[Frontiers in Bioscience S3, 777-787, January 1, 2011]

Skin cancer chemoprevention by α-santalol

Xiaoying Zhang[1], Chandradhar Dwivedi[2]

[1]Department of Biology, Shanghai chempartner Co., LTD, Shanghai, China 201203, [2] Department of Pharmaceutical Sciences, South Dakota State University, Brookings, SD 57007

TABLE OF CONTENT

1. ABSTRACT

Alpha-santalol, a naturally occurring terpenoid, has been shown to have chemopreventive effects on both 7, 12-dimethylbenz(a)anthracene (DMBA)-initiated and 12-O-tetradecanoylphorbol-13-acetate (TPA)-promoted skin cancer development in CD-1 and SENCAR mice, and UVB-induced skin cancer developments in SKH-1 hairless mice in a concentration-dependent manner. Studies have demonstrated that α-santalol could be effective against skin carcinogenesis through both induction of apoptosis *via* caspase activation together with dissipation of mitochondria membrane potential and cytochrome *c* release in A431 cells, and inhibition of cell growth *via* induction of G_2/M phase arrest in both A431 cells and melanoma UACC-62 cells by altering multiple cell cycle regulatory proteins and complexes. This review summarizes the chemopreventive effects and molecular mechanisms of α-santalol on skin cancer development in both animal models and skin cancer cell lines.

2. INTRODUCTION

2.1. Skin cancer

Cancer is the second most common cause of death in the USA accounting for one of every four deaths (1). Among all the cancers, skin cancer is the most common form of cancer in the United States with more than one million cases diagnosed yearly leading to an estimated 11,790 deaths in 2010 (1). The incidence of non-melanoma skin cancer (NMSC), including basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) are the most common malignant neoplasms in human (2). It has been estimated that more than 1 million cases of BCC and SCC are diagnosed each year in the US alone (1), which is equivalent to the incidence of malignancies in all other organs combined (3-4). Therefore, the development of effective chemopreventive or chemotherapeutic agents is a logical approach to address the management of cutaneous malignancies.

Skin is a protective barrier against microbial, chemical, radiation, thermal and electrical insults from the

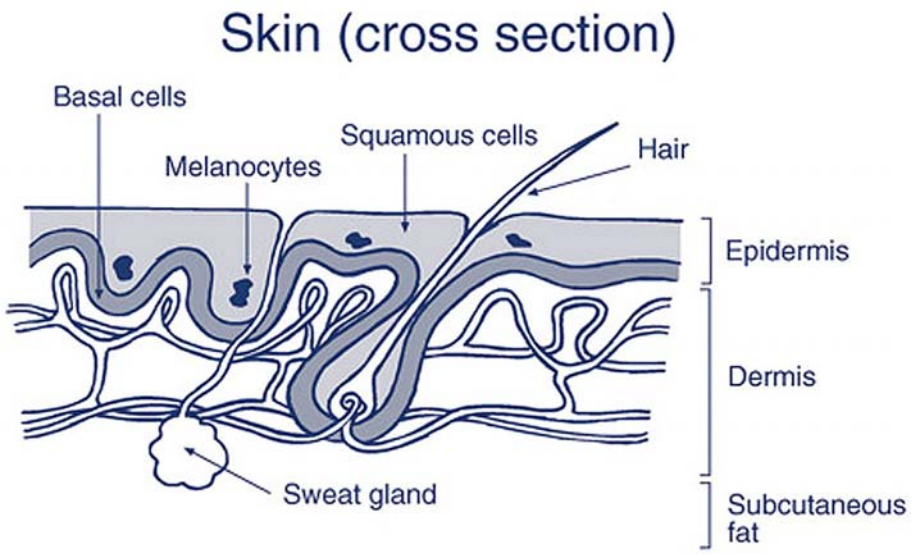

Figure 1. The structure of skin (5).

environment. As shown in Figure 1 (5), skin has three layers differing in function, thickness, and strength. The top layer is called the epidermis which is a tough protective layer that contains three different kinds of cells: basal cells which produce new cells constantly to push older ones up toward the surface of the skin; squamous cells which produce keratin, a tough, protective protein that makes up the majority of the structure of the skin, and melanocytes which produce the skin coloring or pigment known as melanin giving skin its tan or brown color and helping protect the deeper layers of the skin from the harmful effects of the sun. The second layer (located under the epidermis) is called the dermis which contains nerve endings, sweat glands, blood, and hair follicles. A fatty layer of subcutaneous tissue is under these two skin layers known as subcutaneous layer.

In general, skin cancers are named after the type of cell they original from. There are three common types of skin cancer: basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and melanoma. BCC is the most common skin cancer and arises from basal cells of the epidermis where the involved basal cells fail to mature into keratinocytes. SCC involves epidermal keratinizing cells that invade the dermal-epidermal junction. Melanomas are malignant cutaneous tumors that develop from melanocytes, which produce melanin and are responsible for skin color. In general, nonmelanoma skin cancers are readily treatable, rarely metastasize, and only infrequently cause death. Melanoma is a different matter and if not treated successfully it can spread to affect the liver, lungs or brain (6).

There are various risk factors that lead to the development of skin cancer, such as sun damage, genetics, chemical exposure and immunosuppression. Among the factors, chronic exposure to ultraviolet (UV) B light (290-320 nm) is the most recognized cause of skin cancer. The mechanism of cancer induction appears to be both damage to DNA within the epidermis and induction of immunologic changes that inhibit an immune response against the tumor formation (7-8). Thus, people should be taught basic "safe sun" measures: sun avoidance during peak ultraviolet-B hours; proper use of sunscreen and protective clothing; and avoidance of suntanning (9).

In addition to UVB damage, chemical exposure leads to the formation of skin cancer. Both occupational and environmental exposure to some chemicals has resulted in an increased risk of skin cancers. For example, a long-term exposure to arsenic has been shown to induce keratoses that increase the risk of SCC (10).

2.2. Experimental animal models for skin tumor carcinogenesis

Carcinogenesis is a multistage process that leads a normal cell to evolve into a cancer cell (11). The process of carcinogenesis is divided into three stages: initiation, promotion and progression. In this process, initiation is the first step in which a carcinogen interacts with DNA, producing a strand break or, more often, an altered nucleotide called an adduct. In the presence of promoting agents, the initiated cell clonally expands into a visible tumor, often a benign lesion such as a papilloma. The effect of promotion is completely reversible, and if the promoter is removed, disappearance of the expanding clones of cells will result. They will reappear if the promoter is reapplied. The benign lesion must undergo the progression to a malignant neoplasm which involves the acquisition of one or more qualitative changes in the precursor cells. In fact, progression probably involves multiple, heritable changes. These alterations could arise from additional exposures to a carcinogen, spontaneous mutations due to the natural infidelity of enzymes involved in replication, or genomic instabilities induced by initiating mutations which result in an irreversible change in the cell that allows expression of malignant neoplasm (11).

