Modulation of Wnt/β-catenin signaling pathway by bioactive food components

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Introduction

The term ‘Wnt’ was coined by the amalgamation of Drosophila segment polarity gene Wingless (1) and the mouse proto-oncogene Int-1 (2,3). Wnt signaling pathway is known as a key regulator of cellular functions related to tumor initiation and progression, cell proliferation, differentiation, survival and adhesion. Because aberrant Wnt/β-catenin signaling has been observed in a variety of human cancers including a majority of colorectal cancers, about half of prostate cancers and a third of melanomas, inhibitors of its complex signaling pathways are being investigated for therapy as well as chemoprevention of these cancers. During the last decade, several naturally occurring dietary agents have been shown to target intermediates in the Wnt/β-catenin signaling pathway. In this review, we highlight the current understanding of the Wnt/β-catenin signaling pathway and present an analysis of the key findings from laboratory studies on the effects of a panel of dietary agents against a variety of cancers. Promise of these agents for treating and preventing human cancer is then discussed.

Wnt/β-catenin signaling: an overview

The Wnt genes encode a large family of secreted protein growth factors that have been identified in animals from hydra to humans (18). In humans, 19 Wnt genes have been identified and the chromosomal location of each is known (18). During development, Wnt(s) have diverse roles in governing cell fate, proliferation, migration, polarity and death. β-Catenin alternatively is a 781 amino acid protein that is encoded by CTNNB1 gene in humans. β-Catenin together with α- and γ-catenin was originally isolated as a protein associated with the cytoplasmic region of E-cadherin, a transmembrane protein involved in homotypic cell–cell contact (19,20). Cytoplasmic β-catenin levels are normally kept low through continuous proteosome-mediated degradation by a ‘destructive complex’ of adenomatous polyposis coli (APC)/glycogen synthase kinase-3β (GSK-3β)/Axin (Figure 1). When cells receive Wnt signals, the degradation of β-catenin is inhibited and levels of β-catenin build up in the cytoplasm and nucleus (Figure 1) (21). Nuclear β-catenin interacts with transcription factors, such as T-cell factor/lymphoid enhancer-binding factor (Tcf/Lef) where it serves as a transcription regulator for several genes that, in part, regulates tumor formation and progression (Table I) (21). Once in the nucleus, β-catenin is thought to convert the Tcf repressor complex into a transcriptional activator complex. This may occur through displacement of Groucho from Tcf/Lef and recruitment of the histone acetylase cyclic adenosine monophosphate response element-binding protein/p300. Cyclic adenosine monophosphate response element-binding protein may bind to the β-catenin/Tcf complex as a coactivator.

Abbreviations: APC, adenomatous polyposis coli; CRC, Colorectal cancer; CSC, cancer stem cell; ECGC, epigallocatechin-3-gallate; GSK-3β, glycogen synthase kinase-3β; HCC, hepatocellular carcinoma; Lef, lymphoid enhancer-binding factor; LRP, LDL receptor related protein; MITF, microphthalmia-associated transcription factor; NF-κB, nuclear factor-kappa B; SFN, Sulforaphane; Tcf, T-cell factor.

1These authors contributed equally to this work.

Fig. 1. Wnt/β-catenin signaling pathway.
enin signaling is an important event in the genesis of many cancers. In an important role in the regulation of the stability of 

didence for this model has come from mouse models of APC truncation thought to initiate colon adenoma formation (64). Considerable evi-

of other tumors (4,5,63), suggesting that deregulation of Wnt/

62). Mutations in 

and rectum. Truncations in APC, which promotes aberrant activation 

of Wnt pathway indicating that the feedback control is a key feature of 

the colorectal cancers that exhibit wild-type APC (20,49,50).

