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Review Article

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Molecular Mechanisms of Silibinin-Mediated Cancer Chemoprevention with Major Emphasis on Prostate Cancer

Harold Ting,[1] Gagan Deep,[1,2] and Rajesh Agarwal[1,2,3,4]

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Abstract. Despite advances in early detection, prostate cancer remains the second highest cancer mortality in American men, and even successful interventions are associated with enormous health care costs as well as prolonged deleterious effects on quality of patient life. Prostate cancer chemoprevention is one potential avenue to alleviate these burdens. It is a regime whereby long-term treatments are intended to prevent or arrest cancer development, in contrast to more direct intervention upon disease diagnosis. Based on this intention, cancer chemoprevention generally focuses on the use of nontoxic chemical agents which are well-tolerated for prolonged usage that is necessary to address prostate cancer's multistage and lengthy period of progression. One such nontoxic natural agent is the flavonoid silibinin, derived from the milk thistle plant (*Silybum marianum*), which has ancient medicinal usage and potent antioxidant activity. Based on these properties, silibinin has been investigated in a host of cancer models where it exhibits broad-spectrum efficacy against cancer progression both *in vitro* and *in vivo* without noticeable toxicity. Specifically in prostate cancer models, silibinin has shown the ability to modulate cell signaling, proliferation, apoptosis, epithelial to mesenchymal transition, invasion, metastasis, and angiogenesis, which taken together provides strong support for silibinin as a candidate prostate cancer chemopreventive agent.

KEY WORDS: cell cycle; chemoprevention; prostate cancer; signal transduction; silibinin.

PROSTATE CANCER CHEMOPREVENTION

Cancer chemoprevention is a treatment regime centered on the use of chemical agents to reduce cancer risk. These agents may be derived either synthetically or from natural products and are intended for long-term use, generally limiting candidate agents to nontoxic compounds. The rationale for this modality is based on the multistage and often lengthy period of time required to accumulate the cellular damage necessary for carcinogenesis (i.e., dysfunctional proliferation, differentiation, apoptosis, etc.). This then provides an opportunity to inhibit or eliminate initiated cells or localized lesions prior to their development into a fully malignant tumor. This general concept of cancer chemoprevention can be subdivided into primary, secondary, and tertiary cancer chemoprevention depending on the stage of carcinogenesis that is being targeted. Primary chemoprevention focuses on the removal of the initiating cellular dysfunction/s to decrease or eliminate cancer incidence prior to cancer formation. This is an ideal clinical outcome as it has the greatest impact on reducing treatment costs, adverse effects to the patient, and ultimately, mortality due to cancer. Barring this outcome and premalignant lesions already formed, secondary chemoprevention seeks their arrest or elimination, thus slowing or reversing progression of these lesions into malignant ones. Finally, if the previous interventions have not or cannot be enacted and a primary tumor has formed, tertiary chemoprevention seeks to inhibit the progression of tumor into a metastatic cancer as well as to prevent the recurrence of this tumor if it has been treated. In short, a cancer chemoprevention strategy could be applicable to almost all stages of carcinogenesis including post-therapy.

Prostate cancer is well suited for a cancer chemoprevention scheme for several reasons. One reason is the number of people afflicted by this disease. It is the most common cancer diagnosed in men in the United States, and there were approximately 241,740 new prostate cancer cases in 2012 alone (1). As a result, prostate cancer is also the second leading cause of mortality with an estimated 28,170 deaths in 2012 (1). Prostate cancer, when detected in advanced and metastatic stage, results in high mortality with almost three fourths of those diagnosed dying

[1] Department of Pharmaceutical Sciences, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA.
[2] University of Colorado Cancer Center, University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA.
[3] Skaggs School of Pharmacy and Pharmaceutical Sciences, University of Colorado Anschutz Medical Campus, 12850 E. Montview Blvd, Room V20-2118, Box C238, Aurora, Colorado 80045, USA.
[4] To whom correspondence should be addressed. (e-mail: Rajesh.Agarwal@UCDenver.edu)

