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Review

Flavonoid Antioxidant Silymarin and Skin Cancer

RANA P. SINGH and RAJESH AGARWAL

ABSTRACT

Oxidative stress is one of the key players in skin carcinogenesis, and therefore identifying nontoxic strong antioxidants to prevent skin cancer is an important area of research. In both animal and cell culture studies, we have shown that silymarin, a naturally occurring polyphenolic flavonoid antioxidant, exhibits preventive and anticancer effects against skin cancer. For example, silymarin strongly prevents both photocarcinogenesis and skin tumor promotion in mice, in part, by scavenging free radicals and reactive oxygen species and strengthening the antioxidant system. We also found that this effect of silymarin is by inhibiting endogenous tumor promoter tumor necrosis factor α in mouse skin, a central mediator in skin tumor promotion. In mechanistic studies, silymarin inhibits mitogenic and cell survival signaling and induces apoptosis. Furthermore, silymarin effectively modulates cell-cycle regulators and check points toward inhibition of proliferation, and growth arrest in G0–G1 and G2–M phases of the cell cycle. Thus, due to its mechanism-based chemopreventive and anticancer effects in experimental models, silymarin is an important candidate for the prevention and/or therapy of skin cancer, as well as other cancers of epithelial origin in humans. *Antioxid. Redox Signal.* 4, 655–663.

INTRODUCTION

Source and chemical composition of silymarin

SILYMARIN is present in the black shiny seeds (fruit) of milk thistle plant [*Silybum marianum* (L.) Gaertner., Family Asteraceae], which is also known as Mary thistle, St. Mary thistle, marian thistle, lady's thistle, and hold thistle (46). It is indigenous to the Mediterranean region, southwest Europe, South America, and Australia, and also naturalized in North America, especially California.

Silymarin, a flavanolignan complex, was first isolated from the milk thistle seeds in 1968. Silymarin constitutes 4–6% of ripe fruits and primarily consists of three flavanolignans, silybin (silibinin), silychristin (silichristin), and silidianin (74). Silibinin is the most active and abundant constituent present in silymarin (Fig. 1). Other flavanolignans present in lesser proportion are dehydrosilybin, 3-desoxysilichristin, deoxysilydianin (silymonin), siliandrin, silybinome, silyhermin, and neosilyhermin (75). Apart from flavanolignans, the other constituents include apigenin, silybonol [a fixed oil (16–18%), consisting largely of linoleic and oleic acids, plus myristic, palmitic, and stearic acids], betaine hydrochloride, triamine, and histamine (46, 74, 75).

Pharmacological background

Milk thistle seeds have been used in traditional medicine continuously for 2,000 years for liver conditions (first mentioned by Pliny in the 1st century). Eclectic physicians in 19th century America used seeds against liver complications. Reinvestigation of the value of milk thistle in modern practice began with H. Schulz in 1929 and G. Madaus in 1938. Milk thistle extract is also commercially available as crude, ethanolic extracts, tablets, or capsules standardized to 70% silymarin (as silibinin), and marketed as a dietary supplement in the U.S. and Europe. Even in large doses, silymarin does not show any toxic effects and in particular has no harmful effects on the embryo (32, 46, 75). Silymarin is primarily known for its hepatoprotective and antioxidant activity, which has been demonstrated in numerous experimental

Department of Pharmaceutical Sciences, School of Pharmacy, University of Colorado Health Sciences Center, Denver, CO 80262.

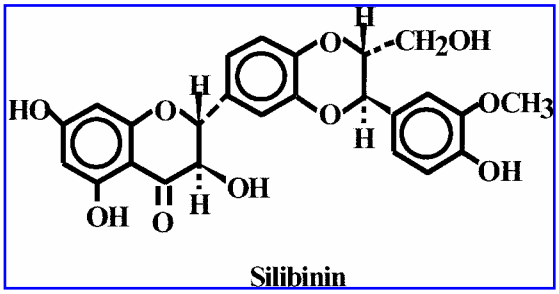

FIG. 1. Chemical structure of silibinin, the most abundant and active component of silymarin.

models of toxic liver damage, as well as clinical studies, including carbon tetrachloride, galactosamine, thioacetamide, and the toxins such as phalloidin and α-amanitin of *Amanita phalloides* (deathcap fungus) (12, 43, 73). Silymarin shows strong free radical-scavenging activity that is severalfold greater than that of vitamin E (72). Recently, our laboratory has established its effectiveness against skin cancer by using different mouse skin carcinogenesis models and cell-culture studies (Table1), which are discussed in detail in the following sections.

