenced by skin pigmentation as well as geographical parameters such as latitude and altitude [15]. Sunscreens are commonly used to protect the skin from UVB radiation, however, the incidence of morbidity from NMSC and expenses for medical care are still increasing, suggesting their ineffectiveness to protect from UVB radiation [16,17]. Many epidemiological studies suggest that more than two-thirds of cancer cases could be prevented by modification in lifestyle factors including dietary pattern [18–20]. In such scenario, it is most likely that the use of non-toxic natural agents targeting initiation, promotion and/or progression could be an additional and more effective approach in controlling NMSC [21–25]. By now, this hypothesis has been tested using many non-toxic naturally occurring agents, including green tea, black tea, epigallocatechin galate, silymarin and silibinin against UVB-induced mouse skin carcinogenesis with encouraging results [24,26–29].

The present review is focused on the efficacy and mechanisms of silibinin mainly against UVB skin carcinogenesis, including studies done with its crude form silymarin. In earlier studies, we used silymarin that showed strong protection against photocarcinogenesis in SKH-1 hairless as well as chemical carcinogenesis in SENCAR mouse skin [27,30–33]. After identifying silibinin as the major bioactive pure compound in flavanolignan mixture silymarin, subsequent studies were done with silibinin only. The encouraging photoprotective effect of which has formed the basis of our ongoing research program 'silibinin and skin cancer prevention'. A brief background of silibinin is provided below before discussing the details of its photoprotective effects.

2. Silibinin

Silibinin is a major bioactive flavanone (Fig. 1) present in milk thistle seeds, which has been used as a traditional medicine for about 2000 years to treat various liver conditions. It was first mentioned by Pliny in the first century. At present, milk thistle extract is sold as dietary supplement throughout Europe, Asia and the USA as a remedy for liver-related ailments. The sale of this dietary supplement is lucrative, amounting to millions of dollars per year [34]. Milk thistle (*Silybum marianum* (L.) Gaertn.; Family: Asteraceae or Compositae) plant is a branching annual or biennial and 1–2 m in height. It is indigenous to Mediterranean region, southwest Europe; cultivated for centuries and naturalised in much of Europe and North America, especially California. Silymarin, a flavanolignan complex, was first isolated from milk thistle seeds in the late 1960s. It constituted ~5% of dry weight of ripe fruits and primarily consisted of silibinin (silybin), silychristin (silichristin), silidianin and dihydrosilybin [35]. Silymarin/silibinin is clinically used as a hepatoprotective agent and to treat alcoholic liver diseases in Europe and recently in Asia also.

Several studies have shown that silibinin and silymarin are very strong antioxidants, capable of scavenging both free radicals and reactive oxygen species (ROS), is approximately 10-fold more potent than vitamin E and can strongly enhance cellular antioxidant defense mechanisms. Their hepatoprotective activity has been demonstrated in numerous experimental models of toxic liver damage, which is based on different mechanisms of action including interaction with hepatic cell membranes, inhibition of malondialdehyde formation, blocking binding sites and hindering the uptake of toxins [36]. In clinical studies, silibinin has shown protective and curative effects on liver damage resulting from highly toxic compounds such as phalloidin and α-amanitin (from *Amanita phalloides*) [37]. It has been used with success in toxic-metabolic liver damage, ranging from fatty liver through to hepatitis and actual hepatic cirrhosis, caused by toxic substances, drugs, or exposure to irradiation [38,39].

In vivo studies of both silibinin and silymarin show that these agents are well tolerated and do not cause any significant adverse health effects. At different doses and modes of administration to laboratory animals,

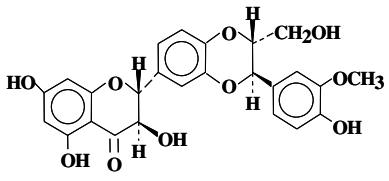

Fig. 1. Chemical structure of naturally occurring flavanone, silibinin, which is isolated from milk thistle (*Silybum marianum*).

both agents have been shown to be non-toxic without any known adverse health effect [36,40]. We also found that silibinin feeding to nude mice at 0.1% (w/w) in diet for 2 months does not result in any apparent sign of toxicity including change in body weight [41]. Similarly, we observed that dietary feeding of silymarin at 0.01–1% (w/w) doses to rats for two weeks and silibinin upto 2 g/kg dose for 16 days in SENCAR mice do not result in any adverse change in body weight gain and diet consumption [42]. These studies indicate the non-toxic nature of silibinin, which is an essential criterion for potential chemopreventive agent.

3. Anticancer efficacy of silibinin against epithelial cancers

Our earlier study identified silibinin as the anticancer agent within silymarin [43]. Over the years, many investigations have used various sources of the primary compound, silibinin, including those as silymarin. There are extensive data, which support silibinin (including silymarin) use as a chemopreventive agent against skin cancer in mouse models [33] and human prostate cancer xenograft growth in nude mouse model [41]. Furthermore, in cell culture studies, silibinin shows growth arrest in many human epithelial cancer cell types including skin [33], prostate [44], breast [43], cervical [43], bladder [45], colon [42] and lung [46]. Recent studies by us also found that silibinin feeding in diet inhibits human colon tumour xenograft growth in nude mice. Others have shown that dietary silymarin feeding prevents chemical carcinogen-induced colon carcinogenesis in rats [47]. Additionally, we have also observed the inhibitory effect of dietary silibinin on animal models of lung [48], bladder and pancreas (unpublished data) malignancies. Several short-term cell culture and long-term animal studies in the past have suggested that silymarin may be a potent anticarcinogenic agent. For example, in *in vitro* carcinogenesis studies, silymarin has been shown to inhibit the formation of transformed rat tracheal epithelial cell colonies arising from exposure to the carcinogen benzo(*a*)pyrene. Furthermore, it confounded phorbol-ester- and other skin tumour promoter-mediated induction of epidermal ornithine decarboxylase (ODC) activity and mRNA expression in mouse skin, and tumour promotion in the mammary gland organ culture initiation-promotion protocol (reviewed in [33]). Overall, these findings suggest a wide range of anticancer activities of silibinin against epithelial cancers.

4. Skin cancer prevention by silibinin

Our studies have shown a strong protective effect of silibinin and silymarin in cell culture as well as in preclinical long-term UVB and chemical-induced skin carcinogenesis mouse models (reviewed in [33]). Since, the focus of this review is on photocarcinogenesis, only a brief mention of the preventive and therapeutic efficacy of silibinin/silymarin in chemical-induced skin carcinogenesis models is included. Our studies show that silymarin inhibits both stages of tumour promotion in DMBA (7,12-dimethylbenz(*a*)-anthracene)–TPA (12-*O*-tetradecanoyl-13 phorbol acetate) and DMBA-MEZ (mezerein) SENCAR mouse skin carcinogenesis models, characterised by prolonged latency period of tumour development; and inhibition of tumour incidence, multiplicity and volume [31]. In other studies where free radical generating agents, such as benzoyl peroxide (BPO) or okadaic acid were used as tumour promoters after DMBA treatment, a similarly strong antitumour promoting effect of silymarin was observed as in the DMBA-TPA protocol [49,50]. Further, in a therapeutic protocol, dietary feeding of silymarin caused regression of established skin tumours in the DMBA-TPA SENCAR mouse model, which was accompanied by inhibition of cell proliferation and induction of apoptotic cell death in tumour cells [32]. Overall, these findings provide evidence for the preventive and therapeutic efficacy of silibinin/silymarin against chemical-induced skin carcinogenesis in mouse models.