within 5 years; however, advances in early detection have resulted in an increase in the treatment of prostate cancer as early and localized disease (1). But the counted success of early detection serves as a double-edged sword as it also comes with a concomitant increase in aggressive intervention where less aggressive treatment might have better served the patient. This is the second factor making prostate cancer a candidate for chemoprevention, situations where adverse side-effects develop in patients as a consequence of treating prostate cancer especially in cases where perhaps they were not necessary. There are a host of treatment options, and each to varying degrees carries the risk of adverse effects. Among these, the principle risks are urinary incontinence, bowel issues, and impotence. In one study, normalized by only reporting patients with normal function prior to treatment, upwards of 58% of patients reported minor to major urinary dysfunction and 27% reported bowel problems 3 years following radical prostatectomy (2). In addition, a whopping 94% reported some sexual dysfunction of which two thirds of these were reported as severe issues. In another study following the effects of radical prostatectomy 2 years following treatment, almost 49% of patients reported urinary issues ranging from complete lack of control to frequent or occasional leakage (3). Similarly, 60% of patients reported severe sexual dysfunction (3). In patients treated with radiation, it was found that urinary issues were only reported in 17% of cases but bowel issues increased in number to 66% of patients and sexual dysfunction remained high at 74% reporting mild or major issues 3 years following treatment (2). Finally, the enormous cost of treating prostate cancer highlights the benefits of the chemoprevention approach. In the USA, an estimated 11.85 billion dollars were spent in 2010 on direct health care costs alone (4). This cost is predicted to grow as a combination of increasing cancer incidence, improved early diagnosis, increasing life expectancy, and higher priced treatments gaining acceptance. In fact, this last factor is estimated to have increased prostate cancer health care costs by more than 350 million dollars from just 2002 to 2005 (5). As this sum only reflects the direct health care costs, it does not include lost worker productivity due to infirmity or death. In addition, the stress of diagnosis (whether it is accurate or a false positive) and the fear of disease recurrence place a heavy burden on the mental well-being of the patient. For these reasons, chemoprevention whereby prostate cancer might be prevented from developing or at least from progressing to a symptomatic level would be ideal to aid in lowering these costs while improving the quality of life of a large number of potential sufferers.

As there is such a clear benefit in reducing the burden of prostate cancer, both societal as well as individual, several classes of agents have been brought forth as potential chemopreventive agents. One agent of interest is finasteride, a synthetic type II 5-α-reductase (5αR) inhibitor used in the treatment of male pattern baldness. The mechanism of action of 5αR is to inhibit androgen receptor (AR) induced signaling by inhibiting the conversion of testosterone into dihydrotestosterone, a higher affinity ligand of AR. As androgen-AR signaling is an important factor in the development and progression of prostate cancer, this activity has the potential to reduce or reverse the development of the disease. In a large-scale study, finasteride was shown to reduce incidence of prostate cancer, but consistent with the long-term response of prostate cancers to other AR-ablating compounds, the tumors that did arise despite finasteride treatment more frequently had high Gleason scores, which is associated with high mortality (6,7). Other potential chemopreventive compounds have been derived from natural products often identified based on their historic usage and more specifically food products based partially on ease of clinical translation. Green tea is one such product, its active ingredient believed to be a mixture of catechins (polyphenols with antioxidant properties), most commonly derived from the plant *Camellia sinensis* (8,9). Green tea has been associated with decreased overall risk of cancer and a high intake was found to be associated with a lower incidence of prostate cancer in men (9,10). Oral administration of green tea catechins reduced PSA levels (9). Another natural product, soy, contains a mixture of isoflavones exerting antioxidant properties. Soy consumption has been associated with a decreased risk of prostate cancer (11), which might be a result of reported inhibition of signaling pathways including AR, Akt, NF-κB, mitogen-activated protein kinases (MAPKs), and Notch signaling (12,13). The tomato (*Solanum lycopersicum*) contains a compound called lycopene which is a carotenoid with strong antioxidant property. Elevated lycopene consumption is associated with low prostate cancer risk (10,14). Another fruit, pomegranate (*Punica granatum*) contains a mixture of polyphenolic compounds that act as antioxidants which have been shown to delay prostate cancer growth in patients diagnosed with prostate cancer (15). A specific polyphenolic antioxidant agent that has been extensively studied for its chemopreventive properties is silibinin.

