Anti-Cancer Properties of Anthraquinones from Rhubarb

Qing Huang,[1] Guodong Lu,[1,2] Han-Ming Shen,[1] Maxey C.M. Chung,[2] Choon Nam Ong[1]

[1]Department of Community, Occupational and Family Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore
[2]Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore

Published online 4 October 2006 in Wiley InterScience (www.interscience.wiley.com).
DOI 10.1002/med.20094

Abstract: Rhubarb has been used as a traditional Chinese medicine since ancient times and today it is still present in various herbal preparations. In this review the toxicological and anti-neoplastic potentials of the main anthraquinones from Rhubarb, *Rheum* palmatum, will be highlighted. It is interesting to note that although the chemical structures of various anthraquinones in this plant are similar, their bioactivities are rather different. The most abundant anthraquinone of rhubarb, emodin, was capable of inhibiting cellular proliferation, induction of apoptosis, and prevention of metastasis. These capabilities are reported to act through tyrosine kinases, phosphoinositol 3-kinase (PI3K), protein kinase C (PKC), NF-kappa B (NF-κB), and mitogen-activated protein kinase (MAPK) signaling cascades. Aloe-emodin is another major component in rhubarb found to have anti-tumor properties. Its anti-proliferative property has been demonstrated to be through the p53 and its downstream p21 pathway. Our recent proteomic study also suggests that the molecular targets of these two anthraquinones are different. However, both components were found to be able to potentiate the anti-proliferation of various chemotherapeutic agents. Rhein is the other major rhubarb anthraquinone, although less well studied. This compound could effectively inhibit the uptake of glucose in tumor cells, caused changes in membrane-associated functions and led to cell death. Interestingly, all three major rhubarb anthraquinones were reported to have *in vitro* phototoxic. This re-evaluation of an old remedy suggests that several bioactive anthraquinones of rhubarb possess promising anti-cancer properties and could have a broad therapeutic potential.

Key words: anthraquinones; *Rheum* palmatum; toxicity; mechanisms; traditional medicine

Contract grant sponsor: NIH-NCCAM; *Contract grant number:* R21-AT-1945; *Contract grant sponsor:* Centre for Environmental and Occupational Health Research, National University of Singapore.
Correspondence to: Prof. Choon Nam Ong, Department of Community, Occupational and Family Medicine, Yong Loo Lin School of Medicine, National University of Singapore, 16 Medical Drive, Singapore 117597, Singapore. E-mail: cofongcn@nus.edu.sg

1. INTRODUCTION

Rhubarb root (Da Huang) is one of the oldest and best-known Chinese herbal medicines, first appearing in the Classic of the Materia Medica, Shen Nong Ben Cao Jing[1] of the Han dynasty, and was classified as a top medicinal plant.[2] The most commonly used species is *Rheum* palmatum or *Rheum* officinale Baill of the *Polygonaceae* family. The traditional use is as a laxative, treatment of constipation jaundice, gastro-intestinal hemorrhage, and ulcers. Currently, many of the Chinese herbal preparations also contain Da Huang, however its toxicity, pharmaceutical potentials and molecular mechanisms have not been well investigated. This review attempts to provide an up-to-date collection on data related to the toxicological and anti-cancer potential of various major anthraquinones of rhubarb, *Rheum* palmatum, with emphasis on the molecular mechanisms of action that have been revealed during the past two decades.

2. STRUCTURAL AND TOXICOLOGICAL PROPERTIES OF RHUBARB ANTHRAQUINONES

The main bioactive constituents of rhubarb are anthraquinone derivatives, including emodin (1, 3, 8-trihydroxy-6-methylanthraquinone, ~2.6%), aloe-emodin (1, 8-dihydroxy-3-hydroxyl-methyl anthraquinone, ~1.8%), rhein (1, 8-dihydroxy-3-carboxyanthraquinone, ~1.9%), chrysophanol (1, 8-dihydroxy-3-methyl-anthraquinone 1.9%), physcion (1, 8-drihydroxy-3-methyl-6-methoxyanthraquinone, ~0.8%), and Danthron (1, 8-dihydroxy-9, 10-anthraquinone, $<0.2\%$) (Fig. 1).[3] Several glycosides such as stilbene, naphthalene, and chromones also can be detected in *R.* palmatum, together with small amounts of tannins. Catechins, gallic acid, and cinnamic acid are also present in rhubarb.

Most of the water-soluble components of rhubarb are readily absorbed after ingestion. This medicinal plant is generally considered low in toxicity, but intoxication could result from over dosage, especially of the fresh herb. Toxic symptoms include nausea, vomiting, dizziness, abdominal colic, and jaundice. Although no recent study has been conducted on the long-term effects of anthraquinones present in rhubarb, an earlier study suggested that they could lead to liver cirrhosis and hypokalemia.[4] It has been recommended that persons with a history of renal stones should avoid rhubarb due to its high-oxlate content.[5] Kemper et al.[6] suggested that the high-tannin content in

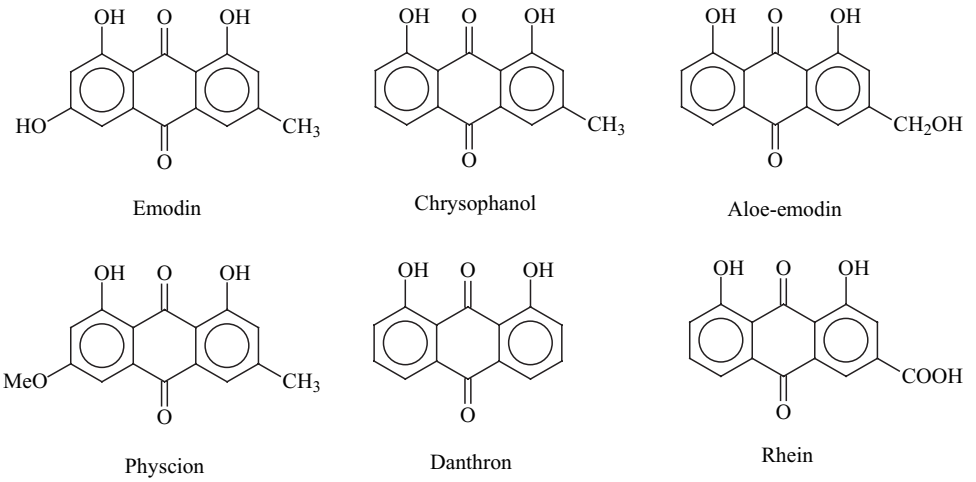

Figure 1. Chemical structures of major anthraquinones in Rhubarb, *Rheum* palmatum.

rhubarb may cause upset stomach, hepatic necrosis, and increased risk of esophageal and nasal cancer.

The debates over carcinogenicity of anthraquinones in anthranoid laxatives have been going on for decades.[7,8] Due to their planar chemical structure, several anthraquinones were postulated to be able to intercalate into DNA.[9] It was hypothesized that chronic abuse of anthranoid-containing laxatives may act as a risk factor for colorectal cancer.[7] Mueller et al.[10] suggested that certain anthraquinones from *Rheum* family and edible vegetables were genotoxic, and emodin and danthron were more potent compared to chrysophanol and physcion. Further studies conducted in mouse lymphoma L5178Y cells suggested that non-covalent DNA-binding[11] and indirect inhibition of topoisomerase II catalytic activity[12] could have contributed to the anthraquinones-induced genotoxicity. In addition, danthron, rhein, and chrysophanol, three anthraquinones with hydroxyl groups in the 1,8-positions (Fig. 1) were found to promote transformation of C3H/M2 mouse fibroblasts initiated by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine or 3-methylcholanthrene.[13] Among all anthraquinones, *in vivo* carcinogenicity was only established with danthron.[14] Since danthron was the main component of many anthranoid laxatives this *in vivo* finding could have led to the withdrawal of the synthetic danthron laxative from the market.[15] However, it was believed that if the concentrations of anthraquinones, estimated daily intake and the genotoxic potency, as well as protective effects of the food matrix were taken into consideration together with a balanced human diet, they do not represent a genotoxic risk.[10] Recent toxicology and carcinogenesis investigations carried out by the National Toxicology Program (NTP)[16] reported that emodin showed no evidence of carcinogenic activity for emodin in male F344/N rats and female B6C3F mice and equivocal evidence of carcinogenic activity in female 344/N rats and male B6C3F mice, however, the reason for which is not clear. As for aloe-emodin, *in vivo* study suggested that this anthraquinone exhibits no effect on inducing DNA-damage in hepatocytes of male Wistar rats and showed no mutagenic activities in bone marrow cells of NMRI mice and Wistar rats.[17] To our knowledge, no *in vivo* report has been found regarding the carcinogenicity of rhein, chrysophanol and physcion, and rhubarb extract. So far, no casual relationship has been demonstrated between rhubarb abuse and colorectal cancer[18] or gastric cancer.[19] Interestingly, *in vitro* studies suggest that emodin, aloe-emodin, and rhein were all found to be phototoxic, most probably through the involvement of singlet oxygen and stable photoproducts.[20] Emodin has been reported to photosensitize human leukemic cells.[21] Wamer et al. found that its phototoxicity was due to the generation of single oxygen upon irritation of UV[22,23] or visible light.[23] Topical application of aloe-emodin on skin of C3H mice enhanced the formation of UV radiation-induced development of melanin-containing skin tumors.[24] Another study conducted on C3H/HeN mice showed that aloe-emodin combined with UV radiation can induce a broader distribution of mutations in *p53* gene.[25] However, till now, no epidemiological or case report could be found linking usage of aloe-emodin or rhubarb with skin cancer.

It is also of concern that ingestion of emodin by pregnant women might have adverse effects. NTP[16] thus in 2002 conducted a study on the maternal toxicity and teratogenicity in rats and the results suggested a lowest observed adverse effect level (LOAEL) of emodin was 1,700 ppm, based on reduction of maternal body weight, reduction of fetal body weight. In a more recent study, Jahnke and colleagues[26] evaluated the developmental toxicity of emodin in rats and mice. Their results suggest that the prenatal mortality, live litter size, fetal sex ratio, and morphological development were unaffected in both rats and mice. At concentration of 6,000 ppm the average fetal body weight per litter was significantly reduced in mice. The LOAEL for maternal and developmental toxicity of rats were similar to the finding of NTP study, at a concentration of 1,700 ppm. The LOAEL for developmental toxicity of emodin in mice was 6,000 ppm. The no observed adverse effect level (NOAEL) was 850 ppm in rats and 2,500 ppm for mice. So far, no study has been carried out on the reproductive capability or teratogenicity of other anthraquinones in rhubarb.

It is interesting to note that although numerous investigations have been conducted on various anthraquinones in rhubarb, and despite their structural similarity, the overall findings do not suggest

that there is a straightforward direct relationship between the chemical structure and cytotoxicity or genotoxicity. Nevertheless, Lu et al. recently used emodin as a parent compound to synthesis a series of different anthrapyrazole derivatives, and their results suggest that those anthraquinone chromophore with positive charged side chains are generally more cytotoxic.[27] Compared to emodin, they had significantly higher DNA-binding affinity and showed higher cytotoxicity against different tumor cells. The derivatives with a mono-cationic alkyl side chain exhibited the highest DNA-binding affinity and cytotoxicity.[28]

3. ANTI-CANCER ACTIVITIES OF RHUBARB ANTHRAQUINONES

So far, literature search suggests that the effect of rhubarb on anti-microbial capability, renal protection and gastrointestinal disorder prevention are most convincing although no comprehensive review has been conducted. Owing to space limitation, this review will focus mainly on the anti-cancer properties of rhubarb and highlight some of the recent studies on the anti-neoplastic effect of the more abundant anthraquinones of Rhubarb, in particular the mechanisms involved.