Wnt signaling can promote the expression of various components of Wnt pathway indicating that the feedback control is a key feature of the regulation of Wnt/β-catenin signaling (Table II). One class of targets that respond to Wnt signaling is Frizzled (Fz). Two cytoplasmic antagonists are also induced by Wnt signaling (51–53). The naked cuticle (naked) gene binds directly to Dsh and inhibits Wnt signaling in drosofila (48) and vertebrates (55). The Axin2 gene is also anoth-

Table I. Target genes of the Wnt/β-catenin signaling pathway

<table>
<thead>
<tr>
<th>Gene</th>
<th>Condition/disease</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>WNT5a</td>
<td>Leukemia</td>
<td>(22)</td>
</tr>
<tr>
<td>DSH/DVL</td>
<td>Lung cancer</td>
<td>(23)</td>
</tr>
<tr>
<td>MITF</td>
<td>Melanomas</td>
<td>(24–27)</td>
</tr>
<tr>
<td>Tcf-1</td>
<td>Human colon cancer</td>
<td>(28)</td>
</tr>
<tr>
<td>Lef-1</td>
<td>Human colon cancer</td>
<td>(29,30)</td>
</tr>
<tr>
<td>CTLA-4</td>
<td>T cells, melanoma</td>
<td>(27)</td>
</tr>
<tr>
<td>APC</td>
<td>CRC</td>
<td>(31)</td>
</tr>
<tr>
<td>AXIN</td>
<td>Several cancers</td>
<td>(5)</td>
</tr>
<tr>
<td>MMP 2/9</td>
<td>T cells</td>
<td>(32,33)</td>
</tr>
<tr>
<td>Tiam1</td>
<td>Colon tumors</td>
<td>(34)</td>
</tr>
<tr>
<td>Endothelin 1</td>
<td>Human colon cancer</td>
<td>(35)</td>
</tr>
<tr>
<td>VEGF</td>
<td>Human colon cancer</td>
<td>(36)</td>
</tr>
<tr>
<td>C-myc</td>
<td>Human colon cancer</td>
<td>(37,38)</td>
</tr>
<tr>
<td>Axin2</td>
<td>Human cancer, tooth agenesis</td>
<td>(40–43)</td>
</tr>
<tr>
<td>Osteocalcin</td>
<td>Mouse</td>
<td>(44)</td>
</tr>
<tr>
<td>BMP4</td>
<td>Xenopus</td>
<td>(45)</td>
</tr>
<tr>
<td>p16ink4A</td>
<td>Melanomas</td>
<td>(46)</td>
</tr>
</tbody>
</table>

CTLA-4, Cytotoxic T-lymphocyte antigen-4; VEGF, Vascular endothelial growth factor; BMP4, Bone morphogenetic protein 4.

(47,48). The oncogenic role of β-catenin was prominent by the discover-

y in which activating mutations in β-catenin was detected in half of the colorectal cancers that exhibit wild-type APC (20,49,50).

Wnt signaling can promote the expression of various components of Wnt pathway indicating that the feedback control is a key feature of the regulation of Wnt/β-catenin signaling (Table II). One class of targets that respond to Wnt signaling is Frizzled (Fz). Two cytoplasmic antagonists are also induced by Wnt signaling (51–53). The naked cuticle (naked) gene binds directly to Dsh and inhibits Wnt signaling in drosofila (48) and vertebrates (55). The Axin2 gene is also another negative regulator that is a direct target of Wnt signaling.

Wnt/β-catenin signaling in cancer

Mutations that result in Wnt/β-catenin signaling pathway being con-

stutitutively active lead to cancer. Axin2 also known as axin-like protein (Axil) or axis inhibition protein 2 (Axin2) or conductin is a protein 

that in humans is encoded by the Axin2 gene. Axin2 apparently plays an important role in the regulation of the stability of β-catenin in the Wnt signaling pathway and is known to display a predisposition to colon cancer (40). Familial adenomatous polyposis is an inherited disorder in which patients display numerous polyps in the colon and rectum. Truncations in APC, which promotes aberrant activation of Wnt/β-catenin signaling pathway leading to adenomatous lesions, are the most frequent cause of familial adenomatous polyposis (59–

62). Mutations in β-catenin and APC have been observed in a variety of other tumors (4,5,63), suggesting that deregulation of Wnt/β-catenin signaling is an important event in the genesis of many cancers. In fact, aberrant Wnt/β-catenin signaling following the loss of APC is thought to initiate colon adena formation (64). Considerable evidence for this model has come from mouse models of APC truncation where nuclear β-catenin was found to be detectable soon after loss of APC (64). Several studies observed that stabilizing mutations in β-catenin in the absence of mutations in APC in ~7% of sporadic human colon carcinomas and thus, these studies provide additional genetic evidence linking APC loss and β-catenin activation (49,64–

66). Furthermore, transgenic mice with stabilized mutant β-catenin develop numerous intestinal adenomas (64,67). Taken together, these studies suggest that dysregulation of β-catenin is a key oncogenic event that follows loss of APC.

