Chapter 8
An Evidence-based Perspective of *Scutellaria Barbata* (Skullcap) for Cancer Patients

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Abstract *Scutellaria barbata* (skullcap) has been used in traditional Chinese and Korean medicine for treating various inflammation illnesses and cancers. *In vitro* studies have demonstrated the anti-mutagenesis (chemo-preventive) effect of skullcap *via* modulating the metabolism of mutagenic compounds such as aflatoxin B_1 and benzo[a]pyrene to reduce their DNA binding efficiency. *In vitro* and *in vivo* studies using flavonoid compound of skullcap in both human and animal models have shown promising anticancer effects. The aqueous extract of skullcap has shown to have the most effective anticancer chemical constituents. *In vitro* studies indicated that skullcap might be effective against all three stages of carcinogenesis (initiation, promotion, and progression). It has also been found to exert anticancer mechanisms such as anti-inflammation, anti-proliferation, induction of apoptosis against numerous cancer cell lines including digestive (liver and colorectal), respiratory (lung and nasopharyngeal), and lymphatic (leukemia) systems, and induction of sex hormone-specific glycolytic necrosis, especially those of the reproductive system (breast, uterine, and prostate) while inactive on normal human mammary epithelial cells. *In vivo* murine model showed aqueous extract of skullcap may enhance macrophage cell line activity leading to inhibition of tumor growth. It has also been shown to inhibit aberrant crypt formation in colon, delay prostate cancer development and progression in transgenic adenocarcinoma of mouse prostate mice, and reduced solid ascites tumor proliferation in the breast of mice. Aqueous extracts of skullcap have been found to have a favorable toxicity profile when used as an oral feeding in Phase 1 and 1B clinical studies in metastatic breast cancer patients. Based on the studies reported on skullcap, the best prospective therapeutic application of skullcap would be in breast, prostate, liver, colorectal, uterine, and lung cancer patients.

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Fig. 8.1 Picture of *Scutellaria barbata* D Don (skullcap or Ban-Zhi-Lian). (http://healingpastures.com/2010/08/02/cancer-fighting-mint-plant, Accessed 26 August 2010)

8.1 Introduction

Scutellaria barbata D. Don (skullcap), Chinese name *Ban-Zhi-Lian* of the Lamiaceae family, is a 12–35 cm tall perennial herb, found along fields and ditches in southern China and Korea (Fig. 8.1). It is commonly known as skullcap, a mint with a mildly bitter taste commonly used in traditional Chinese and Korean medicine for the treatment of a variety of ailments such as appendicitis, hepatitis, snake bites, lung, liver and rectal cancer (Jiangsu New Medical College 1977; Chong and Lee 1988; Zhu 1998; Huang 1999). In China, skullcap has been used clinically in treating breast cancer, lung cancer, liver cancer, digestive tract cancers, and chorioepithelioma. Skullcap has been used in combination with *Oldenlandia diffusa* in patients with liver, rectal and lung cancer. However, it was stated that skullcap alone or in combination with other herbs has not been successful in complete cure of cancer (complete remission) but rather provides symptomatic relief (Chong and Lee 1988). Literature review also shows very limited *in vitro* and *in vivo* research on the inhibitory or anticancer properties and mechanisms of skullcap following the ethnopharmacological approach in the early 1900s. The purpose of this chapter is to describe the early chemopreventive studies of skullcap and follow its development into the isolation and understanding of the various chemical constituents as well as their *in vitro* and *in vivo* effect or mechanism based on research literature.

8.2 Early Studies

8.2.1 Anti-mutagenesis and Anti-carcinogenesis Chemo-prevention Properties

Our early studies found that aqueous extracts (whole) of skullcap possess anti-mutagenic and chemopreventive properties (Wong et al. 1992a, b, c, 1993a, b). These stud-

ies indicate that skullcap contains phytochemicals which inhibited mutagenesis, DNA binding, and metabolism of the pro-carcinogens aflatoxin B_1 (AFB_1) and benzo[a]pyrene bioactivated by Arcoclor 1254-induced rat S9. Inhibition effects of skullcap were also shown in the mutagenicity of AFB_1 on mutant bacteria *Salmonella typhimurium* TA100 using dexamethansone (DXM)-induced rat hepatic S9, on cytochrome P450-linked aminopyrine N-demethylase (APND) activity in DXM-induced hepatic microsomes, and on the metabolism of AFB_1 by DXM-induced S9. Aqeuous extract of skullcap consistently inhibited the mutagenicity of AFB_1 bioactivated by either non-induced or DXM-induced hepatic S9. The effects correlated with the inhibition of cytochrome P450-linked APND activity in DXM-induced S9 mediated metabolism of $[^3H]AFB_1$. These findings suggest that skullcap contains antimutagenic and antitumorigenic property on AFB_1 *via* inhibition of cytochrome p450 isozyme (CYP3)-mediated metabolism of the carcinogens. This data suggests that skullcap is effective against all three stages of carcinogenesis (initiation, promotion, and progression).

