Chemopreventive Efficacy of Silymarin in Skin and Prostate Cancer

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Prevention and therapeutic intervention by phytochemicals are newer dimensions in the arena of cancer management. In this regard, the cancer chemopreventive role of silymarin *(Silybum marianum)* has been extensively studied and has shown anticancer efficacy against various cancer sites, especially skin and prostate. In skin cancer, silymarin treatment inhibits ultraviolet B radiation or chemically initiated or promoted carcinogenesis. These effects of silymarin against skin carcinogenesis have been attributed to its strong antioxidant and anti-inflammatory action as well as its inhibitory effect on mitogenic signaling. Similarly, silymarin treatment inhibits 3, 2-dimethyl-4-aminobiphenyl–induced prostate carcinogenesis and retards the growth of advanced prostate tumor xenograft in athymic nude mice. In prostate cancer, silymarin treatment down-regulates androgen receptor–, epidermal growth factor receptor–, and nuclear factor-κB–mediated signaling and induces cell cycle arrest. Extensive preclinical findings have supported the anticancer potential of silymarin, and now its efficacy is being evaluated in cancer patients.

Keywords: chemoprevention; antioxidant; anti-inflammatory; carcinogenesis; cell cycle; hepatoprotectant

Silymarin in Brief

Cancer incidences are on the rise, and despite substantial progress in chemotherapy and other therapies, additional measures are urgently needed to lower the cancer burden. In this regard, prevention and therapeutic intervention by phytochemicals are new dimensions in cancer management.[1] Administration of phytochemicals is shown to prevent initiation, promotion, and progression stages associated with carcinogenesis in different animal models and is suggested to effectively reduce cancer morbidity and mortality.[1] Most of these phytochemicals are constituents of the human diet or are taken as dietary supplements and have shown little toxicity to normal cells.[2] Because systemic toxicity is a major limitation of both chemotherapy and radiation therapy, these phytochemicals could be used in cancer chemoprevention as well as in the development of alternative approaches to achieve beneficial effects in cancer treatment. Among various groups of phytochemicals, a great magnitude of experimental data have been generated for polyphenolic compounds, generally known as flavonoids, for their role in chemoprevention of various cancers.[3-5] In this regard, silymarin has been extensively studied, both in vivo and in vitro, for its cancer chemopreventive potential against various cancer sites. Silymarin is isolated mainly from the seeds of milk thistle [*Silybum marianum* (L.) Gaertn. (Asteraceae)] and is a mixture of various flavonolignans, the major constituents being silybin A, silibin B, isosilybin A, isosilybin B, silychristin, and silydianin.[6,7] Silymarin is acceptable for human consumption and has been used clinically for more than 3 decades in Europe and recently in Asia and the United States.[8] Silymarin is well known for its hepatoprotective activity both in animal models of liver injuries and in humans.[8,9] In addition to reviewing silymarin, we also focus on cancer chemopreventive and anticancer efficacy of silibinin, which constitutes ~40% (w/w) of silymarin and has shown comparable biological effects to silymarin against both liver toxicity and cancer (Table 1).[7-9]

Silymarin as Cancer Chemopreventive Agent

Silymarin and silibinin possess most of the characteristics of an ideal cancer chemopreventive agent; they are nontoxic to normal cells, selectively inhibit the growth of cancer cells, and are biologically available following its oral administration. Because of these characteristics, silymarin and silibinin have been extensively studied for their anticancer efficacy, and

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DOI: 10.1177/1534735407301441

Table 1. Summary of the Chemopreventive Effect of Silymarin Against Various Cancer Sites

Cancer Sites Against Which Silymarin and Silibinin Have Shown Anticancer Potential In Vitro or In Vivo	*References*
Urinary bladder carcinogenesis	Vinh et al (2002),[13] Tyagi et al (2003),[106] Tyagi et al (2006)[107]
Tongue carcinogenesis	Yanaida et al (2002)[14]
Colon cancer	Agarwal et al (2003),[4] Yang et al (2003),[12] Kohno et al (2002),[15] Volate et al (2005)[16]
Prostate cancer	Dhanalakshmi et al (2002),[3] Davis-Searles et al (2005),[7] Zi et al (1999),[67] Zhu et al (2001),[68] Deep et al (2006),[69] Zi et al (1998),[71] Kohno et al (2005),[72] Singh et al (2002),[73] Singh et al (2003),[76] Bhatia et al (2001),[86] Sharma et al (2001),[87] Tyagi et al (2002),[99] Dhanalakshmi et al (2003),[100] Thelen et al (2004),[108] Zi et al (2000),[109] Tyagi et al (2002)[110]
Cervical cancer	Bhatia et al (1999),[111] Huang et al (2005)[112]
Hepatic cancer	Ramakrishnan et al (2006),[17] Varghese et al (2005)[113]
Skin cancer	Katiyar et al (1997),[29] Mallikarjuna et al (2004),[30] Zi et al (1997),[31] Chatterjee et al (1999),[32] Zhao et al (2000),[33] Zhao et al (1999),[34] Singh et al (2002),[35] Katiyar et al (2002),[44] Ahmad et al (1998),[46] Zi et al (1999),[51] Dhanalakshmi et al (2004),[65] Mallikarjuna et al (2005),[66] Gu et al (2005),[114] Singh et al (2006),[115] Dhanalakshmi et al (2005),[116] Dhanalakshmi et al (2004),[117] Mohan et al (2004)[118]
Leukemia	Manna et al (1999),[54] Kang et al (2001)[119]
Breast cancer	Tyagi et al (2004),[11] Scambia et al (1996),[101] Bhatia et al (1999),[111] Zi et al (1998)[120]
Ovarian cancer	Scambia et al (1996),[101] Giacomelli et al (2002),[102] Gallo et al (2003)[121]
Lung cancer	Singh et al (2004),[122] Sharma et al (2003),[123] Singh et al (2006)[124]

a brief account of their activity against various malignancies is provided in Table 1. In in vitro carcinogenesis studies, silymarin has been shown to inhibit the formation of transformed rat tracheal epithelial cell colonies induced by a chemical carcinogen benzo(*a*)pyrene.[10] Furthermore, silymarin has been shown to exert anticarcinogenic effects against human breast carcinoma MDA-MB468 cells in vitro.[11] Strong antiangiogenic effects of silymarin also have been reported in colon cancer LoVo cells.[12] In in vivo studies, dietary administration of silymarin has been shown to significantly inhibit the incidence of chemical carcinogen–induced urinary bladder neoplasm and preneoplastic lesions.[13] Dietary feeding of silymarin also suppresses 4-nitroqinoline 1-oxide–induced tongue carcinogenesis[14] and azoxymethane-induced colon carcinogenesis in rats.[15,16] More recently, dietary feeding of silymarin has been shown to inhibit N-nitrosodiethylamine–induced hepatocarcinogenesis by modulating the antioxidant defense status of the animals.[17] In addition to these reports, numerous studies by us and others show the efficacy of both silymarin (in earlier studies) and silibinin (in recent studies) against various cancer sites (Table 1). Skin and prostate are the leading sites of cancer-related incidences in the United States, and conclusive studies over the last decade have helped us to better understand the mechanistic aspects of silymarin action against these 2 malignancies both in vivo and in vitro (Table 1); therefore, these aspects are dealt with in detail in this review.