A. In Vitro Evidence

1. Inhibition of Cell Growth

A number of studies have demonstrated that the main anthraquinones of rhubarb, emodin, aloe-emodin, and rhein, inhibit the growth and proliferation of various cancer cells. Emodin has been reported to inhibit proliferation in breast, lung, cervical, colorectal, and prostate cancers cells.[29–33] After comparison with other anthraquinone derivatives, such as emodin 1-O-β-D-glucoside, physcion, and physcion 1-O-β-D-glucoside, C1 and C3 position of emodin is believed to be important for the anti-tumor function.[32] However, emodin can evoke a less or no cytotoxic effect in several normal cells, including HBL-100 cells derived from normal human breast tissue,[30] human fibroblast-like lung WI-38 cells, and three primary cultured rat normal cells.[34] It has also been demonstrated that emodin displays over 25-fold differential cytotoxicity against ras-transformed bronchial epithelial cells to the normal human bronchial epithelial cells.[33] These data suggest that normal cells are more resistant to emodin-induced cytotoxicity than cancer cells. Such specificity of emodin towards malignant cells might be due to its effect targeting on some oncogene signaling transductions, which are constitutively active or amplified in cancer cells.

Aloe-emodin was also able to inhibit cell growth in several tumor cells, including human lung carcinoma,[35] hepatoma,[36,37] and leukemia cell lines.[38] In addition, aloe-emodin exhibits higher cytotoxicity against oral squamous cell carcinoma and salivary gland tumor than against normal human gingival fibroblasts (HGF).[39] Interestingly, aloe-emodin showed a high specificity for neuroectodermal tumor cells.[40,41] According to Pecere and his/her colleagues' report,[40] energy-dependent drug incorporation of aloe-emodin may correspond to the greater sensitivity of neuroectodermal tumor cells.

Rhein, another anthraquinone derivative of rhubarb, has also been reported to display inhibitory effect on the proliferation of human breast, colon, lung, CNS, and glioma cancer cells.[42,43] Recent study by Zhou et al. suggests that rhein exhibited anti-fungal potential but was low in cytotoxicity, compared to other anthraquinones in rhubarb.[44] Taken into consideration rhein concentration is about the same as aloe-emodin in rhubarb (approximately 1.9%) additional studies will be useful to address some of its biological properties.

On the other hand, although sharing very similar chemical structure with emodin and aloe-emodin, chrysophanol, and physcion show no significant effect on the inhibition of cancer cell proliferation.[32,45]

2. Disruption of Cell Cycle

Cells contain various pathways designed to protect them from the genomic instability or toxicity that can result when their DNA is damaged. A pivotal role in this response is played by checkpoint proteins that control the normal passage of cells through the cell cycle. During tumorigenesis, tumor cells frequently loose checkpoint controls and this facilitates the development of the tumor.[46] Thus, one of the important approaches for cancer chemotherapy is to regulate cell-cycle progression.

Effect of the main rhubarb anthraquinones, emodin,[33,34] and aloe-emodin[37,38,41] on G_2/M cell cycle have been demonstrated on various cancer cells, including v-ras-transformed cells,[33] hepatoma,[34,37] leukemia,[38] and neuroectodermal cells.[41] Elevation of p53 and p21 expression were suggested to be a common mechanism involved in these induced G_2/M cell-cycle arrest.[34,37,41,47] However, few reports were found for rhein and other anthraquinones. Similarly, G_1/S cell-cycle arrest was also found in human hepatoma,[37,47] glioma,[48] breast,[30] lung,[36] and colon[49] carcinoma cells upon treatment of rhubarb anthraquinones (emodin,[30,49] aloe-emodin,[36,37,48] and rhein[47]). Our recent proteomic study found that p16-Rb-E2F pathway, which is responsible for G_1/S arrest was affected in aloe-emodin but not in emodin-treated HepG2 cells (Lu et al. unpublished data). This may be one of the mechanisms that reflect that aloe-emodin is more potent in inhibiting hepatoma cell growth than emodin.

3. Induction of Apoptosis

The process of apoptosis is fundamental in the developmental and homeostatic maintenance of complex biological systems. Dysregulation or failure of normal apoptotic mechanisms will contribute to transformation of cells and provide a growth advantage to cancer cells. It is characterized by cell shrinkage, chromatin condensation, DNA fragmentation, and the activation of specific cysteine proteases known as caspases. Two pathways converge on caspase-3: one involving caspase-8 and the other involving mitochondrial release of cytochrome c and activation of caspase-9.[50]

Apoptosis could be a potential general mechanism providing a mechanistic basis for the anti-proliferative and anti-neoplastic effects of emodin. As shown in Table I, a number of studies have demonstrated that emodin is capable of inducing apoptotic cell death in various cancer cells. Several studies indicate that emodin-induced apoptosis is mediated by ROS generated from the semiquinone,[51,52] although there was report that emodin-induced apoptosis is independent of ROS.[45] As we know, quinones are highly redox active molecules, which can form a redox cycle with their semiquinone radicals, leading to the formation of reactive oxygen species that include superoxide anion radical, hydrogen peroxide, and hydroxyl radical.[53–55] The quinoid structure of emodin (Fig. 1) could be activated to the semiquinone radical intermediate, which in turn could react with oxygen to produce ROS. The generation of ROS may contribute to mitochondrial damage, reduction of mitochondrial transmembrane potential, release of cytochrome c and Smac, and subsequent caspase activation and apoptosis.[56]

Similar to emodin, apoptosis can be induced by aloe-emodin in various tumor cells. Pecere and his colleagues[40] reported that aloe-emodin can induce sub-G_1 peak (one of the biomarkers of apoptosis) at 48 hr following G_2/M arrest at 24 hr in neuroectodermal tumor cells. Aloe-emodin was also found to induce apoptosis in many other tumor cells derived from hepatoma, lung, bladder carcinoma, and leukemia. The treatment dose and outcome are summarized in Table II. Similar to emodin, aloe-emodin can induce DNA damage through oxidative stress, and later initiate apoptosis.[57] In addition, aloe-emodin-induced apoptosis in HepG2 cells was found to be ROS-dependent (Lu et al., unpublished data).

Rhein has been shown to inhibit the uptake of glucose uptake in Ehrlich ascites tumor cells. This inhibition has been postulated to be an interaction of rhein with cell membranes that results in an alteration of membrane-associated functions.[58] Further study suggests that this anthraquinone

Table I. Induction of Apoptosis by Emodin, Aloe-Emodin, and Rhein in Human Cancer Cells

Anthra -quinones	Cell types	Treatments	Outcomes	Ref.
emodin	human lung adenocarcinoma cells A549	(10-50μM) up to 72 hrs	ROS generation, inactivation of ERK and Akt, decrease of Bcl-2, increase of Bax, disruption of mitochondrial membrane potential, apoptosis	51
emodin	Human myelogenous leukemia cells HL-60	(10-80μM) up to 12 hrs	Decrease of Mcl-1, no change of Bcl-2, Bcl-XL, Bax and Bad, ROS independence, apoptosis	45
emodin	Human lung squamous carcinoma cells CH27; human lung non-small carcinoma cells H460	(1-50μM) up to 72 hrs	Increase and translocation of Bak and Bax, no change of Bcl-XL, activation of caspase-3, 9, 8, decrease of PKC δ and ε, apoptosis	118,135
emodin	Human cervical carcinoma cells HeLa	As_2O_3 combined with (0.5-10μM) emodin up to 72 hrs	Generation of ROS, inhibition of NF-κB and AP-1, sensitization of HeLa cells to As_2O_3-induced apoptosis	87
Aloe -emodin	Neuroblastoma cells (IMR-32, IMR-5, AF8, SJ-N-KP and SK-N-BE (2c))	~10μM, 30¯M	Cytotoixc effect in vitro and in vivo; Induction of apoptosis, Increase of p53, Bax and Bcl-2. Induction of apoptosis	40,41
Aloe -emodin	Human hepatocellular carcinoma cell HepG2 and Hep3B	37 and 74μM up to 48hr	Activation of p53 (only in HepG2 cells) and p21. Increase of FAS receptor expression (only in HepG2 cells). Increase of BAX expression. Induction of apoptosis.	37
Aloe -emodin	Human transformed glial cells U-373MG and SVG	40 μM up to 72hr	Delay in entry and exit S phase. Decrease of PKC activity. Induction of apoptosis. Decrease of survivin.	48
Aloe -emodin	Human bladder cancer cell T24		Activation of p53 and p21, ctivation of caspase-3,. increase Fas/APO1 receptor and Bax expression, but inhibit Bcl-2 expression	28
rhein	Human hepatoma cells **HepG2**	(0-400μM) up to 72 hrs	P53 increase, enhancement of CD95 and CD95 ligands, apoptosis	47
rhein	Human myelogenous leukemia cells **HL-60**	(0-100μM) up to 8 hrs	Loss of mitochondrial membrane potential, cytochrome c release, Bid cleavage, ROS independence, apoptosis	53

induced enhancement in CD95 and its ligands, and subsequent apoptotic effect in HepG2 cells.[47] In addition, this anthraquinone induces apoptosis in HL-60 cells through the mitochondrial death pathway by causing the loss of mitochondrial membrane potential, cytochrome c release, and cleavage of *Bid* protein.[53] Earlier study has suggested that rhein is able to accumulate within the mitochondria and affect mitochondrial membrane permeability.[59] It is believed that rhein is a suitable substrate for one-electron-reducing enzymes and an effective redox cycler, thus leading to the production of ROS and induction of apoptosis.[54] Since mitochondrial functions are critical for cell survival, when the mitochondrial membrane potential is lost, ATP production ceases and the cell dies.