The most frequently used and reliable preclinical animal model of photocarcinogenesis is the SKH-1 hairless mouse strain [5]. This model allows the observation of the initiation, promotion and progression stages of skin carcinogenesis when mice are irradiated with UVB at least twice or thrice a week at appropriate dose levels. After UVB exposure, epidermal hyperplasia is evident representing high-risk mice, further irradiation leads to the development of benign papillomas and most of them progress to squamous cell carcinomas (SCC). These characteristics of the model closely mimic the human form of NMSC development in response to sunlight exposure representing 96% of human form of skin cancer (remaining 4% is melanomas). Therefore these models have been used extensively to study mechanisms as well as prevention of photocarcinogenesis.

An early photocarcinogenesis study was done with topically applied silymarin, in which it inhibited UVB-induced tumour initiation, promotion and complete carcinogenesis in SKH-1 hairless mouse skin [27]. In this study, silymarin caused an increase in the latency period of tumour appearance; and a decrease in tumour incidence, multiplicity and volume. In a recently completed study, we used silibinin both via topical as well as dietary administration in order to investigate the interaction of silibinin at the molecular level in skin epidermal cells and assess the possible sunscreen effect, if any, in its preventive efficacy against photocarcinogenesis in SKH-1 hairless mouse skin [29]. Three different protocols were used, in which silibinin was applied

topically prior to or immediately after UVB, or fed in diet. Silibinin treatments resulted in a moderate decrease in tumour incidence and increased the latency period (appearance of first tumour) by up to 4 weeks, and it affected tumour multiplicity and tumour volume strongly [29]. The most profound effect of silibinin was observed on tumour volume in which silibinin showed a decrease by 80–97% in volume per mouse or per tumour at the end of 25 weeks of the study. These studies show convincingly a strong protective effect of both topically applied (prior to or immediately after UVB) as well as dietary-fed silibinin against UVB-induced tumourigenesis in mouse skin. We also observed that dietary silibinin was well tolerated by mice in terms of food consumption and did not show the loss in body weight, and that topical and dietary silibinin treatments did not exert any adverse health effects in mice. Moreover, dietary silibinin also prevented the loss of body weight caused by UVB treatment in these mice.

5. Silibinin inhibits UVB-induced DNA damage in mouse skin

DNA damage is the foremost requirement for the initiation of photocarcinogenesis. In UV skin tumourigenesis, 80–90% of carcinogenic dose of sunlight is derived from UVB radiation [5]. UVB is known to directly interact with DNA forming a variety of photoproducts including thymine dimers, 6–4 photoproducts, cytosine photohydrates, DNA-DNA or DNA-protein cross-links and DNA strand breaks [5,7]. Moreover, the formation of thymine dimers is frequent and the most frequently studied form of UVB-induced DNA damage, and occurs three times more often than other photoproducts [51]. Cyclobutane/thymine dimers, if left unrepaired, cause CC to TT or C to T mutations after DNA replication, which have been frequently observed in the tumour suppressor *p53* gene in NMSC, actinic keratoses and sun/UV-damaged skin [5]. These studies suggest that inhibition of UVB-induced DNA damage by chemopreventive agents could reduce the incidence of skin cancer.

In animal studies, thymine dimer formation usually peaks at 1 h after UVB exposure, suggesting that the initiation step is quicker than the prolonged promotion and progression steps in photocarcinogenesis [52]. In our studies, silibinin showed strong inhibition of UVB-induced thymine dimer formation in SKH-1 hairless mouse skin epidermis as assessed by immunostaining. More importantly, the inhibitory effect of silibinin was almost similar irrespective of whether it was topically applied prior to or immediately after UVB exposure of mouse skin, accounting for 85% and 76% inhibition, respectively [53]. Further, we have observed that dietary

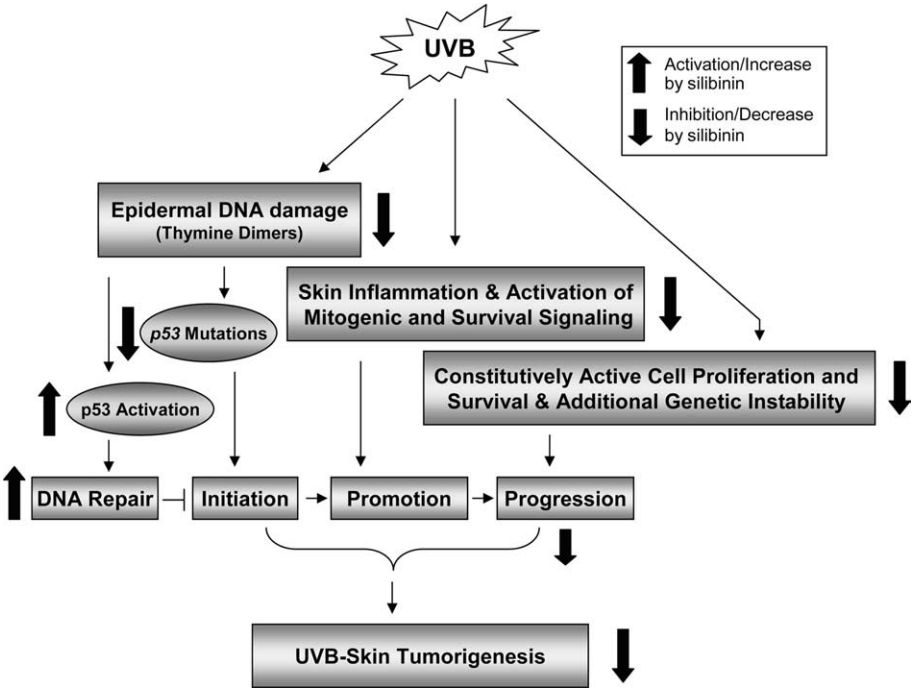

Fig. 2. Effect of silibinin on UVB-induced DNA damage, alterations in p53 and different stages of photocarcinogenesis. UVB causes DNA damage such as thymine dimer formation in mouse skin epidermal cells leading to mutations in tumour suppressor genes such as *p53* and/or oncogenes, which, if not repaired, can form 'initiated cells'. These cells can undergo through promotion and progression phases in response to UVB exposure involving many biochemical and molecular changes to form basal and squamous cell carcinoma of skin during photocarcinogenesis. The activation/increasing and inhibitory/decreasing effects of silibinin on different biochemical and biological events are indicated by upward and downward arrows, respectively.

fed silibinin also reduces the number of UVB-induced thymine dimer-positive cells in mouse skin epidermis [54]. These findings clearly suggest that silibinin interacts at the molecular level to prevent UVB-induced DNA damage in mouse skin and that could, in part, be responsible for the inhibitory effect of silibinin on UVB-induced skin tumour initiation (Fig. 2).