Silymarin and Skin Cancer

Skin Cancer Incidence and Causes

There are 3 main types of skin cancers: basal cell carcinoma, squamous cell carcinoma, and melanoma. More than 1 million cases of combined basal and squamous cell cancers occur annually and account for 95% of all skin cancers in the United States alone.[18] An estimated 10 590 deaths attributable to skin cancers were expected to occur in 2005.[18] Melanoma, originating from pigment cells known as melanocytes, is the most serious form of skin cancer; it causes 75% of all skin cancer deaths and was diagnosed in about 59 580 persons in the United States in the year 2005.[18] Risk factors for skin cancer include sun sensitivity; a history of excessive sun exposure including sunburns; exposure to tanning booths and to disease that suppresses the immune system; a past history of basal cell or squamous cell skin cancer; and occupational exposure to coal tar, pitch, creosote, arsenic compounds, or radium.[18]

Silymarin and Photocarcinogenesis

Excessive exposure to solar ultraviolet (UV) radiation is the major etiologic factor for skin cancer.[19] The UV wavelengths of sunlight most effective in producing skin cancer lie within UVC (200-290 nm) and UVB (290-320 nm) ranges[20]; however, because the ozone layer in the earth's atmosphere filters out all of the UVC, it has little, if any, biological relevance to skin cancer.[21] In addition to the strong skin carcinogenic effects of UVB, animal studies have clearly demonstrated

that UVA (320-400 nm) is capable of producing skin cancer; however, when compared with UVB, UVA-induced skin cancer in mice requires much greater exposure and a longer latency period before tumors are evident.[21,22] Therefore, UVB is the most frequently used photocarcinogen in animal studies.[23,24] UVB is strongly absorbed by cellular DNA in skin and results in several different types of DNA damage; cyclobutane pyrimidine dimers and 6-4 photoproducts are the most important DNA lesions with respect to photocarcinogenesis.[25,26] In addition, oxidative stress involving generation of free radicals and reactive oxygen species (ROS) and depletion of antioxidant machinery involved in removing these moieties are important consequences of UVB exposure to skin.[27,28] These oxidative reactions could also lead to DNA damage and to several other biochemical and molecular events that ultimately favor tumorigenesis.[27,28] Therefore, agents that could protect from UVB-caused DNA damage and/or possess strong antioxidant properties may be useful against photocarcinogenesis. In this regard, silymarin, with its strong antioxidant property, has been assessed for its efficacy against photocarcinogenesis at initiation, promotion, and complete carcinogenesis stages.[29]

In the studies assessing protection at tumor initiation stage (UVB initiated), application of silymarin for 14 days before UVB exposure reduced the percentage of mice with tumors, the numbers of tumors per mouse, and tumor volume per mouse compared with vehicle-treated controls.[29] In studies assessing the protective effect of silymarin during tumor promotion stage (7,12-dimethylbenz(a)anthracene [DMBA]–initiated and UVB-promoted protocol), its application before each UVB exposure resulted in an extended latency period by an additional 3 weeks before the onset of the first tumor and in reduced tumor incidence, multiplicity, and tumor volume throughout the treatment period.[29] A much more profound effect of silymarin was observed in studies involving complete carcinogenesis by UVB. Topical application of silymarin for 14 days before UVB exposure as a tumor initiator and then again during UVB-induced tumor promotion both delayed the latency period by 9 weeks and caused highly significant protection in terms of both tumor incidence and tumor multiplicity throughout the treatment period.[29] Silymarin treatment also resulted in a highly significant reduction in the occurrence of sunburn and the number of apoptotic cells formed after UVB exposure.[29] Furthermore, silymarin treatment also inhibited UVB-caused cutaneous edema formation.[29] Our work also showed that silymarin treatment inhibits UVB-caused increases in activity and messenger RNA (mRNA) expression of ornithine decarboxylase in mouse epidermis.[29] Similarly, we have reported that topical application of silibinin before or immediately after UVB exposure or its dietary feeding results in a strong protection against photocarcinogenesis, in terms of tumor multiplicity, tumor volume per mouse, and tumor volume per tumor.[30] These effects of silibinin were attributable to inhibition of DNA synthesis, cell proliferation, cell cycle progression, and an induction of apoptosis.[30]

Silymarin and Skin Chemical Carcinogenesis

Silymarin has also shown promising results as a chemopreventive and/or therapeutic agent in various skin chemical carcinogenesis models.[31-35] In a mouse skin chemical carcinogenesis model, animals are topically exposed on the dorsal side with a chemical carcinogen, such as DMBA, for tumor initiation that involves irreversible mutation in a critical proto-oncogene, Ha-ras, which controls normal cellular growth and differentiation.[36-38] In the promotion stage, carcinogen-initiated skin is repeatedly exposed to skin tumor promoters, such as phorbol ester, which causes many important epigenetic alterations in initiated cells, facilitating their clonal expansion and leading to the formation of benign tumors or papillomas.[36-38] It has also been well established that mouse skin tumor promotion involves 2 stages known as stage I and stage II tumor promotion.[36,38,39] The early stage of promotion is reversible, whereas promotion in the late stage and progression by which the benign tumors convert to the malignant form are irreversible phases of the carcinogenesis process.[37,38]

We have shown that silymarin inhibits both stage I and stage II of tumor promotion, specifically in the DMBA-12-*O*-tetra-decanoylphorol 13-acetate (TPA) and DMBA-mezerein (MEZ) SENCAR mouse skin carcinogenesis models, respectively.[32] Application of silymarin before that of TPA resulted in an exceptionally high protection during stage I of tumor promotion in a dose-dependent manner and was evident in terms of a prolonged latency period in tumor development and a strong inhibition in tumor incidence, tumor multiplicity, and tumor volume throughout the experiment. At the termination of the study, these 3 parameters showed 75%, 97%, and 96% inhibition, respectively, when compared with vehicle controls.[32] With regard to stage II tumor promotion, silymarin showed 26%, 63%, and 54% protection in tumor incidence, multiplicity, and volume, respectively.[32] A more profound protective effect of silymarin was observed when it was applied during both stage I and stage II protocols.[32]

In another study in which a free radical–generating tumor promoter, benzoyl peroxide, was used on DMBA-initiated mouse skin, silymarin showed strong antitumor-promoting effects similar to the DMBA-TPA protocols.[33] Interestingly, topical application of silymarin caused complete protection against a non-phorbol ester tumor promoter (okadaic acid)–induced tumor promotion in DMBA-initiated SENCAR mouse skin.[31] Silymarin almost completely inhibited TPA-caused skin edema and induction of epidermal hyperplasia in SENCAR mice. More interestingly, the application of silymarin before TPA did not show any appreciable increase in skin edema or epidermal hyperplasia even after TPA was applied 2 or 3 times.[32] Mechanistically, these results are supported by the facts that silymarin decreased proliferation cell nuclear antigen (PCNA)–positive cells in TPA-induced epidermal proliferation in mouse skin[32] and in a cell culture study inhibited [methyl-^{3}H] thymidine incorporation (a measure of DNA synthesis) in human epidermoid carcinoma A431 cells.[32] Silymarin also significantly inhibits ornithine decarboxylase mRNA levels and activity induced by TPA and other tumor promoters in SENCAR mouse epidermis.[40]

We have also reported the therapeutic efficacy of silymarin against DMBA-TPA–induced skin tumors in SENCAR mice.[35] Silymarin feeding significantly inhibited the tumor growth and also caused regression of established tumors.[35] Overall, these studies suggest the efficacy of silymarin as a skin cancer preventive and therapeutic agent.