SILIBININ

Silibinin (Fig. 1a) is derived from the seeds (Fig. 1b) of milk thistle (*Silybum marianum*; Asteraceae) which has its origins in the Mediterranean region where for millennia it has been used as a remedy for a variety of ailments, particularly of the liver, gall bladder, and kidneys. More recently, milk thistle has been found to be effective in treating hepatic injury due to bile duct inflammation, cirrhosis, fatty liver, mushroom poisoning, and viral hepatitis (16). Perhaps as a consequence of this long-standing medicinal use, the characteristic purple-red flowers of the milk thistle (Fig. 1c) can now be found growing worldwide. In modern times, the usage of the whole milk thistle has been supplemented with a standardized extract of milk thistle seeds called silymarin. This extract is composed of a complex mixture of several flavonolignans and other compounds. The flavonoid silibinin is the principle active ingredient found in silymarin and is by far the most abundant component, along with the stereoisomers dihydrosilybin, isosilybin, silychristin, and silydianin. Silibinin, in turn, is composed of an approximately equimolar mixture of two diastereomers (silybin A and silybin B). As a polyphenolic compound, silibinin is fairly water insoluble and thus is often administered within capsules. Once in the GI tract, silibinin is absorbed, circulated, conjugated in the liver, and excreted, much of it in the bile (17). In mice, plasma concentrations of free silibinin peak at 30 min and in tissues at 60 min, whereupon it decays with a half-life of 57 to 127 min; however, the concentrations of conjugated silibinin peak at 1 h and decay with a half-life of 45 to 94 min (18). Silibinin exhibits low toxicity as reported in studies where animals were intravenously injected with silymarin. A 50% lethal dose (LD_{50}) required high concentrations of silymarin, depending on specific experimental conditions: mice tolerate 400–1,050 mg/kg, rats 385–920 mg/kg,

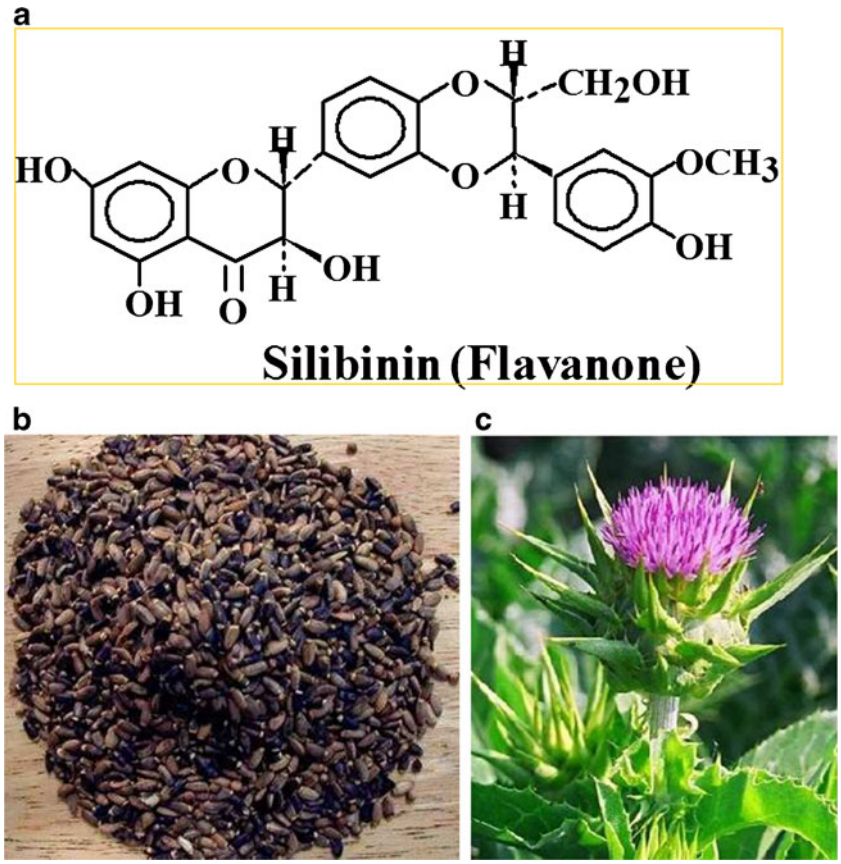

Fig. 1. **a** Chemical structure of silibinin, **b** milk thistle seeds, and **c** flower of the *S. marianum*; Asteraceae

and rabbits and dogs 140–300 mg/kg (19–21). When silymarin was delivered orally, the required values of silymarin to achieve toxicity were, in some cases, over 10 g/kg (19–21). In two human trials, a commercial silibinin formulation, silibinin phytosome, was administered orally to prostate cancer patients at 13 g daily for a mean of 20 days and 2.5–20 g/daily for 28 days, respectively (22,23). Consistent with silibinin's target organ in clinical usage, the most common adverse event at high doses was asymptomatic hepatotoxicity followed by low-grade hyperbilirubinemia (grades 1–2) and diarrhea (22,23). There was one case of a grade 4 postoperative thromboembolic event (out of 19 total treated patients within the two trials). Together these studies provide compelling evidence for well-tolerated administration of high doses of silibinin in human patients.