6. Silibinin inhibits skin inflammation

Skin inflammation is marked by the infiltration and accumulation of neutrophils in response to UV radiation and chemical tumour promoters [55]. Most of the studies on skin inflammation were done with silymarin where we observed strong inhibition of tumour promoter-induced arachidonic acid metabolism accompanied by inhibition of myeloperoxydase, lipoxygenase and cyclooxygenase activities in mouse skin [30]. Increased myeloperoxydase activity is associated with neutrophil infiltration, while increased metabolism of arachidonic acid via lipoxygenase and cyclooxygenase (COX) forming hydroxyeicosatetrenoic acid (HETE) and prostaglandin (PG) metabolites, is associated with skin inflammation and tumour promotion [56,57]. Many inflammatory agents and tumour promoters are known to activate inducible COX (COX-2) leading to formation of PG metabolites in mouse epidermis. Silymarin selectively inhibits TPA-induced COX-2 expression and activity without affecting COX-1 expression in mouse epidermis [30]. In additional studies, silymarin inhibited tumour promoter-induced expression of inflammatory cytokines TNFα and IL-1α in mouse epidermis [30,49]. In a short-term study, silymarin also inhibited the formation of UVB-caused cutaneous edema in mouse skin [27]. These findings showing inhibitory effects of silymarin on arachidonic acid metabolism and on the expression of inflammatory cytokines provide a rationale for its anti-inflammatory effect, which might also be linked to the inhibition of tumour promotion in skin tumourigenesis.

7. Silibinin inhibits UVB-induced epithelial cell proliferation and damage in skin

Basal cells in skin epidermis with sustained UV-signature mutation represent 'initiated cells' [58]. During tumour promotion, these cells undergo clonal expansion forming actinic keratoses in human or benign papillomas in mouse skin [5]. In animal experiments, UVB is known to cause increased cell proliferation in SKH-1 hairless mouse skin leading to epidermal hyperplasia [59]. Proliferating cell nuclear antigen (PCNA), a co-factor of DNA polymerase, is a widely accepted qualitative as well as quantitative molecular marker of cellular proliferation [60]. Our studies show that silibinin strongly inhibited UVB-induced PCNA-positive cells in mouse skin epidermis [53]. This inhibitory effect of silibinin on UVB-induced cell proliferation was observed in both acute and chronic UVB exposure protocols [29,53]. Furthermore, this antiproliferative effect on skin epithelial cells was evident after topical application of silibinin, pre- or post-UVB exposure, as well as in dietary feeding protocols [29,53]. In one of our acute UVB exposure studies, topical application of silibinin prior to or immediately after UVB exposure at 180 mJ/cm^2 resulted in up to 52% inhibition in UVB-induced epidermal cell proliferation after 1–12 h of UVB exposure in SKH-1 hairless mice [53]. These results suggest that inhibiting cell proliferation could be one of the mechanisms by which silibinin inhibits UVB-induced skin tumour promotion.

Consistent with its inhibitory effect on UVB-caused DNA damage, silibinin inhibits UVB-induced apoptotic and sunburn cell formation in mouse skin. In acute UVB exposure, silibinin treatment prior to or immediately after UVB exposure inhibited UVB-induced apoptosis, as observed by both TUNEL and H&E staining. This accounted for up to 70% decrease in UVB-induced apoptotic sunburn cell population [53]. UVB-mediated apoptosis involves caspase-dependent pathway [61]. Under similar condition and consistent with inhibition of skin damage pre- or post-treatment with silibinin, a remarkable decrease in the number of UVB-induced cleaved caspase 3-positive cells in mouse skin was shown [29,53]. Similar to the inhibitory effect of silibinin on UVB-caused sunburn and apoptotic cells formation in SKH-1 hairless mouse skin epidermis, in an earlier short-term study, silymarin also showed comparable responses [27]. Overall, these findings provide a solid indication of the preventive effects of silibinin on UVB-induced epidermal cell proliferation and skin damages, which could, in part, account for the chemopreventive efficacy of silibinin against photocarcinogenesis (Fig. 2).

8. Effect of silibinin on UVB-induced p53 activation

The tumour suppressor p53 is a tightly regulated protein, and known to play a crucial role in response to DNA damage or oncogene activation by arresting cell proliferation when the damage/stress is mild or by inducing apoptosis when the damage/stress is severe [62–64] (Figs. 2 and 5). One important cellular response occurring after UVB exposure or UVB-caused DNA damage is the phosphorylation of p53 at various serine sites that increases the half-life of p53 causing its stabilization and accumulation [59,64]. This is followed by an increase in nuclear level of p53 where it forms a homotetrameric complex, the active form of the transcription factor, that binds to target genes having p53 specific

DNA-binding sites and acts as transcriptional activator or suppressor [64–66]. Ubiquitylation and degradation by 26 s proteosome is another important post-translational modification that regulate the level of p53 [67]. Furthermore, p53 is highly mutated in its specific DNA binding core domain during the cancer development resulting in a full length inactive mutant p53 protein as observed in many different form of cancers including skin cancer [68]. Mutations in p53 gene have been observed in 50–60% of NMSC in humans, and the mutation sites are mostly similar to that induced by UVB in mouse skin as well as skin tumours [68]. These findings establish the critical role of p53 in initiation as well as subsequent progression of photocarcinogenesis.

Based on our results showing inhibition of UVB-caused DNA damage by silibinin, the role of p53 in preventive efficacy of silibinin against photocarcinogenesis was anticipated. Similar to earlier reports showing p53 accumulation in mouse epidermis following 8–12 h of UVB exposure [52], we also observed an increase in p53-positive cells in SKH-1 mouse skin after UVB exposure, which was further increased by pre- or post-topical application or dietary feeding of silibinin on mouse skin [29,53]. Silibinin also increased the level of p53 in UVB-induced tumours in long-term study [29]. In cell culture experiments, we have observed that silibinin increases UVB-induced p53 protein by serine 15 phosphorylation and stabilization in tumour promoter sensitive epidermal JB6 cells, which are associated with apoptotic cell death [69]. However, at lower UVB dose, silibinin protects HaCaT cells from under going apoptosis suggesting its role in cell cycle arrest for possible DNA repair [70]. This is in accord with the reports showing that the level of p53 accumulation depends on the extent of DNA damage and that lower levels of p53 correspond to cell cycle arrest [71]. In animal experiments, we have also observed inhibitory effect of silibinin on mutant p53 protein (unpublished data). In our ongoing studies, now we have focused our attention to dissect out the detail mechanisms of p53 regulation by silibinin in response to UVB and its biological significance in cell cycle regulation, apoptosis and DNA repair. Nonetheless, these findings suggest that p53 could be an important mediator of preventive efficacy of silibinin against photocarcinogenesis (Figs. 2 and 5).