SILYMARIN AND ULTRAVIOLET (UV) SKIN CARCINOGENESIS

UV solar radiation has long been known as a ubiquitous risk factor for basal cell and squamous cell carcinomas (nonmelanoma skin cancer), which account for more than a million cases of skin cancer in the U.S.A. (2, 6). UV radiation is genotoxic and causes DNA damage, and depletion and impairment of the cellular antioxidant system, which is generally dependent on the dose and time of UV exposure. On the molecular level, UVA (320-400 nm) and UVB (290–320 nm) cause different types of potentially premutagenic DNA lesions, some of which may lead to malignant transformation (15, 55). Therefore, 290–400 nm UV wavelength possesses immense potential of initiation, as well as promotion, of skin carcinogenesis.

Intracellular chromophores such as nicotinamide coenzymes or riboflavin absorb UVA radiation, resulting into the generation of reactive oxygen species (ROS), which cause strand breaks and oxidation of bases in DNA, and lesions in cellular lipids and proteins (18, 21, 64). UVB specifically creates cyclobutane pyrimidine dimers and [6–4] photoproducts (covalent bonds between adjacent pyrimidine of the same DNA strand) in DNA (Fig. 2) (65). Cellular antioxidant status plays an important role in modulating the effects of unrepaired DNA lesions and cellular sensitivity to the DNA-damaging effects of solar UV radiation. Wild-type p53 protein also provides protection against UV mutagenesis by inducing G1 arrest and providing additional time for the cell to repair damaged DNA or to promote apoptosis (Fig. 2) (36, 45). It has been reported that UVA decreases intracellular glutathione status and subsequently increases UVA sensitivity of keratinocytes (5). The DNA-damaging effect of UVA

TABLE 1. SUMMARY OF THE EFFECTS OF SILYMARIN ON BIOLOGICAL RESPONSES ASSOCIATED WITH SKIN CARCINOGENESIS IN ANIMAL AND CELL CULTURE STUDIES

Biological response	*Effect of silymarin*
Tumor initiation (UVB)	Inhibited
Tumor promotion (UVB, phorbol esters, and non-phorbol esters)	Inhibited
Complete carcinogenesis (UVB)	Inhibited
Skin edema and hyperplasia	Inhibited
TNFα expression	Inhibited
IL-1α expression	Inhibited
ODC expression and activity	Inhibited
COX-2 expression and activity	Inhibited
Lipoxygenase activity	Inhibited
EGFR activation	Inhibited
Shc activation	Inhibited
Shc binding to EGFR	Inhibited
MAPK/ERK1/2 activation	Inhibited
SAPK/JNK1 activation	Induced
Cip1/p21 and kip1/p27	Induced
CDK 1, 2, and 6	Inhibited
Cyclin A, B, E, and D1	Inhibited
CDKI/CDK binding	Inhibited
CDK and cyclin kinase activities	Inhibited
Cell proliferation	Inhibited
Cell-cycle progression	Arrested
Apoptosis	Induced

See text for abbreviations.