BROAD SPECTRUM CANCER CHEMOPREVENTIVE EFFICACY OF SILIBININ

Flavonoids possess antioxidant activity which has been reported to result in diverse biologically protective properties such as inhibiting inflammation, neoplasia, hepatic injury, and other ailments (24). Consistent with other flavonoids, silibinin has been found to be a very potent antioxidant, buttressing native cellular antioxidant mechanisms such as glutathione (GSH) and superoxide dismutase by scavenging free radicals, and reactive oxygen species (ROS) (25,26). This may in part explain silibinin's effectiveness in addressing hepatic injury whether as a result of disease or exposure to toxins as this antioxidant activity may eliminate the oxidative stress associated with hepatic insults preventing the induction of lipid peroxidation (and thus cell death). As a consequence of the general anticancer properties associated with flavonoids collectively, as well as the strong antioxidant potential of silibinin specifically, there has been significant interest in adapting silibinin for use as a chemopreventive agent. In fact, silibinin has been widely investigated for anti-cancer efficacy in a broad range of cancers models.

Based on these factors, and the diseases silibinin has been historically used to treat, it is perhaps not a surprise that silibinin has been found to have an inhibitory effect on cancers of several digestive and excretory organs. Silibinin was found to inhibit cell proliferation and invasion in various hepatocarcinoma cell lines (27–30) as well as in a mouse xenograft model (31). These effects appear to be a consequence of inducing apoptosis as well as cell cycle arrest in hepatocarcinoma cells. Silibinin inhibited ERK1/2 signaling, downregulated survivin, highly expressed in cancer protein-1, the E2F1/DP1 complex, cyclin D1, cyclin D3, cyclin E, cyclin-dependent kinase (CDK)2, and CDK4 levels and induced CDK inhibitor (CDKI) Kip1/p27 in hepatocarcinoma HEPG2 cells (27,28). Furthermore, silibinin altered Akt signaling, downregulated phosphorylation of retinoblastoma (Rb), upregulated histone acetylation, and activated caspases 3 and 9 in hepatoma HuH7 cells (30). In addition, silibinin reduced vascular endothelial growth factor (VEGF) secretion, metalloproteinase-2 (MMP-2), and CD34 in hepatic cancer cells suggesting inhibition of angiogenesis in hepatic tumors (29,30). In pancreatic cancer cell lines, silibinin inhibited their proliferation in a dose- and time-dependent fashion which translated to a decrease in tumor

volume in a mouse xenograft model (32). Again, this inhibition appeared to be a function of both increased apoptosis as well as cell cycle arrest by silibinin. In gastric cancer cells, silibinin dose-dependently inhibited TNF-α-induced secretion of metalloproteinase-9 (MMP-9) (33). Silibinin was found to be deliverable to the human colorectal mucosa in high amounts through ingestion of nontoxic doses of silibinin (34), and consistent for use as a chemopreventive agent, silibinin was found to be beneficial in early colon tumorigenesis (35), reducing loss of differentiation of carcinomas in mice (36), while also inhibiting colon cancer stem-like cells (37). Silibinin potently inhibited the growth of HT-29 and LoVo cells both *in vitro* as well as in xenograft models, strongly inducing G1 and more modestly G2-M cell cycle arrest (38,39). This was associated with decreased levels of cyclins (A, B1, D1, D3, and E), cell division cycle 25C (cdc25C), and Cdc2/p34; decreased activity of cyclin-dependent kinases (1,2,4,6); and phosphorylated Rb in conjunction with increased levels of CDKIs (Cip1/21 and Kip1/p27) (38,39). Silibinin also induced apoptosis associated with increased activation of caspases 3 and 9 as well as poly(ADP-ribose) polymerase (PARP) in LoVo cells (38); however, silibinin-induced apoptosis was independent of caspases activation in HT-29 cells (39). Furthermore, the invasive potential of LoVo cells was reduced by silibinin which was associated with a decrease in MMP-2 (40). Silibinin treatment also led to a decrease in polyp size and number in $APC^{min/+}$ mice, a model of familial adenomatous polyposis (41,42). This phenomenon was associated with decreased β-catenin, c-Myc, phospho-glycogen synthase kinase-3β, and phospho-Akt (41,42). Silibinin-mediated reduction in colorectal carcinoma proliferation and concomitant increase in apoptosis were associated with inhibition of ERK1/2 and Akt (43). Silibinin-mediated angiogenesis inhibition was associated with decreased VEGF, cyclooxygenase (COX), hypoxia-inducible factor-1α (HIF-1α), inducible nitric oxide synthase (iNOS), nitrotyrosine and nitrite levels, and an increased VEGFR-1 (Flt-1) expression (41,43–45). Silibinin inhibited CDK8 and β-catenin signaling which inhibited SW480 tumor growth (46) and initiated an autophagic-mediated survival response in SW480 and SW620 cells (47). Silibinin also suppressed 1,2-dimethylhydrazine (DMH)-induced colon carcinogenesis in rat models via modulating xenobiotic metabolizing enzymes and increasing enzymatic antioxidants to detoxify carcinogens (48,49). This action translated to decreased oxidative stress and subsequent lipid peroxidation, abrogating DMH-induced neoplasia (50).