Chemical- and UVB radiation-induced skin tumors in mice are two typical models reflecting two risk factors of skin cancer and are often used to study mechanisms and efficacy of putative cancer chemopreventive agents in skin tumor development. Classically, murine skin tumors can be experimentally produced by either a single carcinogenesis protocol, or two-stage system carcinogenesis protocol which is commonly used in various laboratories (12-15). The two-stage system of mouse skin carcinogenesis is characterized as follows: initiation is a rapid, irreversible event treated by a single topical application of a subthreshold dose of a carcinogen, e.g., 7, 12-dimethylbenz(a)anthracene (DMBA); no tumors can be caused by this treatment, but the formation of tumor development can be induced by repeated applications of a noncarcinogenic agent called a promoter, e.g., 12-O-tetradecanoylphorbol-13-acetate (TPA) (14-15).

In the DMBA-initiated and TPA-promoted skin tumor mouse model, initiation, the first stage of carcinogenesis, is characterized by exposure to a mutagenic carcinogen, DMBA. The effects of this exposure are irreversible and just one exposure is sufficient to permanently alter cells. Promotion, the second stage of carcinogenesis, involves repeated exposure to a promoter, TPA that permits and increases the development of benign tumors, or papillomas in an initiated cell population. Progression, the third stage of carcinogenesis, is marked by conversion of papillomas into carcinomas (16-17).

Over-exposure to ultraviolet radiation from the sun can cause sunburn, skin damage and ultimately, skin cancer. Solar ultraviolet radiation is conventionally divided into UVA (320-400 nm), UVB (280-320 nm) and UVC (200-280 nm). UVB and UVC are potentially the most dangerous of the three. However, UVC does not penetrate the earth's atmosphere. In addition, the level of UVC reaching the earth's surface is controlled largely by the amount of ozone in the atmosphere (18-19).

UVB can act in mouse skin models as a complete carcinogen, meaning that UVB can function as an initiator as well as a promoter (20-21). The initiation process in UVB-induced skin carcinogenesis seems to involve producing distinctive mutations of DNA. Mutations due to direct absorption of UV light by DNA are predominantly C→ T transitions at dipyrimidine sites, including CC → TT double-base mutations (22). Tumor promotion by UVB is thought to be because of alterations in signaling molecules including epidermal growth factor (EGF) receptors (23), mitogen-activated protein kinases (MAPKs) (24) and phosphatidylinositol 3-kinase (PI3K) (25) that give rise to changes in specific proteins to increase cell proliferation. Tumor progression by UVB operationally involves the conversion of a benign tumor to a malignant tumor. These seem to be further gene alteration, including changes in gene copy number, gene mutations and gene rearrangements (26).

2.3. Chemoprevention of cancer

The most desirable way of eliminating the impact of cancer in human is by prevention, since chemotherapy and surgery have not been fully effective against the high incidence of most of the cancers (27). Chemoprevention of cancer is a means of cancer control in which the occurrence of this disease is prevented by administration of one or several chemical compounds (27).

Chemopreventive agents can be divided into two categories. The first category includes blocking agents. There are inhibitors that prevent carcinogenic agents from reaching or reacting with critical target sites by directly inhibiting full carcinogens, initiators or promoters. There are a different set of compounds such as the phenols, tannins, flavones, curcumin, which inhibit tumor promotion by inhibiting the components in arachidonic acid cascade, antioxidant activity and hormonal response modulation (28). The second category of chemopreventive agents includes suppressing agents which prevent the evolution of the neoplastic process in cells by preventing carcinogen activation, increasing detoxification, and trapping reactive carcinogenic species. The most extensively studied suppressing agents are the retinoids and selenium salts (29).

Chemoprevention of cancer focuses on two general categories of risk group. One of these is the general population and the other is subjects who are at increased risk of cancer. Individuals in the general population are not ill, and if they do develop cancer, it can be at any one of a large number of organ sites. Accordingly, the chemopreventive agents used must have trivial or no toxicity. Chemopreventivion of cancer in individuals at high risk has less stringent toxicity requirements. Organ specificity is clear in most instances in individuals at high risk so that targeting of agents is defined (29). In the general population, the first priority is to use blocking agents, which prevent attack on host tissues by carcinogenic agents. The priority of use of chemopreventive agents for high-risk individuals would be suppressing agents, since high risk individuals have already been exposed to carcinogenic stimuli so that it is necessary to prevent evolution of the neoplastic process in those cells already attached (29).

2.4. Natural agents for skin cancer chemoprevention

Recently, much attention has been devoted to identifying cancer chemopreventive phytochemicals of natural products, minerals and vitamins (30). It is appreciated as plausible strategy for cancer chemoprevention for two main reasons: the first one is that they do not require toxicological evaluation and secondly unlike environmental factors which are difficult to control these can be controlled by modifying various dietary habits (31). Among these phytochemicals, many of them have been shown chemopreventive effects against skin carcinogenesis including green tea, resveratrol, curcumin and silymarin.

For example, oral administration of green tea polyphenols (GTPs) reduced UVB-induced tumor incidence, tumor multiplicity, and tumor growth *in vivo* through affecting several biomarkers that are involved in UV carcinogenesis, including inhibition of angiogenic factors and recruitment of cytotoxic T cells in the tumor

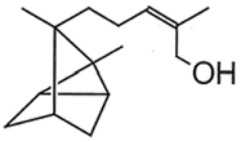

Figure 2. The structure of α-santalol.

microenvironment (32-34). (-)-Epigallocatechin-3-gallate (EGCG), a major constituent of green tea polyphenols derived from the leaves of the *Camellia sinensis* prevented photocarcinogenesis in mice *in vivo* studies and also suppressed extracellular signals and cell proliferation through EGF receptor in human epidermoid carcinoma A431 cells *in vitro* systems (4, 32, 35).

Resveratrol (trans-3,4'5 trihydroxystibene) is another naturally occurring polyphenolic phytoalexin found in grapes. It was found that resveratrol treatment resulted in strong chemopreventive effects against both UVB-induced skin carcinogenesis (36) and DMBA-induced skin carcinogenesis in mice (37) which may be through regulation of apoptosis and cell survival in mouse skin tumors (37-38), modulations in survivin (36) and suppression of COX-2 expression by blocking the activation of mitogen-activated protein kinases (MAPK) and activator protein-1 (AP-1) (39-40).