Mechanisms of Silymarin Action in Inhibiting Skin Cancer

As summarized below, the anticancer effects of silymarin and silibinin have been suggested to be attributable to antioxidant and anti-inflammatory properties, inhibitory effect on epidermal growth factor receptor (EGFR) signaling, alteration in cell cycle progression, and apoptotic effect on cancer cells.

Antioxidant Potential of Silymarin

Oxidative stress is one of the major contributors in skin tumor promotion.[27,28,38] It has been shown that exposure of mouse or human skin or derived epidermal keratinocytes to tumor promoters like UVB or phorbol esters generates a strong oxidative stress and down-regulates the antioxidant system (Figure 1).[34,38,41] The oxidative stress condition, if not eliminated, leads to generation of free radicals and ROS, such as superoxide anion, hydroxyl radical, peroxyl radical, alkoxyl radical, hydroperoxyl radical, and hydrogen peroxide, which can either directly or indirectly modify a number of biologically important molecules including DNA, protein, and lipid-rich membranes, causing various diseases including skin cancer.[38,41,42] In this regard, the process of lipid peroxidation in biological membranes is a useful system to evaluate the oxidant and antioxidant activity of endogenous as well as xenobiotic agents.[43] We have shown that silymarin inhibits malondialdehyde formation in epidermal microsomes in a dose-dependent manner.[32] Similarly, silymarin strongly inhibits TPA- and benzoyl peroxide (BPO)–caused lipid peroxidation in mouse skin epidermis,[33,34] supporting its strong in vivo antioxidant activity.

Our studies have also shown that silymarin strongly reverses TPA-caused depletion of epidermal enzyme activities of superoxide dismutase, catalase, and glutathione peroxidase.[34] Another study showed that silymarin significantly inhibits UVB-induced production of H_2O_2.[44] Together, these inhibitory effects of silymarin on oxidative stress possibly shift the equilibrium of carcinogen metabolism, gene expression, and enzyme activity toward the retardation or abolition of the process of skin carcinogenesis, which could be one of the mechanisms of silymarin activity in preventing a wide range of carcinogens and tumor promoter–induced skin cancers in mice.

Inhibitory Effect of Silymarin on EGFR-Mediated Mitogenic Signaling

Enhanced tyrosine kinase activity caused by overexpression of receptor and/or protein tyrosine kinases leads to constitutive mitogenic and cell survival signaling, resulting in uncontrolled growth of cancer cells.[45] For example, an aberrant expression of the erbB family of receptor tyrosine kinases has been implicated in several human malignancies including skin cancer.[45] Different skin tumor promoters, such as TPA, chrysarobin, okadaic acid, and UVB radiation, have been shown to activate EGFR in mouse skin.[38] The EGFR signaling pathway plays an important role in carcinogenesis and is also activated by oxidative stress, which has been implicated in skin tumor promotion.[38] 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 skin cancer.[38,45]

Our recent studies have shown that silymarin inhibits both ligand-caused 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.[46] Phosphorylated Shc acts as an adaptor for other SH-2–containing proteins in cell signaling involving the Grb2-SOS-ras-raf

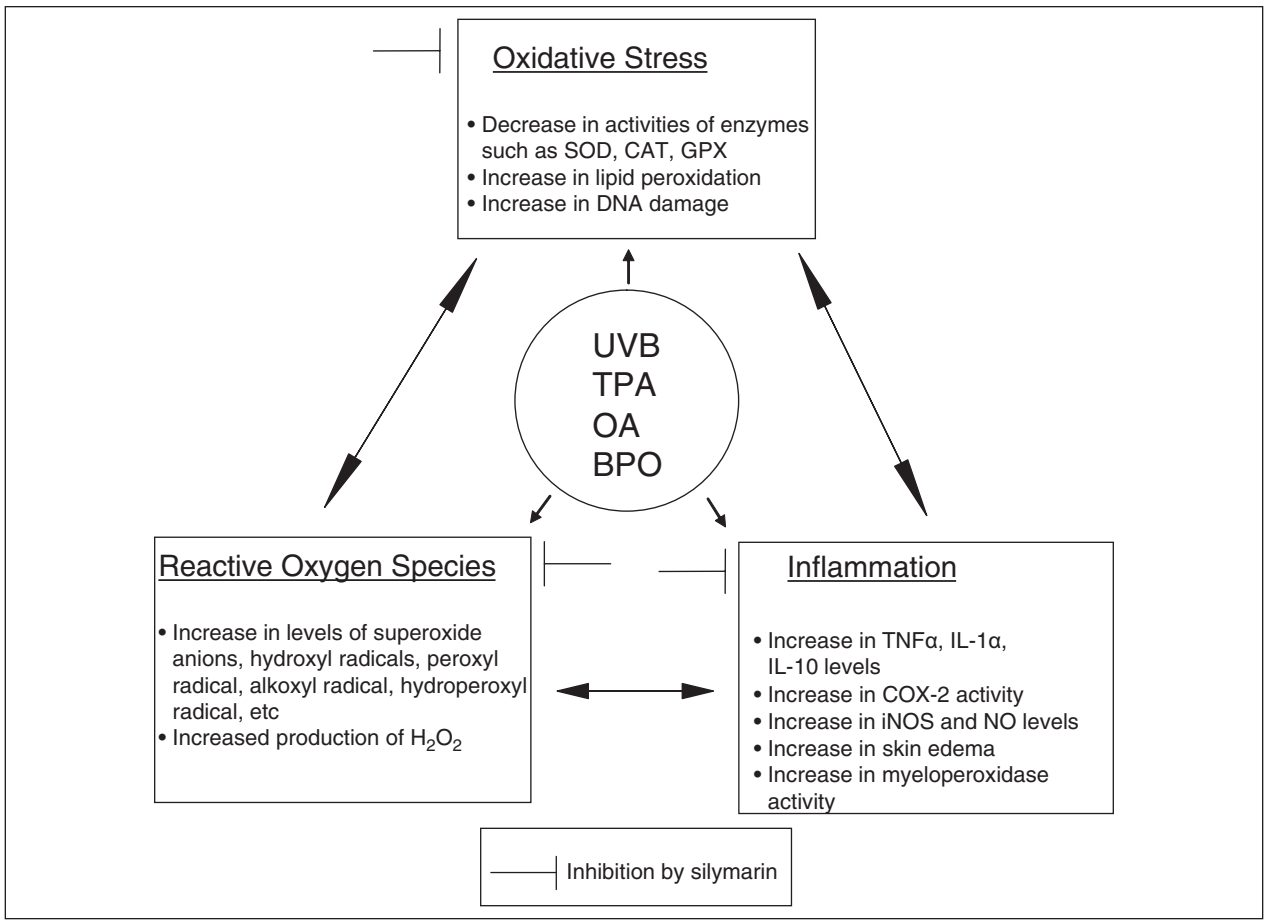

Figure 1 Model representing the inhibitory effect of silymarin on oxidative stress, production of reactive oxygen species, and inflammation caused by UVB and other chemical promoters, such as 12-O-tetra-decanoylphorol 13-acetate (TPA), okadaic acid (OA), and benzoyl peroxide (BPO). SOD, superoxide dismutase; CAT, catalase; GPX, glutathione peroxidase; TNF, tumor necrosis factor; IL, interleukin; COX-2, cyclooxygenase-2; iNOS, inducible nitric oxide synthase; NO, nitric oxide.

pathway[47] as well as phosphatidylinositol 3–kinase and phospholipase C pathways, ultimately activating mitogen activated protein kinase (MAPK), leading to the activation of various transcription factors for cell growth and proliferation (Figure 2).[47,48] MAPK/extracellular signal-regulated kinase (ERK1/2) is an essential element of mitogenic signaling and is often constitutively active in various cancers, including skin cancer.[49,50] In this regard, lower doses (50-75 μg/mL) of silymarin inhibited the epidermal growth factor–induced activation of ERK1/2 in starved A431 cells, whereas the higher doses of silymarin did not show any effect on ERK1/2 activation but activated c-Jun amino-terminal kinase (JNK) signaling.[51] The inhibitory effect of silymarin on the activation of the EGFR-Shc-ERK1/2 pathway indicates that this mitogenic signaling cascade is one of the targets of silymarin in its skin cancer chemopreventive efficacy.