Consistent with its effect in models of digestive organ cancers, silibinin was also found to inhibit excretory organ cancers. Silibinin treatment decreased renal cancer 786-O cell proliferation and invasiveness (51), while inhibiting proliferation and increasing apoptosis in renal cancer Caki-1 cells (52). This action was associated with inhibition of epidermal growth factor (EGF), ERK1/2, and survivin expression with concomitant upregulation of p53 expression and caspase cleavage (52). Silibinin feeding reduced the size of 786-O renal tumors in mice xenografts which was associated with decreased expression of MMP-2, MMP-9, and urokinase-type plasminogen activator (u-PA), and activation of p38 and ERK1/2 (51). Silibinin also enhanced the sensitivity of −786-O renal cell carcinoma cells towards 5-fluorouracil and paclitaxel (51). Treatment of SN12K1 cells with silibinin reduced cell viability and DNA synthesis resulting in apoptosis (53). Likewise, silibinin-fed SCID mice injected with SN12K1 cells exhibited a reduction in tumor size (54). Consistent with these results, several studies have shown that silibinin inhibits growth as well as induces apoptosis in several urinary bladder cancer cell lines which were associated with an increase in p53 expression, downregulation of survivin, cyclin D1, ERK1/2 phosphorylation and nuclear phospho-p65, cleavage of caspases, PARP, and Cip1/p21, and mitochondrial release of cytochrome *c*, Omi/HtrA2, and apoptosis-inducing factor (55–60). This silibinin-mediated inhibition was also observed in rat models of urinary bladder cancer reducing lesions (60).

Silibinin was also found to reduce oral cancer cell invasion as a consequence of decreased MMP-2 and u-Pa expression, decreased ERK1/2 activation, and increased tissue inhibitor of metalloproteinase-2 (TIMP-2), and plasminogen activator inhibitor-1 (PAI-1) expression (61). Likewise, in laryngeal squamous cell carcinoma SNU-46 cells, silibinin induced apoptosis (62). Furthermore, silibinin inhibited proliferation, invasion, and angiogenesis in lung carcinoma while simultaneously inducing apoptosis (63–65). Proliferation of Anip973 cells was inhibited by silibinin (66), which in non-small cell lung cancer cell lines corresponded to inhibition of CDK2, CDK4, and Rb phosphorylation, as well as induction of apoptosis by activation of the caspase cascade pathway (63,67). Similar to oral cancer, silibinin treatment concentration- and time-dependently decreased MMP-2 and u-Pa expression through inhibition of either ERK1/2 or Akt phosphorylation along with increasing TIMP-2 expression which together translated to an inhibition of invasiveness in the aggressive human lung adenocarcinoma A549 cells (68,69). Silibinin was reported to decrease expression of COX-2, iNOS, MMP-2, and MMP-9 and inhibit activation of ERK1/2, NF-κB, STAT-1, and STAT-3 in mouse lung epithelial LM2 cells (70). Reduction of iNOS elicited by silibinin treatment was also found in A549 cells (71). In the A/J mouse model of lung cancer, silibinin treatment reduced the number, growth, progression, and angiogenesis of induced tumors which was associated with downregulated VEGF, COX-2, iNOS, HIF-1α, STAT-3, and NF-κB, and increased Ang-2 and Tie-2 (64,65). Furthermore, silibinin enhanced sensitivity of A549 cells to doxorubicin through reduction of NFκ-B-mediated chemoresistance (72). In glioblastoma models, silibinin was shown to inhibit growth and invasiveness and induce apoptosis (73,74). Silibinin was also reported to inhibit EGFR activation in a rat glioma cell line stably expressing human EGFR (75). NF-κB-mediated stimulation of MMP-9 in glioblastoma U87 cells was found to be abrogated by silibinin treatment which served to attenuate invasiveness (74). Silibinin was found to induce caspase-mediated apoptosis by activating MAPKs as well as reverting sensitivity to TRAIL signaling in otherwise resistant glioma cells by modulating components of the death receptor-mediated apoptotic pathway (73,76). Interestingly, in the glioblastoma cell line, U87MG, silibinin appeared to partially synergize with arsenic trioxide treatments to increase apoptosis while inhibiting cell proliferation, metabolism, and mRNA expression of several proteinases (77) suggesting the possibility of combinatorial treatments to arrest cancer.