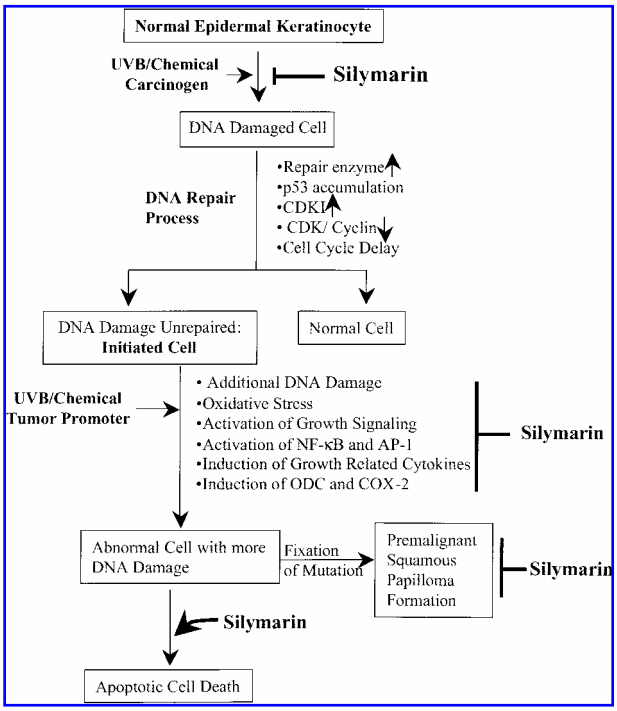

FIG. 2. Mechanistic basis of protective effect of silymarin on skin tumor initiation and promotion induced by UV radiation and chemical carcinogen/promoters. See text for abbreviations.

can be reduced by improving the regulation of intracellular antioxidant status by suitable antioxidants (5).

Our studies have shown a strong protective effect of silymarin in short-term, as well as long-term, UVB-induced skin carcinogenesis in the mouse model. In long-term studies, topical application of silymarin inhibited tumor initiation, promotion, and complete carcinogenesis induced by UVB radiation in SKH-1 hairless mouse skin (38). In the promotion stage, application of silymarin prior to each UVB exposure increased the latency period of tumor appearance; this effect was more profound in UVB complete carcinogenesis protocol in which UVB was used as both initiator and promoter of skin carcinogenesis. Furthermore, the strong protection provided by silymarin against UVB radiation was evident by the drastic decrease in tumor incidence, tumor multiplicity and tumor volume in silymarin-treated groups in all three protocols (38). The important observation was that silymarin did not show any sign of toxicity in terms of loss of body weight or mortality. In short-term study, silymarin inhibited the formation of UVB-caused sunburn cells, apoptotic cells, and cutaneous edema (38).

Some of the biochemical and molecular events associated with UVB-induced tumor promotion (Fig. 2) include generation of free radicals, ROS, depletion of antioxidant systems, acute inflammation, induction of cyclooxygenase (COX), and increased expression and activation of ornithine decarboxylase (ODC), in which oxidative stress is regarded as a major contributor in tumor development (Fig. 2) (1, 49, 58, 69). We have shown that silymarin inhibits lipid peroxidation and provides significant protection against UVB-induced depletion of catalase activity. Therefore, silymarin can effectively terminate the harmful biochemical reactions by scavenging free radicals and ROS, and by strengthening the cellular antioxidant status. Silymarin also inhibits UVB-caused induction of COX and ODC activity, and ODC mRNA expression, which provides an insight into the molecular mechanisms of the protective effect of silymarin against UV skin carcinogenesis.

SILYMARIN AND CHEMICAL SKIN CARCINOGENESIS

Carcinogenesis has been demonstrated by experimental and epidemiological studies to be a multifactorial, multigenic, and multiphasic process composed of three major sequential stages, namely, initiation, promotion, and progression (50). A single exposure of carcinogenic agent, *viz.,* 7, 12-dimethylbenz(a)anthracene (DMBA), to epidermal cells may result in a small subset of initiating cells carrying irreversible mutation in critical gene(s), *i.e.,* proto-oncogenes and tumor suppressor genes controlling normal cellular growth and differentiation (8, 50, 80). In the promotion stage, repeated applications of promoters such as phorbol esters that are generally nonmutagenic bring about many important epigenetic alterations in initiated cells facilitating the clonal expansion of initiated phenotype, leading to the formation of benign tumors or papillomas. The early stage of promotion is reversible, whereas promotion in late stage and progression represent the irreversible phases of the carcinogenesis process (50).