Silymarin Causes Cell Cycle Arrest and Apoptotic Cell Death

Deregulated cell cycle progression and inhibition of apoptosis have been described as hallmarks of cancer cells,[52] and therefore chemopreventive agents that could affect the deregulated cell cycle and induce apoptosis should be effective in inhibiting the growth of cancer cells.[53] In this regard, silymarin has shown dose- and time-dependent growth inhibition and death of human epidermoid carcinoma A431 cells.[51] Quantitative and qualitative analyses of cell death showed that apoptosis is a major contributor in cell death caused by silymarin at higher doses. For example, in quantitative analysis, silymarin treatment (75-150 μg/mL) caused 48% to 78% apoptotic cell death in A431 cells.[51] Apoptotic effect of silymarin at these doses was also evident in terms of a classic DNA ladder formation in A431 cells. The apoptotic effect

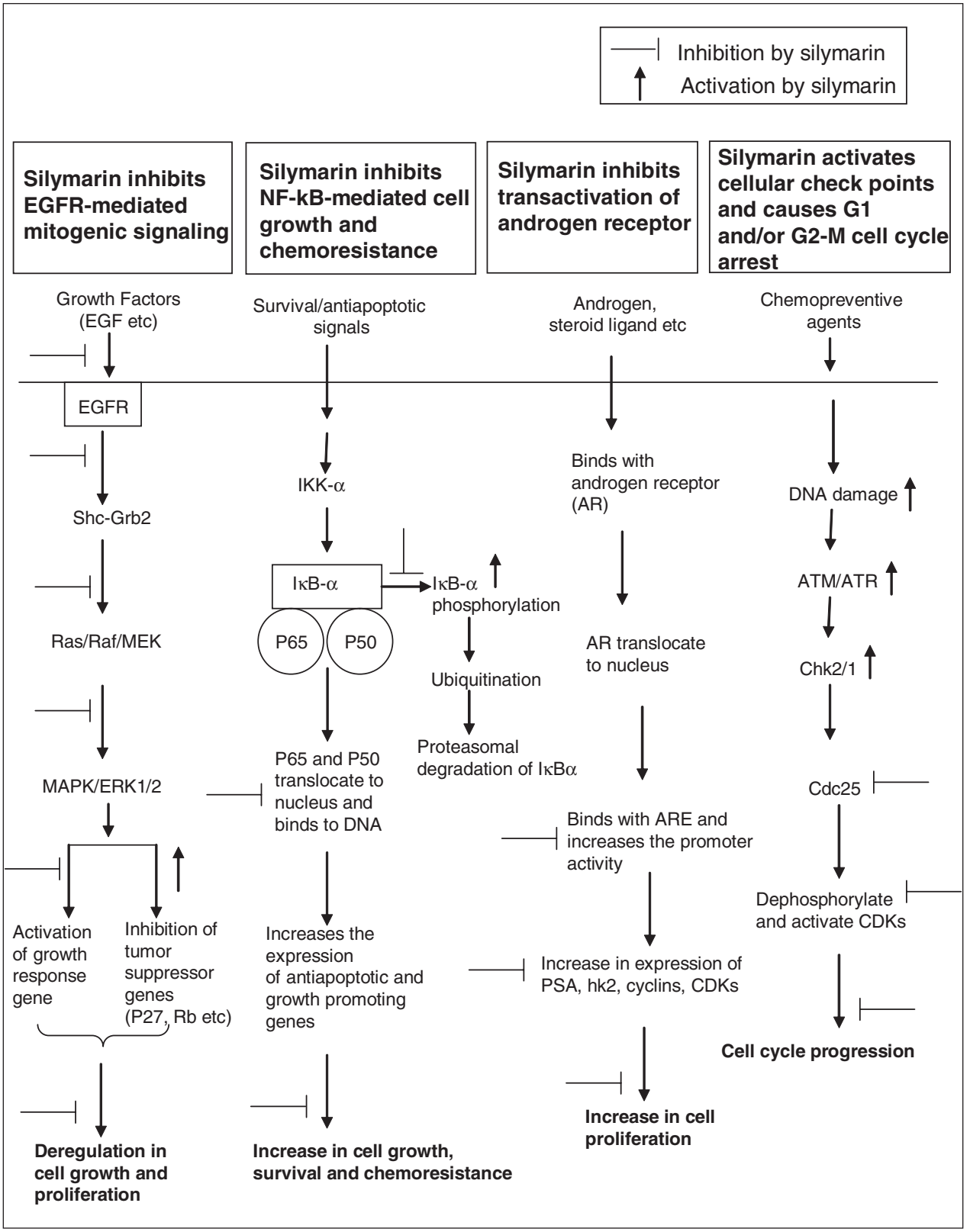
Inhibition by silymarin
Activation by silymarin
Silymarin inhibits EGFR-mediated mitogenic signaling
Silymarin inhibits NF-kB-mediated cell growth and chemoresistance
Silymarin inhibits transactivation of androgen receptor
Silymarin activates cellular check points and causes G1 and/or G2-M cell cycle arrest
Growth Factors (EGF etc)
Survival/antiapoptotic signals
Androgen, steroid ligand etc
Chemopreventive agents
EGFR
Shc-Grb2
Ras/Raf/MEK
MAPK/ERK1/2
Activation of growth response gene
Inhibition of tumor suppressor genes (P27, Rb etc)
Deregulation in cell growth and proliferation
IKK-α
IκB-α
P65
P50
IκB-α phosphorylation
Ubiquitination
Proteasomal degradation of IκBα
P65 and P50 translocate to nucleus and binds to DNA
Increases the expression of antiapoptotic and growth promoting genes
Increase in cell growth, survival and chemoresistance
Binds with androgen receptor (AR)
AR translocate to nucleus
Binds with ARE and increases the promoter activity
Increase in expression of PSA, hk2, cyclins, CDKs
Increase in cell proliferation
DNA damage
ATM/ATR
Chk2/1
Cdc25
Dephosphorylate and activate CDKs
Cell cycle progression

Figure 2

of silymarin has been suggested to occur via an increase in both JNK phosphorylation and its kinase activity at higher doses.[51] Similarly, in our in vivo study, we reported an increased apoptosis following silibinin treatment of mice.[30] In this study, we showed that skin tumor samples from silibinin treatment groups in a photocarcinogenesis study in SKH-1 hairless mice harbor an activation of JNK and an increase in cleaved caspase 3 levels.[30] Silibinin treatment also inhibited protein kinase B phosphorylation and decreased the survivin levels in these skin tumor samples.[30] Together, these studies suggest that induction of apoptosis in cancer cells is one of the key mechanisms through which silymarin and silibinin exert their growth inhibitory effect on tumor cells and that these effects of silymarin and silibinin play a major role in their overall skin cancer chemopreventive effects.