The Bcl-2 family protein contains anti-apoptotic (Bcl-2, Bcl-XL) and proapoptotic (Bax, Bak) proteins and they are well-characterized regulators of apoptosis. To further explore the mitochondria-related pathway involved in anthraquinone-induced apoptosis, several groups examined the role of Bcl-2 family members. Emodin treatment significantly increases the expression of proapoptotic proteins, Bax and Bak, and causes Bax mitochondrial translocation and subsequent apoptosis.[52] In

Table II. The Anti-Cancer Properties of Emodin and Aloe-Emodin Using *In Vivo* Animal Models

Animal Models	Treatments	Outcomes	Ref.
PC3-AR xenografts in nude mice	40mg/kg/d emodin, i.p.	Reduce the volume of tumors	31
C3(1)/SV40 transgenic mice	40mg/kg/d emodin, i.p.	Prolong survival, maintain body weight gain, lower incidence of tumor invasion	
EC/CUHK1 xenografts in nude mice	30mg/kg/d emodin, i.p.	Decrease of tumor weight	85
	20-30mg/kg/d emodin combined with arsenic, i.p.	Decrease of tumor size and weight	
Two-stage carcinogenesis test of mouse skin tumors	0.0025% emodin in drinking water	Decrease of papilloma numbers	99
Oxygen-induced retinopathy mouse model	15-30mg/kg/d emodin, i.p.	Decrease of retinal neovascularization	74
Glioma xenograft in nude mice	50 mg/kg/d emodin, oral gavage	Decrease of MMP secretion	70
MDA-MB-361 xenografts in nude mice	40mg/kg/d emodin alone or combined with taxol, i.p.	Inhibit tumor growth, prolong survival	98
IMR5 neuroblastoma xenograft in SCID mice	50mg/kg/d aloe-emodin, i.p.	Decrease of tumor size	40
Matrigel plug mouse model (*in vivo* angiogenesis model)	Matrigel containing VEGF-A (100 ng) with or without emodin (50 μM) was injected into C57BL/6 mice	less blood vessel formation, reduced hemoglobin content	76

addition, anti-apoptotic protein Bcl-2 was also involved in emodin-induced apoptosis: first, emodin causes a significant decrease in Bcl-2 expression; second, ectopic expression of Bcl-2 markedly blocks emodin-induced apoptosis.[51] In addition to mitochondria, PKC-δ and ε may also be involved in emodin-induced apoptosis. It appears that PKC is affected after the activation of caspase-3 in the emodin-induced apoptosis.[55] Meanwhile, as for aloe-emodin, anti-apoptotic Bcl-2 members (Bcl-2, Bcl-XL) have been found to be downregulated upon its treatment on CH27[35] and H460 cells.[36] In contrast, proapoptotic members (Bax and Bak) were found to be upregulated in hepatocellular carcinoma cells.[37] Translocation of Bax and Bak from cytosol to mitochondria is an important event to initiate mitochondria-mediated apoptosis. CH27 cell exhibited such translocation of Bax and Bak upon treatment of aloe-emodin.[35]

These results suggest that anthraquinones (emodin, aloe-emodin, and rhein) can activate apoptotic cell death in different tumor cells and the mitochondrial-dependent pathway was suggested to be the main apoptotic process.

No apoptosis reports of danthron, physcion, and chrysophanol were found so far.

4. Anti-Metastasis

The high mortality rates associated with cancer are caused by the metastatic spread of tumor cells from the original site. There are at least four inter-related biological events required for tumor

metastasis: angiogenesis, cell adhesion, cell invasion (extracellular matrix degradation and cell migration), and cell proliferation. One critical aspect of the anti-cancer activity of emodin is its inhibitory effect on cancer metastasis. Emodin potentially interferes with tumor metastasis progression at several pivotal points.

First, emodin inhibits TNF-α-induced expression of cell surface adhesion proteins (ICAM-1, VCAM-1, ELAM-1), which is essential in cell adhesion, migration, and inflammation.[56] This inhibition is mainly due to suppression of emodin on NF-κB activation. Another interesting finding of our group is that emodin exhibits significant inhibition on the adhesion of various cancer cells. This inhibition was achieved through reducing cholesterol content in the membrane lipid rafts, preventing rafts clustering and subsequent colocalization of integrin β1 and focal adhesion complex proteins, and eventually disrupting the lipid rafts-associated integrin-signaling pathway.[60] Moreover, recent studies suggest that the characteristics of membrane lipids of cancer and non-cancer cells are rather different. For example, relative higher content of cholesterol and saturated fatty acids, including sphingolipids, was found in cancer cells.[61,62] Moreover, in cancer cells the structure and function of lipid rafts may be modified in such a way that it would enhance cancer cell survival.[63] Thus, in this case, due to its disruptive effect on cholesterol in the lipid rafts, emodin might have a stronger inhibitory effect on cancer cells than normal cells, although further investigations are needed to confirm this.

Second, emodin inhibits EGF-induced cell migration in various cancer cells.[64] In the search for the underlying mechanisms, PI3K was found to be the molecular target for emodin. Emodin significantly suppressed the PI3K-mediated Cdc42 and Rac1 activation and subsequent cytoskeletal changes, including filopodia and lamellipodia formation. Moreover, emodin could block cell migration in cells transfected with constitutively active (CA)-Cdc42 and CA-Rac1 by interference with the formation of Cdc42/Rac1 and the p21-activated kinase (PAK) complex. These data suggest that emodin inhibits cancer cell migration via suppressing the PI3K-Cdc42/Rac1-PAK signaling pathway.[64] In addition, as a $p56^{lck}$ inhibitor, emodin decreases urokinase-type plasminogen activator and cell motility induced by hypoxia/reoxygenation (H/R).[65,66]

Third, emodin inhibits the invasiveness of human cancer cells by suppressing MMP-9 expression[67–69] through inhibiting AP-1 and NF-κB signaling pathways.[67,70] It has been shown that emodin first suppresses the phosphorylation of IκBα and NF-κB activation and then followed by decreasing the phosphorylation of ERK1/2 and JNK, and subsequently AP-1 activation. Through these pathways, emodin decreases the MMP-9 secretion and cell invasion in 12-o-tetradecanoyl phorbol-13-acetate (TPA)-treated cells. On the other hand, overexpression of Her-2/neu oncogene could enhance tumor metastatic potential, including the invasive ability to penetrate the basement membrane.[71] Thus, as a Her-2/neu tyrosine kinase inhibitor, emodin has been shown to suppress the metastasis-associated properties in Her-2/neu transformed NIH3T3 cells.[72] Recently, emodin was reported to suppress the expression of syndecan-1 through inhibiting NF-κB activation in malignant glioma cells, thus capable of blocking the invasion of tumor cells into the surrounding normal brain tissues.[73]

Fourth, mainly due to its inhibition on casein kinase II (CKII), emodin shows significant protective effect from angiogenesis and retinal neovascularization.[74] It stabilizes retinal endothelial cell tubes on basement membrane matrix and inhibited secondary sprouting, cell migration, and proliferation. In addition, emodin also causes G_2/M arrest in bovine aortic vessel endothelial cells[75] and G_1 arrest in human umbilical vein endothelial cells,[76] which contributes its dramatic inhibitory effect on angiogenesis. More recently, emodin was found to inhibit the basal secretion of MMP-2 and VEGF-A-stimulated urokinase-type plasminogen activator receptor expression, which is critical during angiogenesis process.[76] Inhibition of phosphorylation of receptor 2 (KDR/Flk-1) and downstream effector molecules, including FAK, ERK1/2, p38, Akt, and endothelial nitric oxide synthase, was found to be a possible underlying mechanism of the anti-angiogenic activity of emodin toward VEGF-A-induced angiogenesis *in vitro* and *in vivo*.[76]

All these findings strongly suggest that emodin interferes with the progression of cancer metastasis through inhibiting cell adhesion, migration, invasion, and angiogenesis (Fig. 2). Such inhibition is through different, and at least partially, crosslinked mechanisms, such as NF-κB, AP-1/MAPK signaling, and PI3K. Clarification of these emodin-targeted signaling transductions might contribute to the studies of developing emodin into a new potential anti-tumor drug.

Although no studies have been conducted on the anti-metastatic potential of rhein, some related reports imply that rhein might have certain inhibiting effects in the cascade of tumor metastasis. For example, rhein suppressed the IL-1α-induced production of pro-MMPs-1, -3, -9, and -13 but increased tissue inhibitor of metalloproteinase-1 (TIMP-1) synthesis in rabbit chondrocytes.[77] Similarly, rhein led to a strong decrease of the MMP-3/TIMP-1 ratio in human chondrocytes.[78] These observations suggest that rhein might also have a suppressive effect on ECM degradation via its inhibition on the production and activity of pro-MMPs. Such suppressive effect might be a consequence of rhein's inhibition on several critical signaling pathways, such as MAPKs, AP-1,[79,80] and NF-κB.[81] Further investigations are needed to clarify this hypothesis.

Similarly, although no related work has been carried out to clarify the possible effect of aloe-emodin on cancer metastasis, its inhibition of PKC isozymes,[48] p38,[36] and ERKs,[82] which play critical role in the development of cancer metastasis, lead to the hypothesis that aloe-emodin might interfere with metastasis process as well. And the recent proteomic study suggest that upregulation of nucleoside diphosphate kinase A (a metastasis suppressor) may potentiate aloe-emodin with anti-metastasis activities (Lu et al., submitted). Detailed studies are needed to determine whether

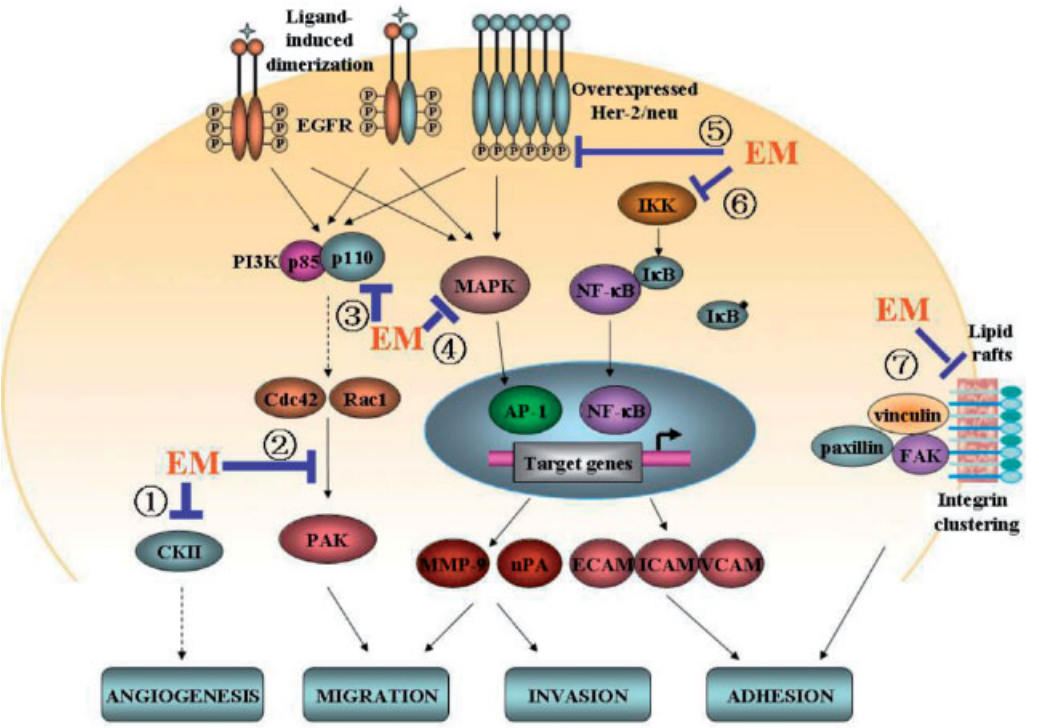

Figure 2. Mechanisms involved in anti-metastatic effect of emodin (EM). EM exhibits anti-metastatic effect mainly via the following signaling transduction: ①, inhibition of angiogenesis through specific suppression of CKII; ②③, suppression of cell migration via inhibiting PI3K-Cdc42/Rac1-PAK pathway; ④⑤, prevention of cell invasion through suppressing Her-2/neu kinase activity and MAPK signaling pathway; ⑥⑦, inhibition of cell adhesion via suppressing the NF-kappa;B-mediated protein expression of adhesion molecules, as well as disrupting the lipid raft-associated integrin-signaling pathway.

aloe-emodin could be a candidate in developing potential chemotherapeutic agents against cancer metastasis.

So far, no anti-metastatic studies of danthron, chrysophanol, and physicon have been carried out.

5. Sensitization Activity

Chemoresistance is of outstanding importance for the limited results of chemotherapy in various tumors. Different combinations of chemotherapeutic drugs may offer great potential for improving anti-tumor responses in various carcinomas.