9. Effect of silibinin on UVB-induced mitogenic signalling

Mitogen-activated protein kinases (MAPKs) are activated by a wide variety of extracellular stimuli, including UVB. They are important upstream regulators of many transcription factors including AP1, and control cellular events such as proliferation and apoptosis [72–74]. MAPKs are serine/threonine protein kinases and consist of extracellular signal-regulated protein kinase (ERK) 1/2, c-Jun amino-terminal kinase (JNK)1/2 and p38, which are activated individually or in tandem depending on the nature of the stimuli [72]. MAPKs signalling regulate the extent and nature of UVB radiation-induced cellular responses [74]. In this context, ERK1/2 activation causes both cell proliferation and survival, and JNK1/2 and p38 are known to exert both anti- and pro-apoptotic functions [72–74]. JNK1/2 and p38 also mediate various forms of cellular stress including damage repair mechanisms and cell growth arrest [72,75]. Overall, UV radiation-caused activation of MAPK family protein kinases and their roles in cell proliferation, cell cycle, apoptosis and cell survival regulation influencing UVB-induced tumourigenesis, have been well studied in recent years by many investigators (Fig. 3).

In an acute UVB exposure protocol, we observed that topical or dietary silibinin inhibited UVB-induced activation of ERK1/2, JNK1/2 and p38 signalling in SKH-1 hairless mouse skin [76]. However, in a chronic UVB exposure protocol, topical or dietary silibinin showed further increase in UVB-induced activation of ERK1/2, JNK1/2 and p38 kinases in tumour samples, but also inhibited activation of these molecules in chronically-UVB exposed non-tumour bearing skin [29]. Since ERK1/2 activation has been reported to inhibit cell cycle progression via cyclin-dependent kinase inhibitors (CDKIs) upregulation [77], and activation of JNK1/2 and p38 is widely reported to accompany apoptosis induction [75], our findings suggest dual roles of MAPK

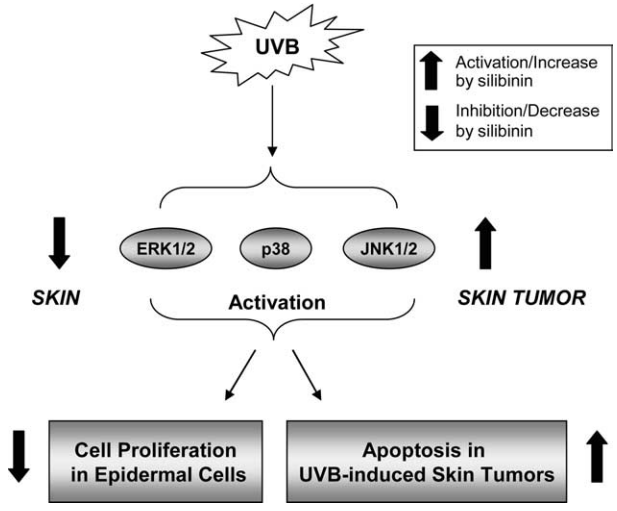

Fig. 3. Differential effects of silibinin on UVB-induced MAPKs signalling in normal skin and UVB-induced skin tumours. According to our proposed hypothesis, inhibition of UVB-induced MAPKs signalling in skin by silibinin would lead to its antiproliferative action; while its further activation in skin tumours would cause apoptotic death of tumour cells. The activating/increasing and inhibitory/decreasing effects of silibinin on different biochemical and biological events are indicated by upward and downward arrows, respectively. MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated protein kinase; JNK, c-Jun amino-terminal kinase.

pathways in silibinin efficacy against photocarcinogenesis (Fig. 3).

10. Effect of silibinin on UVB-induced apoptotic and survival signalling

Photocarcinogenesis involving DNA damage could generate enough stress to induce apoptotic deletion of the damaged cells through activation of apoptotic proteins such as Bax or by down-regulation of antiapoptotic proteins such as bcl-2 and cellular inhibitor of apoptosis in p53-dependent or -independent manner [66,78,79]. Apoptotic pathways are presumably activated when cellular response pathways are failed for DNA repair and cell survival [79,80]. Failure to trigger apoptosis in DNA damaged cells do not only predispose to the development of malignancies, but also increases resistance of growing tumours to irradiation and chemotherapeutic as well as chemopreventive agents [79]. UVB-induced apoptosis is mainly executed through the activation of intracellular caspases, which cause cleavage of substrates leading to final demise of the cell.

Our extensive studies with UVB irradiation show differential effects of silibinin on apoptosis induction in UVB-exposed mouse skin and UVB-induced mouse skin tumours [29,53]. In the former case, it inhibits apoptosis while in the latter it promotes apoptosis; and both these events most likely predispose for chemopreventive efficacy of silibinin against photocarcinogenesis. Our studies involving topical application of silymarin or both topical and dietary administration of silibinin show strong protection from acute exposure of UVB-caused sunburn and apoptotic cell formation in SKH-1 hairless mouse skin [27,53]. However, silibinin was found to promote apoptosis in mouse skin when mice were chronically exposed to UVB for 25 weeks [29]. Similarly, the number of apoptotic cells was increased following silibinin treatment in UVB-induced skin tumours [29]. These observations are based on both terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) and cleaved-caspase 3 immunostaining of uninvolved skin and skin tumours that showed 2–4-fold increase in apoptotic cells in silibinin plus UVB groups as compared to UVB group alone [29]. A thorough analysis of these findings engenders the suggestion that silibinin may act as sensor for apoptotic induction depending on levels of DNA damage by UVB exposure.

In addition to antiapoptosis, survival signalling plays a prominent role in the development of cancer. PI3K-Akt signalling is known to render the survival of tumour cells exquisitely responsive to levels of growth factors [81,82]. It has been observed that p53 also plays an important role in Akt-down regulation predisposing

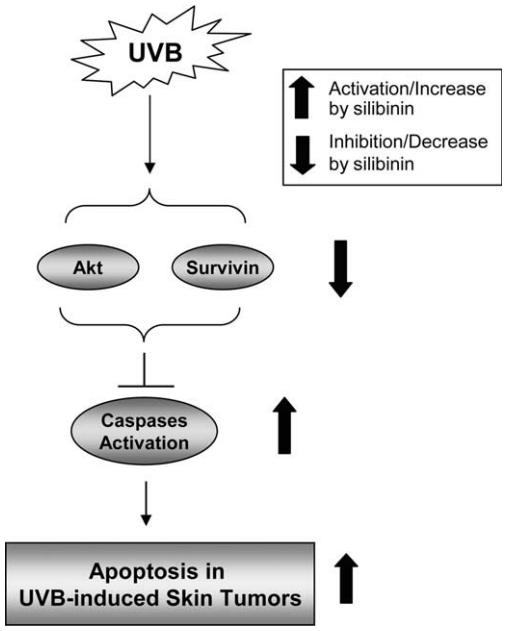

Fig. 4. Effect of silibinin on UVB-induced survival signalling in photocarcinogenesis. UVB activates Akt signalling and survivin for tumour cell survival, which are inhibited by silibinin for resulting in cellular apoptotic death through caspase-mediated pathway. The activating/increasing and inhibitory/decreasing effects of silibinin on different biochemical and biological events are indicated by upward and downward arrows, respectively.