Curcumin is a yellow ingredient obtained from the root of turmeric plant, *Curcuma longa*. Topical application prior to carcinogen challenge has shown to enhance glutathione content, inhibit lipid peroxidation and arachidonic acid metabolism and also shown to decrease the ornithine decarboxylase (ODC) activity. In the recent efforts to understand the molecular mechanism behind the chemopreventive activity of curcumin has illustrated that curcumin inhibited induction of COX-2 and attenuated NF-κB activation by inhibiting IκB phosphorylation (41). It is observed that curcumin inhibited DMBA-initiated and TPA-promoted skin tumor development in mice in *in vivo* studies and also induced apoptosis in human melanoma cells through Fas receptor/caspase-8 pathway in *in vitro* studies (42-43).

Silymarin is a polyphenolic flavonoid, isolated from the seeds of Silybum marianum obtained as a mixture of sevaral flavonolignans, silybin, silydianin and silychristin, silibinin of which silybin is the most active one. In a study conducted by Katiyar (44) that topical application of silymarin to female SKH-1 hairless mice prior to chemical and photocarcinogenesis had a significant protective effect. Initial studies by Ahmad *et al.* (45) reported that activation of EGFR is an early mechanism associated with photocarcinogenesis. Mechanistic studies by Mallikarjuna *et al.* (46) further illustrated the TNF-an inhibitory effects of silymarin. Similar studies conducted by this group on silibinin have shown protective action against UVB induced skin damage. The findings from this study suggests that topical application of silibinin before and immediately after UVB exposure on SKH-1 hairless mice has photoprotective effects mediated by inhibition of DNA synthesis, cell proliferation and induction of apoptosis by the modulation of p53 and cyclin-dependent kinase (CDK)-cyclin-CDK inhibitor levels (47-49) .

3. SKIN CANCER CHEMOPREVENTION BY α-SANTALOL IN ANIMAL MODELS

3.1. Skin cancer chemoprevention by sandalwood oil

The essential oil, emulsion or paste of sandalwood (*Santalum album* L) has been used for centuries in India for the treatment of inflammatory and eruptive skin diseases. The essential oil of sandalwood is distilled from the small chips and billets cut out of the heart wood of sandalwood. The oil is of a light yellow color and possesses a characteristic pleasant odor (50-51).

Studies have been shown that oral feeding of sandalwood oil can induce the activity of glutathione S-transferase (GST) and the level thiol (SH) group in the liver. GST, a phase II enzyme, on induction by endogenous and exogenous chemicals plays an important role in protecting macromolecules against reactive carcinogenic species. It catalyzes conjugation reactions involving conversion of reactive electrophilic species into thioesters of glutathione which are subsequently transformed into readily excretable substances (50).

Studies (51) from our laboratory showed that topical application of 5% sandalwood oil significantly decreased papilloma incidence by 67% and multiplicity by 96% in DMBA-initiated and TPA-promoted skin tumors in female CD-1 mice. Sandalwood oil also showed a significant decrease in ODC activity and incorporation of thymidine into epidermal DNA induced by TPA in CD-1 mice. Further studies (52) from our laboratory suggested that the topical application of sandalwood oil significantly ($P < 0.05$) decreased skin tumor incidence and multiplicity in a concentration- and time-dependent manner. Among the three time schedules (0.5, 1 and 2 h before carcinogen applications), topical application of sandalwood oil (at all concentrations) 1 h before TPA produced most effective chemopreventive effects. Although there were no significant differences among 5% sandalwood oil pre-treatment groups and 3.75%, 2.5% sandalwood oil treatment groups on papilloma incidence, the topical application of sandalwood oil at 5% 1 h before TPA provided maximum chemopreventive effects.

The major constituent (90% or more) of the sandalwood oil is santalol, which is available as a racemic mixture of two isomers α- and β-santalol. The other constituents include aldehydes, ketones, isovaleric aldehydes, santanone, esters and free acids (52). NMR and GC-MS analysis have identified α-santalol (Figure 2), a naturally occurring terpenoid constituting about 61%, as a major component of sandalwood (53). Thus, effects of α-santalol on skin cancer development and its possible mechanisms of action were further investigated from our laboratory.

3.2. Effects of α-santalol in DMBA-TPA induced skin cancer development in mice

Based on the findings from sandalwood oil, isolation of α-santalol from sandalwood oil provided a different direction for identifying chemopreventive agents against skin cancers.

First, the effects of α-santalol in DMBA-initiated and TPA-promoted skin cancer development in both female CD-1 and SENCAR mice were investigated in our laboratory. The results from this investigation indicated that α-santalol inhibited skin papilloma development in both tumor incidence and multiplicity only during the promotion phase of DMBA and TPA protocol in both CD-1 and SENCAR strains of mice. α-Santalol treatment did not have any significant effects during initiation phase. Thus, α-santalol may not affect the binding of DMBA to DNA during initiation phase. Induction of epidermal ODC activity and DNA synthesis are some of the prominent effects of TPA treatment on skin. As expected, α-santalol treatment significantly decreased ($P < 0.05$) TPA-induced ODC activity and incorporation of ^{3}H-thymidine in DNA in the skin of both strains of mice. Because α-santalol treatment did not influence DMBA-induced initiation, most likely the effects of α-santalol on TPA-induced promotion are not because of the blocking of absorption of TPA. Preliminary experiments in our laboratory using GC-MS have detected α-santalol within 5 min in serum, skin, and liver of animals receiving topical application of α-santalol suggesting that α-santalol gets absorbed, and the chemopreventive effects on TPA-induced promotion are likely caused by systematic absorption rather than simply blocking the penetration of TPA (53).

Dose-response study indicated that α-santalol at three different concentrations (1.25%, 2.5% and 5%) significantly decreased DMBA-TPA induced skin tumor incident and multiplicity, TPA-induced ODC activity and TPA-induced DNA synthesis in CD-1 mice. Moreover, α-santalol at 5% resulted in a significantly higher effect on decreasing skin tumor incidence and multiplicity, inhibiting of TPA-induced ODC activity and inhibition of DNA synthesis as compared to α-santalol application at 1.25% and 2.5% in CD-1 mice (54).

The effects of α-santalol on skin papilloma incidence and multiplicity are very similar to the effects of sandalwood oil as reported earlier from our laboratory (51-52). α-Santalol may be the most important constituent in sandalwood oil for these chemopreventive effects.

3.3. Effects of α-santalol in UVB-induced skin cancer development in mice

In addition to chemical-induced skin cancer, UVB-induced skin cancer is major the cause of human skin cancer. Therefore, the effects of α-santalol on UVB radiation-induced skin cancer development in female SKH-1 mice also have been investigated. Results from this investigation showed a strong chemopreventive potential of α-santalol against photocarcinogenesis in three different protocols (DMBA-initiated and UVB-promoted; UVB-initiated and TPA-promoted and UVB-initiated and UVB-promoted). When the effect was assessed against UVB-caused tumor initiation, α-santalol showed a strong reduction in both tumor incidence and multiplicity. Similar protective effects, though to a lesser extent, were also observed when α-santalol was applied before each UVB exposure in a tumor promotion protocol. Most effective observation was the α-santalol's effect on UVB-caused complete tumorigenesis, specifically a 72% reduction in tumor multiplicity. Furthermore, α-santalol treatment significantly decreased UVB-induced ODC activity in SKH-1 mice epidermis which may contribute to the inhibitory effects of α-santalol on UVB-induced skin cancer mouse models (55).