As a Her-2/neu tyrosine kinase inhibitor, emodin suppresses the proliferation of Her-2/neu-overexpressing lung cancer cells and sensitizes these cells to chemotherapeutic drugs, such as cisplatin, doxorubicin, and VP16.[83] Emodin acts in synergy with celecoxib to suppress the growth of rat cholangiocarcinoma, which arises from the epithelial lining cells of intra- and extra-hepatic bile ducts, in a cyclooxygenase-2 independent manner.[84] This combination treatment decreases the phosphorylation level of Akt, leading to increased caspase-9 and -3 mediated apoptosis. In addition, emodin has been reported to sensitize different tumor cells to arsenic trioxide, including EC/CUHK1 (human esophageal carcinoma cell), HeLa, U937, and NB4 (human myeloid leukemic cell).[85–87] It is worthy mentioning that non-tumor human fibroblasts are not responsive to the combination treatment of emodin and arsenic trioxide.[85] Another report revealed that emodin, as a CKII inhibitor, sensitizes cancer cells to TRAIL-induced apoptosis through suppressing CKII activity and subsequent NF-κB-mediated expression of Bcl-xL and c-FLIP.[88]

Aloe-emodin has also been reported to potentiate the inhibitory effect of some common chemotherapeutic agents (cisplatin, doxorubicin, and 5-fluoroucil) in Merkel carcinoma cells.[89] However, the mechanism of the synergistic effect of aloe-emodin is less studied. Furthermore, aloe-emodin can sensitize programmed cell death in Merkel cells when co-treated with tyrosine kinase inhibitor ST1 571.[89] Emodin is commonly used as tyrosine kinase inhibitor and coexisted with aloe-emodin in Rhubarb, but no further studies ever been conducted on whether emodin can sensitize aloe-emodin-induced cell death. In addition, similar to emodin, aloe-emodin has also been reported to sensitize tumor cells to arsenic.[85]

Meanwhile, rhein shows synergistic inhibitory effect with mitomycin C,[90] although no detailed mechanism was investigated.

The underlying mechanisms involved in these sensitization effects might be: (a) inhibition of Her-2/neu or CKII kinase; (b) generation of ROS; (c) suppression of NF-κB, and AP-1 activation. Since emodin and other anthraquinones have similar structures to that of mitochondrial ubiquinone, many reports demonstrated the effect of emodin as a free radical generator.[51,87,91] Thus, ROS generation by emodin has been suggested to play an important role in its synergistic effect with other chemotherapeutic agents. Yang et al.[85] noted that the increased ROS induced by emodin/aloe-emodin and arsenic combination treatment mediates simultaneously dual regulation, both the enhancement of pro-apoptosis and the inhibition of anti-apoptosis. The enhancement of pro-apoptosis includes the collapse of the mitochondrial transmembrane potential, the release of cytochrome c, and the activation of caspases 9 and 3. The inhibition of anti-apoptosis includes suppressing AP-1[87] and NF-κB activation, and expression of survivin, NF-κB-specific anti-apoptosis protein.[85] The antioxidant, *N*-acetyl-L-cysteine, attenuates this sensitization effect, whereas dicoumarol, an inhibitor of NADPH dyhydrogenase, enhances the synergistic effect of emodin and arsenic trioxide.[92] After examining the cDNA microarray-based global transcription profiling, the same group of workers revealed that the increased ROS level generated by emodin contributes to the significant expression alteration of genes, which is important in relative signal transduction, organelle functions, cell-cycle checkpoint, and cytoskeleton. These changes finally sensitize the cancer cells to arsenic cytotoxicity.[93] Such observations further demonstrate that ROS generation is critical in the process of sensitization effect of emodin/aloe-emodin with chemotherapeutic agents.

On the other hand, emodin failed to show significant chemosensitizing effect to carboplatin, an alkylating anti-neoplastic agent, in primary cells from an ovarian carcinoma overexpressing Her-2/neu and topoisomerase IIα.[94] This might be due to the character of high topoisomerase IIα expression level in this cancer cell line. It is reported that the inhibition of topoisomerase IIα significantly decreased the clonogenic survival of the cells.[95] High topoisomerase IIα might influence chemosensitivity by changing the accessibility of DNA sequences for alkylation and subsequent repair.

Similarly, controversies arise as there are also reports that indicated aloe-emodin can inhibit the anti-proliferation of certain agents. Mijatovic et al.[96] reported that aloe-emodin can prevent interferon-γ and interleukin-1-induced cytoxicity in mouse fibrosarcoma cell (L929) (although aloe-emodin itself can inhibit L929 cell growth) and this effect has been linked to the inhibition of auto-toxic nitric oxide (NO) release. Paradoxically, aloe-emodin cannot protect NO-releasing chemical SIN-1-induced tumor cell death. And only in cytokine-activated cells these effects is observable. On the other hand, the same authors[97] also reported that aloe-emodin (20 μM) can slightly reduce the cytotoxicity activity of cisplatin in murine fibrosacroma L929 and rat glioma C6 cells. The inhibition of ERK kinase was believed to be a protective effect. Since ERK convey both cell death and cell survival signals, aloe-emodin's action on tumor cell may depend on cell type and treatment.

B. In Vivo Evidence

A number of studies have been carried out to test the anti-tumor effect of emodin using *in vivo* animal models. When administered intraperitoneally, emodin was found to reduce tumor weight in the EC/CUHK1 (a cell line derived from esophageal carcinoma)[85] or PC3-AR (an androgen receptor-overexpressing PC3 cell line) xenografts in nude mice.[31] Moreover, C3(1)/SV40 transgenic mice, a strain that will develop androgen receptor-positive prostate cancer in male, was used as a model to further explore emodin's anti-tumor effect *in vivo*.[31] Emodin-treated mice had significant longer survival and better body weight gain than the control group. It is worthy mentioning that emodin not only has low drug toxicity but also maintains the physical activity of transgenic mice by preventing tumor progression.

In addition, in terms of anti-metastasis, emodin is found to be effective in reducing incidence of tumor invasion to the periurethral muscle structure in C3(1)/SV40 transgenic mice, suggesting the preventive effect of emodin on tumor invasion.[31] Consistently, decrease of MMP-9 secretion is observed after emodin treatment in a nude mice glioma xenograft model.[70] Meanwhile, an *in vivo* study using a mouse oxygen-induced retinopathy model shows that emodin markedly decreases the extent of retinal neovascularization.[74] This implies that emodin might reduce tumor growth by adversely affecting tumor neovasculature. In addition, emodin was reported to suppress blood vessel formation in mouse Matrigel plug angiogenesis model. Less blood vessel formation and decreased hemoglobin content was observed after emodin treatment.[76] These findings, consistent with *in vitro* evidence, further support the anti-metastatic effect of emodin.

As a tyrosine inhibitor, emodin has been shown to inhibit the growth the HER-2/neu-overexpressing MDA-MB-361 breast cancer cells, and also, sensitize these cells to taxol in a nude mice xenograft model.[98] Since the tyrosine kinase activity of HER-2/neu is required for cell growth and taxol resistance, the synergistic effect of emodin plus taxol on tumor growth in mice might be due to decreasing tyrosine phosphorylation of p185neu. Meanwhile, emodin sensitizes the EC/CUHK1 cell-derived tumors to arsenic trioxide with no apparent systemic toxicity and side effects.[85] The combined treatment of emodin and arsenic trioxide attenuates the antioxidant system of the tumor cells and enhances apoptosis, and this may contribute to its anti-cancer efficacy. Moreover, tumor cells are usually more susceptible to the synergistic effect of emodin and arsenic trioxide, whereas non-tumor counterparts are not responsive. This selectivity might give a good reason for the low side effects and system toxicity *in vivo*.

In addition, emodin exhibits potent inhibitory effect on two-stage carcinogenesis test of mouse skin tumors induced by nitric oxide donor, (±)-(E)-methyl-2-[(E)-hydroxyimino]-5-nitro-6-methoxy-3-hexeneamide as an initiator and TPA as a promoter. Also, emodin shows similar suppressive effect on tumor-promotion induced by 7, 12-dimethylbenz[α]anthracene and TPA.[99] This is consistent with the earlier reports that emodin shows high anti-tumor promoting activity using a short-term *in vitro* assay of the EBV-EA activation induced by TPA.[100,101] Taken together, these studies further support the cancer chemopreventive property of emodin.

In contrast to the extensive *in vivo* studies that have been carried out on emodin, only few studies on the *in vivo* anti-neoplastic effect of aloe-emodin have been reported. Kupchan and his co-workers[102,103] found that aloe-emodin exhibited tumor-inhibitory activity in mice against P-388 lymphocytic leukemia. However, the authors stated that this anti-leukemic activity was observed only when aloe-emodin was administered as a suspension in acetone-Tween 80. In contrast, Pecere et al.[40] reported that treatment of 50 mg/kg/day aloe-emodin significantly reduced neuroectodermal tumors cells (IMR5) growth without acute or chronic toxic effects. Under the same condition, aloe-emodin failed to inhibit the growth of human colon carcinoma cells, LoVo109, suggesting that the anti-neoplastic effect of aloe-emodin was specific to neuroectodermal tumors cells.

4. MOLECULAR MECHANISMS INVOLVED IN THE ANTI-CANCER EFFECTS OF RHUBARB ANTHRAQUINONES

A. NF-κB

NF-κB, one of the main molecular targets of chemopreventive phytochemicals, is a transcription factor involved in multiple cellular processes, including cytokine gene expression, cellular adhesion, apoptosis, and metastasis.[104] Because of the immunosuppressive and anti-inflammatory effects of emodin, researchers became interested in how emodin may regulate NF-κB signaling pathways leading to inflammation. Kumar et al. reported that emodin inhibits NF-κB activation induced by TNF. This inhibition is not due to its chemical modification of NF-κB subunits, but its suppressive effect of degradation of IκB, an inhibitory subunit of NF-κB molecules.[56] A recent report revealed that emodin inhibits the expression of IKK-α and IKK-γ, which is essential in phosphorylating IκBα and subsequently activating NF-κB.[105] NF-κB activation involves the participation of several intermediates, such as protein tyrosine kinases (PTK), reactive oxygen intermediates, and proteases. Also, IKKs, RIP, NIK, TRAF-2 play a critical role in regulating NF-κB activation.[106] Thus, the underlying mechanism on how emodin inhibits the activation of NF-κB is rather limited. Further investigation is still needed to explore the exact role of NF-κB in the bioactivity of emodin.

On the other hand, contradicting results on emodin and NF-κB has also been reported. For example, Chen et al. reported that emodin failed to block NF-κB binding and transcriptional activation associated with decreased p65 proteins in the nucleus induced by lipopolysaccharide (LPS) in macrophage RAW264.7 cells.[107] This discrepancy may be due to different methodology or different cell lines they used.

There are few studies on aloe-emodin's effect on activation of NF-κB. Mijatovica et al.[96] reported that aloe-emodin could not affect INF-γ and IL-1-induced IκBα phosphorylation (key factor for NF-κB activation) at any time point in L929 (murine fibrosarcoma cell) and C6 (rat astrocytoma) cells, However, the possibilities that aloe-emodin might interfere downstream of NF-κB activation cannot be excluded in that paper.