cells to rapid apoptosis in response to a combination of cellular stress and decreased survival signals [83]. In a recent study, we have shown high levels of Akt (Ser473) phosphorylation in UVB-induced skin tumours, and almost complete down-regulation of Akt (ser473) activation in the tumours obtained from the silibinin-treated groups. This suggests the possible involvement of down-regulation of Akt signalling in apoptosis upregulation by silibinin, in its efficacy against photocarcinogenesis [29] (Fig. 4). Survivin, an inhibitor of apoptosis, is another molecule known to interact with caspases and suppress apoptosis (reviewed in [84,85]). Survivin expression in mouse skin is reported to prevent papilloma regression and promote tumour progression [86]. In our studies, silibinin decreased survivin level in UVB-induced tumours (Fig. 4), suggesting the involvement of multi-signalling pathways, including p53, Akt and survivin in apoptosis regulation by silibinin in photocarcinogenesis.

11. Effect of silibinin on UVB-induced cell cycle progression-related events

There is evidence that enhanced expression of cell cycle regulatory proteins such as cyclin-dependent kinases (CDKs) and cyclins, and/or decreased or loss of expression of CDKIs are causally involved in

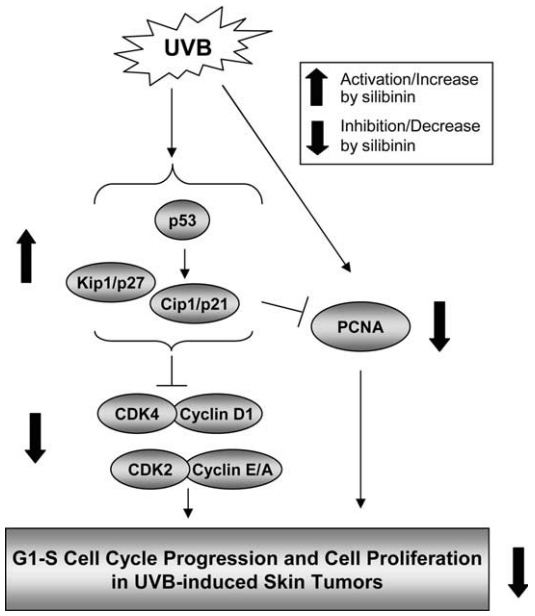

Fig. 5. Effect of silibinin on UVB-induced cell cycle regulatory proteins in photocarcinogenesis. UVB alters CDKI–CDK–cyclin as well as p53 and PCNA in favor of G1-S phase cell cycle progression and increased cell proliferation in tumour cells, which are modulated by silibinin to halt cell cycle progression as well as cell proliferation in its anti-photocarcinogenic efficacy. The activating/increasing and inhibitory/decreasing effects of silibinin on different biochemical and biological events are indicated by upward and downward arrows, respectively. CDK, cyclin-dependent kinase; CDKI, CDK inhibitor; PCNA, proliferation cell nuclear antigen.

photocarcinogenesis [62,87,88] (Fig. 5). Cell cycle regulation plays an important role in maintaining the genetic integrity of the cell, when it is challenged by biological or environmental genotoxins such as UVB radiation [62]. A prolonged G1 phase of the cell cycle is characteristic of UVB-induced DNA damaged cells, which allows cells enough time to repair DNA damage [62,65]. This is usually accompanied by an increase in CDKI, Cip1/p21 protein, the universal inhibitor of cell cycle progression, in p53-dependent or -independent manner, which plays a crucial role in the adaptive response after UVB exposure to prevent the progression of cells through the S phase of cell cycle and inhibit proliferation in DNA defective cells [78]. Therefore, it has been suggested that the agents causing increase in G1 arrest after UVB exposure could be helpful in allowing sufficient time to cells for the correction of UVB-induced DNA damage.

In an acute UVB exposure protocol, our studies showed that silibinin up-regulates UVB-induced level of Cip1/p21 protein in SKH-1 hairless mouse skin epidermis [53]. Cip1/p21 is also known to inhibit cell proliferation by direct binding with PCNA thereby inhibiting the expression of growth responsive genes [89]. As expected, silibinin also inhibited UVB-induced cell proliferation in skin epidermal cells [53]. Since silibinin caused similar increases in p53 protein and in Cip1/p21, there is a strong possibility that the increase by silibinin in UVB-activated p53–Cip1/p21-mediated events impact on its antiphotocarcinogenic efficacy.

In a chronic UVB exposure protocol, tumour tissues from the UVB group showed an increase in levels of cyclins A, E and D1 and CDK2; which were decreased when mice were treated with silibinin prior to or immediately following UVB, or fed in diet [29]. Silibinin also increased the protein levels of CDKIs, Cip1/p21 and Kip1/p27 in tumours, which are known to negatively regulate G1-S transition by inhibiting CDK–cyclin kinase activity [29,90]. Kip1/p27 is upregulated in response to antiproliferative signals [91]. Overall, silibinin-induced expression of Cip1/p21 and Kip1/p27 was accompanied by a decrease in CDK2, CDK4, cyclin A, cyclin E and cyclin D1 levels, and decreased CDK–cyclin kinase activity leading to cell cycle arrest in tumour cells. These findings suggest that inhibition of cell cycle progression with a concomitant decrease in cell proliferation might be one of the mechanisms of chemopreventive efficacy of silibinin against UVB-induced skin tumourigenesis (Fig. 5).

12. Silibinin accelerates repair of UVB-induced DNA damage in skin

One of the most important characteristics of UVB-caused carcinogenesis is DNA damage and mutagenesis, as exemplified by the formation of thymine dimers, better known as 'hot spots' of UVB mutagenesis [8,92]. The primary biological response to DNA damage in living cells is DNA repair. A dynamic equilibrium is maintained between the extent of DNA damage and the efficiency of DNA repair [92,93]. If the DNA repair system is faulty or inefficient, the mutation frequency increases leading to an enhanced susceptibility of cancer risk [94]. This phenomenon is best understood in a hereditary form of human disease called xeroderma pigmentosum (XP), in which DNA repair system (nucleotide excision repair) is defective with a striking increased risk of individuals to develop skin cancer upon exposure to sunlight.

In our study, silibinin treatment resulted in a remarkable decrease in UVB-caused thymine dimer-positive cells in mouse skin as early as 1 h after UVB irradiation [53]. We also observed an enhanced rate of decrease in thymine dimer-positive cells in presence of silibinin after a single UVB exposure in mouse skin (unpublished data). The decrease in DNA damage kinetics by silibinin suggests the possible involvement of the DNA repair system in the chemopreventive efficacy of silibinin against photocarcinogenesis. However, more studies are needed to investigate the effect of silibinin on DNA repair mechanisms such as nucleotide excision repair, base excision repair and mismatch repair, which operate in the presence of base damage caused by UVB radiation [92].