Anti-tumor-promoting effect of silymarin in chemical skin carcinogenesis

Most of the antioxidants have been reported to have efficacy against tumor promotion, as oxidative stress is suggested to be an obligate event in tumor promotion (26, 48, 77). In this regard, silymarin, a polyphenolic flavonoid antioxidant, has shown promising results as a chemopreventive and/or therapeutic agent in various carcinogenesis models (16). We have shown that silymarin inhibits both stage I and stage II of tumor promotion in the DMBA-TPA (12-*O*-tetradecanoylphorbol 13-acetate) and DMBA-MEZ (mezerein) SENCAR mouse skin carcinogenesis model, respectively (16). The protective effect of silymarin against stage I tumor promotion was found to be dose-dependent, accompanied by a prolonged latency period of tumor development and strong inhibition of tumor incidence, tumor multiplicity, and tumor volume throughout the experiment (accounting for 75, 97, and 96% inhibition, respectively, at the termination of experiment) (16). A more profound protective effect was observed in stage II tumor promotion, and complete stage I and II tumor promotion protocols in which both TPA and MEZ were used as stage-specific tumor promoter in DMBA-initiated mouse skin (16). In another chemical skin carcinogenesis study, in which free radical generating tumor promoter, benzoyl peroxide, was used on DMBA-initiated mouse skin, silymarin showed strong anti-tumor-promoting effects similar to the DMBA-TPA protocol (82). Similarly, silymarin showed complete protection against a non-phorbol ester tumor promoter (okadaic acid)-caused tumor promotion in DMBA-initiated SENCAR mouse skin (83). In another study, silymarin almost completely inhibited TPA-caused skin edema and induction of epidermal hyperplasia in SENCAR mice. More interestingly, the preapplication of silymarin before TPA did not show any appreciable increase in skin edema or epidermal hyperplasia even after TPA was applied two or three times (16). On the molecular level, these results are supported by the facts that silymarin decreases PCNA (proliferation cell nuclear antigen)-positive cells in TPA-induced epidermal proliferation in mouse skin (16), and in a cell culture study inhibits [methyl-^{3}H]thymidine incorporation (DNA synthesis) in human epidermoid carcinoma A431 cells (16).

Phorbol esters have been shown to cause oxidative stress as one of the mechanisms of tumor promotion. The oxidative stress condition, if not eliminated, leads to generation of free radicals and ROS, which attack DNA, protein, and lipid-rich membranes (57). The process of lipid peroxidation in biological membranes is a useful system to evaluate the oxidant/antioxidant activity of endogenous, as well as xenobiotic, agents (31, 34). In this regard, our recent studies show that silymarin inhibits malondialdehyde formation in epidermal microsome in a dose-dependent manner. Similarly, silymarin strongly inhibits TPA-caused lipid peroxidation in mouse skin epidermis (16), supporting its strong *in vivo* antioxidant activity and suggesting that it could be one of the possible mechanisms of its action against skin tumor promotion. Together, these finding suggested that silymarin could be useful in preventing a wide range of carcinogen and tumor promoter-induced skin cancer. Other mechanistic rationales of anticancer action of silymarin are discussed separately in the following sections.

MECHANISTIC STUDIES IN MICE

In our ongoing investigations, we have focused our efforts to explore the mechanistic basis of the anti-tumor-promoting activity of silymarin in the mouse skin carcinogenesis model. The biochemical and molecular events studied are the expression and/or activity of antioxidant and inflammatory enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), lipoxygenase, and COX, and cytokines such as tumor necrosis factor α (TNFα) and interleukin 1α (IL-1α), which play important roles in skin tumorigenesis (Fig. 2).