Also, rhein inhibited interleukin-1β-induced degradation of IκBα, thus inhibiting NF-κB activation.[81]

B. Tumor Suppressor Gene p53

Tumor suppressor gene *p53* is an important molecule in the process of apoptosis and cell-cycle arrest. Many tumor cells evade apoptosis and cell-cycle arrest via mutation of p53. In response to stress

stimuli, such as DNA damage, p53 is stabilized, which leads to its nuclear translocation and transactivation of many target genes (e.g., *p21*, *Bax*, *CD95*). In certain cells, activation of p53 leads to apoptosis and activation of another tumor suppressor gene *p21*, which contributes to the arrest of cell in G_1 phase by inhibition of cyclin-cdk complex. Cells with wild-type and deficient/mutant p53 have been used to study the function of p53 in aloe-emodin-induced anti-neoplastic effects. Kuo and his colleagues[37] reported that aloe-emodin can induce apoptosis in both HepG2 (wild-type p53) and Hep3B cells (p53 deficient). In HepG2 cells, induction of G_1/S arrest was accompanied with upregulation of p53 and p21. Although p53-deficient Hep3B was more resistant to aloe-emodin, the p53-independent activation of p21 is believed to be responsible for aloe-emodin-induced apoptosis. On the other hand, Perce[41] reported that neuroblastoma cells, SJ-N-KP (wide-type p53) were sevenfold more sensitive to aloe-emodin than SK-N-BE(2c) cells (deficient in p53 nuclear transcriptional activity). While translocation of p53 to mitochondria by aloe-emodin treatment preceding release of cytochrome c may be responsible for aloe-emodin-induced apoptosis in SK-N-BE(2c) which has mutant p53 transcriptional activity. These two studies suggested that p53 is a key protein governing the cell sensitivity to aloe-emodin. In contrast, cells with deficient or mutated p53, other pathways (e.g., p21 and mitochondria-derived cell death) could also be activated by aloe-emodin to induce apoptosis.

In addition, emodin was shown to induce accumulation of p53 in HepG2/C3A cells with the resultant increase in p21 expression and cell-cycle arrest.[34] Although the underlying mechanism on how emodin increases p53 level is still under investigation, several recent reports reveal that this might be through emodin's inhibitory effect on the COP9 signalosome (CSN) associated kinases CK2 and PKD.[108,109] CSN is a multimeric protein complex associated with CK2 and PKD. p53 can be phosphorylated by the CSN kinases, and subsequently degraded by Ub/26S proteasome system.[110] Emodin might suppress the activity of CSN-associated kinases, recombinant CK2 and PKD, inhibit the CSN-directed phosphorylation and ubiquitin degradation on p53, and finally lead to p53 accumulation.

Similar to emodin and aloe-emodin, rhein significantly increased the expression of p53 and p21 protein, which caused cell-cycle arrest, and the expression of CD95, which might be responsible for the apoptotic effect induced by rhein.[47]

C. Inhibition of Key Kinases in Cancer Development

The proto-oncogene HER-2/neu encodes a 185 kd transmemebrane tyrosine kinase growth factor receptor with homology to epidermal growth factor receptor (EGFR).[111,112] The enhancement of HER-2/neu tyrosine kinase activity increase malignant phenotypes. Therefore, inhibition of the HER-2/neu tyrosine kinase activity may lead to suppression of cell transformation and tumor growth. Emodin preferentially suppressed autophosphorylation and transphosphorylation activities of HER-2/neu tyrosine kinase, which leads to a decrease of p185neu phosphorylation in HER-2/neu-overexpressing breast cancer cells,[30,83] although emodin can also suppress EGF-induced tyrosine phosphorylation of EGFR at high concentrations.[98] Moreover, emodin induces a significant differentiation change in MDA-MB-453 and AU-565 human breast cancer cells, which express high level of $p185^{neu}$. On the other hand, no significant change could be observed in the MCF-7 cells, which expresses basal levels of $p185^{neu}$. Zhang and her coworkers further demonstrated that $p185^{neu}$ tyrosine kinase activity is important for the chemoresistant phenotype of HER-2/neu-overexpressing cancer cells. Emodin could sensitize these cells to chemotherapeutic drugs, such as cisplatin, doxorubicin, etoposide, or paclitaxel *in vitro*[83] and *in vivo*.[72] After examining the relationship between the chemical structure and the activity of emodin and nine anthraquinone derivatives, they identified that one methyl, one hydroxy, and one carbonyl groups are important for biological effect of emodin.[72] At the same time, using mutation-activated HER-2/neu transformed 3T3 cells, they found that emodin could suppress transformation phenotypes, including anchorage-dependent and

-independent growth, and metastasis-associated properties.[72] However, as far as literature is available no studies have been conducted on aloe-emodin and rhein on HER-2 oncogene.

Protein kinase CKII, probably the most pleiotropic member of the protein kinase family, plays important roles in the process of cell proliferation, transformation, and differentiation.[113,114] Unlike most of the Ser/Thr protein kinases whose substrates contain consensus sequences generally determined by basic and prolyl residues, substrates of CKII share unique phosphoacceptor sites specified by clusters of acidic residues.[113,114] Emodin was first reported to be a specific inhibitor of CKII by Yim et al.,[115] who were originally interested in emodin's inhibitory effect on cdc2, a Ser/Thr protein kinase essential in the process of cell cycle. They unexpectedly found that emodin acts as a competitive inhibitor of CKII with respect to ATP, with an IC50 value of 2 μM. A follow-up structure study of the complex between emodin and CKII crystals revealed that emodin could penetrate into the active site of CKIIα, overlap the position occupied by ATP, and fill a hydrophobic pocket between the *N*-terminal and the *C*-terminal lobes. This leads to its inhibition of the binding of the natural co-substrates of CKII, in a competitive manner.[116] In addition, the crucial relevance of hydrophobic interactions was studied to determine the affinity of emodin for its binding site on CKII. Mutating two non-polar residues, Val66 and Ile174, result in reducing the inhibitory effect of emodin, as judged from increased IC50 values.[117] The importance of hydroxyl group 3 in anchoring emodin with CKII through polar interactions is further proven by the observation that the two analogues of emodin, 1,8-dihydroxy-anthraquinone and chrysophanic acid, both lacking this group, did not show obvious inhibitory effect on CKII activity.[117] Meanwhile, the inhibitory IC50 of CKII by aloe-emodin was over 10-fold higher than that of emodin,[117] suggesting that the inhibitory effect of aloe-emodin on cell proliferation was unlikely to act through the inhibition of CKII kinase.

In addition to Her-2/neu tyrosine kinase and protein kinase CKII, emodin also shows inhibitory effect on some other kinases. Among them, emodin acts as a modest inhibitor of PKC.[115,118,119] Protein kinase C (PKC) has been implicated in the regulation of apoptosis, as well as cell proliferation and differentiation. PKCδ, one of its isoforms is found to be at the downstream of caspase-3 and proteolytic activation of PKCδ is responsible for apoptotic execution.[120,121] Emodin inhibits the translocation of PKCα from cytosol to plasma membrane in human peritoneal mesothelial cells.[122] In addition, the decrease in the expression of PKCδ and ε may play a critical role in emodin-induced apoptosis.[118] On the other hand, aloe-emodin exhibit similar inhibition on PKCδ and ε in CH27[118] and H460 cells.[36] In addition, decrease of PKC activity after aloe-emodin treatment has also been found in glial cells Aced.[48] But unlike emodin, decrease of PKCδ and ε by aloe-emodin treatment could not be recovered by caspase-3 inhibitor,[118] suggesting other mechanism may be responsible. It has been suggested that PKC inhibition by anthraquinones includes: binding of quinones to the PKC regulatory domains; binding between SH groups of PKC cysteine residues and the quinone moiety; quinine-sensitized photodamage of PKC through photosensitization.[123]

Compared to its analogue emodin, aloe-emodin is less well reported with respect to the inhibition of kinase activity, although it has also been reported to inhibit tyrosine-phosphorylation.[124] However, the mechanisms have not been well elucidated.

The mitogen-activated protein kinase (MAPK) signaling pathways, play central role in regulating cell proliferation, apoptosis, and migration.[125,126] The MAPK members consist of three major classes; the *c-jun N*-terminal kinases (JNKs), the extracellular signal-regulated proteins kinase (ERKs) and p38. Emodin has been found to decrease TPA-induced phosphorylation of JNK and ERK, but not p38 kinase.[67] Rhein showed similar inhibitory effect on MAPK activation induced by TPA[79] or interleukin-1β.[80] Both emodin and rhein subsequently suppressed AP-1 transactivation. Nevertheless, a report noted that rhein could induce phosphorylation of JNK and p38 in HL-60 cells, and this induction was blocked by catalase and NAC.[53] In addition, exposure of H460 cells to 40 μM aloe-emodin resulted in the decrease of ERK and p38 protein levels.[36] However, pretreatment of p38 MAP kinase inhibitor (SB202190) prevented aloe-emodin-induced p38 degradation. In rat C6 glioma cells,[82] induction of differentiation, but not apoptosis was accompanied with ERK inhibition,

while JNK, p38, and IκB pathways were not involved. Treatment of PD98059 (MEK inhibitor) mimicked aloe-emodin-induced anti-proliferation and differentiation effects. However, PD98059 cannot induce apoptosis or autophagy like aloe-emodin in C6 cells. Interestingly, inhibition of ERK by aloe-emodin has also been linked with decrease of cisplatin-induced C6 cell death.[82] Thus, aloe-emodin's toxicity on tumor cell may depend on cell type and treatment concentration or way of treatment due to the complicated ERK involvement in both apoptotic and survival pathway.

D. Other Anti-Cancer Mechanisms of Rhubarb Anthraquinones

Glutathione S-transferase P1-1 (GSTP1-1) is a phase II drug metabolism enzyme. It is also involved in carcinogenesis and resistance of cancer cells to oxidative stress and chemotherapeutic drugs. Emodin decreases TNFα- and TPA-induced *GSTP1-1* gene expression through inhibiting NF-κB and AP-1 binding onto GSTP1-1 promoter in K562 and U937 leukemia cells.[127] This could contribute to the reduction of the incidences of glutathione-related drug resistance in human cancers.

Emodin inhibits androgen receptor (AR) transcriptional activity by preventing AR nuclear translocation. This results in AR degradation through proteasome-mediated pathway in a ligand-independent manner.[31] Through this mechanism, emodin suppresses prostrate cancer cell growth *in vitro* and prolong the survival of prostate cancer-producing C3(1)/SV40 transgenic mice *in vivo*.

A recent proteomic study,[128] showed that the release of nucleophosmin from the nucleus to cytosol might be associated with aloe-emodin-induced H460 cell death. However, whether this translocation and degradation of nucleophosmin is the cause or effect of aloe-emodin-induced apoptosis is still yet to be elucidated. Our recent proteomic study using hepatocellular carcinoma cells HepG2 revealed a number of up- and downregulated proteins involved in antioxidation (e.g., peroxidoxin 2, 4, 6) and cell-cycle arrest (e.g., p16) prior to cell death. These changes were further confirmed by cell study and time-course Western blot (Lu et al. unpublished data).