β-Catenin mutations and interstitial deletions have been reported in both primary hepatocellular carcinomas (HCC) and hepatoma cell lines (68–70). Ser 37 mutation was rarely observed in HCC but alter-

ations affecting the putative phosphorylation residues were common.

Studies suggest that 50% of HCC that develop in transgenic mice expressing c-myc or H-ras in the liver contain β-catenin mutations, indicating that β-catenin activation can cooperate with ras or myc in progression of HCC (69,70). Forty-eight percent of sporadic hepatoblastomas, a type of childhood malignant liver tumors, exhibit β-catenin mutations and exon 3 deletions (71). Wilms tumor, a common childhood renal cancer, has been shown to harbor mutations in β-catenin. An interesting finding in these studies was that 90% of these tumors have β-catenin mutation at codon 45 (72–74).

Wnt/β-catenin signaling in melanomas. A single amino acid substitu-

tion at the N-terminus of β-catenin was identified as a mela-

noma-specific antigen (75) and this sequence is part of a sequence recognized by a melanoma-specific tumor-infiltrating lymphocyte. This study was the first to suggest a mutation in β-catenin could result in cancer. Abnormally high levels of β-catenin have been observed in a subset of melanomas and are usually associated with mutation in CTNNB1 (50). β-catenin mutations were reported to occur at Ser 37, a residue rarely reported in colon cancer (50,76). However, similar to colon cancer, overexpression of APC reduced the levels of β-catenin in these lines (50). Studies suggests that deregulation of Wnt signaling may ultimately lead to deregulation of microphthalmia-associated transcription factor-M (MITF-M) expression resulting in improper cell functions (differentiation, proliferation and survival) (6,77). MITF is essential for melanocyte differentiation and its heterozygous mutations cause auditory–pigmentary syndromes. Functional cooperation of MITF with Lef1 results in synergistic transactivation of an early melano-

blast marker dopachrome tautomerase gene promoter (24). Lef1 also interacts with PAX3 and Groucho-related corepressor Grg4 in melanocytes. During active Wnt/β-catenin signaling, Grg4 and Pax3 are removed from the dopachrome tautomerase gene promoter and no longer interact with Lef1 thus resulting in a loss of Pax3-mediated repression (78). Another factor that has been implicated in the control of proliferation and strongly upregulated in melanomas is the POU domain transcription factor Brm-2 (79–81). In melanoma cells, down-

regulation of Brm-2 is associated with changes in morphology and loss of melanocytic and neural crest markers, like MITF and tyrosinase (81). A decrease in the production of Brm-2 is associated with a loss of tumorigenicity of these cells in nude mice (79). An active Lef1/Tcf-binding site has been identified in the Brm-2 promoter (81), implying that Wnt/β-catenin signaling can induce BRN-2 protein, which is in-

volved in cell proliferation and another protein (MITF) that is involved in cell differentiation.

Wnt/β-catenin signaling in colorectal cancer. Colorectal cancer (CRC) is the third most common cause of cancer death in the USA (64,82). One of the central players, in the series of molecule changes resulting in CRC, is APC which was identified as the responsible germline mutation in familial adenomatous polyposis patients (62,83). Eighty percent of non-inherited CRC cases carry mutations in APC, an important component in the Wnt/β-catenin signaling path-

way. Since mutations in CRC occur at the early stages of colorectal

Table II. Targets of Wnt signaling that serve as components of the Wnt signaling pathway

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Interact with</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fz (Frizzled)</td>
<td>Wnt</td>
<td>(51–53)</td>
</tr>
<tr>
<td>Dsh2</td>
<td>Wnt</td>
<td>(54)</td>
</tr>
<tr>
<td>Arrow/LRP</td>
<td>Wnt</td>
<td>(55,56)</td>
</tr>
<tr>
<td>naked</td>
<td>Dsh</td>
<td>(41)</td>
</tr>
<tr>
<td>Axin2</td>
<td>β-catenin</td>
<td>(57)</td>
</tr>
<tr>
<td>β-TCP</td>
<td>β-catenin</td>
<td>(58)</td>
</tr>
<tr>
<td>Tcf-1</td>
<td>Tcf</td>
<td>(28,58)</td>
</tr>
<tr>
<td>LEF1</td>
<td>β-catenin</td>
<td>(30)</td>
</tr>
</tbody>
</table>