Along with inducing apoptosis, silymarin also induces cell cycle arrest in cancer cells. For example, silymarin treatment for 12 hours resulted in G2-M arrest in A431 cells; longer treatment caused both G0-G1 and G2-M arrest.[51] Cyclin-dependent kinases (CDKs) and their associated cyclins are known to play a major role in cell cycle regulation. Our studies reveal that silymarin decreases the expression as well as the kinase activity of cyclin E, D1, A, and B and CDK1, 2, and 6 in A431 cells.[51] Silymarin also increases the protein expression of $p21^{Cip\ 1}$ and $p27^{Kip\ 1}$ and their binding with CDK1, 2, and 6.[51] Similar effects of silibinin were also reported by us recently in photocarcinogenesis studies in an SKH-1 mouse model,[30] which further validated cell culture observations with silymarin and suggested that the modulation of cell cycle regulators causing a cell cycle arrest is an additional mechanism of action of silymarin and silibinin in their efficacy in the prevention and therapeutic intervention of skin cancer.

Anti-inflammatory Effect of Silymarin

Various studies have shown that silymarin possesses strong anti-inflammatory potential.[29,38,44,54] The cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-1α have been shown to be associated with skin inflammation and tumor promotion.[55,56] The up-regulation of TNF-α, an endogenous tumor promoter, is one of the common mechanisms of tumor promotion that mediates the effect of exogenous tumor promoters, including phorbol ester (eg, TPA) as well as nonphorbol ester (eg, okadaic acid) (Figure 1). It has been suggested that inhibition of TNF-α mRNA expression and its release could be an important strategy in cancer chemoprevention.[57] We have shown that silymarin causes exceptionally high to complete inhibition of okadaic acid– and TPA-induced mRNA expression of TNF-α in SENCAR mouse skin.[31] Similarly, a single topical application of TPA on mouse skin induces high expression of IL-1α mRNA.[56] 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.[34] The findings that silymarin inhibits the expression of IL-1α as well as TNF-α provide a convincing mechanistic basis for anti-inflammatory and anti–tumor-promoting activities of silymarin against skin tumorigenesis.

Additionally, in tumor promotion, leukocyte (macrophage and neutrophil) infiltration has been observed in response to the application of tumor promoters onto mouse skin.[58] The infiltration and accumulation of neutrophils are characteristic features of TPA- and UV radiation–caused skin inflammation and are also used to measure the intensity of skin inflammation.[58] Silymarin also possesses a strong ability to inhibit UVB-, TPA-, and BPO-caused increases in myeloperoxidase activity, which is a marker of neutrophil infiltration.[33,34,44]

The TPA-caused skin inflammation is also mediated by lipoxygenase and cyclooxygenase (COX) enzymes, ultimately leading to the formation of hydroxyeicosatetraeonic acid (HETE) and prostaglandin metabolites.[59,60] Constitutive expression of 8-lipoxygenase has been shown in skin papillomas,[61] indicating that 8-lipoxygenase–catalyzed arachidonic acid metabolite 8-HETE plays an important role in skin tumor promotion.[61] In this regard, silymarin inhibits the TPA-caused increase in lipoxygenase activity, as evidenced by a decrease in 8-HETE formation in mouse skin.[34] COX, like lipoxygenase, also plays a critical role in cell proliferation, skin inflammation, and skin tumor promotion.[60,62] Recently, it has been reported that TPA-caused induction of COX activity is only attributable to constitutive overexpression of inducible COX (COX-2) in mouse epidermal tumors (Figure 1).[63] The elevated level of prostaglandin E_2 has been positively associated with the expression of COX-2 when mouse epidermis is exposed to TPA.[34] Silymarin inhibits both TPA-caused COX-2 expression and COX activity in terms of prostaglandin E_2, prostaglandin $F_{2\alpha}$, and prostaglandin D_2 formation in mouse skin epidermis.[34] This effect of silymarin on COX-2 was selective because it did not alter COX-1 expression, and silymarin also did not show any effect on COX-1 and COX-2 levels when applied alone on mouse epidermis.[34] Similarly, the topical application of silymarin before UVB irradiation results in a highly significant inhibition of UVB-caused induction of epidermal COX activity measured by the inhibition in prostaglandin metabolite formation.[29]

Together, our findings suggest that silymarin could be explored as a cancer preventive agent, targeted toward COX-2 modulation in epithelial cancers.

In other studies, it has been shown that UVB treatment results in a significant increase in inducible nitric oxide synthase (iNOS) expression and the production of nitric oxide (NO), which plays an important role in the induction of inflammation (Figure 1). Silymarin treatment significantly inhibits the expression of iNOS as well as production of NO in UV-treated skin.[44] Thus, the anti-inflammatory effect of silymarin might occur partly through its inhibitory effect on iNOS and NO production. Overall, the anti-inflammatory action of silymarin and silibinin further explains their chemopreventive effects against skin cancer.

Silymarin and DNA Repair

One of the most important characteristics of UV-caused carcinogenesis is DNA damage, which includes DNA strand break, thymine dimers, 6-4 photoproducts, cytosine photohydrates, and DNA-DNA or DNA-protein cross-link formation.[64] The formation of thymine dimers is frequent and is the most extensively studied form of UVB-induced DNA damage.[25,26] Cyclobutane pyrimidine dimers, if left unrepaired, lead to CC to TT or C to T mutations after DNA replication.[25,26] It has been suggested that inhibition of UVB-induced DNA damage by chemopreventive agents could reduce the incidence of skin cancer.[64] One of the key pathways through which silymarin inhibits the process of carcinogenesis is by scavenging free radicals, which otherwise cause DNA damage, and strengthening the antioxidant potential of the cell.[33,34,29,44] However, it remains to be seen whether silymarin also activates DNA repair machinery. In this direction, we have reported that topical application of silibinin results in a remarkable decrease in UV-caused thymine dimer–positive cells, but we did not find any change in the levels of mismatch repair enzyme MSH2 with silibinin treatment, suggesting the potential involvement of other DNA repair mechanisms.[65] In other studies, silibinin treatment activated p53, which is a key molecule in regulating DNA repair machinery along with cell cycle and apoptosis.[65] Furthermore, we have observed that dietary silibinin also reduces the number of UVB-induced thymine dimer–positive cells in mouse skin epidermis.[66] Further work is in progress to evaluate the effect of silibinin on various DNA repair pathways following UVB exposure and to further define its mechanism of action in terms of a decrease in thymine dimer–positive cells and its association with the overall chemopreventive efficacy of silibinin against photocarcinogenesis.