13. Conclusions

Plants and plant-products have been in use by humans throughout the world for centuries as traditional cure for various diseases including cancer. In recent times, many dietary and non-dietary phytochemicals are being investigated to explore and provide scientific basis for their possible anticancer effects at different levels in various models of carcinogenesis. Extensive studies with silibinin for its cancer chemopreventive efficacy suggest that it modulates many cellular, biochemical and molecular events causally liked with the development of cancer including photocarcinogenesis. The strong protective effect of silibinin against photocarcinogenesis is the corollary of favorable chemopreventive effects including; (a) inhibition of skin inflammation, DNA damage, epithelial cell proliferation and sunburn; (b) alteration of mitogenic, apoptotic and survival signalling; (c) activation of p53; (d) induction of cell cycle arrest; and (e) enhanced repair of DNA damage. These mechanism-based non-toxic photoprotective effects of silibinin form a genuine case supporting its clinical evaluation in high-risk human populations, and its further development as a skin cancer chemopreventive drug. In our current research program, studies are in progress to further define and explore the different molecular events involved in the prevention of skin cancer by silibinin.

Conflict of interest statement

None declared.

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References

1. Singh RP, Agarwal R. SENCAR mouse skin tumourigenesis model. In Teicher BA, ed. *Tumour models in cancer research*. Totowa (NJ), Humana Press, 2001. pp. 359–380.
2. Zoumpourlis V, Solakidi S, Papathoma A, *et al*. Alterations in signal transduction pathways implicated in tumour progression during multistage mouse skin carcinogenesis. *Carcinogenesis* 2003, **24**, 1159–1165.
3. Harris RB, Alberts DS. Strategies for skin cancer prevention. *Int J Dermatol* 2004, **43**, 243–251.
4. McKenzie R, Connor B, Bodeker G. Increased summertime UV radiation in New Zealand in response to ozone loss. *Science* 1999, **285**, 1709–1711.
5. Bowden GT. Prevention of non-melanoma skin cancer by targeting ultraviolet-B-light signalling. *Nat Rev Cancer* 2004, **4**, 23–35.
6. Gloster Jr HM, Brodland DG. The epidemiology of skin cancer. *Dermatol Surg* 1996, **22**, 217–226.
7. Matsui MS, DeLeo VA. Photocarcinogenesis by ultraviolet A and B. In Mukhtar H, ed. *Skin cancer: mechanisms and human relevance*. Boca Raton (FL), CRC Press, 1995. pp. 21–30.
8. Kanjilal S, Ananthaswamy HN. In Weber R, Miller M, Goepfert H, eds. *Basal and squamous cell skin cancers of the head and neck*. Baltimore, Williams and Wilkins, 1996. pp. 25–26.
9. Pourzand C, Tyrrell RM. Apoptosis, the role of oxidative stress and the example of solar UV radiation. *Photochem Photobiol* 1999, **70**, 380–390.
10. Ziegler A, Jonason AS, Leffell DJ, *et al*. Sunburn and p53 in the onset of skin cancer. *Nature* 1994, **372**, 773–776.
11. Gilchrest BA. A review of skin ageing and its medical therapy. *Br J Dermatol* 1996, **135**, 867–875.
12. Cruz JR, Leverkus PDM, Dougherty I, *et al*. Thymidine dinucleotides inhibit contact hypersensitivity and activate the gene for tumour necrosis factor alpha1. *J Invest Dermatol* 2000, **114**, 253–258.
13. Kulms D, Schwarz T. Molecular mechanisms of UV-induced apoptosis. *Photodermatol Photoimmunol Photomed* 2000, **16**, 195–201.
14. Matsumura Y, Ananthaswamy HN. Toxic effects of ultraviolet radiation on the skin. *Toxicol Appl Pharmacol* 2004, **195**, 298–308.
15. Chin L. The genetics of melanoma: lessons from mouse and man. *Nat Rev Cancer* 2003, **3**, 559–570.
16. Wolf P, Donawho CK, Kripke ML. Effect of sunscreens on UV radiation-induced enhancement of melanoma growth in mice. *J Nat Cancer Inst* 1994, **86**, 99–105.
17. Pinnell SR. Cutaneous photodamage, oxidative stress, and topical antioxidant protection. *J Am Acad Dermatol* 2003, **48**, 1–19.
18. Ames BN, Gold LS, Willett WC. The causes and prevention of cancer. *Proc Natl Acad Sci USA* 1995, **92**, 5258–5265.
19. Potter JD. Cancer prevention: epidemiology and experiment. *Cancer Lett* 1997, **114**, 7–9.
20. Sporn MB, Suh N. Chemoprevention: an essential approach in controlling cancer. *Nat Rev Cancer* 2002, **2**, 537–543.
21. Bickers DR, Athar M. Novel approaches to chemoprevention of skin cancer. *J Dermatol* 2000, **27**, 691–695.
22. Bode AM, Dong Z. Signal transduction pathways: targets for chemoprevention of skin cancer. *Lancet Oncol* 2000, **1**, 181–188.
23. Hong JT, Kim EJ, Ahn KS, *et al*. Inhibitory effect of glycolic acid on ultraviolet-induced skin tumourigenesis in SKH-1 hairless mice and its mechanism of action. *Mol Carcinog* 2001, **31**, 152–160.
24. Lu YP, Lou YR, Lin Y, *et al*. Inhibitory effects of orally administered green tea, black tea, and caffeine on skin carcinogenesis in mice previously treated with ultraviolet B light (high-risk mice): relationship to decreased tissue fat. *Cancer Res* 2001, **61**, 5002–5009.
25. Conney AH. Tailoring cancer chemoprevention regimens to the individual. *J Cell Biochem* 2004, **91**, 277–286.
26. Huang MT, Xie JG, Wang ZY, *et al*. Effects of tea, decaffeinated tea, and caffeine on UVB light-induced complete carcinogenesis in SKH-1 mice: demonstration of caffeine as a biologically important constituent of tea. *Cancer Res* 1997, **57**, 2623–2629.
27. Katiyar SK, Korman NJ, Mukhtar H, *et al*. Protective effects of silymarin against photocarcinogenesis in a mouse skin model. *J Natl Cancer Inst* 1997, **89**, 56–66.
28. Lu YP, Lou YR, Xie JG, *et al*. Topical applications of caffeine or (−)-epigallocatechin gallate (EGCG) inhibit carcinogenesis and selectively increase apoptosis in UVB-induced skin tumours in mice. *Proc Natl Acad Sci USA* 2002, **99**, 12455–12460.
29. Mallikarjuna G, Dhanalakshmi S, Singh RP, *et al*. Silibinin protects against photocarcinogenesis via modulation of cell cycle regulators, mitogen-activated protein kinases, and Akt signalling. *Cancer Res* 2004, **64**, 6349–6356.