Several studies have also revealed that silibinin offers protection from photo-carcinogenesis in skin cancer models. A key mechanism by which silibinin mitigates UVA- and UVB-induced dysfunction is activation of the DNAPK-p53 pathway, inhibiting DNA synthesis, cellular proliferation, and apoptosis

and inducing cell cycle arrest and repair in response to UV-induced DNA damage which together serves to inhibit tumor appearance and growth (78–81). This response is in part mediated by inhibition of ERK1/2, with concomitant increase of p53 and p21/Cip1 (82,83). Furthermore, silibinin was found to abrogate ATP and GSH depletion, ROS production, and lipid peroxidation in UVA-irradiated human keratinocytes, corresponding to inhibition of UVB-induced PARP and caspase 9 cleavage (84,85). These effects operated in conjunction with inhibition of inflammatory mediators such as COX-2, STAT-3, and NF-κB and angiogenic mediators such as HIF-1α and iNOS (86). In MG-63 cells, silibinin treatment reduced osteosarcoma invasiveness which was associated with inhibition of focal adhesion kinase, ERK1/2 activation, and uPA and MMP-2 expression (87). Similarly, in HT1080 cells, silibinin treatment activated p38 and JNK pathways and inhibited ERK and Akt pathways resulting in autophagy (88).

In breast cancer models, silibinin induced apoptosis in MCF-7 cells which synergized with inhibition of insulin growth factor receptor (IGFR) (89,90) and also inhibited metastasis of MDA-MB-231 cells (91). In addition, silibinin dose-dependently decreased expression of EGFR ligand-induced CD44, 12-*O*-tetradecanoylphorbol-13-acetate-induced MMP-9 and VEGF, as well as activation of ERK1/2 (92–94). Interestingly, silibinin induced reactive nitrogen species and ROS generation in MCF-7 cells (95). These phenomena translated to induction of tumor growth arrest and apoptosis in silibinin-treated HER-2/neu transgenic mice (96). In accordance with these findings, silibinin increased apoptosis and induced G2-M cell cycle arrest of A2780/taxol cells, enhancing their sensitivity to paclitaxel, which was associated with the downregulation of survivin and P-glycoproteins (97). In turn, mice xenografts with A2780 cells exhibited a reduction in angiogenic activity in response to silipide (silibinin phytosomes) treatment as a consequence of downregulation of VEGF receptor 3 and upregulation of Ang-2 (98). Together, the abovementioned studies clearly demonstrated the broad spectrum chemopreventive and anticancer efficacy of silibinin. Next, we have focused on silibinin efficacy and mechanism of its action against prostate cancer cells.

MOLECULAR MECHANISMS FOR SILIBININ CHEMOPREVENTIVE EFFICACY AGAINST PROSTATE CANCER

Silibinin has been shown to potently inhibit prostate cancer through targeting multiple cell signaling pathways, decreasing proliferation, inducing apoptosis, and inhibiting invasion, metastasis, and angiogenesis. The specific molecular targets of silibinin that induce broad-spectrum efficacy against prostate cancer are summarized in Fig. 2.

SILIBININ EFFECTS ON CELL SIGNALING IN PROSTATE CANCER CELLS

Silibinin has been shown to disrupt several signaling pathways known to be important in the development and progression of prostate cancer. Treatment of prostate cancer cells with silibinin abrogated constitutive activation of STAT-3 in DU145 cells (99), disrupted EGFR signaling in LNCaP and DU145 cells (100,101), targeted IGFR signaling in PC3 cells (102), the Wnt/β-catenin pathway in PC3 and DU145 cells (103), and AR signaling in LNCaP cells both directly by reducing nuclear localization of the receptor (104) and indirectly through downregulation of a co-activator, prostate epithelium-derived Ets transcription factor (105,106). Disruption of EGF signaling by silibinin in prostate cancer cells was associated with a decrease in secreted transforming growth factor-α and modulation of MAPK activity of both ERK1/2 and JNK1/2 (101). Disruption of the Wnt/β-catenin pathway involved modulation of a co-receptor, the low-density lipoprotein receptor-related protein-6 (LRP6) (103). Silibinin inhibited the promoter activity, mRNA, basal expression, as well as phosphorylation of LRP6 (103). Silibinin also dose-dependently induced mRNA for insulin-like growth factor-binding protein-3 (IGFBP-3) which translated into higher concentrations of IGFBP-3 in PC3 conditioned medium (102). In accordance with this finding, silibinin feeding of mice was found to upregulate both circulating plasma and tumor levels of IGFBP-3 and a decreased loss of differentiation in their tumors (36,107,108). Finally, silibinin was found in several studies to broadly alter NF-κB signaling (109,110). It inhibited the constitutive activation of NF-κB found in prostate carcinoma DU145 cells, decreasing IKKα kinase activity, the resultant ratio of phospho-IκBα to IκBα, and ultimately, the translocation of p50 and p65 NF-κB subunits to the nucleus (109).