8.2.2 Immune Enhancing and Anti-inflammation Effect of Skullcap

A later study showed that oral feeding with the aqueous herbal extract inhibited the growth of transplanted murine renal cell carcinoma (RenCa) in Balb/c mice significantly (Wong et al. 1996). *In vitro* data of the same study revealed that it enhanced the phagocytic oxidative burst in murine macrophage, J774 cells. This suggests its ability to inhibit tumor growth observed *in vivo*. An aqueous extract of skullcap was also shown to have greater antimutagenic effect in inhibiting DNA damage of peripheral lymphocytes when injected into cigarettes (Han et al. 1997). Our later study revealed skullcap treatment could elevate H_2O_2 and hydroxyl radical production in the macrophage-like RAW 264.7 mouse peritoneal cell line. It showed that skullcap modulated COX-2 (cyclooxygenase-2) and inducible NO (nitric oxide) synthase protein expression, as well as the activity of prostaglandin E_2 and stable oxidation products of NO *in vitro*. This may be related to the formation of reactive oxygen species (Harris et al. 2003). Together these early studies aroused and led to continuous research interest in the chemical isolation and specific anticancer effect and mechanism of skullcap since the early 2000s.

8.3 Chemical Constitution

Skullcap has been reported to contain several bioactive flavonone compounds such as scutellarein, scutellarin, carthamidin, isocarthamidin, wogonin, apigenin, luteolon, pheophoride-*a*, and various clerodane diterpeonoids (Zhu 1998; Kim et al. 2005; Chan et al. 2006; Dai et al. 2006a, b; 2007a, b, 2008, 2009a, b, 2010). Tables 8.1 and 8.2 list more detail chemical compounds and extracts of skullcap with their respective *in vitro* and *in vivo* anticancer properties (effect and mechanism) classified

Table 8.1 Anticancer properties of chemical constitution of *Scutellaria barbata* (skullcap) on murine *in vitro* and *in vivo* studies according to organ system

Organ system (Cancer cell line/Tissue/Animal model) Chemical constitution	Effect/Mechanism	Reference
Integumentary:		
Skin		
Aqueous extract	Inhibition of tumorigenesis (DMBA-induced) in mouse skin cancer model	Suh et al. 2007
Lymphatic:		
Macrophage-like		
(RAW 264.7) Aqueous extract (whole)	Elevation of H_2O_2 and hydroxyl radical in macrophage-like RAW 264.7 mouse peritoneal cell line Modulation of cyclooxygenase-2 (COX-2) and inducible nitric oxide (NO) synthase protein expression, and prostaglandin E_2 and stable oxidation products of NO	Harris et al. 2003
Macrophage		
(J744) Aqueous extract (whole)	Enhancing J744 macrophage cell oxidative burst	Wong et al. 1996
Respiratory:		
Lung		
(LLC) Luteolin	Induction of apoptosis in Lewis lung carcinoma LLC cells *via* caspase activation and extracellular signal-regulated kinase ERK/Akt inhibition Reduction of proliferation cell nuclear antigen (PCNA) Enhancement of Annexin-V-positive cell growth and G1 DNA Activation of caspase-3 and -9, and PARP cleavage Increasing the ratio of Bax/Bcl-2 Reduction of mitochondrial membrane potential Inhibition of growth of LLC cells implanted on the flank of mice	Kim et al. 2007b
(LLC) Aqueous extract	Inhibition of growth	Shoemaker et al. 2005
Digestive:		
Colon		
Aqueous extract (whole)	Inhibition of azoxymethane (AOM)-induced aberrant crypt foci (ACF) formation in C57BL/6 mice	Wong et al. 2010
Liver		
(H22) Aqueous extract (ESB)	Inhibition of proliferation and induction of apoptosis of mouse hepatoma cells through loss of mitochondrial transmembrane potential, release of cytochrome c, and activation of caspase-3	Dai et al. 2008a

Table 8.1 (continued)

Organ system (Cancer cell line/Tissue/Animal model) Chemical constitution	Effect/Mechanism	Reference
(H22) Aqueous extract (ESB)	Induction of apoptosis *via* reduction of mitochondrial transmembrane potential of mouse hepatoma cells by serum containing ESB	Dai et al. 2008b
(H22) Aqueous extract (ESB)	Inhibition of proliferation and induction of apoptosis of hepatoma cells	Dai et al. 2008c
(Hep3B) Pheophorbide-a $C_{35}H_{36}N_4O_5$	Reduction of tumor size (57%) in nude mice model	Tang et al. 2006
Urinary:		
Kidney		
(RenCa) Aqueous extract (whole)	Inhibition of tumor growth of transplanted RenCa cancer cells in Balb/c mice	Wong et al. 1996
Reproductive (female):		
Mammary adenocarinoma		
(EAC- Ehrlich ascites tumor cells) Chloroform fraction: Phytol, wogonin, luteolin, hispidulin	Inhibition of solid Ascites tumor proliferation and prolonging life span in mice	Yu et al. 2007
Mammary gland tissue Aqueous extract	Inhibition of preneoplastic lesions development in carcinogen (DMBA)-induced mouse mammary glands in culture	Suh et al. 2007
Breast		
(MCNeuA) Aqueous extract	Inhibition of murine breast carcinoma MCNeuA cell growth (while relatively inactive on normal human mammary epithelial huMEC cells)	Shoemaker et al. 2005
(MCNeuA) Aqueous extract	Inhibition of cell growth Apoptosis	Campbell et al. 2002
Reproductive (male):		
Prostate		
(TRAMP-C1) Aqueous extract (whole)	Induction of apoptosis *via* DNA fragmentation	Wong et al. 2009
Prostate		
Aqueous extract (whole)	Delaying prostate cancer development & progression in TRAMP mice Activation of caspase-3 in TRAMP mice prostate tissue	Wong et al. 2009