Silymarin protects against oxidative stress

Oxidative stress is one of the major contributors in skin tumor promotion. The increase in free radicals and highly reactive oxygen species (ROS) such as superoxide anion radical, hydroxyl radical, and peroxyl radical, alkoxyl radical, hydroperoxyl radical, hydrogen peroxide leads to oxidative stress that can either directly or indirectly modify a number of biologically important molecules, causing various diseases, including skin cancer (28, 78). It has been shown that exposure of mouse or human skin or derived epidermal keratinocytes to tumor promoters generates a strong oxidative stress and down-regulates the antioxidant system (1, 14). In oxidative stress, superoxide anions are formed by transfer of a single electron to oxygen. If these are not scavenged by the SOD-catalase/GPx system (via reduced glutathione/oxidized glutathione redox cycle), it results in the formation of hydroxyl radicals via the Fe^{2+}/Fe^{3+}/lactoferrin reaction, which damages critical cellular macromolecules (40, 41). Our studies show that silymarin strongly inhibits TPA-caused depletion of epidermal enzyme activities of SOD, catalase, and GPx (81). The observed protective effect of silymarin on TPA-caused depletion of antioxidant enzyme activity was greater on SOD followed by catalase and GPx (81). The profound effect of silymarin in reducing the oxidative stress can shift the equilibrium of carcinogen metabolism, gene expression, and enzyme activity toward the inhibition of the process of skin carcinogenesis.

Inhibitory effect of silymarin on myeloperoxidase, lipoxygenase, and COX

In tumor promotion, neutrophil infiltration has been observed in response to application of tumor promoters on mouse skin. The infiltration and accumulation of neutrophil are characteristic features of TPA- and UV radiation-caused skin inflammation, and are also used to measure the intensity of skin inflammation (9, 70). Silymarin shows a strong ability to complete inhibition of the TPA-caused increase in myeloperoxidase activity (81), which is closely associated with neutrophil infiltration. The TPA-caused skin inflammation is mediated by increased metabolism of arachidonic acid, induced by lipoxygenase and COX, ultimately leading to the forma tion of hydroxyeicosatetraenoic acid (HETE) and prostaglandin (PG) metabolites (1, 24, 27). Recently, constitutive expression of 8- lipoxygenase was shown in skin papillomas. This provides the evidence that 8-lipoxygenase catalyzed arachidonic acid metabolite 8-HETE plays an important role in tumor promotion (10). Consistent with this, silymarin inhibits the TPA-caused increase in lipoxygenase activity in terms of 8-HETE formation in mouse skin (81).

COX, like lipoxygenase, also plays a critical role in skin inflammation, cell proliferation, and skin tumor promotion (27, 30, 37). More recently, it has been reported that TPA-caused induction of COX activity is only due to constitutive overexpression of inducible COX (COX-2), in mouse epidermal tumors (51). The elevated level of PGE2 has been associated with increased expression of COX-2, when mouse epidermis is exposed to TPA. Silymarin inhibits both TPA-caused COX-2 expression, and COX activity in terms of PGE_2, $PGF_{2\alpha}$, and PGD_2 formation in mouse epidermis (81). The inhibition of TPA-induced COX-2 expression by silymarin is selective as it does not alter COX-1 (constitutive COX) expression, and also does not show any effect on both COX-1 and COX-2 levels, when applied alone on mouse epidermis. Therefore, our findings suggest that silymarin could be explored as a cancer preventive agent, targeted toward COX-2 modulation in epithelial cancers.

Silymarin inhibits expression of cytokines TNFα and IL-1α

Cytokines such as TNFα and IL-1α have been shown to be associated with skin inflammation and tumor promotion (Fig. 3) (29, 60). The induction of TNFα, an endogenous tumor promoter, is one of the common mechanisms of tumor promotion that mediates the effect of phorbol ester (*e.g.,* TPA) as well as non-phorbol ester (*e.g.,* okadaic acid) tumor promoters. It has been suggested that inhibition of TNFα mRNA expression and its release can play an important role in cancer chemoprevention (71 and references therein). We have shown that silymarin causes exceptionally high to complete inhibition of TPA- and okadaic acid-induced expression of TNFα mRNA in a dose-dependent manner, in SENCAR mouse skin (83).

A single topical application of TPA on mouse skin induces high expression of IL-1α mRNA (42, 60). Consistent with inhibition of skin inflammation and tumor promotion, silymarin strongly inhibits the TPA-caused increase in IL-1α mRNA expression and corresponding IL-1α protein level in mouse skin (81). The finding that silymarin inhibits the expression of IL-1α, as well as TNFα, provides a reliable mechanistic basis for antiinflammatory and anti-tumor-promoting activities of silymarin against skin tumorigenesis.