Silymarin and Prostate Cancer

Prostate Carcinogenesis and the Role of Silymarin Against Prostate Cancer

Prostate cancer (PCA) is the second most common malignancy (after nonmelanoma skin cancers) in American men and is the second leading cause of cancer-related deaths.[18] In the United States, about 30 350 deaths and 232 090 incidences of PCA were reported in 2005.[18] Despite the high incidence of PCA, its etiology is not well defined. Genesis of human PCA is a multistep process where a normal epithelial prostate cell undergoes several cellular, biochemical, and genetic alterations leading to the formation of low-grade followed by high-grade prostatic intraepithelial neoplasia (PIN). Additional genetic and epigenetic changes in high-grade PIN lead to clinical PCA that is initially androgen dependent,[2] and accordingly androgen deprivation and 5α-reductase blockers are extensively used to control and manage this malignancy at the early stage.[2] These approaches lead to the inhibition of PCA growth; however, within a short time period, perhaps 1 to 3 years, cancer regrowth occurs that is totally androgen independent and causally involves genetic alterations including those in receptor tyrosine kinases and growth factor ligands.[2]

Among the widely accepted risk factors for PCA are age, race, ethnicity, and geographical dependence.[2] This malignancy is low in the Asian population and high in the Scandinavian countries, with the highest incidence and mortality rates occurring in African American men, the latter being 2-fold higher than in Caucasian American men.[2] The low incidences in the Asian population have been attributed to consumption of diets low in animal proteins and fat, high in starch and fiber, and rich in "weak plant estrogens."[67] Some of these phytochemicals possess weak estrogenic, antiestrogenic, and antioxidant activity and therefore possess the potential for influencing hormone-dependent cancers including PCA.[67] Among various phytoestrogens, silymarin and silibinin have attracted a lot of attention for their anticancer efficacy against human PCA. Recent studies have reported that silymarin and its constituents including silibinin inhibit prostate-specific antigen (PSA) levels regulated by both serum and androgen, causing strong inhibition of human prostate carcinoma LNCaP cell growth.[67,68] Both silymarin and silibinin have been shown to inhibit the growth of various human and rodent PCA cells independent of their androgen responsiveness.[7,67-71] The anticancer efficacy of silymarin and silibinin against PCA has also been demonstrated in various in vivo models, which are discussed in detail in the next section.

Silymarin and Prostate Chemical Carcinogenesis

Kohno et al[72] recently showed the protective effect of silymarin against 3,2-dimethyl-4-aminobiphenyl (DMAB)–induced prostate cancer. In this study, the male F344 rats were given a dose of DMAB (25 mg/kg body weight) every other week for 20 weeks. Thereafter, animals were fed a diet containing 100 and 500 ppm silymarin for 40 weeks. Following these treatments, rats were sacrificed, and prostate tissue was processed for histopathologic diagnosis. The prostatic lesions, including PIN, were histopathologically diagnosed. Histologically, there were no pathologic alterations in liver, kidney, lung, and heart in silymarin-treated rats, suggesting that this agent is nontoxic at the doses and duration of treatment in this study. The mean body weight and the liver, prostate, and bilateral testicular weights also did not change significantly with silymarin treatment.[72] In terms of prostate carcinogenesis, DMAB exposure induced PIN and adenocarcinomas in the ventral lobe of the prostate, and silymarin feeding significantly reduced the incidences of both PIN and adenocarcinomas induced by DMAB.[72] Treatment with silymarin alone (500 ppm) did not result in any prostatic neoplasm. Thus, the study showed that dietary administration of silymarin during the promotion phase of DMAB-induced prostatic carcinogenesis inhibits the incidence of prostatic adenocarcinomas. Silymarin treatment also suppresses the high proliferative activity of cells initiated with carcinogen, significantly inhibits both PCNA and cyclin D1 labeling indices, and increases apoptotic index in the preneoplasm and/or carcinomas of the prostate.[72] Thus, this study clearly showed the efficacy of silymarin against chemical carcinogen–induced prostate carcinogenesis.

Silymarin and Prostate Tumor Xenograft Model

The tumor xenograft model has been extensively used to assess the cancer preventive and therapeutic efficacy of various phytochemicals.[73-75] In a recent study, we showed the inhibitory effect of dietary feeding of silibinin on the growth of DU145 tumor xenograft in athymic nude mice.[73] Silibinin feeding significantly decreased the tumor volume and wet weight of the tumors. Silibinin showed higher efficacy once its feeding was started 3 weeks before the tumor cell implantation. The in vivo anticancer effect of silibinin against DU145 xenograft was associated with increased plasma levels of insulin-like growth factor–binding protein-3.[73] Furthermore, this effect of silibinin was related to its inhibitory effect on cell proliferation, increased apoptosis, and inhibition of angiogenesis in DU145 tumor xenografts.[76]

Silymarin and TRAMP Model

A transgenic adenocarcinoma of mouse prostate (TRAMP) model has been developed in the C57BL/6 strain of mice using the minimal probasin promoter to drive the expression of SV40 early genes (T_1t) (Tag) specifically to prostatic epithelium.[77] The probasin promoter allows for the temporal and spatial regulation of transgene expression within the ventral and dorsal prostate lobes of sexually mature male mice.[77] TRAMP mice develop progressive stages of prostatic disease with time from early lesions of intraepithelial hyperplasia to late-stage metastatic adenocarcinomas.[77] We treated TRAMP mice having palpable tumor at the age of 20 weeks with purified diet containing 0.5 and 1% (w/w) silibinin until 31 weeks of age.[78] Silibinin feeding decreased the weight of tumor + prostate + seminal vesicle when compared with control mice. Silibinin feeding also significantly decreased tumor angiogenesis and proliferation and increased apoptosis in prostate tumor tissue samples in the TRAMP model.[78]

Molecular Targets of Silymarin Action in Prostate Cancer

Both silymarin and silibinin possess strong anticancer efficacy against human androgen-dependent and androgen-independent prostate carcinoma cells (Table 1). Recent work has shown various molecular targets of silymarin and silibinin actions in prostate carcinoma cells, which are discussed below.

Androgen Receptor

The androgen receptor (AR) is a key mediator of androgen signaling, responsible for the growth and development of normal prostate as well as prostate cancer.[68,79] AR consists of an N-terminal transactivation domain, a DNA-binding domain, a hinge region that contains the bipartite nuclear translocation signal involved in AR nuclear translocation, and a ligand-binding domain (LBD), which is responsible for ligand binding and transactivation.[79] The AR, in its inactive state, associates with heat-shock proteins, and unliganded AR is mainly located to the cytoplasm.[79] On binding of androgens, the AR undergoes a conformational change within the LBD and dissociates from the heat-shock proteins.[79] Activated ARs then form homodimers and translocate to the nucleus (Figure 2), where they bind to specific DNA sequences termed androgen-responsive elements (AREs) to initiate gene transcription of androgen-regulated genes, such as PSA, a molecular marker clinically used to detect prostate cancer and monitor recurrence and progression.[7,67,68,79] Several studies have shown that the androgen refractory nature of prostate cancer is caused mainly by AR overexpression, AR mutations, or

posttranslational activation of the receptor or its coactivators.[79,80] These changes allow AR to be activated by low levels of adrenal androgens, other steroids, and even antiandrogen or ligand-independent mechanisms.[79,81] Therefore, approaches that target reducing the circulating levels of androgen or the use of antiandrogens could be a major reason for the overall failure of endocrine therapy. For these reasons, ablation of AR itself from prostate cancer cells would be a better therapeutic strategy for advanced prostate cancer.[68,82-84] It has been shown that silymarin treatment inhibits AR-mediated signaling. Zhu et al[68] showed that silymarin treatment inhibits the androgen-stimulated cell proliferation as well as androgen-stimulated secretion of PSA and human glandular kallikrein. Furthermore, silymarin treatment was shown to diminish the transactivation of AR (Figure 2). This decrease was attributable to inhibition of nuclear levels of AR by silymarin treatment. In earlier work, we showed that silibinin treatment inhibits growth and induces differentiation in androgen-dependent prostate cancer LNCaP cells.[67] We have also shown that silibinin treatment inhibits the intracellular as well as secreted form of PSA in these cells.[67] Overall, these studies suggest that silymarin and silibinin have inhibitory effects on AR-mediated signaling and thus could play a key role against prostate cancer progression.