LRP, LDL receptor related protein.
carcinogenesis, it means the functional loss of APC is a prerequisite for further progression toward malignancy (84). Reintroduction of wild-type APC results in decreased levels of β-catenin. Studies suggest that transfection of APC in colon cancer cells reduces the abnormally high Tcf reporter plasmid activity (85). Interestingly, rare cell lines without APC mutations also displayed β-catenin/Tcf transcriptional activity as measured by luciferase reporter assay (49). Sequencing the β-catenin gene (CTNNB1) in two APC–/–β-catenin activated cell lines revealed mutations at the GSK-3β consensus phosphorylation site of β-catenin: a deletion of Ser 45 in HCT116 cell line and a Ser 55 to Thr substitution in SW-48 cells. Expression of β-catenin stability leads to a dominant effect on Tcf/β-catenin transcription (49). Mutations in β-catenin gene have been observed in ~50% of CRCs without APC mutations, representing <10% of overall CRCs. These mutations occur mainly in the microsatellite-instable tumors (65,86). Immunolocalization of β-catenin in colon carcinomas showed a strong nuclear enrichment at the invasion front, whereas in large parts of the central tumor area, β-catenin was detected in the cytoplasm and at the membrane, indicating that high levels of nuclear β-catenin play a role in the transition to the invasive state of tumor cells (87–89).

**Wnt/β-catenin signaling in prostate cancer**

Prostate cancer is one of the few cancers where the aberrant Wnt/β-catenin signaling appears to be a late event in tumorogenesis. Approximately a third of prostate tumors have aberrant Wnt/β-catenin signaling (90). Mutations in APC, β-catenin and β-TrCP have been detected in prostate cancer (90–92). A study using single-strand conformation polymorphism in a panel of 104 prostate cancers identified five mutations in the regulatory site of β-catenin (91). Four of these sites affected putative phosphorylation sites of β-catenin and one affected a residue adjacent to Ser 33, similar to those seen in colon cancer (65). The expression levels of Wnt ligands (Wnt 1, Wnt 2 and Wnt 5a) were positively correlated with Gleason scores as well as to the cellular level of β-catenin and serum prostate specific antigen levels (93–95). Studies by Truica et al. (96) have demonstrated that β-catenin interacts with androgen receptor and enhances the transcriptional activity for androgen receptor in LNCaP cells suggesting a possible mechanism of cross talk between Wnt and androgen signaling pathways. Several other studies using yeast two-hybrid, *in vitro* and *in vivo* protein binding assays, have confirmed the interaction between Wnt and androgen receptor signaling pathways (93,97,98).

**Wnt/β-catenin signaling in other diseases**

Misregulation of Wnt/β-catenin signaling pathway leads to a variety of abnormalities. A mutation in LRP receptor related protein has been identified that leads to increased bone density at defined locations, such as the jaw and palate (99,100). A single amino acid substitution makes LRP5 insensitive to the inhibition of Wnt signaling (99,101). Frame shift and missense mutations in LRP5 resulted in loss of function in LRP5 insensitive to the inhibition of Wnt signaling (99,101). Sequencing data suggest the role of vitamin D in exerting antiproliferative and prodifferentiating effects on a variety of stem and progenitor cells (113,114). Genistein has been reported to act synergistically with a vitamin D derivative to inhibit the growth of prostate cells and regulate genes that are involved in stem cells renewal (115–117). A recent study by Kakkarla et al. (118) showed that curcumin inhibits Wnt/β-catenin signaling in mammary stem cells by inhibiting their self-renewal capability. Another study by Yu et al. (119) showed that curcumin in combination with the therapeutic cocktail of levocovarin, 5-fluorouracil and oxaliplatin (FOLFOX) eliminated colon CSC population suggesting that curcumin by itself or together with the conventional chemotherapeutic could be an effective treatment strategy for preventing the emergence of chemoresistant colon cancer cells by reducing/eliminating CSCs.

** Naturally occurring compounds targeting Wnt/β-catenin signaling**

Naturally occurring compounds represent attractive candidates for development as chemopreventive agents. Naturally occurring dietary agents circumvent the need to introduce foreign compounds into healthy asymptomatic individuals. Dietary agents are also in general less toxic, more accessible and less expensive than synthetic agents. In the past few years, several laboratories have identified numerous phytochemicals that have useful biological properties; however, only few groups have directly evaluated the ability of these agents to disrupt β-catenin-mediated Wnt signaling. Flavanoids (genistein), curcumin, epigallocatechin-3-gallate (EGCG), resveratrol, lupeol, retinoids, lycopene, duguolin and SPN are described here.