SILIBININ INHIBITS PROLIFERATION OF PROSTATE CANCER CELLS

Multiple studies have shown that silibinin inhibits prostate cancer cell proliferation (111–114). In addition, mice fed with silibinin exhibited decreased tumor growth both in xenograft as well as transgenic models of prostate cancer (107,108,115–117). These phenomena were in part due to potent cell cycle arrest induced by silibinin in prostate cancer cells (111). Silibinin mediated G1 arrest in prostate cancer cells by modulating a plethora of elements in the cyclins–CDKs–CDKIs pathway: decreasing protein levels of cyclin D1, cyclin D3, cyclin E, CDK4, CDK6, and CDK2, and kinase activity of CDK2 and CDK4, increasing CDKIs Kip1/p27 and Cip1/p21, and sequestering cyclin D1 and CDK2 in the cytoplasm (108,111,113,118,119). In addition, silibinin induced a marked increase in Rb levels, principally in the hypophosphorylated retinoblastoma Rb/p107 and Rb2/p130, as well as a marked decrease in levels of the transcription factors, E2F3, E2F4, and E2F5 which altogether serves to inhibit cell cycle progression (113,118). Furthermore, silibinin mediated G2-M arrest by modulating the Chk2–Cdc25C–Cdc2/cyclin B1 pathway and decreasing levels of cyclin A, cyclin B1, both total and phosphorylated Cdc2, Cdc25B, and Cdc25C phosphatases, and inhibiting Cdc2 kinase activity (111,120,121). The inhibition of Cdc25C phosphatases combined with increased checkpoint kinase-2 phosphorylation resulted in the translocation of nuclear Cdc25C to the cytoplasm as a result of increased phosphorylation (111). This was accompanied by an increased binding with 14–3-3β (111). In addition, silibinin has been reported to inhibit both telomerase as well as DNA topoisomerase IIα activity in LNCaP and DU145 cells, respectively (105,122). Interestingly, both mitoxantrone and doxorubicin were found to synergize with silibinin in inhibiting prostate cancer cell proliferation (121,123), and cisplatin and carboplatin were found to synergize with silibinin in inducing G2-M arrest corresponding to potent

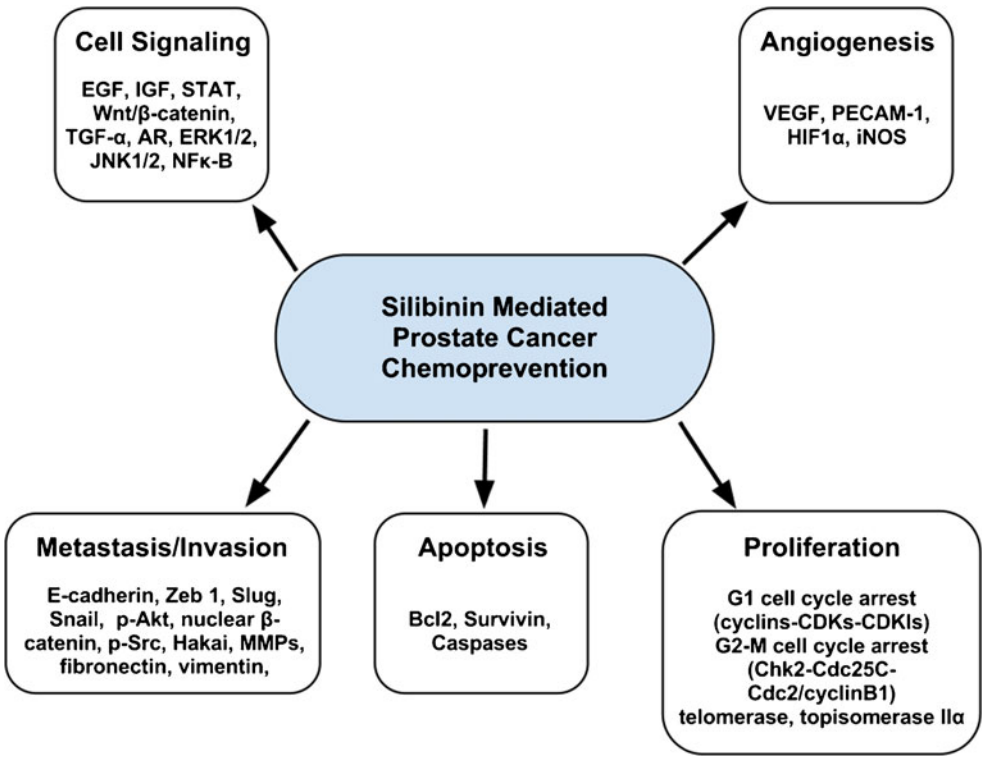

Fig. 2. Schematic representation of the molecular mechanisms for silibinin-mediated prostate cancer chemoprevention

downregulation of Cdc2, cyclin B1, and Cdc25c (124). Together, these findings suggest the potential for combinatorial treatments to arrest prostate cancer progression.