Dose-response studies also showed that α-santalol inhibited UVB-initiated and UVB-promoted skin tumor development in a concentration-dependent manner. α-Santalol pretreatment at 2.5% and 5% significantly reduced skin tumor development. However, α-santalol at 1.25% did not have any significant effect on UVB-induced skin tumor development. Thus, the minimum possible concentration of α-santalol which could potentially reduce UVB-induced skin tumor development was 2.5%. However, 5% of α-santalol provided an optimal chemoprevention as compared to other relative lower concentrations (56).

Since UVB-initiated and UVB-promoted skin carcinogenesis mouse model mimics human NMSC (55, 57-58), the results obtained are encouraging, and warrant more studies assessing α-santalol efficacy against NMSC in pre-clinical models and associated mechanisms of action. It is anticipated that successful outcomes from such studies would help conduct a clinical trial with this agent in human population suffering with NMSC or a pre-cancerous stage actinic keratoses for squamous cell carcinoma skin cancer (59).

4. MECHANISMS OF ACTION OF α-SANTALOL IN SKIN CANCER CHEMOPREVENTION

4.1. General mechanisms of action of cancer chemopreventive agents

The formation of cancer cells is caused by imbalance between cell death and cell growth. Cell death is caused by apoptosis which is a programmed death. Excessive apoptosis can cause disease, e.g. neurodegeneration, autoimmune diseases, AIDS and ischemia-associated injury. On the other hand, deficient apoptosis can also cause diseases, e.g. cancer (60). Cell growth can be regulated by cell cycle and signaling pathways that control cellular proliferation and survival.

Chemopreventive agents prevent formation of cancer by multiple mechanisms. Studies focused on blocking the activation of carcinogens and induction of detoxification pathway in 1980s (27, 61). However, studies in recent years are more focused on the modulation of cell survival pathways such as cell cycle arrest and induction of apoptosis as well as the inhibition of inflammatory mediators (62).

4.2. Effects of α-santalol on apoptosis

The term of apoptosis or a programmed cell death, which is taken from the Greek word for falling down in Greek, was first used by Kerr *et al.* (63) in 1972 to indicate a novel type of cell death that was involved several characteristics that distinguished it from necrotic cell death.

During the past 10 years, our knowledge of the signaling pathways that regulate apoptosis has risen dramatically (64). Virtually all anticancer drugs can kill tumor cells by inducing apoptosis. Thus, the understanding of apoptosis has provided the basic for novel targeted therapies that can prevent the occurrence of cancer, induce death in cancer cells or sensitize them to established cytotoxic agents and radiation therapy (65).

In a classical apoptosis as shown in Figure 3, apoptotic cell death can be initiated by either death receptor pathway (extrinsic pathway) or mitochondrial pathway (intrinsic pathways).The extrinsic signaling pathways that initiate apoptosis involve transmembrane receptor-mediated interactions. These involve death receptors that are members of the tumor necrosis factor (TNF) receptor gene superfamily. Activation of death receptors *via* ligands leads to caspase-8 activity. Two routes have been identified to activate caspase-3 by caspase-8. In one route, caspase-8 directly processes pro-caspase-3 in the downstream. In another route, caspase-8 cleaves Bid, a member of the Bcl-2 family. The truncated Bid then translocates to mitochondria and stimulates release of cytochrome *c*, which activates caspase-9 together with Apaf-1. The activated caspase-9 causes processing of pro-caspase-3 to the activated form. The intrinsic signaling pathways that initiate apoptosis involve a diverse array of non-receptor-mediated stimuli that produce intracellular signals that act directly on targets within the cell and are mitochondrial-initiated events. The stimuli that initiate the intrinsic pathway produce intracellular signals that may act in either a positive or negative fashion such as pro-apoptotic proteins and anti-apoptotic members of the Bcl-2 family. All of these stimuli cause changes in the inner mitochondrial membrane that result in an opening of the mitochondrial permeability transition (MPT) pore, loss of mitochondrial transmembrane potential and release of cytochrome *c* from the mitochondria to cytosol. The cytochrome *c* then binds and activates Apaf-1 as well as procaspase-9. Activation of caspase-9 in turn activates caspase-3 (65-66).

The extrinsic and intrinsic pathways both end at the point of the execution phase, considered the final pathway of apoptosis. It is the activation of execution caspases such as caspase-3, -6 and -7 that begin this phase of apoptosis. Execution caspases activate cytoplasmic endonulease, which degrades nuclear material, and proteases that degrade the nuclear and cytoskeletal proteins. Execution caspases cleave various substrates including cytokeratins, PARP, and others that ultimately cause the morphological and biochemical changes seen in apoptotic cells. Caspase-3, the most important of the execution caspases, specifically activates the caspase-activated deoxyribonuclease (CAD). In proliferating cells CAD is complexed with its inhibitor, ICAD. In apoptotic cells, activated caspase-3 cleaved ICAD to release CAD. CAD then degrades chromosomal DNA within the nuclei and causes chromatin condensation (65, 67).

Both *in vitro* and *in vivo* mechanistic studies of α-santalol were consistent with the notion that induction of apoptosis is an important strategy for cancer prevention. *In vitro* study has showed that treatment of human epidermoid carcinoma A431 cells with α-santalol at concentrations of 25-50 μM resulted in a concentration- and time-dependent apoptosis as early as 3 h post-treatment. As shown in Figure 3, induction of apoptosis caused by α-santalol treatment is associated with caspase-3 activation and poly (ADP-ribose) polymerase (PARP) cleavage through activation of upstream caspase-8 and caspase-9. Further, the treatment of cells with α-santalol also led to disruption of the mitochondrial membrane potential and cytochrome c release into the cytosol, thereby implicating the involvement of the mitochondrial pathway. Pre-treatment of cells with caspase-8 or -9 inhibitor, pan caspase inhibitor or cycloheximide totally blocked α-santalol caused caspase-3 activity and PARP cleavage, but only partially reversed apoptotic cell death suggesting an involvement of both caspase-dependent and –independent pathways in α-santalol induced apoptosis (68).

In vivo mechanistic study from our laboratory showed that pretreatment of α-santalol 1 h prior to UVB exposure for 30 weeks significantly ($P < 0.05$) increased the expressions of apoptotic proteins such as caspase-3 and caspase-8 and tumor suppressor protein p53 (69).

Both *in vitro* and *in vivo* studies suggest that α-santalol provides chemopreventive effects against skin cancer possible through induction of apoptosis and tumor suppressor protein.