**Flavonoids**

The flavonoids comprise a large class of low-molecular weight natural products of plant origin ubiquitously distributed in foods. They are recognized as dietary constituents having a variety of biological effects among which there are some that have been associated with protective activity in the development of degenerative diseases, such as atherosclerosis or cancer (120–123). Several studies suggest that an intake of flavonoids results in reduced cancer risk (17,124,125). Dietary agents are also in general less toxic, more accessible and less expensive than synthetic agents. In the past few years, several laboratories have identified numerous phytochemicals that have useful biological properties; however, only few groups have directly evaluated the ability of these agents to disrupt β-catenin-mediated Wnt signaling. Genistein (notably genistein) upregulated the expression of GSK-3β, enhanced GSK-3β binding to β-catenin and increased β-catenin phosphorylation, suggesting that genistein could inactivate Wnt/β-catenin signaling thereby inhibiting prostate cancer growth (126,127). Genistein...
also diminished Wnt 1-induced proliferation and decreased the expression of Wnt targets viz. c-myc and cyclin D1 (127–129).

Curcumin

Curcumin or diferuloylmethane [1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], a yellow pigment, is a major component of turmeric and a member of the ginger Curcuma longa (Zingiberaceae). Turmeric has been used in ayurvedic medicine in India for several thousands of years (130). The therapeutic benefits of curcumin have been reported as an analgesic, antiinflammatory, antibacterial, antiviral, antiparasitic, antioxidant, antiarthritic, antihypercholesterolemic and antihypertensive (131–133). Its anti-inflammatory activity is used in the treatment of a variety of diseases ranging from asthma (by down-regulating hyalurondase activity) to Alzheimer’s disease (reducing expression of β-amyloid) and human immunodeficiency virus (17,134–136). Dietary curcumin has been shown to prevent cancer in the skin, forestomach, duodenum and colon in mice and the tongue, colon, mammary and sebaceous glands in rats (137,138). Furthermore, curcumin has also been associated with regression of established malignancy in humans (17,127,139–141). Curcumin specifically inhibits the expression of cyclooxygenase-2, which plays an important role in colon carcinogenesis (130,142). Studies suggest that curcumin is not toxic to humans up to a dose of 8000 mg/day when taken orally for 3 months, suggesting a biological effect in the area of chemoprevention (130). Curcumin treatment inhibits the messenger RNA expression of Wnt target genes c-myc, c-fos, c-jun and iNOS in a variety of cancer cells (143,144). Studies suggest that curcumin treatment resulted in a dose-dependent decrease in β-catenin transcriptional activity in a variety of CRC cell lines (145,146). Curcumin treatment (40 μM dose) resulted in the increased degradation of cytoplasmic β-catenin and decreased nuclear β-catenin levels (146). Studies by Ryu et al. (146) also demonstrate a dose-dependent decrease in expression of nuclear p300 coactivator upon curcumin treatment. This is especially significant since nuclear β-catenin forms a complex with Tcf4 and p300 coactivator to generate a transcriptional active complex (147).

EGCG and green tea polyphenols

Tea, derived from the plant Camellia sinensis, is the most popular beverage consumed by two-thirds of the world’s population (17). Green, black and oolong tea are the main commercial types and these differ in how they are processed and fermented. Green tea contains epigallocatechin-3-gallate (EGCG), epigallocatechin, epicatechin-3-gallate and epicatechin. These compounds grouped as catechins are strong radical scavengers and metal chelators in a number of in vivo and chemical-based assays (148). An increasing number of studies have also demonstrated these antioxidative effects in vivo. Studies have shown that tea treatment can induce Phase II metabolism and antioxidant enzymes in both animal models and humans (85,149–151). Several studies have shown that the effect of EGCG and other tea polyphenols on key cell signaling pathways may be dependent on polyphenol-mediated reactive oxygen species. Green tea was found to inhibit the formation of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine-induced colonic aberrant crypts in the rat as well as intestinal polyps in the APC-Min mouse (152,153). Decrease in tumor burden was associated with downregulation of β-catenin expression in the intestine as well as markedly lowered expression of β-catenin/Tcf target genes (c-jun and cyclin D1). These findings are important because they suggest the possibility for intervention by tea against the early stages of human CRC, involving the β-catenin/APC pathway and the alteration of Wnt target genes (65,85,151). Studies suggest that β-catenin/Tcf4 transcriptional activity was inhibited in a concentration-dependent fashion upon treatment with EGCG (154) in HEK293 cells induced with β-catenin/Tcf4 (154). Wnt/β-catenin signaling was found to be inhibited by EGCG in a dose-dependent manner in breast cancer cells. Treatment with EGCG resulted in upregulation of HMG-box containing protein 1, an important antagonist of Wnt signaling (127,155). EGCG reduced both proliferation and invasiveness of breast cancer cells through induction of HMG-box containing protein 1 and the subsequent downregulation of Wnt/β-catenin signaling.