MECHANISTIC STUDIES IN CELL CULTURE

Inhibitory effects of silymarin on mitogenic and cell survival signaling

In cancer cells, enhanced tyrosine kinase activity due to overexpression of receptor and/or protein tyrosine kinases leads to constitutive mitogenic and cell survival signaling, resulting in uncontrolled growth of tumor cells (44). Aberrant expression of the erbB family of receptor tyrosine kinases has

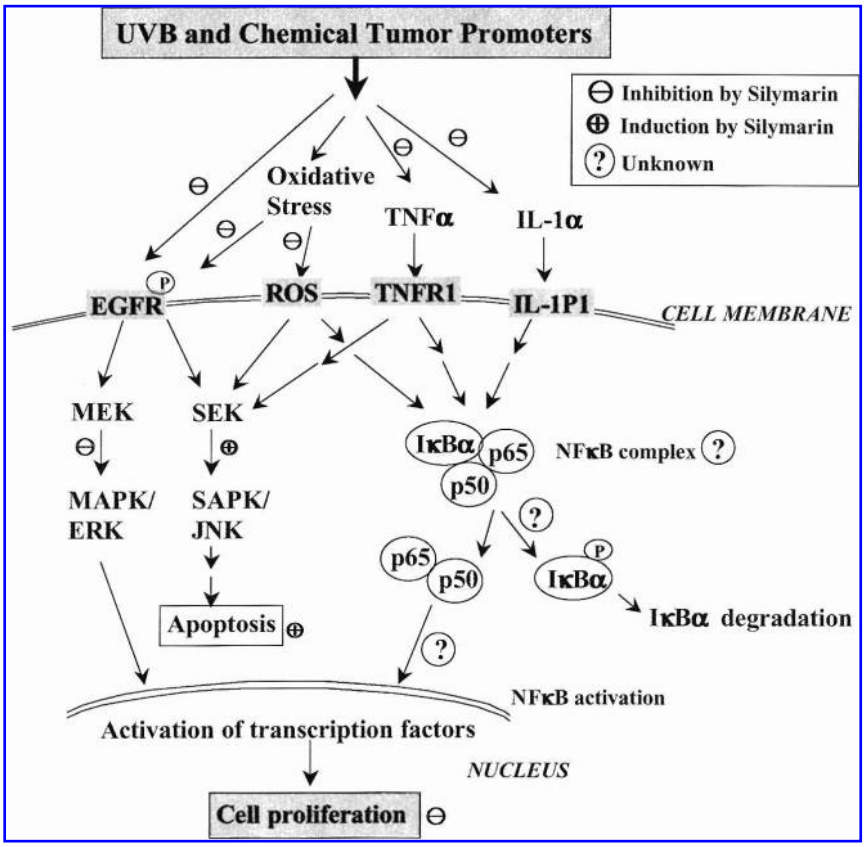

FIG. 3. Proposed mechanisms of antiproliferative and apoptotic activities of silymarin against UVB and chemical skin tumor promotion. See text for details.

been implicated in several human malignancies including skin cancer (35, 39, 44). Different skin tumor promoters, such as TPA, okadaic acid, chrysarobin, and as UVB radiation, have been shown to activate epidermal growth factor receptor (EGFR) in mouse skin (23, 25, 56, 76). This signaling pathway plays an important role in oncogenesis, and is activated by oxidative stress, which has been implicated in skin tumor promotion (Fig. 3) (11, 34, 66). Therefore, identification of potential agents that can inhibit the tyrosine phosphorylation of EGFR and its intrinsic kinase activity has emerged as a novel approach to control various diseases, including skin cancer (11, 34, 66). Our recent studies have shown that silymarin inhibits both the ligand activation of receptor tyrosine kinase EGFR and its intrinsic kinase activity, and subsequently inhibits the activation of an immediate downstream target Shc, an adaptor protein containing src homology-2 (SH-2) domain (3). Following tyrosine phosphorylation, Shc acts as an adaptor for other SH-2-containing proteins in cell signaling involving the Grb2-SOS-ras-raf pathway (13, 47, 52). This pathway, as well as phosphatidylinositol 3-kinase and phospholipase Cγ pathways, ultimately activates mitogen-activated protein kinase (MAPK), leading to activation of various transcription factors for cell growth and proliferation (Fig. 3) (13, 22, 47, 52).