5. SUMMARY AND CONCLUSIONS

Anthraquinones are an important group of bioactive components that are found not only in rhubarb, but also in many other species of medicinal herbs, such as aloe, senna, and purslane. From all the *in vitro* and *in vivo* data currently available, in addition their anti-fungal and anti-bacterial effects they also show potential protective effects for the gastrointestinal and renal systems.[129–134] In addition, recent studies have indicated that a number of anti-neoplastic effects could be found among several well-studied anthraquinones, including emodin, aloe-emodin, and rhein. Induction of apoptosis is commonly reported among emodin and aloe-emodin, which involve disruption of mitochondria membrane potential, cytochrome c release, and activation of caspase 3. These two anthraquinones were also able to induce cell-cycle arrest, involving an increase in p53 expression level and accompanied by upregulation of p21. Most of the anthraquinones are also able to cause sensitization effect with other chemotherapeutic agents. Furthermore, some anthraquinones have been reported to inhibit the activities of certain key enzymes and transcription factor, such as HER-2/neu, CKII, PKC, and NF-κB. It is also worthwhile mentioning that in many cases tumor cells seem to be more sensitive to these anthraquinones than normal cells. In addition, emodin has been shown to interrupt the progression of tumor metastasis through multiple signaling pathways. This strongly suggests that emodin could be a promising candidature for the research and development of new anti-tumor drugs.

Although the complimentary or antagonistic actions of various anthraquinones in rhubarb and the cross-communications among the various pathways are yet to be elucidated, so far, this old remedy appears to offer encouraging evidence of its *in vitro* and *in vivo* anti-tumor effect. With the unraveling of more and more molecular mechanisms involved, these anthraquinones can be developed either as chemotherapeutic drug by itself or in combination. Recent findings also suggest

that they could be used as sensitizers in chemotherapy-based combination regimens.[83–88] The strategy of enhancing the efficacy of anti-cancer therapy, without significant side effects is a rather novel approach which warrants further investigation. However, being small molecule inhibitors, it should be taken into account that the inhibitors might not be specific for certain kinases and their possible interactions with other known or unknown kinases, some of which may also be expressed only in certain tissues. To reach the magnitude and specificity of effective cancer treatment, cooperative investigations need to be carried out in the areas of oncology, chemistry, and pharmacology.

ACKNOWLEDGMENTS

This study was supported in part by the NIH-NCCAM startup grant no. R21-AT-1945 and the Centre for Environmental and Occupational Health research, National University of Singapore. We thank Ms. Lay Ha Chua for the assistance in preparing this manuscript.

REFERENCES

1. Yang SZ. The divine farmer's materia medica: (A translation of the Shen Nong Ben Cao Jing). Boulder, CO: Blue Poppy Press; 1997.
2. Li SC. Ben Cao Gang Mu. Beijing, China: People's Health Publishing Press; 1982.
3. Cai Y, Sun M, Xing J, Corke H. Antioxidant phenolic constituents in roots of *Rheum officinale* and *Rubia cordifolia*: structure-radical scavenging activity relationships. J Agric Food Chem 2004;52:7884–7890.
4. Berning H, Fischer R. Chronic abuse of laxatives and their effect on mineral metabolism. Chemical Abstracts. 1962;56:4066.
5. McGuffin MHC, Upton R, Glodberg A. American herbal preoducts association's botanical safety handbook. Boca Raton, New York: CRC Press; 1997.
6. Kemper KJ. Rhubarb root. 1999.
7. van Gorkom BA, de Vries EG, Karrenbeld A, Kleibeuker JH. Review article: Anthranoid laxatives and their potential carcinogenic effects. Aliment Pharmacol Ther 1999;13:443–452.
8. Organization WH, editor. WHO monographs on selected medicinal plants. Vol. 1. Geneva: World Health Organization; 1999. pp 231–240.
9. Swanbeck G. Interaction between deoxyribonucleic acid and some anthracene and anthraquinone derivatives. Biochim Biophys Acta 1966;123:630–633.
10. Mueller SO, Schmitt M, Dekant W, Stopper H, Schlatter J, Schreier P, Lutz WK. Occurrence of emodin, chrysophanol and physcion in vegetables, herbs and liquors. Genotoxicity and anti-genotoxicity of the anthraquinones and of the whole plants. Food Chem Toxicol 1999;37:481.
11. Mueller SO, Lutz WK, Stopper H. Factors affecting the genotoxic potency ranking of natural anthraquinones in mammalian cell culture systems. Mutat Res 1998;414:125.
12. Mueller SO, Stopper H. Characterization of the genotoxicity of anthraquinones in mammalian cells. Biochim Biophys Acta 1999;1428:406.
13. Wolfle D, Schmutte C, Westendorf J, Marquardt H. Hydroxyanthraquinones as tumor promoters: Enhancement of malignant transformation of C3H mouse fibroblasts and growth stimulation of primary rat hepatocytes. Cancer Res 1990;50:6540–6544.
14. Mori H, Sugie S, Niwa K, Yoshimi N, Tanaka T, Hirono I. Carcinogenicity of chrysazin in large intestine and liver of mice. Jpn J Cancer Res 1986;77:871–876.
15. XXX. Laxatives: Replacing danthron. Drug Ther Bull 1988;26:53–56.
16. NTP. Toxicology and carcinogenesis studies of EMODIN (CAS NO. 518-82-1) feed studies in F344/N rats and B6C3F1 mice. Natl Toxicol Program Tech Rep Ser 2001;493:1–278.
17. Heidemann A, Volkner W, Mengs U. Genotoxicity of aloeemodin in vitro and in vivo. Mutat Res 1996;367:123.
18. Siegers CP. Anthranoid laxatives and colorectal cancer. Trends Pharmacol Sci 1992;13:229–231.
19. Mantani N, Sekiya N, Sakai S, Kogure T, Shimada Y, Terasawa K. Rhubarb use in patients treated with Kampo medicines—A risk for gastric cancer? Yakugaku Zasshi 2002;122:403–405.
20. Vargas F, Fraile G, Velasquez M, Correia H, Fonseca G, Marin M, Marcano E, Sanchez Y. Studies on the photostability and phototoxicity of aloe-emodin, emodin and rhein. Pharmazie 2002;57:399.

21. Hartley JA, Forrow SM, Souhami RL, Reszka K, Lown JW. Photosensitization of human leukemic cells by anthracenedione antitumor agents. Cancer Res 1990;50:1936–1940.
22. Vath P, Wamer WG, Falvey DE. Photochemistry and phototoxicity of aloe emodin. Photochem Photobiol 2002;75:346.
23. Wamer WG, Vath P, Falvey DE. In vitro studies on the photobiological properties of aloe emodin and aloin A. Free Radic Biol Med 2003;34:233.
24. Strickland FM, Muller HK, Stephens LC, Bucana CD, Donawho CK, Sun Y, Pelley RP. Induction of primary cutaneous melanomas in C3H mice by combined treatment with ultraviolet radiation, ethanol and aloe emodin. Photochem Photobiol 2000;72:407.
25. Badgwell DB, Walker CM, Baker WT, Strickland FM. Ethanol and aloe emodin alter the p53 mutational spectrum in ultraviolet radiation-induced murine skin tumors. Mol Carcinog 2004;39:127.
26. Jahnke GD, Price CJ, Marr MC, Myers CB, George JD. Developmental toxicity evaluation of emodin in rats and mice. Birth Defects Res B Dev Reprod Toxicol 2004;71:89–101.
27. Lu Y, Huang ZS, Tan JH, Diang Y, Bu XZ, An LK, Ma L, Fu LW, Gu LQ. Synthesis and cytotoxicity of emodin derivatives. Chinese J Org Chem 2005;25:944–948.
28. Lin JG, Chen GW, Li TM, Chouh ST, Tan TW, Chung JG. Aloe-emodin induces apoptosis in T24 human bladder cancer cells through the p53 dependent apoptotic pathway. J Urol 2006;175:343–347.
29. Chang CJ, Ashendel CL, Geahlen RL, McLaughlin JL, Waters DJ. Oncogene signal transduction inhibitors from medicinal plants. In Vivo 1996;10:185.
30. Zhang L, Chang CJ, Bacus SS, Hung MC. Suppressed transformation and induced differentiation of HER-2/neu-overexpressing breast cancer cells by emodin. Cancer Res 1995;55:3890.
31. Cha TL, Qiu L, Chen CT, Wen Y, Hung MC. Emodin down-regulates androgen receptor and inhibits prostate cancer cell growth. Cancer Res 2005;65:2287–2295.
32. Kuo YC, Sun CM, Ou JC, Tsai WJ. A tumor cell growth inhibitor from *Polygonum hypoleucum* Ohwi. Life Sci 1997;61:2335.
33. Chan TC, Chang CJ, Koonchanok NM, Geahlen RL. Selective inhibition of the growth of ras-transformed human bronchial epithelial cells by emodin, a protein-tyrosine kinase inhibitor. Biochem Biophys Res Commun 1993;193:1152.
34. Shieh DE, Chen YY, Yen MH, Chiang LC, Lin CC. Emodin-induced apoptosis through p53-dependent pathway in human hepatoma cells. Life Sci 2004;74:2279.
35. Lee HZ, Hsu SL, Liu MC, Wu CH. Effects and mechanisms of aloe-emodin on cell death in human lung squamous cell carcinoma. Eur J Pharmacol 2001;431:287–295.
36. Yeh FT, Wu CH, Lee HZ. Signaling pathway for aloe-emodin-induced apoptosis in human H460 lung nonsmall carcinoma cell. Int J Cancer 2003;106:26–33.
37. Kuo PL, Lin TC, Lin CC. The antiproliferative activity of aloe-emodin is through p53-dependent and p21-dependent apoptotic pathway in human hepatoma cell lines. Life Sci 2002;71:1879–1892.
38. Chen HC, Hsieh WT, Chang WC, Chung JG. Aloe-emodin induced in vitro G2/M arrest of cell cycle in human promyelocytic leukemia HL-60 cells. Food Chem Toxicol 2004;42:1251–1257.
39. Shi YQ, Fukai T, Sakagami H, Kuroda J, Miyaoka R, Tamura M, Yoshida N, Nomura T. Cytotoxic and DNA damage-inducing activities of low molecular weight phenols from rhubarb. Anticancer Res 2001;21:2847–2853.
40. Pecere T, Gazzola MV, Mucignat C, Parolin C, Vecchia FD, Cavaggioni A, Basso G, Diaspro A, Salvato B, Carli M, Palu G. Aloe-emodin is a new type of anticancer agent with selective activity against neuroectodermal tumors. Cancer Res 2000;60:2800–2804.
41. Pecere T, Sarinella F, Salata C, Gatto B, Bet A, Dalla Vecchia F, Diaspro A, Carli M, Palumbo M, Palu G. Involvement of p53 in specific anti-neuroectodermal tumor activity of aloe-emodin. Int J Cancer 2003;106:836–847.
42. Cichewicz RH, Zhang Y, Seeram NP, Nair MG. Inhibition of human tumor cell proliferation by novel anthraquinones from daylilies. Life Sci 2004;74:1791–1799.
43. Floridi A, Gentile PF, Bruno T, Fanciulli M, Paggi MG, Zeuli M, Benassi M. Cytotoxic effect of the association of BCNU with rhein or lonidamine on a human glioma cell line. Anticancer Res 1991;11:789–792.
44. Zhou X, Song B, Jin L, Hu D, Diao C, Xu G, Zou Z, Yang S. Isolation and inhibitory activity against ERK phosphorylation of hydroxyanthraquinones from rhubarb. Bioorg Med Chem Lett 2006;16:563–568.
45. Chen YC, Shen SC, Lee WR, Hsu FL, Lin HY, Ko CH, Tseng SW. Emodin induces apoptosis in human promyeloleukemic HL-60 cells accompanied by activation of caspase 3 cascade but independent of reactive oxygen species production. Biochem Pharmacol 2002;64:1713.
46. Deane NG, Parker MA, Beauchamp RD. Cell proliferation: A matter of time and place. Surgery 2005;138:1–7.