Resveratrol

Resveratrol, a dietary polyphenol, that has been shown to possess potent antioxidant as well as anti-inflammatory properties, mediates induction of antioxidant enzymes and modulates lipid metabolism while attenuating hepatic lipid peroxidation (156–158). It has been shown that resveratrol increases hepatic glutathione content, scavenges free radicals and induces enzymes of phase II hepatic metabolism. It also inhibits the transcription factor nuclear factor-kappa B (NF-kB), which induces the inflammatory cascade (158–160). Resveratrol has been shown to reduce the nuclear translocation of NF-κB (161) as well as interfere with its transcription (162). Additionally, resveratrol also produces a significant reduction in the expression of several proinflammatory cytokines (156,162). Studies suggest that resveratrol inhibits skin tumorigenesis through the regulation of phosphatidylinositol-3-kinase and Akt proteins, which have been implicated in development and progression of cancer (127,163). Resveratrol has shown to significantly decrease the level of β-catenin in the nucleus of colon cancer cells. The decreased nuclear localization of β-catenin by resveratrol treatment could be due to reduced expression of lgs and pygol, which are regulators of β-catenin localization (164). In addition to the solid tumor, resveratrol also inhibits proliferation and induced cell cycle arrest and apoptosis in Waldenstrom’s macroglobulinemia cells. These effects of resveratrol were found to be mediated via the downregulation of Akt, mitogen-activated protein kinase and Wnt signaling pathways (165). Several studies suggest that resveratrol-induced apoptosis is associated with the activation of the p33 in a dose- and time-dependent manner (127,165). Resveratrol also modulates DNA double-strand break repair pathways in p53-dependent manner (166), suggesting the regulatory effect of resveratrol on p53 signaling.

Lupeol

Lupeol is a well-studied dietary triterpene. Lupeol (C30H50O) has a melting point of 215–216°C and a molecular weight of 426. It is found in several fruits (olives, figs, mangoes, strawberries and grapes), vegetables (green peppers, white cabbage and tomato) and medicinal plants (American ginseng, Shea butter plant, Tamarindus indica, Crataeva nurvala and Bombax ceiba). Lupeol was used extensively by native tribes of North America, China, Africa and the Caribbean islands (167). Lupeol has been shown to exhibit various pharmacological activities under in vitro and in vivo conditions. These include its beneficial effect against inflammation, cancer, arthritis, diabetes, heart diseases, renal toxicity and hepatic toxicity (reviewed in ref. 167–185). It is noteworthy to mention that lupeol exhibits strong anti-mutagenic activity under in vitro and in vivo systems (179,186). Studies by Nigam et al. (179) have demonstrated that application of lupeol (200 μg per mouse) prevents 7,14-dimethylbenz(a)anthracene-induced DNA damage (DNA strand breaks) in mouse skin. Recently, lupeol was shown to inhibit benzo-a-pyrene genotoxicity in a mouse model (180). In benzo-a-pyrene-treated animals, a significant induction of chromosomal aberration and micronuclei was recorded, with a decrease in mitotic index, and significant decrease in benzo-a-pyrene-induced clastogenicity was recorded upon lupeol administration (180). It has demonstrated that lupeol inhibits tumor promotion in a murine mouse model of the skin (181). Topical administration of lupeol (40 mg per animal per thrice a week) was shown to significantly decrease tumor burden in mice (181). The antitumor effects of lupeol were observed to be associated with its potential to modulate NF-κB and phosphatidylinositol-3-kinase/Akt pathway which play an important role in tumor progression. Our laboratory has studied the beneficial effects of lupeol in a variety of cancers, such as prostate, pancreas and melanoma (182–184,187,188). A recent study suggested that lupeol caused inhibition in the growth and proliferation of human prostate cancer cells irrespective of their androgen status (187). Recent studies from our laboratory have suggested the inhibition of human metastatic melanoma cells in vitro and in a xenograft mouse model (182).
laboratory has also demonstrated that lupeol has a significant growth inhibitory potential on melanoma cells that exhibit constitutive Wnt/β-catenin signaling (189). We have also demonstrated that lupeol inhibits the localization of β-catenin into the nucleus and decreases the phosphorylation status of β-catenin at important serine sites (ser 552 and ser 675). Recent studies suggest that phosphorylation of β-catenin at Ser-552 or Ser-675 abets β-catenin to localize to the nucleus and induce transcription of various downstream targets (190–192).