Table 8.2 Anticancer properties of chemical constitution of *Scutellaria barbata* (skullcap) on human *in vitro* and *in vivo* studies classified according to organ system

Organ system (Cancer cell line/Tissue/ Clinical study) Chemical constitution	Effect/Mechanism	Reference
Circulatory & Lymphatic:		
Leukemia		
(HL-60) Neo-clerodane diterpenoids: barbatellarines A (1), B (2)	Weak cytotoxic against human leukemia HL-60 cells by barbatellarine B	Lee et al. 2010
Flavonoids: Apigenin, luteolin	Induction of G1 arrest and apoptosis in promyeloctyic leukemia HL-60 cell line Inhibition of Cyclin A, D1, D2, D3, E; CDK 2, 4, 6 Up-regulation of p21, CDK inhibitors Down-regulation of retinoblastoma protein phosphorylation	Kim et al. 2007a
(KG-1) Aqueous extract (whole)	Anti-proliferation and induction of apoptosis in this acute myelogenous leukemia cell line	Chui et al. 2005
(U937) Methylene Chloride fraction	Induction of apoptosis (activating caspase-3 and -9, cleaving PARP, increasing the ratio of Bax/Bcl-2, and releasing cytochrome c *via* the mitochondrial signaling pathways)	Cha et al. 2004
(Red blood cell)		
Polysaccharide B3-PS1 (1,700KDa) Gal 4.3:Glc 1.6:Man 1.1:Ara 1.0+Rha, Fuc, Xyl	Anti-complementary in hemolytic assay (C1q, C1r, C1s, C2, C3, C4, C5, C9)	Wu and Chen 2009
(Peripheral lymphocytes)		
Aqueous extract	Inhibition of damage of DNA in lymphocytes caused by the total particle material extract of cigarette tar and greater antimutagenesis effect when the herb was injected into cigarettes ahead	Han et al. 1997
Respiratory:		
Lung		
(A549)		
Flavonoid (wogonin)	Inhibition of phorbol 12-myristate 13-acetate (PMA)-induced *COX-2* gene expression *via* inhibition of *c-Jun* expression and *AP-1* activation in this epithelial cancer cell line	Chen et al. 2008

Table 8.2 (continued)

Organ system (Cancer cell line/Tissue/ Clinical study) Chemical constitution	Effect/Mechanism	Reference
Aqueous extract (whole)	Inhibition of cell growth	Shoemaker et al. 2005
Aqueous extract (whole)	Anti-proliferation and induction of apoptosis	Chui et al. 2005
Ethanol extract (30%)	Cytotoxic and Induction of apoptosis Induction of gene expression (5-fold change in 10 genes involved in DNA damage, cell cycle control, nucleic acid binding and protein phosphorylation) by *c*DNA microarray analysis	Yin et al. 2004
(SPC-A-1)		
Ethanol extract (75%)	Induction of apoptosis by up-regulation of caspase-3 expression and down-regulation of survival genes (*survinin*) expression	Wei et al. 2007
Nasopharyngeal		
(HONE-1)		
Neo-clerodane diterpenoids : Scutehenanine H (1) & 6-(2,3-epoxy-2-isopropyl-n-propoxyl) barbatin C (2)	Cytotoxic against the cell line	Dai et al. 2010
Scutehenanines A-D (1,4,5,6), 6-O-acetylscutehenanine A (2), 6-O-(2-carbonyl-3-methylbutanoyl)scutehenanine A (3)		Dai et al. 2009a
Scutebarbatine O (1), 6-O-nicotinoylscutebarbatine G (2)		Dai et al. 2009b
6,7-dibenzoyloxybarbatin C (1, barbatin D), 6-(2-acetoxy-3-methylbutanoloxy)-7-(2-carbonyl-3-methylbutanoyloxy) barbatin C (2, barbatin E)		Dai et al. 2008
Ent-clerodane diterpenoids (1–4)		Dai et al. 2007a
Scutebarbatines I-L (1–4)		Dai et al. 2007b

Table 8.2 (continued)

Organ system (Cancer cell line/Tissue/ Clinical study) Chemical constitution	Effect/Mechanism	Reference
Barbatins A-C (1–3), Scutebarbatine B (4)		Dai et al. 2006
Scutebarbatine C-F (1–4)		Dai et al. 2006b
Digestive:		
Oral epidermoid carcinoma		
(KB)		
Colorectal carcinoma		
(HT29)		
[Chemicals same as those of nasopharyngeal above]	Cytotoxic against these two cancer cell lines	[References same as above]
Stomach		
(AGS) Ethanol (30%) extract	Inhibition of growth of gastric AGS cancer cell line	Yin et al. 2004
Pancreas		
(Panc-1) Aqueous extract	Inhibition of growth of pancreatic carcinoma cells (while relatively inactive on normal human mammary epithelial huMEC cells)	Shoemaker et al. 2005
Colon		
(LoVo) Methanol extract (chemically standardized with: scutellarein, sutellarin, carthamidin, isocarthamidin, wogonin)	Induction of cell death (increase in sub G1 phase) in colon adenocarinoma LoVo cells using a functional proteomic approach	Goh et al. 2005
Liver		
(Hep-G2) Pheophorbide-a $C_{35}H_{36}N_4O_5$	Reversal of P-glycoprotein multidrug resistance mediated (reduction of expression at both transcriptional and translational level) in hepatoma Hep-G2 cell line Induction of cell cycle arrest at G2/M phase and inhibition of cylcin-A1 and cdc2	Tang et al. 2007
(Hep-G2) Ethanol extract	Inhibition of cell proliferation, blockage of cell cycle induction (decreasing S phase and increasing G0/G1phase), and induction of apoptosis (up-regulation of *Fas* expression related to the activation of *FNFR* superfamily) of hepatocellular Hep-G2 cell	Lin et al. 2006a