4.3. Effects of α-santalol on cell cycle

Cell cycle transition is an ordered, tightly-regulated process that involves multiple checkpoints that assess extracellular growth signals, cell size, and DNA integrity. The somatic cell cycle is divided into four distinct phases as shown in Figure 3 (70). During two of these phases, the cells execute the basic events in cell division like generation of a single and faithful copy of its genetic material (synthetic or S phase) and partitioning of all the cellular components between the two identical daughter cells (mitosis or M phase). The two other phases of cell cycle represent gap periods (G_1 and G_2), during which the cells prepare themselves for the successful completion of the S and M phases, respectively. When the cells cease proliferation, then they exit the cycle and enter a non-dividing, quiescent state, known as G_0. In addition, the cell cycle may be arrested at the G_1 or G_2 checkpoints that assess cell size, extracellular growth signals, and DNA integrity.

The essence of the regulation of the cell cycle progression is a family of enzymes, called the cyclin-dependent kinases (CDKs). The active forms of CDKs are a complex of at least two proteins, a kinase and a cyclin (71). These complexes undergo changes in the kinase and cyclin components that drive the cell from one stage of the cell cycle to another (72). In mammalian cells, a succession of kinase subunits (CDK4, CDK6, CDK2, and CDC2) are expressed along with a succession of cyclins (cyclin D, E, A, and B), as the cells progress from G_1 to mitosis. CDK4 and CDK6 complexed with one of several D-type cyclins

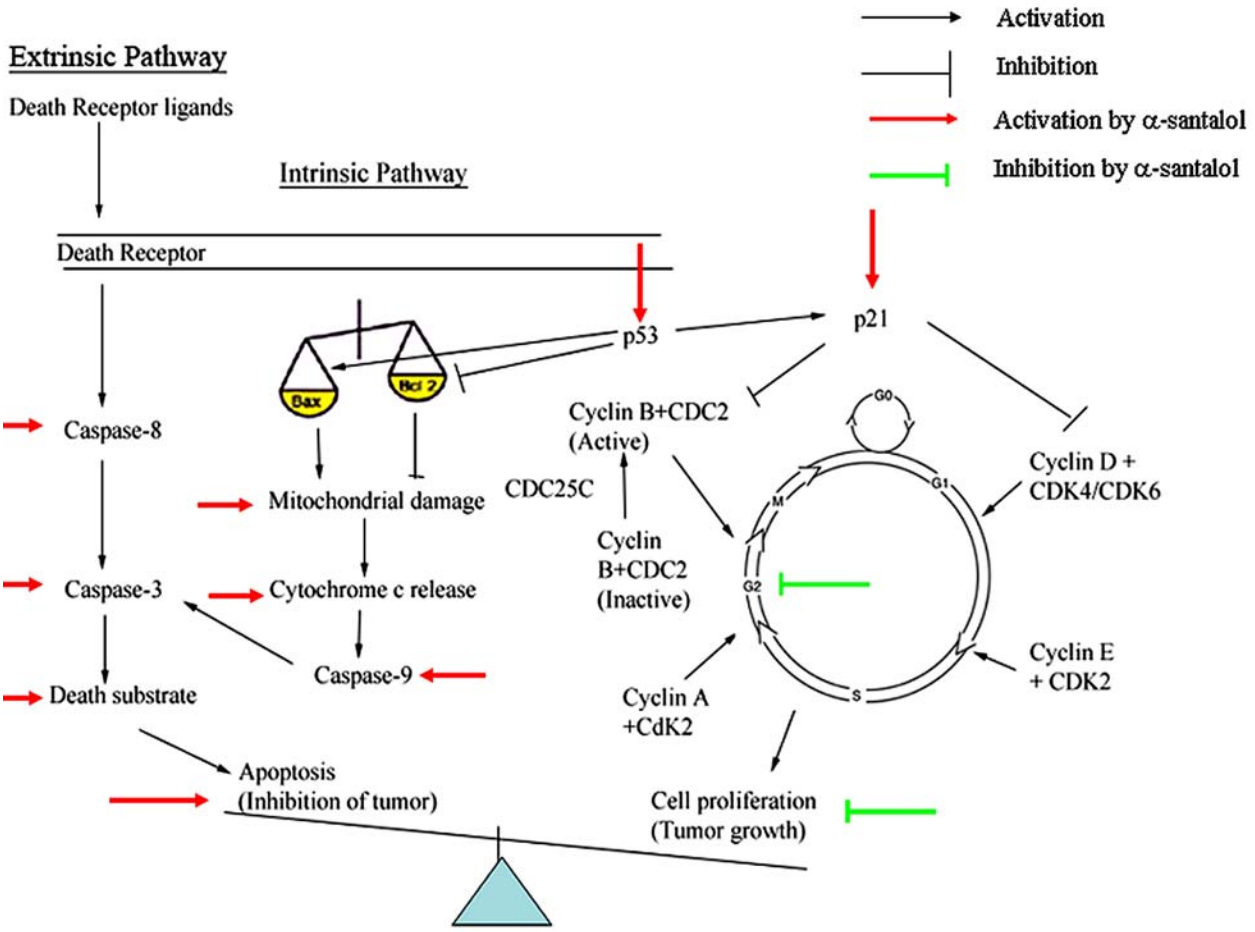

Figure 3. Effects of α-santalol on apoptosis and cell cycle in skin cancer cell lines, possible mechanisms of action of α-santalol against skin cancer. α-Santalol provides potential chemopreventive effects against skin cancer possibly by both induction of apoptosis through both extrinsic and intrinsic pathway and inhibition of cell growth through G2/M phase cell cycle arrest.

function early in the G_1 phase, probably in response to growth factors. CDK2 that complexed with cyclin E, cyclin A, or both is essential for the G_1-S transition and DNA replication, respectively. CDC2 that complexed with cyclin A and cyclin B is essential for mitosis.

Cell cycle checkpoints have been defined as "biochemical pathways that ensure dependence of one process upon another process that is biochemically unrelated" (73). Their activation in response to DNA damage either lead to cell cycle arrest, so as to allow repair of DNA damage, or lead to cell death by apoptosis or terminal growth arrest. DNA damaging agents trigger checkpoints that produce arrest in G_1 and G_2 stages of the cell cycle. Cells can also arrest in S, which amounts to a prolonged S phase with slowed DNA synthesis. Cell cycle arrest, also referred to as delay, is produced by a variety of factors that may be intrinsic or extrinsic and may affect several different checkpoints (74). Many genes are involved in the regulation of DNA damage and related to the DNA damage checkpoint pathways such as p53 and p21, both of which have been used to study the interaction of drugs at the molecular levels.