47. Kuo PL, Hsu YL, Ng LT, Lin CC. Rhein inhibits the growth and induces the apoptosis of Hep G2 cells. Planta Med 2004;70:12–16.
48. Acevedo-Duncan M, Russell C, Patel S, Patel R. Aloe-emodin modulates PKC isozymes, inhibits proliferation, and induces apoptosis in U-373MG glioma cells. Int Immunopharmacol 2004;4:1775–1784.
49. Kamei H, Koide T, Kojima T, Hashimoto Y, Hasegawa M. Inhibition of cell growth in culture by quinones. Cancer Biother Radiopharm 1998;13:185.
50. Fischer U, Schulze-Osthoff K. Apoptosis-based therapies and drug targets. Cell Death Differ 2005;12:942–961.
51. Su YT, Chang HL, Shyue SK, Hsu SL. Emodin induces apoptosis in human lung adenocarcinoma cells through a reactive oxygen species-dependent mitochondrial signaling pathway. Biochem Pharmacol 2005;70:229–241.
52. Jing X, Ueki N, Cheng J, Imanishi H, Hada T. Induction of apoptosis in hepatocellular carcinoma cell lines by emodin. Jpn J Cancer Res 2002;93:874–882.
53. Lin S, Fujii M, Hou DX. Rhein induces apoptosis in HL-60 cells via reactive oxygen species-independent mitochondrial death pathway. Arch Biochem Biophys 2003;418:99–107.
54. Kagedal K, Bironaite D, Ollinger K. Anthraquinone cytotoxicity and apoptosis in primary cultures of rat hepatocytes. Free Radic Res 1999;31:419–428.
55. Lee HZ. Protein kinase C involvement in aloe-emodin- and emodin-induced apoptosis in lung carcinoma cell. Br J Pharmacol 2001;134:1093–1103.
56. Kumar A, Dhawan S, Aggarwal BB. Emodin (3-methyl-1,6,8-trihydroxyanthraquinone) inhibits TNF-induced NF-kappaB activation, IkappaB degradation, and expression of cell surface adhesion proteins in human vascular endothelial cells. Oncogene 1998;17:913.
57. Lee HZ, Lin CJ, Yang WH, Leung WC, Chang SP. Aloe-emodin induced DNA damage through generation of reactive oxygen species in human lung carcinoma cells. Cancer Lett 2006;239:55–63.
58. Castiglione S, Fanciulli M, Bruno T, Evangelista M, Del Carlo C, Paggi MG, Chersi A, Floridi A. Rhein inhibits glucose uptake in Ehrlich ascites tumor cells by alteration of membrane-associated functions. Anticancer Drugs 1993;4:407–414.
59. Bironaite D, Ollinger K. The hepatotoxicity of rhein involves impairment of mitochondrial functions. Chem Biol Interact 1997;103:35–50.
60. Huang Q, Shen HM, Shui G, Wenk MR, Ong CN. Emodin inhibits tumor cell adhesion through disruption of the membrane lipid Raft-associated integrin signaling pathway. Cancer Res 2006;66:5807–5815.
61. Meng X, Riordan NH, Riordan HD, Mikirova N, Jackson J, Gonzalez MJ, Miranda-Massari JR, Mora E, Trinidad Castillo W. Cell membrane fatty acid composition differs between normal and malignant cell lines. P R Health Sci J 2004;23:103–106.
62. Freeman MR, Solomon KR. Cholesterol and prostate cancer. J Cell Biochem 2004;91:54–69.
63. Rakheja D, Kapur P, Hoang MP, Roy LC, Bennett MJ. Increased ratio of saturated to unsaturated C18 fatty acids in colonic adenocarcinoma: Implications for cryotherapy and lipid raft function. Med Hypotheses 2005;65:1120–1123.
64. Huang Q, Shen HM, Ong CN. Emodin inhibits tumor cell migration through suppression of the phosphatidylinositol 3-kinase-Cdc42/Rac1 pathway. Cell Mol Life Sci 2005;62:1167–1175.
65. Mahabeleshwar GH, Das R, Kundu GC. Tyrosine kinase, p56lck-induced cell motility, and urokinase-type plasminogen activator secretion involve activation of epidermal growth factor receptor/extracellular signal regulated kinase pathways. J Biol Chem 2004;279:9733–9742.
66. Mahabeleshwar GH, Kundu GC. Tyrosine kinase p56lck regulates cell motility and nuclear factor kappaB-mediated secretion of urokinase type plasminogen activator through tyrosine phosphorylation of IkappaBalpha following hypoxia/reoxygenation. J Biol Chem 2003;278:52598–52612.
67. Huang Q, Shen HM, Ong CN. Inhibitory effect of emodin on tumor invasion through suppression of activator protein-1 and nuclear factor-kappaB. Biochem Pharmacol 2004;68:361.
68. Wang XH, Zhen YS. Inhibitory effect of emodin on metastasis-associated properties of PG cells from a human highly metastatic giant lung carcinoma cell line. Chinese J Cancer 2001;20:789.
69. Zhu F, Liu XG, Liang NC. Effect of emodin and apigenin on invasion of human ovarian carcinoma HO-8910PM cells in vitro. Ai Zheng 2003;22:358.
70. Kim MS, Park MJ, Kim SJ, Lee CH, Yoo H, Shin SH, Song ES, Lee SH. Emodin suppresses hyaluronic acid-induced MMP-9 secretion and invasion of glioma cells. Int J Oncol 2005;27:839–846.
71. Yu D, Wang SS, Dulski KM, Tsai CM, Nicolson GL, Hung MC. c-erbB-2/neu overexpression enhances metastatic potential of human lung cancer cells by induction of metastasis-associated properties. Cancer Res 1994;54:3260–3266.

72. Zhang L, Lau YK, Xi L, Hong RL, Kim DS, Chen CF, Hortobagyi GN, Chang C, Hung MC. Tyrosine kinase inhibitors, emodin and its derivative repress HER-2/neu-induced cellular transformation and metastasis-associated properties. Oncogene 1998;16:2855.
73. Watanabe A, Mabuchi T, Satoh E, Furuya K, Zhang L, Maeda S, Naganuma H. Expression of syndecans, a heparan sulfate proteoglycan, in malignant gliomas: Participation of nuclear factor-kappaB in upregulation of syndecan-1 expression. J Neurooncol 2006;77:25–32.
74. Ljubimov AV, Caballero S, Aoki AM, Pinna LA, Grant MB, Castellon R. Involvement of protein kinase CK2 in angiogenesis and retinal neovascularization. Invest Ophthalmol Vis Sci 2004;45:4583.
75. Wang XH, Wu SY, Zhen YS. Inhibitory effects of emodin on angiogenesis. Yao Xue Xue Bao 2004;39: 254.
76. Kwak HJ, Park MJ, Park CM, Moon SI, Yoo DH, Lee HC, Lee SH, Kim MS, Lee HW, Shin WS, Park IC, Rhee CH, Hong SI. Emodin inhibits vascular endothelial growth factor-A-induced angiogenesis by blocking receptor-2 (KDR/Flk-1) phosphorylation. Int J Cancer 2006;118:2711–2720.
77. Tamura T, Kosaka N, Ishiwa J, Sato T, Nagase H, Ito A. Rhein, an active metabolite of diacerein, down-regulates the production of pro-matrix metalloproteinases-1, -3, -9 and -13 and up-regulates the production of tissue inhibitor of metalloproteinase-1 in cultured rabbit articular chondrocytes. Osteoarthritis Cartilage 2001;9:257–263.
78. Sanchez C, Mathy-Hartert M, Deberg MA, Ficheux H, Reginster JY, Henrotin YE. Effects of rhein on human articular chondrocytes in alginate beads. Biochem Pharmacol 2003;65:377–388.
79. Lin S, Li JJ, Fujii M, Hou DX. Rhein inhibits TPA-induced activator protein-1 activation and cell transformation by blocking the JNK-dependent pathway. Int J Oncol 2003;22:829–833.
80. Martin G, Bogdanowicz P, Domagala F, Ficheux H, Pujol JP. Rhein inhibits interleukin-1 beta-induced activation of MEK/ERK pathway and DNA binding of NF-kappa B and AP-1 in chondrocytes cultured in hypoxia: A potential mechanism for its disease-modifying effect in osteoarthritis. Inflammation 2003;27:233–246.
81. Mendes AF, Caramona MM, de Carvalho AP, Lopes MC. Diacerhein and rhein prevent interleukin-1beta-induced nuclear factor-kappaB activation by inhibiting the degradation of inhibitor kappaB-alpha. Pharmacol Toxicol 2002;91:22–28.
82. Mijatovic S, Maksimovic-Ivanic D, Radovic J, Miljkovic D, Harhaji L, Vuckovic O, Stosic-Grujicic S, Mostarica Stojkovic M, Trajkovic V. Anti-glioma action of aloe emodin: The role of ERK inhibition. Cell Mol Life Sci 2005;62:589–598.
83. Zhang L, Hung MC. Sensitization of HER-2/neu-overexpressing non-small cell lung cancer cells to chemotherapeutic drugs by tyrosine kinase inhibitor emodin. Oncogene 1996;12:571.
84. Lai GH, Zhang Z, Sirica AE. Celecoxib acts in a cyclooxygenase-2-independent manner and in synergy with emodin to suppress rat cholangiocarcinoma growth in vitro through a mechanism involving enhanced Akt inactivation and increased activation of caspases-9 and -3. Mol Cancer Ther 2003;2:265.
85. Yang J, Li H, Chen YY, Wang XJ, Shi GY, Hu QS, Kang XL, Lu Y, Tang XM, Guo QS, Yi J. Anthraquinones sensitize tumor cells to arsenic cytotoxicity in vitro and in vivo via reactive oxygen species-mediated dual regulation of apoptosis. Free Radic Biol Med 2004;37:2027.
86. Yang J, Tang XM, Li H, Shi GY, Zhu P, Jin HF, Yi J. Emodin sensitizes HeLa cell to arsenic trioxide induced apoptosis via the reactive oxygen species-mediated signaling pathways. Shi Yan Sheng Wu Xue Bao 2003;36:465.
87. Yi J, Yang J, He R, Gao F, Sang H, Tang X, Ye RD. Emodin enhances arsenic trioxide-induced apoptosis via generation of reactive oxygen species and inhibition of survival signaling. Cancer Res 2004;64: 108.
88. Ravi R, Bedi A. Sensitization of tumor cells to Apo2 ligand/TRAIL-induced apoptosis by inhibition of casein kinase II. Cancer Res 2002;62:4180–4185.
89. Fenig E, Nordenberg J, Beery E, Sulkes J, Wasserman L. Combined effect of aloe-emodin and chemotherapeutic agents on the proliferation of an adherent variant cell line of Merkel cell carcinoma. Oncol Rep 2004;11:213–217.
90. Huang YH, Zhen YS. Rhein induces apoptosis in cancer cells and shows synergy with mitomycin. Yao Xue Xue Bao 2001;36:334–338.
91. Huang HC, Chang JH, Tung SF, Wu RT, Foegh ML, Chu SH. Immunosuppressive effect of emodin, a free radical generator. Eur J Pharmacol 1992;211:359.
92. Jing YW, Yi J, Chen YY, Hu QS, Shi GY, Li H, Tang XM. Dicoumarol alters cellular redox state and inhibits nuclear factor kappa B to enhance arsenic trioxide-induced apoptosis. Acta Biochim Biophys Sin (Shanghai) 2004;36:235.
93. Wang XJ, Yang J, Cang H, Zou YQ, Yi J. Gene expression alteration during redox-dependent enhancement of arsenic cytotoxicity by emodin in HeLa cells. Cell Res 2005;15:511–522.