Table 8.2 (continued)

Organ system (Cancer cell line/Tissue/ Clinical study) Chemical constitution	Effect/Mechanism	Reference
(Hep-G2) Ethanol extract (30%)	Inhibition of growth of liver Hep-G2	Yin et al. 2004
(Hep3B) Pheophorbide-*a* $C_{35}H_{36}N_4O_5$	Inhibition of cell proliferation and induction of apoptosis (*via* the releasing of cyc-tochrome c mitochondria and decreasing pro-caspase-3 and -9) in hepatocellular carcinoma Hep3B cells	Tang et al. 2006
(Hep3B) Pheophorbide- *a* $C_{35}H_{36}N_4O_5$	Anti-proliferation (*via* sub-G1 cell cycle arrest) and induction of apoptosis (*via* the suppression of Bcl-2, releasing in Hep3B of cytochrome c to cytosol, and activation of pro-caspase-3 and -9) (viral-induced) cells	Chan et al. 2006
(Hep3B) Aqueous extract (whole)	Anti-proliferation and induction of apoptosis in Hep3B hepatocellular carcinoma and Hep-G2 hepatoblastoma	Chui et al. 2005
(Hep-G2, BEL-7402) Chloroform fraction: Phytol, wogonin, luteolin, hispidulin	Cytotoxic to both cell lines Induction of apoptosis in hepatocellular carcinoma BEL-7402 cells with a lower cytotoxic effect on normal liver cell line (L-O2) Activation of caspase-9 and cytochrome c Decrease *S* phase content	Yu et al. 2007
(QGY-7701) Aqueous extract (ESB)	Inhibition of cell proliferation, activation of anti-oncogene *Bcl-2*, induction of apoptosis and inhibition of G to S phase cell cycle progression in hepatocellular carinoma QGY-7701 cells	Lin et al. 2006b
Urinary **Kidney** (ACHN) *n*-hexane, ethyl acetate, & chloroform (most effective) fractions	Cytotoxic against the renal adenocarcinoma cells	Yu et al. 2007
Reproductive (female): **Breast** (Metastatic breast cancer) Aqueous extract (BZL101)	Oral feeding (40 g/day) was safe, well-tolerated in phase 1B does escalation trial of human metastatic breast cancer	Perez et al. 2010

Table 8.2 (continued)

Organ system (Cancer cell line/Tissue/ Clinical study) Chemical constitution	Effect/Mechanism	Reference
(Metastatic breast cancer) Aqueous extract (BZL101)	Favorable toxicity profile & promising efficacy in Phase I clinical trial of advanced breast cancer	Rugo et al. 2007
Breast		
(MCF-7, MDA-MB-231) Aqueous extract (BZL101)	Phenotype specific anti-proliferative gene expression responses in human breast (early stage estrogen sensitive MCF-7 *vs* late estrogen insensitive MDA-MB-231) reproductive cancer cells Induction of G(1) cell cycle arrest & ablated expression of regulators Cyclin D1, CDK2, CDK4, growth factor stimulatory pathways & estrogen receptor-α expression in estrogen sensitive MCF-7 breast cancer cells (ablation of promoter activities)	Marconett et al. 2010
(MDA-MB-231) Pheophorbide-a (photo-activated) $C_{35}H_{36}N_4O_5$	Activation of mitochondria-mediated apoptosis through the suppression of extracellular signal-regulated kinase (ERK)-mediated autophagy in estrogen receptor-negative human breast adenocarcinoma cells MDA-MB-231 by pheophorbide-a based photodynamic therapy (Pa-PDT) Triggering mitogen-activated protein kinase (MAPK) pathway Activation of *c-June N*-terminal kinase (JNK) Inhibition of ERK	Bui-Xuan et al. 2010
(MDA-MB-231) Brucea javanica extract *(Scutellaria barbata + Gleditsia sinensis + Radix Sophorae Tonkinesis)*	Induction of apoptosis (*via* mitochrondrial dependent pathway associated with caspase-3 activity) in human breast carcinoma MDA-MB231 cell	Lau et al. 2005
(MDA-MB-231) Aqueous extract (whole)	Anti-proliferation and induction of apoptosis	Chui et al. 2005
(SK-BR-3) Neoclerodane diterpenoids: Scutebatas A-G (1–7)	Weak cytotoxicity against human mammary cancer cells SK-BR-3 by Scutebata A	Zhu et al. 2010

Table 8.2 (continued)