Based on the role of cell cycle on cancers, the effects of α-santalol on cell cycle were investigated by employing two human skin cancer cell lines: p53 mutated human epidermoid carcinoma A431 cells and p53 wild-type human melanoma UACC-62 cells. Results showed that α-santalol at 50-100 μM decreased cell growth from 24 h treatment and α-santalol at 50 μM-75 μM induced G_2/M phase cell cycle arrest from 6 h treatment in both A431 and UACC-62 cells. α-Santalol altered expressions of cell cycle proteins such as cyclin A, cyclin B1, Cdc2, Cdc25c, p-Cdc25c and Cdk2 and changed bindings of cyclin A/Cdk2 and cyclin B/Cdc2 in both A431 and UACC-62 cells. All of these proteins and complexes are critical for G_2/M transition. α-Santalol treatment up-regulated the expression of p21 and suppressed expressions of mutated p53 in A431 cells; whereas, α-santalol treatment increased expressions of wild-type p53 in UACC-62 cells. Knockdown of p21 in A431 cells, knockdown of p21 and p53 in UACC-62 cells did not affect cell cycle arrest caused by α-santalol. Furthermore, α-santalol caused depolymerization of microtubules similar to vinblastine in UACC-62 cells. Taken together, α-santalol

may cause metaphase of mitosis arrest in A431 cells through up-regulation of cyclin B/Cdc2 complex; whereas, in UACC-62 cells, α-santalol induced cell arrest in G_2 phase by down-regulation of both cyclin A/Cdk2 and cyclin B/Cdc2 complexes resulting in microtubule depolymerization (75).

These data suggest that in addition to induction of apoptosis by α-santalol observed in *in vitro* and *in vivo* studies (68-69), α-santalol also inhibit cell proliferation through induction of G_2/M phase arrest in different skin cancer cell lines which might be contributing to its overall cancer preventive efficacy in various mouse skin cancer models.

5. CONCLUSION AND FUTURE DIRECTIONS

α-Santalol is a major component of sandalwood oil (*Santalum album* Linn, Indian sandalwood) which has been traditionally used in the treatment of various skin ailments. Studies from our laboratory demonstrate that topical application of α-santalol significantly reduces tumor incidence and tumor multiplicity in both chemical-induced and UVB-induced skin cancer mouse models. Topical application of α-santalol at 5% exhibits relative higher chemopreventive effects against skin cancer as compared to other lower concentrations. Furthermore, α-santalol elicits its chemopreventive effects through multiple mechanisms. As shown in Figure 3, α-santalol regulates cell proliferation, cell cycle arrest at G2/M phase and apoptosis through both extrinsic and intrinsic pathway. Based on present *in vivo* data, α-santalol appears to be a promising preventive agent against non-melanoma skin cancer. Furthermore, current *in vitro* study also suggests that α-santalol may be effective for the prevention and treatment of both non-melanoma skin cancer and melanoma skin cancer. Therefore, future studies on the effects of α-santalol on melanoma cancers and the effects of α-santalol for the treatment of skin cancer in animal models are needed to fully explore the value of α-santalol on skin cancer.

6. ACKNOWLEDGEMENTS

This study was supported by a grant from National Institutes of Health, and 2010 Research Initiative Center funded by the State of South Dakota as Translational Cancer Research Center (TCRC).

7. REFERENCES

1. Cancer Facts & Figures-2009. In: American Cancer Society, (2009)

2. S. M. Meeran, S. K. Mantena, C. A. Elmets and S. K. Katiyar: (-)-Epigallocatechin-3-gallate prevents photocarcinogenesis in mice through interleukin-12-dependent DNA repair. *Cancer Res*, 66(10), 5512-20 (2006)

3. M. F. Naylor, A. Boyd, D. W. Smith, G. S. Cameron, D. Hubbard and K. H. Neldner: High sun protection factor sunscreens in the suppression of actinic neoplasia. *Arch Dermatol*, 131(2), 170-5 (1995)

4. N. Yusuf, C. Irby, S. K. Katiyar and C. A. Elmets: Photoprotective effects of green tea polyphenols. *Photodermatol Photoimmunol Photomed*, 23(1), 48-56 (2007)

5. Sunsmart: In: http://www.sunsmart.com.au/skin_cancer/types_of_skin_cancer,

6. C. Hamm, S. Verma, T. Petrella, K. Bak and M. Charette: Biochemotherapy for the treatment of metastatic malignant melanoma: a systematic review. *Cancer Treat Rev*, 34(2), 145-56 (2008)

7. D. S. Preston and R. S. Stern: Nonmelanoma cancers of the skin. *N Engl J Med*, 327(23), 1649-62 (1992)

8. R. D. Granstein: Evidence that sunscreens prevent UV radiation-induced immunosuppression in humans. Sunscreens have their day in the sun. *Arch Dermatol*, 131(10), 1201-4 (1995)

9. A. F. Jerant, J. T. Johnson, C. D. Sheridan and T. J. Caffrey: Early detection and treatment of skin cancer. *Am Fam Physician*, 62(2), 357-68, 375-6, 381-2 (2000)

10. R. Marks: An overview of skin cancers. Incidence and causation. *Cancer*, 75(2 Suppl), 607-12 (1995)

11. J. C. Barrett and R. W. Wiseman: Cellular and molecular mechanisms of multistep carcinogenesis: relevance to carcinogen risk assessment. *Environ Health Perspect*, 76, 65-70 (1987)

12. R. K. Boutwell: Some biological aspects of skin carcinogenesis. *Prog Exp Tumor Res*, 4, 207-250 (1984)

13. J. J. Reiners, Jr., S. Nesnow and T. J. Slaga: Murine susceptibility to two-stage skin carcinogenesis is influenced by the agent used for promotion. *Carcinogenesis*, 5(3), 301-7 (1984)

14. J. DiGiovanni, T. J. Slaga and R. K. Boutwell: Comparison of the tumor-initiating activity of 7,12-dimethylbenz[a]anthracene and benzo[a]pyrene in female SENCAR and CS-1 mice. *Carcinogenesis*, 1(5), 381-9 (1980)

15. R. K. Boutwell: The function and mechanism of promoters of carcinogenesis. *CRC Crit Rev Toxicol*, 2(4), 419-43 (1974)

16. J. C. Barrett: Mechanisms of multistep carcinogenesis and carcinogen risk assessment. *Environ Health Perspect*, 100, 9-20 (1993)

17. E. M. Griner and M. G. Kazanietz: Protein kinase C and other diacylglycerol effectors in cancer. *Nat Rev Cancer*, 7(4), 281-94 (2007)

18. K. H. Kraemer: Sunlight and skin cancer: another link revealed. *Proc Natl Acad Sci U S A*, 94(1), 11-4 (1997)

19. H. Soehnge, A. Ouhtit and O. N. Ananthaswamy: Mechanisms of induction of skin cancer by UV radiation. *Front Biosci*, 2, d538-51 (1997)