30. Zhao J, Sharma Y, Agarwal R. A Flavonoid antioxidant, silymarin, affords significant inhibition against 12-*O*-tetradecanoylphorbol 13-acetate-caused modulation of antioxidant and inflammatory enzymes, and cyclooxygenase 2 and interlukin-1α expression in SENCAR mouse epidermis: Implications in the prevention of stage I tumour. *Mol Carcinog* 1999, **26**, 321–333.
31. Lahiri-Chatterjee M, Katiyar SK, Mohan RR, *et al*. A flavonoid antioxidant, silymarin, affords exceptionally high protection against tumour promotion in the SENCAR mouse skin tumourigenesis model. *Cancer Res* 1999, **59**, 622–632.
32. Singh RP, Tyagi AK, Zhao J, *et al*. Silymarin inhibits growth and causes regression of established skin tumours in SENCAR mice via modulation of mitogen-activated protein kinases and induction of apoptosis. *Carcinogenesis* 2002, **23**, 499–510.
33. Singh RP, Agarwal R. Flavonoid antioxidant silymarin and skin cancer. *Antioxid Redox Signal* 2002, **4**, 655–663.
34. Blumenthal M. Herbs sales down 3% in mass market retail stores: sales in natural food stores still growing, but at lower rate. *HerbalGram* 2000, **49**, 68.
35. Wagner H, Diesel P, Seitz M. Chemistry and analysis of silymarin from *Silybum marianum* (L) Gaertn. *Arzneimittelforsch* 1974, **24**, 466–471.
36. Wellington K, Jarwis B. Silymarin: a review of its clinical properties in the management of hepatic disorders. *BioDrugs* 2001, **15**, 465–489.
37. Trost W, Halbach G. Anti-phalloidine and anti-alpha-amanitine action of silybin in comparison with compounds similar to structural parts of silybin. *Experientia* 1978, **34**, 1051–1052.
38. Trinchet JC, Coste T, Levy VG, *et al*. Treatment of alcoholic hepatitis with silymarin. A double-blind comparative study in 116 patients. *Gastroenterol Clin Biol* 1989, **13**, 120–124.
39. Pares A, Planas R, Torres M, *et al*. Effects of silymarin in alcoholic patients with cirrhosis of the liver: results of a controlled, double-blind, randomized and multicenter trial. *J Hepatol* 1998, **28**, 615–621.
40. Vogel G, Trost W, Braatz R. Studies on the pharmacodynamics, including site and mode of action, of silymarin: the antihepatotoxic principle from *Silybum marianum* (L) Gaertn. *Arzneimittelforsch* 1975, **25**, 82–89.
41. Singh RP, Dhanalakshmi S, Tyagi AK, *et al*. Dietary feeding of silibinin inhibits advance human prostate carcinoma growth in athymic nude mice and increases plasma insulin-like growth factor-binding protein-3 levels. *Cancer Res* 2002, **62**, 3063–3069.
42. Agarwal C, Singh RP, Dhanalakshmi S, *et al*. Silibinin upregulates the expression of cyclin-dependent kinase inhibitors and causes cell cycle arrest and apoptosis in human colon carcinoma HT-29 cells. *Oncogene* 2003, **22**, 8271–8282.
43. Bhatia N, Zhao J, Wolf DM, *et al*. Inhibition of human carcinoma cell growth and DNA synthesis by silibinin, an active constituent of milk thistle: comparison with silymarin. *Cancer Lett* 1999, **147**, 77–84.
44. Singh RP, Agarwal R. Prostate cancer prevention by silibinin. *Curr Cancer Drug Targets* 2004, **4**, 1–11.
45. Tyagi AK, Agarwal C, Singh RP, *et al*. Silibinin down-regulates survivin protein and mRNA expression and causes caspases activation and apoptosis in human bladder transitional-cell papillomas RT4 cells. *Biochem Biophys Res Commun* 2003, **312**, 1178–1184.
46. Sharma G, Singh RP, Chan DC, *et al*. Silibinin induces growth inhibition and apoptotic cell death in human lung carcinoma cells. *Anticancer Res* 2003, **23**, 2649–2655.
47. Kohno H, Tanaka T, Kawabata K, *et al*. Silymarin, a naturally occurring polyphenolic antioxidant flavonoid, inhibits azoxymethane-induced colon carcinogenesis in male F344 rats. *Int J Cancer* 2002, **101**, 461–468.
48. Singh RP, Mallikarjuna GU, Sharma G, *et al*. Oral silibinin inhibits lung tumour growth in athymic nude mice and forms a novel chemocombination with doxorubicin targeting nuclear factor kappaB-mediated inducible chemoresistance. *Clin Cancer Res* 2004, **10**, 8641–8647.
49. Zi X, Mukhtar H, Agarwal R. Novel cancer chemopreventive effects of a flavonoid antioxidant silymarin: inhibition of mRNA expression of an endogenous tumour promoter TNFα. *Biochem Biophys Res Commun* 1997, **239**, 334–339.
50. Zhao J, Lahiri-Chatterjee M, Sharma Y, *et al*. Inhibitory effect of a flavonoid antioxidant silymarin on benzoyl peroxide-induced tumour promotion, oxidative stress and inflammatory responses in SENCAR mouse skin. *Carcinogenesis* 2000, **21**, 811–816.
51. Tornaletti S, Pfeifer GP. UV damage and repair mechanisms in mammalian cells. *Bioessays* 1996, **18**, 221–228.
52. Lu YP, Lou YR, Yen P, *et al*. Time course for early adaptive responses to ultraviolet B light in the epidermis of SKH-1 mice. *Cancer Res* 1999, **59**, 4591–4602.
53. Dhanalakshmi S, Mallikarjuna GU, Singh RP, *et al*. Silibinin prevents ultraviolet radiation-caused skin damages in SKH-1 hairless mice via a decrease in thymine dimer positive cells and an up-regulation of p53–p21/Cip1 in epidermis. *Carcinogenesis* 2004, **25**, 1459–1465.
54. Mallikarjuna G, Dhanalakshmi S, Singh RP, *et al*. Dietary feeding of silibinin prevents early biomarkers of ultraviolet B radiation-induced carcinogenesis in SKH-1 hairless mouse epidermis. *Cancer Epidemiol Biomark Prev* 2005, **14**, 1344–1349.
55. Mukhtar H, Agarwal R. Skin cancer chemoprevention. *J Invest Dermatol Sym Proc* 1996, **1**, 209–214.
56. Fischer SM, Slaga TJ. *Arachidonic acid metabolism and tumour promotion*. Boston, Martinus Nijhoff Publishing, 1985., pp. 1–263.
57. DiGiovanni J. Multistage carcinogenesis in mouse skin. *Pharmacol Ther* 1992, **54**, 63–128.
58. Li G, Ho VC. p53-dependent DNA repair and apoptosis respond differently to high- and low-dose ultraviolet radiation. *Br J Dermatol* 1998, **139**, 3–10.
59. Ouhtit A, Muller HK, Davis DW, *et al*. Temporal events in skin injury and the early adaptive responses in ultraviolet-irradiated mouse skin. *Am J Pathol* 2000, **156**, 201–207.
60. Ramzi ST, Maruno M, Khaskhely NM, *et al*. An assessment of the malignant potential of actinic keratoses and Bowen's disease: p53 and PCNA expression pattern correlate with the number of desmosomes. *J Dermatol* 2002, **29**, 562–572.
61. Mass P, Hoffmann K, Gambichler T, *et al*. Premature keratinocyte death and expression of marker proteins of apoptosis in human skin after UVB exposure. *Arch Dermatol Res* 2003, **295**, 71–79.
62. Liebermann DA, Hoffman B, Steinman RA. Molecular controls of growth arrest and apoptosis: p53-dependent and independent pathways. *Oncogene* 1995, **11**, 199–210.
63. Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. *Nature* 2000, **408**, 307–310.
64. Bode AM, Dong Z. Post-translational modification of p53 in tumourigenesis. *Nat Rev Cancer* 2004, **4**, 793–805.
65. Huang LC, Clarkin KC, Wahl GM. Sensitivity and selectivity of the DNA damage sensor responsible for activating p53-dependent G1 arrest. *Proc Natl Acad Sci USA* 1996, **93**, 4827–4832.
66. Mullauer L, Gruber P, Sebinger D, *et al*. Mutations in apoptosis genes: a pathogenetic factor for human disease. *Mutat Res* 2001, **488**, 211–231.
67. Fuchs SY, Adler V, Buschmann T, *et al*. Mdm2 association with p53 targets its ubiquitination. *Oncogene* 1998, **17**, 2543–2547.
68. Olivier M, Hussain SP, Caron de Fromentel C, *et al*. TP53 mutation spectra and load: a tool for generating hypotheses on the etiology of cancer. *IARC Sci Publ* 2004, **157**, 247–270.
69. Dhanalakshmi S, Agarwal C, Singh RP, *et al*. Silibinin up-regulates DNA-PK-dependent p53 activation to enhance UVB-