Organ system (Cancer cell line/Tissue/ Clinical study) Chemical constitution	Effect/Mechanism	Reference
(SK-BR-3, BT474) Aqueous extract (BZL101)	Selectively induction of cell death in human breast cancer SK-BR-3 and BT474 cells but not in non-transformed mammary epithelial cells (MCF10A) *via* induction of reactive oxygen species (ROS) Inhibition of glycolysis selectively in tumor cells (decrease in the enzymatic activities within the glycolytic pathway and the inhibition of lactate production) Induction of oxidative damage leads to hyperactivation of poly(ADP-ribose)polymerase (PARP), followed by a sustained decrease in levels of NAD and depletion of ATP in cancer cells Induction of expression of genes involved in oxidative response, DNA damage & cell death	Fong et al. 2008
(SK-BR-3, BT474, MDA-MB-231, MDA-MB-361, MDA-MB-453, MDA-MB-468, Du4475) Aqueous extract (BZL101)	Induction of strong growth inhibition and DNA fragmentation (while relatively inactive on normal human fibroblast IMR90 cell lines)	Rugo et al. 2007
(MCF-7, MDA-MB-435S) *n*-hexane, ethyl acetate, & chloroform (most effective) fractions	Cytotoxic against both cell lines	Yu et al. 2007
(MCF-7) Aqueous extract	Inhibition of breast carcinoma cell growth (while relatively inactive on normal human mammary epithelial huMEC cells)	Shoemaker et al. 2005
(MCF-7, MDA-MB-231, SK-BR-3, BT474) Aqueous extract	Inhibition of human breast cancer cells growth	Campbell et al. 2002
Ovarian		
(HOC) Aqueous extract	Function as anti-mutagen, anti-inflammation and anti-proliferation agent. Inhibition of COX-2 and hydroperoxidase expression	Suh et al. 2007
(SKOV-3, CAOV3) Aqueous extract	Induction of apoptosis	Powell et al. 2003

Table 8.2 (continued)

Organ system (Cancer cell line/Tissue/ Clinical study) Chemical constitution	Effect/Mechanism	Reference
Uterine		
(Leiomyoma cells)	Inhibition of leiomyoma growth	Kim et al. 2008
Aqueous extract (evaporated by ethanol)	Down-regulation of the expression of Bcl-2 protein in uterine leiomyoma cells	
Aqueous extract	Reduction of tumor volume in leiomyomal smooth muscle cells *via* regulation of IGF-I production and induction of increased rate of apoptosis	Kim et al. 2005
Aqueous extract	Anti-proliferation (*via* inhibition of expression of proliferating cell nuclear antigen, Cyclin E, cdc2) of HCG-promoted leiomyomal cells	Lee et al. 2004c
Aqueous extract	Inhibition of aromatase activity of leiomyomal cells	Lee et al. 2004b
Aqueous extract	Induction of *c-fos* gene expression in uterine leiomyomal cells *via* increases in adenosine-3',5', cAMP mediated by β2-adrenergic receptors (β2-ARs) which in turn activated the cAMP/protein kinase (PKA) pathway	Lee et al. 2004a
Aqueous extract	Inhibition of uterine leiomyomal smooth muscle cell proliferation *via* the induction of alpha-smooth muscle actin (SMA), calponin h1 and p27	Lee et al. 2004d
Cervix		
(HeLa)		
n-hexane, ethyl acetate, & chloroform (most effective) fractions	Cytotoxic to the cervix carcinoma cell line	Yu et al. 2007
(HeLa)	Function as anti-mutagen, anti-inflammation and anti-proliferation agent.	Suh et al. 2007
Aqueous extract	Inhibition of COX-2 and hydroperoxidase expression	
Reproductive (male):		
Prostate		
(LNCaP, PC-3)	Phenotype specific anti-proliferative gene expression responses in human prostate (androgen sensitive LNCaP *vs* late androgen insensitive PC-3) reproductive cancer cells	Marconett et al. 2010
Aqueous extract (BZL101)	Arresting early stage androgen sensitive LNCaP in G(2)/M phase with corresponding decreases in Cyclin B1, CDK1 & androgen receptor expression	
	Inducing S phase arrest with corresponding ablations in Cyclin A2 & CDK2 expression	

Table 8.2 (continued)

Organ system (Cancer cell line/Tissue/ Clinical study) Chemical constitution	Effect/Mechanism	Reference
(LNCaP, PC-3) Aqueous extract	Inhibition of prostate cancer cell growth (while relatively inactive on normal human mammary epithelial huMEC cells)	Shoemaker et al. 2005
(LNCaP, DU-145) Aqueous extract (whole)	Regulation of caspase-8 activation in LNCaP (androgen dependent) and DU-145 (androgen independent) prostate cancer cells Activation of apoptosis through an upstream androgen-receptor pathway Promotion of apoptosis, anti-inflammation, & anti-proliferation by inhibiting Akt pathway Binding to membrane androgen receptors (mARs) and activation of secondary pathways further lead to apoptosis and inhibit tumor progression	Nguyen et al. 2008
(LNCaP) Aqueous extract (whole)	Induction of apoptosis in LNCaP hormone dependent prostate cancer cells Up-regulation of apoptotic pathway (enhance Bax, p53, Akt, JNK production) and down-regulation of survival pathway	Wong et al. 2009
(LNCaP) Aqueous extract (whole)	Activation of caspase-3, -8, and -9 in LNCaP prostate cancer cell	Wong et al. 2007

according to organ system in animal and human respectively. These chemical constitutions of skullcap include various types of extracts (aqueous, chloroform, ethanol, ethyl acetate, methanol, methylene chloride, and *n*-hexane) and compounds. Among these, clerodane diterpeonoids are the most abundantly isolated compounds (>28) and are potential candidates for further studies of *in vitro* and *in vivo* mechanisms; while aqueous skullcap extract is the most dominant form of extraction with greater and more detailed effect and mechanism. One of such total aqueous extracts, BZL101 is suggested to have greater activity in *in vitro* assays compared to enriched chromatographically isolated pure compounds (Perez et al. 2010). For example, 9 flavonoid phytochemicals showed cytotoxic activity *in vitro*, but only at concentrations far higher than those found in the total aqueous extract. Also, the whole extract is more cytotoxic than any combination of the purified flavonoids. To learn more about the various chemical compounds of skullcap, readers may refer to Tables 8.1 and 8.2 for their specific chemical nature and specific anticancer effects or mechanisms respectively. The following sections will focus on the discussion of some of the anticancer properties and mechanisms of these chemical constituents on animal and human *in vitro* and *in vivo*, classified according to organ system.