SILIBININ INDUCES APOPTOSIS IN PROSTATE CANCER CELLS

Studies have shown that silibinin also initiates apoptosis in prostate cancer cells under certain treatment conditions (99,107,108,124,125). The mechanism appeared to be a consequence of decreased Bcl-2 and survivin levels, caspase activation (caspases 3, 9, and 7), subsequent cytochrome *c* release from mitochondria, and ultimately apoptosis (99,107,108,124). Interestingly, mitoxantrone, doxorubicin, cisplatin, and carboplatin were each found to synergize with silibinin in inducing apoptosis in prostate cancer cells (121,123,124).

SILIBININ INHIBITS INVASION AND METASTASIS OF PROSTATE CANCER CELLS

Multiple studies have revealed that silibinin initiates a shift of treated advanced prostate cancer cells back into an epithelial phenotype and inhibits metastasis (110,116,117,126). It was reported that in PC3, PC3MM2, and C4-2B cells, silibinin upregulated E-cadherin on their cell surface, significantly inhibiting their migratory and invasive potential (126). This phenomenon appeared to be a result of downregulation of epithelial to mesenchymal transition (EMT) regulatory molecules Slug, Snail, phospho-Akt (ser^{473}), nuclear β-catenin, phospho-Src (tyr^{419}), and Hakai (126). This silibinin-induced increase in E-cadherin was also found in a transgenic adenocarcinoma of the mouse prostate (TRAMP) model in which silibinin decreased levels of MMPs, Snail, fibronectin, and vimentin translating into a reduction in cancer metastasis (116,117). Other studies found $ARCaP_M$ cells treated with silibinin exhibited decreased expression of major EMT regulators, the transcription factors ZEB1 and Slug, corresponding with decreased expression of EMT markers, vimentin and MMP-2, together translating into dose- and time-dependent reduction of invasion, motility, and migration (110,127). Along with MMP-2, silibinin has been found to inhibit MMP-9 expression in human prostate carcinoma cell lines (116,117).

SILIBININ EXHIBITS STRONG ANTI-ANGIOGENIC EFFICACY AGAINST PROSTATE CANCER CELLS

Targeting angiogenesis is considered an important element in preventing the growth and progression of solid tumors including prostate cancer. Silibinin was reported to inhibit angiogenesis, decreasing VEGF expression levels and tumor microvessel density in prostate tumors (107,108,116). This anti-angiogenic potential was supported in a study of TRAMP mice where silibinin feeding resulted in decreasing expression of platelet endothelial cell adhesion molecule-1 (PECAM1)/CD-31, VEGF, VEGFR2, HIF-1α, and iNOS (117). This expression pattern corresponded to an increase in glucose and citrate use along with a concomitant decrease in lactate, cholesterol, and phosphatidylcholine levels in prostatic tumors of silibinin-fed TRAMP mice (128). Silibinin treatment of LNCaP and PC3 prostate cancer cells also inhibited their synthesis of HIF-1α both basally as well as induced by hypoxia (129).

CONCLUSIONS

Silibinin, a flavonoid antioxidant derived from the milk thistle has been used for millennia to treat a diverse set of ailments. In more recent times, as a product of this long-term historical usage and aforementioned antioxidant chemistry along with protective properties identified in several other flavonoids, silibinin has been investigated in a host of cancer models. In these studies, silibinin has been found to possess multifactorial anti-cancer efficacy, operating on a broad array of signaling and regulatory mechanisms in diverse milieus. Specifically in regards to prostate cancer, silibinin has been shown to alter cell proliferation, apoptosis, EMT, invasion, metastasis, and angiogenesis. These effects of silibinin have the potential to impact prostate cancer progression encompassing the full range of clinical disease presentation from initial cellular dysfunctions in incipient lesions to advanced metastatic tumors. However, further investigations to confirm the mechanisms of silibinin effect on the prostate cancer microenvironment, as well as to elucidate its efficacious delivery and clinical usage are still needed. Taken together, the evidence provides strong support for the promise of silibinin as a candidate prostate cancer chemopreventive agent.

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