EGFR-Mediated Mitogenic Signaling

Advanced human PCA expresses high levels of EGFR and associated growth factor ligands including transforming growth factor (TGF)-α, which forms an autocrine loop resulting in autonomous PCA cell growth, proliferation, and metastasis.[85] Furthermore, an aberrant expression of other members of the EGFR family of receptors (such as erbB2 and erbB3) was observed with high frequency in PIN as well as in primary and metastatic PCA.[85] As mentioned earlier, one of the EGFR-mediated signaling cascades activates Shc-Grb2-Ras-Raf and ultimately the MAPK/ERK1/2 pathway, which activates transcription factors for growth-responsive genes (Figure 2).[85] Consistent with these reports, constitutive activation of ERK1/2 and associated transcription factors has been observed in human PCA cells.[85] An increase in ERK1/2 activation has also been reported as PCA progresses to a more advanced and androgen-independent malignancy.[85] ERK1/2 signaling has been suggested as a converging point for membrane receptor–as well as nonreceptor-mediated mitogenic signaling, and because this is one of the major mechanisms for human PCA growth and metastasis, inhibition of these signaling events forms an important strategy for both prevention and intervention of human PCA. In this direction, we have studied the effect of silymarin on EGFR-mediated signaling in advanced human PCA DU145 cells.[71] We observed that silymarin inhibits both constitutively active and TGF-α–mediated tyrosine phosphorylation of EGFR and Shc as well as their interaction.[71,86] Further studies using silibinin instead of silymarin showed that it inhibits EGF-caused activation of EGFR in both LNCaP and DU145 cells, together with a similar inhibitory response on ERK1/2 activation, suggesting that silibinin impairs EGFR-Shc-ERK1/2 signaling in prostate carcinoma cells.[87] Mechanistic investigation showed that silibinin inhibits ligand binding to EGFR as well as its internalization.[87] In other experiments with silibinin, we reported its inhibitory effects on EGFR dimerization as well as on DNA synthesis. We have also observed that silibinin inhibits TGF-α mRNA expression and decreases both secreted and cellular levels of TGF-α in both LNCaP and DU145 cells.[85] These studies suggest that silymarin and silibinin down-regulate EGFR signaling in PCA via inhibition in expression and secretion of growth factors, inhibition of growth factor binding to and activation of EGFR, and subsequent impairment of downstream mitogenic events causing anticancer efficacy against PCA cells.

Nuclear Factor-κB–Mediated Signaling

Studies have shown that nuclear factor (NF)-κB is constitutively active in androgen-independent PCA[85] and activates a number of antiapoptotic genes as well as others mediating angiogenesis, invasion, and metastasis.[85] NF-κB has also been implicated in the growth and survival of various types of cancer cells, including PCA. NF-κB is a family of dimeric transcription factor that constitutes p50, p52, p65 (RelA), RelB, and c-Rel, which form various homodimers or heterodimers and bind to a common DNA sequence motif known as κB site.[85] Usually, NF-κB is sequestered in cytoplasm via the inhibitor of κB (IκB) family of proteins, which are phosphorylated at conserved serine sites by inhibitor of IκB kinases (IKKs) and then ubiquitinated and degraded in response to antiapoptotic or cell survival or tumorigenic signals (Figure 2). Free NF-κB then translocates to the nucleus, which can induce a broad range of genes that include inflammatory cytokines (such as TNF-α), chemokines, cell adhesion molecules, growth factors, and interferons.[85] The constitutive activation of NF-κB makes cancer cells resistant to TNF-α–based chemotherapy, and that could be one of the potential causes for the TNF-α insensitivity observed in various cancer cells, including PCA.[85] Overall, these studies suggest that NF-κB signaling is an important cell survival/antiapoptotic molecular mechanism in PCA and that inhibitors

of NF-κB signaling could be useful in the prevention of and therapeutic approaches to PCA. We have observed that silibinin inhibits IKK-α kinase activity and ultimately NF-κB transcriptional activity in human PCA DU145 cells.[3] This effect of silibinin strongly increases the sensitivity of human PCA DU145 cells for TNF-α–induced apoptosis, which otherwise was resistant to apoptosis induction.[3] With regard to silymarin, studies done in non– prostate cancer models have shown that it blocks activation of NF-κB by inhibiting its DNA binding activity,[54,88,89] which was mediated via inhibition of phosphorylation and degradation of IκBα.[54] Together, these findings suggest that the inhibitory effect of silibinin and silymarin on NF-κB signaling in advanced human PCA could be an additional important mechanism of their efficacy.

Cell Cycle Regulators

Several studies have demonstrated a close association between deregulation of cell cycle progression and development of prostate cancer and suggested that inhibition of unchecked cell cycle regulation in cancer cells could be a potential strategy for the management of cancer.[53] The regulation of cell cycle is controlled by a family of cyclins, CDK and CDK inhibitors (CDKIs).[4] G1-S transition is positively controlled by CDKs in association with D-type cyclins for CDK4 and 6 and cyclin E and A for CDK2.[53] These CDK-cyclin complexes phosphorylate the retinoblastoma family of proteins to release E2F transcription factors needed to increase the transcripts for growth-responsive genes.[53] However, CDK-cyclin complexes are negatively controlled by the Kip/Cip family of CDKIs, namely, Kip1/p27 and Cip1/p21, in addition to the INK family of CDKIs.[4,69] In addition to above-listed cyclins, CDK and CDKIs, the G2-M transition is positively regulated by Cdc2 and cyclin B complex, and the Cdc25 family of phosphatases regulates the activity of Cdc2 through dephosphorylation of inhibitory phosphorylation at threonine 14 and tyrosine 15, caused by Wee1 or Myt1.[69,53] These phosphatases are inactivated through their phosphorylation by checkpoint kinases (Chk1/2), which may in turn be activated by upstream kinases ataxia telangiectasia mutated (ATM) and ATM-related kinase (ATR) in response to DNA damage (Figure 2). In our studies with silymarin, we have observed that silymarin causes growth inhibition in human PCA LNCaP, PC3, and DU145 cells.[7,67,69,71,86] This effect of silymarin was associated with an induction of G1 arrest and/or G2-M arrest. Mechanistic investigation suggested that silymarin treatment increases CDKIs (Kip1/p27 and Cip1/p21) and decreases the levels of CDKs (CDK2 and CDK4) and associated kinase activities leading to G1 arrest.[69,71] Recently, we have also reported that silymarin modulates the ATM-Chk1/2-Cdc25-Cdc2-cyclin B1 pathway for G2-M arrest in PC3 cells.[69] We also observed that silymarin alters cytoplasmic versus nuclear localization of CDKs, cyclins, and Cdc25 to control their activity.[69] In other studies, silibinin showed similar results in all the prostate carcinoma cells studied including LNCaP, DU145, and PC3, which were mediated by comparable mechanistic pathways as summarized for silymarin (Table 1). Together, our completed studies thus far have convincingly established the role of silibinin- and silymarin-caused cell cycle arrest as an important mechanism for its efficacy against prostate cancer.