8.4 Anticancer *In Vitro* and *In Vivo* Animal Studies

8.4.1 In Vitro Murine Cancer Cell Line Studies

Guided by ethnopharmacological approach, *in vitro* cell line data in Table 8.1 reveals studies done on the specific effect and mechanism of various chemical constituents of skullcap classified according to organ system. There are 6 murine cancer cell lines, 1 murine tissue (mammary glands in culture), and 4 organ systems studied. These include the lymphatic (macrophage—RAW 264.7 and J744 cancer cells), respiratory (lung—LLC), digestive (liver—H22), and reproductive [(breast—MC-NeuA, and prostate—transgenic adenocarcinoma of mouse prostate (TRAMP)-C1]. Major anticancer mechanisms include anti-inflammation, induction of phagocytosis, induction of apoptosis, and anti-proliferation while inactive on normal human mammary epithelial huMEC cells relatively. Some of the molecular mechanisms include elevation of H_2O_2 and hydroxyl radical, modulation of COX-2, enhancement of macrophage oxidative burst, activation of caspase-3 and -9), activation of poly(ADP-ribose) polymerase (PARP) cleavage, reduction of mitochondrial membrane potential, and release of cytochrome *c*. See Table 8.1 for detail mechanism and references for each cell line respectively.

8.4.2 In Vivo Murine Models

On the other hand, *in vivo* studies show that five mouse organ systems were affected by various chemical constituents of skullcap, including the integumentary

(inhibition of DMBA-induced skin cancer), digestive (inhibition of AOM-induced ACF colon cancer and inhibition of tumor volume in nude mouse transplanted liver Hep3B cancer cells), urinary (inhibition of tumor growth with transplanted kidney RenCa cancer cells), and reproductive (inhibition of solid Ehrlich ascites tumor and prolonging mice life span; delay of prostate cancer development and progression in TRAMP mice, and activation of caspase-3 in the prostate tissue of TRAMP mice). Again, the majority of the effects came from aqueous extract of skullcap (Table 8.1).

The TRAMP model is a spontaneous autochthonous transgenic mouse model. It mimics heterogenic tumor progression in human prostate cancer, providing a relevant pre-clinical model for identifying important pathways in tumorigenesis, androgen independence, and metastasis of prostate cancer (Gingrich et al. 1996, 1997; Gupta et al. 2000). Our *in vivo* data shows a delayed in tumor onset and development in the TRAMP mice. Palpable tumor development in 50% of the mice happened at 25 weeks in the placebo group, 29 weeks in the low-dose (8 mg skullcap daily) and mid-dose (16 mg) treatment groups, and 33 weeks in the high-dose (32 mg) group (log rank, $P = 0.0211$). Hematoxylin and eosin histopathological dorsal prostate tissue also reveals delay of prostate tumor progression and the activation of caspase-3 in the prostate tissue of the skullcap-treated mouse. These findings further suggest the potential efficacy of skullcap as a chemo-preventive and plausible treatment agent in prostate cancer (Wong et al. 2009).

8.5 Anticancer *In Vitro* Human Cell Lines and Clinical Studies

In this section, relevant *in vitro* human cell line and *in vivo* human data on the anticancer effect and mechanism of skullcap as classified by organ system, deserve more detailed description. For most of the systems, especially for reproductive system (breast, prostate, ovarian, and uterine), aqueous extract of skullcap is the most effective and dominant anticancer constituent in both *in vitro* and *in vivo* studies (Table 8.2).

8.5.1 In Vitro Cell Line Studies

Table 8.2 summarizes *in vitro* cell line data in showing the specific effects and mechanisms of the various chemical constituents of skullcap classified according to organ system. There are 34 human cancer cell lines (from 5 different organ systems) and 2 hematopoietic cell lines (red blood cell and peripheral lymphocytes). These include the lymphatic (leukemia—HL-60, KG-1, U937), respiratory (lung—A549, SPC-A-1; nasopharyngeal—HONE-1), digestive (oral—KB; stomach—AGS; pancreas—Panc-1; liver—Hep-G2, Hep3B, BEL-7402, QGY-7701); colon—LoVo; colorectal—HT29), urinary (kidney—ACHN), and reproductive (breast—MCF-7,

MDA-MB-231, MDA-MB-361, MDA-MB-435S, MDA-MB-468, SK-BR-3, BT474; ovarian—HOC, SKOV-3, CAOV3; uterine—leiomyoma cells; cervix—HeLa; prostate—LNCaP, PC-3, DU-145). Major anticancer mechanisms include cytotoxic, anti-inflammation, induction of phagocytosis, induction of apoptosis, anti-proliferation while inactive on normal human mammary epithelial huMEC, and inhibition of glycolysis selectively in tumor cells but not in non-transformed MCF10A cells. Detailed mechanism of each cell line is listed in Table 8.2. BZL101 was reported to induce cell death *via* caspase activation in breast cancer cells but not in non-transformed (benign) mammary epithelial cells MCF10A and normal human fibroblasts IMR90. Hyperactivation of PARP and inhibition of glycolysis are likely the key mechanisms resulting in the energetic collapse and necrotic death that of breast cancer cells (Rugo et al. 2007; Fong et al. 2008).