MAPK/extracellular signal-regulated kinase (ERK) 1/2 are essential elements of mitogenic cell signaling and are constitutively active in various cancers, including skin cancer (20, 53). Interestingly, silymarin inhibited the epidermal growth factor-induced activation of ERK1/2 in starved A431 cells only at lower doses (50–75 μg/ml). The higher doses of silymarin did not show any effect on ERK1/2 activation; instead it activated c-Jun amino-terminal kinase (JNK) signaling as an apoptotic effect of silymarin (Fig. 3) (84). Therefore, the inhibitory effect of silymarin on the activation of EGFR, Shc, and ERK1/2 indicates the signaling pathways targeted by silymarin in skin cancer prevention.

Apoptotic effect of silymarin on skin cancer cells

In cell growth studies, silymarin has shown dose- and time-dependent death accompanied by growth inhibition in human epidermoid carcinoma A431 cells, as well as other cancer cells of epithelial origin (7, 84). Quantitative and qualitative analyses of cell death show that apoptosis is a major contributor in cell death caused by silymarin at higher doses. FACS analysis of fluorescein isothiocyanate staining shows that a 75–150 μg/ml dose of silymarin causes 48–78% apoptotic cell death (84). In a DNA-ladder study, silymarin has shown a dose-dependent increase in DNA fragmentation in mouse skin papilloma SP1 and PA cells (unpublished observation). The MAPK family members, JNK/stress-activated protein kinase (SAPK) are generally activated by environ-

mental stress and contribute to cell death (Fig. 3) (17, 19, 61). Antioxidants have also been shown to activate JNK signaling, thereby contributing to apoptotic cell death. In recent years, it has been suggested that *trans*-acting DNA binding proteins, such as members of the Jun family, are necessary for apoptosis in some models (59). Consistent with these notions, we observed that silymarin increases both JNK1 phosphorylation and its kinase activity at higher doses, corresponding to its apoptotic effect in A431 cells. The kinetics of JNK activation by silymarin was similar to those described for chemopreventive and chemotherapeutic agents causing apoptotic cell death, such as green tea polyphenols and paclitaxel (4, 79). These findings suggest that the apoptotic efficacy of silymarin can be used in the prevention and therapy of skin cancer.

Silymarin modulates cell-cycle regulation

In mammalian cell-cycle progression, particularly those events that control the progression of quiescent cells through G1, and S phase through G2-M transition, are the targets for alteration during development of neoplasm. Cell-cycle progression is regulated by the combinations of cyclins and cyclin-dependent kinases (CDKs) that form complexes at specific phases of the cell cycle (67 and references therein). G1 cyclin-CDK complexes phosphorylate the retinoblastoma gene product, pRb, which has been implicated in the proliferation-regulation mechanism in keratinocytes and other cell types (33). G2 cyclin-CDK complexes activate the transcription factors responsible for driving the cell into mitosis. In mouse skin tumor promotion, cyclins and E2F family proteins are overexpressed, whereas levels of CDKs $p21^{Cip1}$ and $p27^{Kip1}$ remain constant (63, 68). Cyclin D1, which is involved in cell-cycle progression, has been shown to be overexpressed in mouse skin papillomas and carcinomas (62).