induced apoptosis in mouse epithelial JB6 cells. *J Biol Chem* 2005, **280**, 20375–20383.
70. Dhanalakshmi S, Mallikarjuna GU, Singh RP, *et al*. Dual-efficacy of silibinin in protecting or enhancing ultraviolet B radiation-caused apoptosis in human immortalized keratinocyte HaCaT cells. *Carcinogenesis* 2004, **25**, 99–106.
71. Chen X, Ko LJ, Jayaraman L, *et al*. p53 levels, functional domains, and DNA damage determine the extent of the apoptotic response of tumour cells. *Genes Dev* 1996, **10**, 2438–2451.
72. Seger R, Krebs EG. The MAPK signalling cascade. *FASEB J* 1995, **9**, 726–735.
73. Chen W, Bowden GT. Activation of p38 MAP kinase and ERK are required for ultraviolet-B induced c-fos gene expression in human keratinocytes. *Oncogene* 1999, **18**, 7469–7476.
74. Bode AM, Dong Z. Mitogen-activated protein kinase activation in UV-induced signal transduction. *Sci STKE* 2003, **2003**, 1–15.
75. Boldt S, Weidle UH, Kolch W. The role of MAPK pathways in the action of chemotherapeutic drugs. *Carcinogenesis* 2002, **23**, 1831–1838.
76. Mallikarjuna G, Dhanalakshmi S, Mohan S, *et al*. Silibinin inhibits ultraviolet B radiation-induced mitogenic and survival signalling, and associated biological responses in SKH-1 mouse skin. *Carcinogenesis*, 2005 April 14 [Epub ahead of print].
77. Cheng M, Sexl V, Sherr CJ, *et al*. Assembly of cyclin D-dependent kinase and titration of p27Kip1 regulated by mitogen-activated protein kinase kinase (MEK1). *Proc Natl Acad Sci USA* 1998, **95**, 1091–1096.
78. Ponten F, Berne B, Ren ZP, *et al*. Ultraviolet light induces expression of p53 and p21 in human skin: effect of sunscreen and constitutive p21 expression in skin appendages. *J Invest Dermatol* 1995, **105**, 402–406.
79. Okada H, Mak TW. Pathways of apoptotic and non-apoptotic death in tumour cells. *Nat Rev Cancer* 2004, **4**, 592–603.
80. Cotton J, Spandau DF. Ultraviolet B-radiation dose influences the induction of apoptosis, and p53 in human keratinocytes. *Radiat Res* 1997, **147**, 148–155.
81. Testa JR, Bellacosa A. AKT plays a central role in tumourigenesis. *Proc Natl Acad Sci USA* 2001, **98**, 10983–10985.
82. Gottlieb TM, Leal JF, Seger R, *et al*. Cross-talk between Akt, p53 and Mdm2: possible implications for the regulation of apoptosis. *Oncogene* 2002, **21**, 1299–1303.
83. Bachelder RE, Ribick MJ, Marchetti A, *et al*. p53 inhibits alpha 6 beta 4 integrin survival signalling by promoting the caspase 3-dependent cleavage of AKT/PKB. *J Cell Biol* 1999, **147**, 1063–1072.
84. Altieri DC. The molecular basis and potential role of survivin in cancer diagnosis and therapy. *Trends Mol Med* 2001, **7**, 542–547.
85. Altieri DC. Survivin, versatile modulation of cell division and apoptosis in cancer. *Oncogene* 2003, **22**, 8581–8589.
86. Allen SM, Florell SR, Hanks AN, *et al*. Survivin expression in mouse skin prevents papilloma regression and promotes chemical-induced tumour progression. *Cancer Res* 2003, **63**, 567–572.
87. Morgan DO. Principles of CDK regulation. *Nature* 1995, **374**, 131–134.
88. Sherr CJ, Roberts JM. CDK inhibitors: positive and negative regulators of G1-phase progression. *Genes Dev* 1999, **13**, 1501–1512.
89. Ducoux M, Urbach S, Baldacci G, *et al*. Mediation of proliferating cell nuclear antigen (PCNA)-dependent DNA replication through a conserved p21(Cip1)-like PCNA-binding motif present in the third subunit of human DNA polymerase delta. *J Biol Chem* 2001, **276**, 49258–49266.
90. Fotedar R, Bendjennat M, Fotedar A. Role of p21WAF1 in the cellular response to UV. *Cell Cycle* 2004, **3**, 134–137.
91. Polyak K, Kato JY, Solomon MJ, *et al*. p27Kip1, a cyclin–Cdk inhibitor, links transforming growth factor-beta and contact inhibition to cell cycle arrest. *Genes Dev* 1994, **8**, 9–22.
92. Friedberg EC. How nucleotide excision repair protects against cancer. *Nat Rev Cancer* 2001, **1**, 22–33.
93. Smith ML, Fornace Jr AJ. p53-mediated protective responses to UV irradiation. *Proc Natl Acad Sci USA* 1997, **94**, 12255–12257.
94. Kunkel TA, Bebenek K. DNA replication fidelity. *Annu Rev Biochem* 2000, **69**, 497–529.