Some of the molecular mechanisms include but not limited to induction of G1 and G2/M arrest; inhibition of Cyclins (A, D1, D2, D3, E) and CDKs (2, 4, 6); inhibition of COX-2; enhancement of macrophage oxidative burst; activation of caspase-3, -8, and -9; up-regulation of Bax (Bcl-2-associated X protein), p53 (tumor suppressor protein), Akt, JNK, *Fas,* MAPK (mitogen-activated protein kinase); activation of PARP cleavage; reduction of mitochondrial membrane potential; releasing of cytochrome c *via* the mitochondrial signaling pathways; down-regulation of *Bcl-2* (B cell lymphoma 2), *ERK*; induction of *c-fos* gene expression; induction of expression of genes involved in oxidative response (*GCLM*, *CBS*, *TRAF3*, etc), DNA damage (*TIPARP*, *CADD45a*, etc), cell death (*A20*, *TNF*, etc), xenobiotic response (*CYP1A1*, *CYP1B1*, *HSP70*, etc), and NF-κB pathway (*TNF*, *ICAM1*, *IL-8,* etc). Detailed mechanism of each cell line is listed in Table 8.2. Recent *in vitro* study of breast cancer cells (early stage estrogen sensitive MCF-7 *versus* late estrogen insensitive MDA-MB-231) and prostate cancer cells (androgen sensitive LNCaP *versus* late androgen insensitive PC3) revealed phenotype specific anti-proliferative gene expression responses in these cancer cells (Marconett et al. 2010). Induction of G1 cell cycle arrest and ablated expression of regulators Cyclin D1, CDK2, CDK4, growth factor stimulatory pathways, and estrogen receptor-α expression in estrogen sensitive MCF-7 breast cancer cells (ablation of promoter activities) were observed. The skullcap extract also arrested early stage androgen sensitive LNCaP in G2/M phase with corresponding decreases in Cyclin B1, CDK1, and androgen receptor expression. It also induced S phase arrest with corresponding ablations in Cyclin A2 and CDK2 expression.

8.5.2 Clinical Studies

There were two clinical studies on patients with metastatic breast cancer (Rugo et al. 2007; Perez et al. 2010). An aqueous extract of skullcap BZL101 was found to have favorable toxicity profile and promising efficacy in a Phase I clinical trial in the treatment of advance breast cancer (Rugo et al. 2007). Oral feeding of BZL (40 g/day) was demonstrated to be safe and well-tolerated in Phase IB dose escala-

tion trial of metastatic breast cancer (Perez et al. 2010). Preliminary success in the Phase I clinical trial on 21 metastatic breast cancer patients demonstrated that oral intake of BZL101, an aqueous extract of skullcap (up to 12 g in 350 ml solution per day) had a favorable toxicity profile and encouraging clinical activity in heavily chemotherapy pretreated patients (Rugo et al. 2007). In this study, the mean age of patients was 54 years and the mean number of prior treatments for metastatic disease was 3.9. There was no grade III or IV adverse events (AEs). The most frequently reported skullcap-related grade I and II AEs were: nausea (38%), diarrhea (24%), headache (19%), flatulence (14%), fatigue (10), constipation (10%), and vomiting (10%). At the conclusion of this Phase I clinical study, 16 patients were available for evaluation. Among them, 4 had stable disease (SD) for >90 days (25%), 3/16 had SD for >180 days (19%), 5 had objective tumor regression (1 of that was 1 mm short of a partial remission using the Response Evaluation Criteria in Solid Tumors criteria. A follow-up study using oral feeding on BZL (40 g/day) was demonstrated to be safe and well-tolerated in a Phase IB dose escalation trial of metastatic breast cancer (Perez et al. 2010). In this open-label, Phase IB, multicenter, dose escalation study, all 27 women had histologically confirmed breast cancer and measurable stage IV disease. These patients had a median of 2 prior chemotherapy treatments for metastatic disease, and were treated in four different dose cohorts. At the end, grade 3 and 4 AEs were uncommon. The following dose-limiting toxicities were observed: grade 3 diarrhea, fatigue, rib pain, and grade 4 AST elevation (aspartate aminotransferase liver enzyme and metastases). Among 14 evaluable patients according to the Response Evaluation Criteria in Solid Tumors criteria, 3 were classified with stable disease for >120 days (21%), 1 remains stable for 700+days after 449 days BZL101 treatment. Three patients with objective tumor regression (>0% and <30%) were identified by independent radiology review. The maximum tolerated dose of BZL101 was not reached and it was defined as 40 g/day.