Our studies (Fig. 4) reveal that silymarin inhibits the expression of cyclin A, B, D1, and E, and CDK1, 2, and 6, and decreases the kinase activity associated with CDK1, 2, and 6, and cyclin D1 and E in human epidermoid carcinoma A431 cells (84). Silymarin also increases the protein expression of $p21^{Cip1}$ and $p27^{Kip1}$ and their binding with CDK1, 2, and 6 (84). These molecular effects of silymarin on cell-cycle regulatory molecules result in the accumulation of A431 cells in the G2-M phase of the cell cycle at lesser treatment time (12 h). Interestingly, longer treatment (24 h) with silymarin shows both G0-G1 and G2-M arrest at the expense of the S-phase cell population (84). More detailed mechanistic studies with silymarin are in progress to establish the role and correlation of inhibition/induction of cyclins, CDKs, CDK inhibitors (CDKIs), and associated proteins in premalignant progression.

CONCLUSION AND FUTURE PROSPECTS

Epidemiological studies followed by laboratory studies and vice versa have suggested that consumption of fruits, vegetables, and food products containing naturally occurring polyphenols are beneficial in reducing the risk of cancer. To date, a diversified class of compounds, such as antiinflammatory agents, antioxidants, flavones, phenols, COX and lipoxygenase inhibitors, protein kinase C inhibitors, and retinoids, have been tested and used for chemoprevention and/or therapeutic intervention of various cancers, including skin cancer. This suggests that development of cancer is multiphasic, multigenic, and multifactorial. Therefore, for the control or therapy of cancer, the effectiveness of an agent depends on (a) how many cellular, biochemical, and molecular events are modulated by the agent, (b) how important these events are in causing cancer, (c) to what extent these events are being modulated by that particular agent, and (d) finally, how less toxic is that agent to the normal tissues. Silymarin strongly modulates a large number of critical events implicated in skin carcinogenesis, affording prevention and/or therapy of cancer. Therefore, due to its strong pleiotropic anticancer effect without any apparent signs of toxicity, silymarin could be regarded as one of the best available chemopreventive and/or interventive agents against skin cancer. More, detailed preclinical studies followed by clinical trials, however, are warranted in support of this conclusion. Such studies are in progress in our ongoing program on "silymarin and skin cancer."

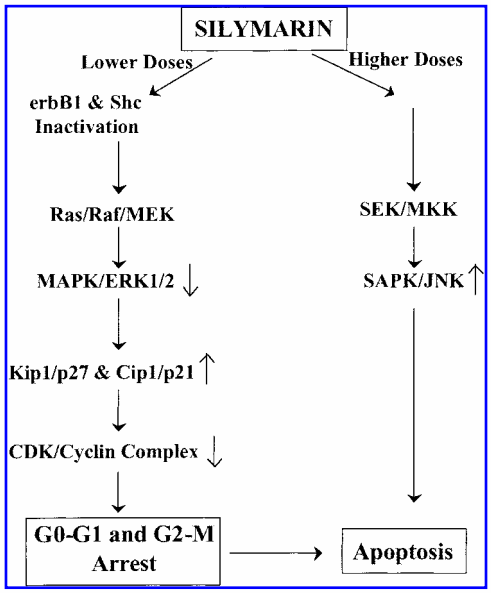

FIG. 4. Proposed mechanisms for the anticancer effect of silymarin in skin cancer. See text for abbreviations.

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ABBREVIATIONS

CDK, cyclin-dependent kinase; CDKI, cyclin-dependent kinase inhibitor; COX, cyclooxygenase; DMBA, 7,12-dimethylbenz(a)anthracene; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; GPx,

glutathione peroxidase; HETE, hydroxyeicosatetraenoic acid; IL-1α, interleukin 1α; JNK, c-Jun amino-terminal kinase; MAPK, mitogen-activated protein kinase; MEZ, mezerein; NF-κB, nuclear factor κB; ODC, ornithine decarboxylase; PG, prostaglandin; ROS, reactive oxygen species; SAPK, stress-activated protein kinase; SH-2, src homology-2; SOD, superoxide dismutase; TNFα, tumor necrosis factor α; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; UV, ultraviolet.

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Address reprint requests to:
Dr. Rajesh Agarwal
Department of Pharmaceutical Sciences
School of Pharmacy
University of Colorado Health Sciences Center
4200 East Ninth Street
Box C238
Denver, CO 80262

E-mail: Rajesh.Agarwal@UCHSC.edu

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