Angiogenesis

Angiogenesis refers to the growth of capillary vessels from existing blood vessels and is obligatory for the growth and progression of solid tumors.[90] Angiogenesis critically depends on several conditions: the endothelial cells must (1) proliferate to provide the necessary number of cells for the growing vessels; (2) secrete matrix metalloproteinases (MMPs), which are required to break down surrounding tissue matrix; and (3) be capable of movement and migration.[91] In addition, the angiogenic stimuli like hypoxia and production of angiogenic cytokines, such as vascular endothelial growth factor (VEGF), must be sustained.[92] Because of the critical dependence of solid tumor growth and metastasis on angiogenesis, therapeutic strategies have been developed targeting various aspects of angiogenic processes.[52,92] We have observed that silymarin treatment inhibits the growth and survival of human umbilical vein endothelial cells (HUVECs).[93] Silymarin also inhibited the secreted and cellular content of MMP-2/gelatinase A in these cells.[93] These effects of silymarin were accompanied by an inhibitory effect on HUVEC tube formation (in vitro capillary differentiation) on a reconstituted extracellular matrix, matrigel.[93] Silymarin treatment also decreased the secreted VEGF level in DU145 prostate and MCF and MDA-MB-468 breast cancer cells.[93] Similarly, silibinin showed a strong, concentration-dependent inhibition of capillary tube formation on matrigel, retraction and disintegration of preformed capillary network, inhibition of matrigel invasion and migration, and a decrease in MMP-2 secretion by HUVECs.[94] Furthermore, our in vivo work has shown that silibinin inhibits microvessel density and inhibits VEGF secretion in prostate tumors.[76] These results suggest the mechanisms for antiangiogenic efficacy of silymarin and silibinin and their usefulness in angioprevention and antiangiogenic therapy of prostate cancer.

Silymarin in Clinical Trials and Combination Chemotherapy

Preclinical data with silymarin have amply proved its efficacy as an anticancer agent. Now the efficacy of silymarin is being evaluated in cancer patients either alone or in combination with other chemotherapeutic agents. In this direction, silymarin was used (along with soy, isoflavones, and lycopene) in clinical trials in men with prostate cancer and rising PSA.[95] Forty-nine patients with a history of prostate cancer and rising PSA levels after radical prostatectomy (n = 34) or radiotherapy (n = 15) participated in a randomized, double-blind, placebo-controlled crossover study.[95] Results showed that treatment decreased the PSA level and increased the PSA doubling time, and no side effect was observed during this study.[95] Our phase I study with silibinin in patients with hormone refractory prostate cancer is also in progress to evaluate the clinical efficacy of silibinin.[96] The completed outcomes thus far show that the oral administration of silibinin in these patients results in biologically relevant serum levels of free silibinin without any major toxicity.[96]

Cancer chemotherapy using anticancer agents provides significant benefits against many cancers, but this is usually accompanied with acute toxicity, especially hepatotoxicity.[97] With many chemotherapy protocols, there are no substitute medications that provide the same effectiveness against the cancer yet preserve liver function. There are also no hepatoprotective medications that allow chemotherapy to continue to be administered while preserving liver function. All of these hepatic toxicities can be dose limiting and prevent a patient from receiving an optimal dose of chemotherapy. This often results in dose reduction or delays in therapy, potentially increasing the risk of relapse of the malignancy. In this regard, silymarin can be used along with chemotherapeutic agents as a hepatoprotectant. Silymarin has been widely used in Europe for the treatment of liver and biliary disorders and for protection from harmful hepatotoxins in adults and children.[8] A survey has found that milk thistle is the most common hepatoprotectant used by patients seen in outpatient gastrointestinal clinics.[9] In this regard, the Commisions E in Germany has approved milk thistle for treating dyspeptic, liver, and gallbladder complaints.[98] Other researchers have suggested that silymarin may play a role in adjuvant cancer therapy. Our in vitro work has shown that silibinin strongly synergizes human prostate carcinoma DU145 cells to doxorubicin, cisplatin, and carboplatin-induced growth inhibition and apoptotic death.[99,100] Similar synergistic effects of silibinin with doxorubicin and cisplatin have also been reported in various breast and ovarian cancer cell lines.[11,101,102]

Anecdotal reports from pediatric patients with cancer have found silymarin to be the most commonly used hepatoprotectant.[97] Silymarin supplementation in adults with viral or alcohol-induced hepatitis and cirrhosis has been associated with decreases in liver transaminase levels, improvements in prognosis, shortened recovery times, and decreases in the length of hospital stay.[9] Thus, the low toxicity associated with silymarin makes it an ideal candidate for combination trials. Invernizzi et al[103] reported the use of silymarin in a 34-year-old woman with promyelocytic leukemia. During 18 months of maintenance chemotherapy with methotrexate and 6-mercaptopurine, the course was complicated by repeated interruptions and dose modifications for liver toxicity. When the patient was given 800 mg of silymarin in conjunction with chemotherapy, during the 4 months of treatment, the patient had normal liver transaminase levels, and there was no further interruption of therapy and no side effects reported.[103] To evaluate the role of silymarin as an adjuvant in cancer chemotherapy, a phase II randomized pilot study has been undertaken in patients with acute lymphoblastic leukemia receiving hepatotoxic chemotherapy.[104] This study will examine the effect of silymarin on liver function, oxidative damage, and serum silibinin values in patients receiving chemotherapy.[104] Furthermore, silymarin has been suggested as one of the key elements of a core nutraceutical program suggested for cancer management.[105] However, human clinical trials have not used standardized forms of milk thistle, and researchers have administered the whole herb, silymarin or silibinin. The form used in most human clinical trials is silibinin bound to a phosphatidylcholine complex, which facilitates absorption and increases bioavailability. Accordingly, there is a need to ensure that silymarin used in clinical trials is standardized and subject to quality control.

Conclusions and Future Perspective

Silymarin, a crude mixture of various flavonolignans, and a relatively pure form of this supplement, silibinin, have been shown to exert strong anticancer and cancer chemopreventive efficacy against various cancer sites, especially prostate and skin cancer. The efficacy of these agents seems to involve multiple mechanisms as well as targets ranging from their antioxidant potential, which alleviates the oxidative stress induced during carcinogenesis, to the fact that they modulate androgen receptors, EGFR, NF-κB, cyclin-CDKs, CDKIs, VEGF, and MMPs, mostly in favor of inhibiting the carcinogenesis process as well as the advanced stages of the malignancy. The common mechanisms of silymarin action in skin and prostate cancer include inhibition of EGFR-mediated mitogenic signaling, induction of cell cycle arrest

via modulating the expression of cell cycle regulators, and alteration of the expression of key molecules regulating angiogenesis. In this regard, more work is needed to elucidate the effect of silymarin on various biomarkers and signaling pathways in more prostate and skin cancer cell lines and animal models. Furthermore, little work has been done to evaluate the efficacy of silymarin against other cancers such as hepatic, brain, breast, pancreatic, esophageal, and colon. The efficacy and nontoxicity of silymarin warrant more studies against these malignancies. Recent studies have also shown that epigenetically regulated mechanisms like chromatin methylation and acetylation play important roles in the process of carcinogenesis, and therefore it will be interesting to examine whether silymarin modulates any such mechanisms activating the expression of tumor suppressor genes or inhibition of various oncogenes.

Preclinical results have also shown that silymarin and silibinin cause synergistic effects on cancer cell growth inhibition and apoptotic death by various chemotherapeutic agents, suggesting that these agents should be evaluated in more clinical trials along with other chemotherapeutic agents. In this regard, clinical studies have shown that silymarin treatment in combination with chemotherapeutic agents reduces the toxicity associated with chemotherapy. Silymarin treatment has also shown favorable results in clinical trials in prostate cancer patients and has been suggested as an integral part of a nutraceutical program tailored for cancer management. More clinical trials are needed to evaluate the clinical efficacy of standardized silymarin as well as silibinin against various human cancers in general and against skin and prostate cancers in particular.

Acknowledgments

The original studies in our program on skin and prostate cancer prevention by silymarin/silibinin are supported by NCI, NIH RO1 grants CA64514 and CA102514. We thank Dr Rana P. Singh for helpful suggestions in preparing the text and critical reading of the final draft.

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