**Retinoids**

Vitamin A is converted in vivo to a number of different metabolites collectively referred to as retinoids. Retinoids are potent regulators of cell proliferation and differentiation that are applicable to cancer therapy and prevention (193,194). The use of retinoids as an important chemopreventive agent has been highlighted by several in vitro studies: preclinical animal model experiments, epidemiologic evidence and clinical trials (195). Retinoids have been shown to inhibit the function of the oncogenic AP-1 and Wnt/β-catenin signaling pathways as well as stabilize components of adherens junctions (196). GSK-3β, an important component of the Wnt/β-catenin signaling pathway, plays a key role in retinoid-induced degradation of cyclin D1. In human bronchial epithelial cells, mutation of Thr286 made cyclin D1 resistant to retinoid treatment and pharmacological inhibitors of GSK-3β antagonized the effect of retinoids (193,197).

**Other natural products**

Lycopene is a bioactive red-colored pigment naturally occurring in plants. Interest in lycopene is increasing due to increasing evidence proving its preventive properties toward numerous diseases. In vitro, in vivo and ex vivo studies have demonstrated that lycopene-rich foods are inversely associated to diseases, such as cancers, cardiovascular diseases, diabetes and others (198–200). Experimental studies have shown that lycopene inhibits cell growth in breast, prostate and endometrial cancer cells with regulation of cell cycle-related genes (201,202). The inhibition of NF-κB DNA-binding activity by lycopene was mediated through the downregulation of IκB phosphorylation, NF-κB expression and NF-κB p65 subunit translocation from cytosol to nucleus (203). Studies suggest that the consumption of lycopene is inversely related to human prostate cancer risk (127). Lycopene reduced localization of β-catenin in the nucleus perhaps by antagonizing the effects of GSK-3β phosphorylation (109).

Deguelin is a rotenoid from Mandulea sericea (Family: Leguminosae). This agent has shown great potential as a chemopreventive and therapeutic agent against several types of cancers, including colon, lung and breast (204–209). Deguelin induces apoptotic death by downregulating cell survival pathways, including Akt, mitogen-activated protein kinase and surviving in a variety of human breast cancer cells (206). Research studies suggest that deguelin has been shown to suppress hypoxia-inducible factor-1α/heat shock protein 90 interaction and expression in radiosensitive lung cancer cells (204,210).

SFN is an isothiocyanate that is abundantly present in cruciferous vegetables like broccoli and cauliflower. SFN has been found to inhibit carcinoma-induced mammary gland tumorigenesis (211), colonic aberrant crypt foci (212,213), stomach tumors (214) and lung cancer (215) in rats/mice. Several animal model studies suggest that SFN can inhibit carcinogenesis (211,212,216). A study by Hu et al. (217) suggests that ApcMin/+ mice fed with SFN-supplemented diet developed significantly fewer and smaller polyps.

**Summary**

Aberrant Wnt/β-catenin signaling is observed in a variety of cancers and plays an important role in cancer progression. The data from in vitro, in vivo and preclinical human studies highlight the importance of natural products that exert their inhibitory effects on carcinogenesis and cancer progression by downregulating Wnt/β-catenin signaling pathway. We believe that these natural products that are non-toxic in nature could be used either alone or in combination with conventional therapeutics for the prevention and/or treatment of a variety of human malignancies. Appropriate and relevant animal model studies along with novel clinical trials are needed to fully appreciate the value of these and other natural products for improving the quality of human health.

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