20. I. Willis, J. M. Menter and H. J. Whyte: The rapid induction of cancers in the hairless mouse utilizing the principle of photoaugmentation. *J Invest Dermatol*, 76(5), 404-8 (1981)

21. G. T. Bowden: Prevention of non-melanoma skin cancer by targeting ultraviolet-B-light signalling. *Nat Rev Cancer*, 4(1), 23-35 (2004)

22. D. E. Brash, J. A. Rudolph, J. A. Simon, A. Lin, G. J. McKenna, H. P. Baden, A. J. Halperin and J. Ponten: A role for sunlight in skin cancer: UV-induced p53 mutations in squamous cell carcinoma. *Proc Natl Acad Sci U S A*, 88(22), 10124-8 (1991)

23. Y. S. Wan, Z. Q. Wang, Y. Shao, J. J. Voorhees and G. J. Fisher: Ultraviolet irradiation activates PI 3-kinase/AKT survival pathway via EGF receptors in human skin *in vivo*. *Int J Oncol*, 18(3), 461-6 (2001)

24. W. Chen, Q. Tang, M. S. Gonzales and G. T. Bowden: Role of p38 MAP kinases and ERK in mediating ultraviolet-B induced cyclooxygenase-2 gene expression in human keratinocytes. *Oncogene*, 20(29), 3921-6 (2001)

25. Y. Kabuyama, M. Hamaya and Y. Homma: Wavelength specific activation of PI 3-kinase by UVB irradiation. *FEBS Lett*, 441(2), 297-301 (1998)

26. S. H. Yuspa: The pathogenesis of squamous cell cancer: lessons learned from studies of skin carcinogenesis--thirty-third G. H. A. Clowes Memorial Award Lecture. *Cancer Res*, 54(5), 1178-89 (1994)

27. L. W. Wattenberg: Chemoprevention of cancer. *Cancer Res*, 45(1), 1-8 (1985)

28. M. B. Sporn and N. Suh: Chemoprevention of cancer. *Carcinogenesis*, 21(3), 525-30 (2000)

29. L. W. Wattenberg: Chemoprevention of cancer by naturally occurring and synthetic compounds. In: *Cancer chemoprevention*. Ed L. W. Wattenberg, M. Lipkin, C. W. Boone&G. J. Kelloff. CRC Press, Boca Raton (1992)

30. M. M. Manson: Cancer prevention -- the potential for diet to modulate molecular signalling. *Trends Mol Med*, 9(1), 11-8 (2003)

31. M. S. Baliga and S. K. Katiyar: Chemoprevention of photocarcinogenesis by selected dietary botanicals. *Photochem Photobiol Sci*, 5(2), 243-53 (2006)

32. S. K. Mantena, S. M. Meeran, C. A. Elmets and S. K. Katiyar: Orally administered green tea polyphenols prevent ultraviolet radiation-induced skin cancer in mice through activation of cytotoxic T cells and inhibition of angiogenesis in tumors. *J Nutr*, 135(12), 2871-7 (2005)

33. S. Katiyar, C. A. Elmets and S. K. Katiyar: Green tea and skin cancer: photoimmunology, angiogenesis and DNA repair. *J Nutr Biochem*, 18(5), 287-96 (2007)

34. N. Bouzari, Y. Romagosa and R. S. Kirsner: Green tea prevents skin cancer by two mechanisms. *J Invest Dermatol*, 129(5), 1054 (2009)

35. Y. C. Liang, S. Y. Lin-shiau, C. F. Chen and J. K. Lin: Suppression of extracellular signals and cell proliferation through EGF receptor binding by (-)-epigallocatechin gallate in human A431 epidermoid carcinoma cells. *J Cell Biochem*, 67(1), 55-65 (1997)

36. M. H. Aziz, S. Reagan-Shaw, J. Wu, B. J. Longley and N. Ahmad: Chemoprevention of skin cancer by grape constituent resveratrol: relevance to human disease? *Faseb Journal*, 19(9), 1193-5 (2005)

37. P. Roy, N. Kalra, S. Prasad, J. George and Y. Shukla: Chemopreventive potential of resveratrol in mouse skin tumors through regulation of mitochondrial and PI3K/AKT signaling pathways. *Pharm Res*, 26(1), 211-7 (2009)

38. N. Kalra, P. Roy, S. Prasad and Y. Shukla: Resveratrol induces apoptosis involving mitochondrial pathways in mouse skin tumorigenesis. *Life Sci*, 82(7-8), 348-58 (2008)

39. J. K. Kundu, K. S. Chun, S. O. Kim and Y. J. Surh: Resveratrol inhibits phorbol ester-induced cyclooxygenase-2 expression in mouse skin: MAPKs and AP-1 as potential molecular targets. *Biofactors*, 21(1-4), 33-9 (2004)

40. J. K. Kundu, Y. K. Shin, S. H. Kim and Y. J. Surh: Resveratrol inhibits phorbol ester-induced expression of COX-2 and activation of NF-kappaB in mouse skin by blocking IkappaB kinase activity. *Carcinogenesis*, 27(7), 1465-74 (2006)

41. K. S. Chun, Y. S. Keum, S. S. Han, Y. S. Song, S. H. Kim and Y. J. Surh: Curcumin inhibits phorbol ester-induced expression of cyclooxygenase-2 in mouse skin through suppression of extracellular signal-regulated kinase activity and NF-kappaB activation. *Carcinogenesis*, 24(9), 1515-24 (2003)

42. M. T. Huang, Z. Y. Wang, C. A. Georgiadis, J. D. Laskin and A. H. Conney: Inhibitory effects of curcumin on tumor initiation by benzo[a]pyrene and 7,12-dimethylbenz[a]anthracene. *Carcinogenesis*, 13(11), 2183-6 (1992)

43. J. A. Bush, K. J. Cheung, Jr. and G. Li: Curcumin induces apoptosis in human melanoma cells through a Fas receptor/caspase-8 pathway independent of p53. *Exp Cell Res*, 271(2), 305-14 (2001)

44. S. K. Katiyar: Silymarin and skin cancer prevention: anti-inflammatory, antioxidant and immunomodulatory effects (Review). *Int J Oncol*, 26(1), 169-76 (2005)

45. N. Ahmad, H. Gali, S. Javed and R. Agarwal: Skin cancer chemopreventive effects of a flavonoid antioxidant silymarin are mediated via impairment of receptor tyrosine kinase signaling and perturbation in cell cycle progression. *Biochem Biophys Res Commun*, 247(2), 294-301 (1998)

46. X. Zi, H. Mukhtar and R. Agarwal: Novel cancer chemopreventive effects of a flavonoid antioxidant silymarin: inhibition of mRNA expression of an endogenous tumor promoter TNF alpha. *Biochem Biophys Res Commun*, 239(1), 334-9 (1997)