8.6 Discussion

8.6.1 Prospective Therapeutic Application and Direction for Cancer Patients

8.6.1.1 Breast Cancer

Breast cancer is the most common cancer among females in the United States (Jemal et al. 2010). The recent Phase I and Phase IB clinical studies involving the use of skullcap in advanced breast cancer patients indicate a promising therapeutic application of skullcap (Rugo et al. 2007; Perez et al. 2010). This data in collaboration with the known mechanisms of skullcap understood through *in vitro* studies (as described in Sect. 8.5.1 and Table 8.2), along with the two posi-

tive preliminary clinical results suggest that skullcap aqueous extract may have a promising application in hormone related female cancers like ovarian, uterine, cervical and breast cancer, despite originally believed to be less effective in treatment of reproductive organ cancers (Chong and Lee 1988; Zhu 1998; Huang 1999).

8.6.1.2 Prostate Cancer

Prostate cancer is the most common form of cancer and the second leading cause of cancer death in American men (Jemal et al. 2010). Data from of our *in vitro* and *in vivo* animal studies suggested a few possible cancer prevention and inhibition mechanism of skullcap on prostate cancer (Wong et al. 2009). Phytochemicals in the whole aqueous extract of skullcap might work together to induce programmed cell death in prostate cancer cells through the expression of p53 and Bax, which activating the apoptosis pathway. Skullcap might also affect cancer progression through the regulation of Akt, phosphorylated Akt, and JNK to suppress the survival pathway *in vitro*.

Prostate cancer is an ideal subject for clinical trials of cancer prevention due to its prevalence, long natural history, relative ease of prostate gland biopsies, and the relative availability of surrogate tumor markers. Patients with early prostate-specific antigen elevation ('prostate-specific antigen-only' disease progression) and with early disease should be ideal candidates for novel investigational therapies such as skullcap (Smith and Kantoff 2001). The preliminary success of Phase I and IB clinical trials on the safety and efficacy of high dosage (40 g/day) aqueous extracts of skullcap (Rugo et al. 2007; Perez et al. 2010) suggest that a Phase I clinical study of the efficacy skullcap on prostate cancer would be rewarding. Prostate cancer like breast cancer is a hormonally-related tumor, and thus skullcap may also be effective. There is currently still no cure in Western medicine for advanced prostate cancer leading many patients to seek alternative medicines, such as traditional Chinese medicine (Tang and Eisenbrand 1992; The Pharmacopoeia Commission of PRC 2000). Further translational studies of skullcap and its selective cytotoxicity in prostate cancer cells and non-transformed prostate epithelial cells may reveal other plausible mechanisms for skullcap, such as induction of reactive oxygen species and inhibition of glycolysis in cancer cells would give more support for the clinical trial of skullcap in prostate cancer patients.

8.6.1.3 Liver, Colorectal, Uterine, and Lung Cancers

Scientific literature reviews yield another group of promising candidates for skullcap treatment including liver cancer (9 *in vitro* studies with 1 murine and 3 human cancer cell lines), colorectal cancer (9 *in vitro* studies with 2 human cell lines and 1 *in vivo* murine ACF model), uterine (6 *in vitro* studies with leiomyoma cells), and

lung cancer (7 *in vitro* studies with 1 murine and 2 human cell lines) (Table 8.2). These studies have shown a favorable anticancer effect. Besides aqueous extract, phenorbide-*a* ($C_{35}H_{36}N_4O_5$), ethanol extract, and chloroform extract (phytol, wogonin, luteolin, and hispidulin) are especially effective (cytotoxic, anti-proliferation, induction of apoptosis and expression of Bcl-2) against liver cancer cells but with a low cytotoxic effect on normal liver L-O2 cell lines (Chan et al. 2006; Lin et al. 2006a, b; Tang et al. 2006, 2007; Yu et al. 2007). Following ethnopharmacological approach and with further positive translational studies plus animal studies, skullcap could be a promising therapeutic anticancer alternative for these types of patients.

8.6.2 Prospective and Challenges

According to Newman and Cragg (2007), about 30% of all new chemical compounds discovered in the last 20 years are derived from natural products and a further 20% are derivatives of these natural products. Additionally, over 60% of drugs approved for cancer treatment were derived from natural products (Newman et al. 2003). Since the approval of the first botanical drug in 2006, the Food and Drug Administration in USA has received over 350 botanical investigational new drug applications and the aqueous extract of skullcap, BZL101 was one of the earliest botanical investigational new drugs issued (Perez et al. 2010). With the present understanding of skullcap's *in vitro* anticancer mechanisms (Tables 8.1 and 8.2) and the success of second Phase I clinical trial revealed that BZL101, a Phase II clinical trial for women with MBC is planned (Perez et al. 2010). Further positive clinical trial results would certainly facilitate the bringing of skullcap from bench to clinical use for breast cancer patients.

The Food and Drug Administration in USA defines dose by the total mass of the extract skullcap, BZL101, and not by the cumulative mass of the active compounds, and the aqueous extract of skullcap (BZL101 is the one of the well studied example) is the most effective type of extract. The challenge for future studies would be defining the relationship of response of pharmacokinetic profiles with the known active chemical components of skullcap. The drug development pathway of BZL101 is different from traditional pharmacognosy (identifying a single active chemical with significant enhanced activity per extract mass) in that the biological response appears to be dependent on simultaneous cytotoxic activity by a group of compounds rather than by just one (Perez et al. 2010). Since the mechanism for the biological response of skullcap has been studied and potential clinical response of BZL101 has been observed, studies on biomarkers for responses, or for patient selection, can be carried out to potentially replace traditional pharmacological analyses aiding clinicians to better understand the therapeutic index of BZL101. These would aid in the similar study and development of other aqueous extracts of skullcap.

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