94. Hengstler JG, Lange J, Kett A, Dornhofer N, Meinert R, Arand M, Knapstein PG, Becker R, Oesch F, Tanner B. Contribution of c-erbB-2 and topoisomerase IIalpha to chemoresistance in ovarian cancer. Cancer Res 1999;59:3206.
95. Stevnsner T, Bohr VA. Studies on the role of topoisomerases in general, gene- and strand-specific DNA repair. Carcinogenesis 1993;14:1841–1850.
96. Mijatovic S, Maksimovic-Ivanic D, Radovic J, Popadic D, Momcilovic M, Harhaji L, Miljkovic D, Trajkovic V. Aloe-emodin prevents cytokine-induced tumor cell death: The inhibition of auto-toxic nitric oxide release as a potential mechanism. Cell Mol Life Sci 2004;61:1805–1815.
97. Mijatovic S, Maksimovic-Ivanic D, Radovic J, Miljkovic D, Kaludjerovic GN, Sabo TJ, Trajkovic V. Aloe emodin decreases the ERK-dependent anticancer activity of cisplatin. Cell Mol Life Sci 2005;62:1275–1282.
98. Zhang L, Lau YK, Xia W, Hortobagyi GN, Hung MC. Tyrosine kinase inhibitor emodin suppresses growth of HER-2/neu-overexpressing breast cancer cells in athymic mice and sensitizes these cells to the inhibitory effect of paclitaxel. Clin Cancer Res 1999;5:343.
99. Koyama J, Morita I, Tagahara K, Nobukuni Y, Mukainaka T, Kuchide M, Tokuda H, Nishino H. Chemopreventive effects of emodin and cassiamin B in mouse skin carcinogenesis. Cancer Lett 2002;182:135.
100. Koyama J, Morita I, Tagahara K, Ogata M, Mukainaka T, Tokuda H, Nishino H. Inhibitory effects of anthraquinones and bianthraquinones on Epstein–Barr virus activation. Cancer Lett 2001;170:15–18.
101. Koyama J, Tagahara K, Osakai T, Tsujino Y, Tsurumi S, Nishino H, Tokuda H. Inhibitory effects on Epstein–Barr virus activation of anthraquinones: Correlation with redox potentials. Cancer Lett 1997;115:179–183.
102. Kupchan SM. Novel plant-derived tumor inhibitors and their mechanisms of action. Cancer Treat Rep 1976;60:1115–1126.
103. Kupchan SM, Karim A. Tumor inhibitors. 114. Aloe emodin: Antileukemic principle isolated from *Rhamnus frangula* L. Lloydia 1976;39:223–224.
104. Bharti AC, Aggarwal BB. Nuclear factor-kappa B and cancer: Its role in prevention and therapy. Biochem Pharmacol 2002;64:883.
105. Li HL, Chen HL, Li H, Zhang KL, Chen XY, Wang XW, Kong QY, Liu J. Regulatory effects of emodin on NF-kappaB activation and inflammatory cytokine expression in RAW 264.7 macrophages. Int J Mol Med 2005;16:41–47.
106. Liu ZG. Molecular mechanism of TNF signaling and beyond. Cell Res 2005;15:24–27.
107. Chen Y, Yang L, Lee TJ. Oroxylin A inhibition of lipopolysaccharide-induced iNOS and COX-2 gene expression via suppression of nuclear factor-kappaB activation. Biochem Pharmacol 2000;59:1445.
108. Fullbeck M, Huang X, Dumdey R, Frommel C, Dubiel W, Preissner R. Novel curcumin- and emodin-related compounds identified by in silico 2D/3D conformer screening induce apoptosis in tumor cells. BMC Cancer 2005;5:97.
109. Uhle S, Medalia O, Waldron R, Dumdey R, Henklein P, Bech-Otschir D, Huang X, Berse M, Sperling J, Schade R, Dubiel W. Protein kinase CK2 and protein kinase D are associated with the COP9 signalosome. EMBO J 2003;22:1302.
110. Bech-Otschir D, Kraft R, Huang X, Henklein P, Kapelari B, Pollmann C, Dubiel W. COP9 signalosome-specific phosphorylation targets p53 to degradation by the ubiquitin system. EMBO J 2001;20:1630–1639.
111. Menard S, Casalini P, Campiglio M, Pupa SM, Tagliabue E. Role of HER2/neu in tumor progression and therapy. Cell Mol Life Sci 2004;61:2965–2978.
112. Chen JS, Lan K, Hung MC. Strategies to target HER2/neu overexpression for cancer therapy. Drug Resist Updat 2003;6:129–136.
113. Unger GM, Davis AT, Slaton JW, Ahmed K. Protein kinase CK2 as regulator of cell survival: Implications for cancer therapy. Curr Cancer Drug Targets 2004;4:77–84.
114. Olsten ME, Litchfield DW. Order or chaos? An evaluation of the regulation of protein kinase CK2. Biochem Cell Biol 2004;82:681–693.
115. Yim H, Lee YH, Lee CH, Lee SK. Emodin, an anthraquinone derivative isolated from the rhizomes of *Rheum palmatum*, selectively inhibits the activity of casein kinase II as a competitive inhibitor. Planta Med 1999;65:9.
116. Battistutta R, Sarno S, De Moliner E, Papinutto E, Zanotti G, Pinna LA. The replacement of ATP by the competitive inhibitor emodin induces conformational modifications in the catalytic site of protein kinase CK2. JBiol Chem 2000;275:29618.
117. Sarno S, Moro S, Meggio F, Zagotto G, Dal Ben D, Ghisellini P, Battistutta R, Zanotti G, Pinna LA. Toward the rational design of protein kinase casein kinase-2 inhibitors. Pharmacol Ther 2002;93:159–168.

118. Lee HZ. Protein kinase C involvement in aloe-emodin- and emodin-induced apoptosis in lung carcinoma cell. Br JPharmacol 2001;134:1093.
119. Fredenhagen A, Mett H, Meyer T, Buchdunger E, Regenass U, Roggo BE, Petersen F. Protein tyrosine kinase and protein kinase C inhibition by fungal anthraquinones related to emodin. J Antibiot (Tokyo) 1995;48:1355.
120. Tsujio I, Tanaka T, Kudo T, Nishikawa T, Shinozaki K, Grundke-Iqbal I, Iqbal K, Takeda M. Inactivation of glycogen synthase kinase-3 by protein kinase C delta: Implications for regulation of tau phosphorylation. FEBS Lett 2000;469:111–117.
121. Tanaka T, Tsujio I, Nishikawa T, Shinosaki K, Kudo T, Takeda M. Significance of tau phosphorylation and protein kinase regulation in the pathogenesis of Alzheimer disease. Alzheimer Dis Assoc Disord 2000;14:S18–S24.
122. Chan TM, Leung JK, Tsang RC, Liu ZH, Li LS, Yung S. Emodin ameliorates glucose-induced matrix synthesis in human peritoneal mesothelial cells. Kidney Int 2003;64:519.
123. Diwu Z, Zimmermann J, Meyer T, Lown JW. Design, synthesis and investigation of mechanisms of action of novel protein kinase C inhibitors: Perylenequinonoid pigments. Biochem Pharmacol 1994;47:373–385.
124. Schorkhuber M, Richter M, Dutter A, Sontag G, Marian B. Effect of anthraquinone-laxatives on the proliferation and urokinase secretion of normal, premalignant and malignant colonic epithelial cells. Eur J Cancer 1998;34:1091–1098.
125. Reddy KB, Nabha SM, Atanaskova N. Role of MAP kinase in tumor progression and invasion. Cancer Metastasis Rev 2003;22:395–403.
126. Bode AM, Dong Z. Signal transduction pathways: Targets for chemoprevention of skin cancer. Lancet Oncol 2000;1:181–188.
127. Duvoix A, Delhalle S, Blasius R, Schnekenburger M, Morceau F, Fougere M, Henry E, Galteau MM, Dicato M, Diederich M. Effect of chemopreventive agents on glutathione S-transferase P1-1 gene expression mechanisms via activating protein 1 and nuclear factor kappaB inhibition. Biochem Pharmacol 2004;68:1101.
128. Lee HZ, Wu CH, Chang SP. Release of nucleophosmin from the nucleus: Involvement in aloe-emodin-induced human lung non small carcinoma cell apoptosis. Int J Cancer 2005;113:971–976.
129. Wojcikowski K, Johnson DW, Gobe G. Medicinal herbal extracts—Renal friend or foe? Part one: The toxicities of medicinal herbs. Nephrology (Carlton) 2004;9:313–318.
130. Wojcikowski K, Johnson DW, Gobe G. Herbs or natural substances as complementary therapies for chronic kidney disease: Ideas for future studies. J Lab Clin Med 2006;147:160–166.
131. Zhang X, Thuong PT, Jin W, Su ND, Sok DE, Bae K, Kang SS. Antioxidant activity of anthraquinones and flavonoids from flower of Reynoutria sachalinensis. Arch Pharm Res 2005;28:22–27.
132. Li X, Wang H. Chinese herbal medicine in the treatment of chronic kidney disease. Adv Chronic Kidney Dis 2005;12:276–281.
133. Wang GL, Hou QY, Zhang J, Xu JM, Peng JF, Lin RC. Studies on the chemical constituents of the stems of Alyxia sinensis (I). Zhongguo Zhong Yao Za Zhi 2002;27:125–127.
134. Alam MM, Javed K, Jafri MA. Effect of Rheum emodi (Revand Hindi) on renal functions in rats. J Ethnopharmacol 2005;96:121–125.
135. Lee HZ. Effects and mechanisms of emodin on cell death in human lung squamous cell carcinoma. Br JPharmacol 2001;134:11.

Qing Huang *received her Ph.D. training at the National University of Singapore (NUS). She is now a research fellow and her research interests include the anti-tumor activities of traditional Chinese medicine, especially their anti-metastasis function.*

Guodong Lu *is a graduate student at the National University of Singapore. His main interest is to understand the molecular mechanism of anthraquinones-induced anti-hepatoma effects by using proteomic strategies.*

Han-Ming Shen *received his medical training in China and obtained his Ph.D. from the National University of Singapore (NUS) in 1996 and has been an assistant professor in NUS since 2002. His main research interests include oxidative stress in cell death signaling and natural products as novel anti-cancer agents.*

Maxey C.M. Chung *obtained his Ph.D. from the Victoria University of Wellington, New Zealand in 1978. He joined the National University of Singapore in 1983 after doing his post-doctoral work in the John Curtin of Medical Research, The Australian National University, Australia. Currently he holds a joint appointment as*

associate professor in the Departments of Biochemistry and Biological Sciences. His research interest is in the application of proteomics in the discovery of cancer biomarkers.

Choon Nam Ong *obtained his Ph.D. from the University of Manchester, UK, in 1977 and has been a professor at the Yong Loo Lin Medical School, NUS since 1980. His main research interests are environmental carcinogens, identification of biomarkers, and cancer chemoprevention. Currently, he is the deputy director of the Office of Life Sciences of NUS.*