47. X. Zi and R. Agarwal: Modulation of mitogen-activated protein kinase activation and cell cycle regulators by the potent skin cancer preventive agent silymarin. *Biochem Biophys Res Commun*, 263(2), 528-36 (1999)

48. R. P. Singh and R. Agarwal: Mechanisms and preclinical efficacy of silibinin in preventing skin cancer. *European Journal of Cancer*, 41(13), 1969-1979 (2005)

49. G. Mallikarjuna, S. Dhanalakshmi, R. P. Singh, C. Agarwal and R. Agarwal: Silibinin protects against photocarcinogenesis via modulation of cell cycle regulators, mitogen-activated protein kinases, and Akt signaling. *Cancer Res*, 64(17), 6349-56 (2004)

50. S. Banerjee, A. Ecavade and A. R. Rao: Modulatory influence of sandalwood oil on mouse hepatic glutathione S-transferase activity and acid soluble sulphydryl level. *Cancer Lett*, 68(2-3), 105-9 (1993)

51. C. Dwivedi and A. Abu-Ghazaleh: Chemopreventive effects of sandalwood oil on skin papillomas in mice. *European Journal of Cancer Prevention*, 6(4), 399-401 (1997)

52. C. Dwivedi and Y. Zhang: Sandalwood oil prevent skin tumour development in CD1 mice. *European Journal of Cancer Prevention*, 8(5), 449-55 (1999)

53. C. Dwivedi, X. Guan, W. L. Harmsen, A. L. Voss, D. E. Goetz-Parten, E. M. Koopman, K. M. Johnson, H. B. Valluri and D. P. Matthees: Chemopreventive effects of alpha-santalol on skin tumor development in CD-1 and SENCAR mice. *Cancer Epidemiol Biomarkers Prev*, 12(2), 151-6 (2003)

54. C. Dwivedi, E. R. Maydew, J. J. Hora, D. M. Ramaeker and X. Guan: Chemopreventive effects of various concentrations of alpha-santalol on skin cancer development in CD-1 mice. *European Journal of Cancer Prevention*, 14(5), 473-6 (2005)

55. C. Dwivedi, H. B. Valluri, X. Guan and R. Agarwal: Chemopreventive effects of alpha-santalol on ultraviolet B radiation-induced skin tumor development in SKH-1 hairless mice. *Carcinogenesis*, 27(9), 1917-22 (2006)

56. A. Bommareddy, J. Hora, B. Cornish and C. Dwivedi: Chemoprevention by alpha-santalol on UVB radiation-induced skin tumor development in mice. *Anticancer Research*, 27(4B), 2185-8 (2007)

57. F. R. de Gruijl and P. D. Forbes: UV-induced skin cancer in a hairless mouse model. *Bioessays*, 17(7), 651-60 (1995)

58. P. D. Forbes: Relevance of animal models of photocarcinogenesis to humans. *Photochem Photobiol*, 63(4), 357-62 (1996)

59. R. B. Harris and D. S. Alberts: Strategies for skin cancer prevention. *Int J Dermatol*, 43(4), 243-51 (2004)

60. H. J. Guchelaar, A. Vermes, I. Vermes and C. Haanen: Apoptosis: molecular mechanisms and implications for cancer chemotherapy. *Pharm World Sci*, 19(3), 119-25 (1997)

61. J. S. Bertram, L. N. Kolonel and F. L. Meyskens, Jr.: Rationale and strategies for chemoprevention of cancer in humans. *Cancer Res*, 47(11), 3012-31 (1987)

62. S. Y. Sun, N. Hail, Jr. and R. Lotan: Apoptosis as a novel target for cancer chemoprevention. *J Natl Cancer Inst*, 96(9), 662-72 (2004)

63. J. F. Kerr, A. H. Wyllie and A. R. Currie: Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer*, 26(4), 239-57 (1972)

64. P. Geserick, C. Drewniok, M. Hupe, T. L. Haas, P. Diessenbacher, M. R. Sprick, M. P. Schon, F. Henkler, H. Gollnick, H. Walczak and M. Leverkus: Suppression of cFLIP is sufficient to sensitize human melanoma cells to TRAIL- and CD95L-mediated apoptosis. *Oncogene*, 27(22), 3211-20 (2008)

65. I. M. Ghobrial, T. E. Witzig and A. A. Adjei: Targeting apoptosis pathways in cancer therapy. *CA Cancer J Clin*, 55(3), 178-94 (2005)

66. H. J. Brady: Apoptosis and leukaemia. *Br J Haematol*, 123(4), 577-85 (2003)

67. S. Elmore: Apoptosis: a review of programmed cell death. *Toxicol Pathol*, 35(4), 495-516 (2007)

68. M. Kaur, C. Agarwal, R. P. Singh, X. M. Guan, C. Dwivedi and R. Agarwal: Skin cancer chemopreventive agent, alpha-santalol, induces apoptotic death of human epidermoid carcinoma A431 cells via caspase activation together with dissipation of mitochondrial membrane potential and cytochrome c release. *Carcinogenesis*, 26(2), 369-380 (2005)

69. B. L. Arasada, A. Bommareddy, X. Y. Zhang, K. Bremmon and C. Dwivedi: Effects of alpha-santalol on proapoptotic caspases and p53 expression in UVB irradiated mouse skin. *Anticancer Research*, 28(1A), 129-132 (2008)

70. S. van den Heuvel: Cell-cycle regulation. *WormBook*, 1-16 (2005)

71. M. T. Park and S. J. Lee: Cell cycle and cancer. *J Biochem Mol Biol*, 36(1), 60-5 (2003)

72. J. M. Raleigh and M. J. O'Connell: The G(2) DNA damage checkpoint targets both Wee1 and Cdc25. *J Cell Sci*, 113 (Pt 10), 1727-36 (2000)

73. S. J. Elledge: Cell cycle checkpoints: preventing an identity crisis. *Science*, 274(5293), 1664-72 (1996)

74. K. A. Schafer: The cell cycle: a review. *Vet Pathol*, 35(6), 461-78 (1998)

75. X. Zhang, W. Chen, R. Guillermo, G. Chandrasekher, R. S. Kaushik, A. Young, H. Fahmy and C. Dwivedi: Alpha-santalol, a chemopreventive agent against skin cancer, causes G2/M cell cycle arrest in both p53-mutated human epidermoid carcinoma A431 cells and p53 wild-type human melanoma UACC-62 cells. *BMC Res Notes,* 3, 220 (2010)

Key Words: Chemoprevention, Skin, Cancer, Alpha-santalol, Review

Send correspondence to: Chandradhar Dwivedi, Department of Pharmaceutical Sciences, College of Pharmacy, Box 2202C, Brookings, SD 57007,South Dakota State University, Tel: 605-688-4247, Fax: 605-688-5993, E-mail: Chandradhar.Dwivedi@sdstate.edu