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Kava, a Tonic for Relieving the Irrational Development of Natural Preventive Agents

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The last two decades have witnessed explosive growth in the study of natural and other cancer chemopreventive agents (1,2). Extensive pre-clinical data have been generated for natural agents, and many (such as green tea, curcumin, phenyl isothicyanate, indole-3-carbinol [I3C], silibinin, lycopene, genistein, selenium, and vitamins A, E and D) are currently in different phases of clinical testing (3). The definitive clinical prevention trials of natural agents completed thus far have been largely negative, suggesting that detailed mechanistic and efficacy studies are necessary to supplement the epidemiological data before clinically testing novel natural compounds (3). Therefore, investigators increasingly are studying the mechanisms of cancer chemopreventive agents (natural or synthetic, including molecular-targeted) in order to substantiate their potential efficacy.

A substantial body of work showing a broad spectrum of natural-agent mechanisms has raised important issues. For example, what is a relevant, achievable dose *in vivo* for targeting relevant pathways or targets (versus the potentially high, unachievable doses studied *in vitro*)? Which of the multiple mechanisms are potential causes of toxicity? A multiplicity of mechanisms certainly could enhance natural agent effects, but it is important also to try to identify specific relevant or predominant mechanism(s) that are critical for preventive activity in specific carcinogenic systems. Besides giving scientific credibility, mechanistic insight will facilitate clinical development by elucidating key pathway(s) and target(s) to be monitored in dose-finding early-phase clinical trials (4). It also helps in selecting patient populations based on appropriate prevention settings and potential sensitivity to the agent's preventive and/or toxic effects. Understanding relevant mechanisms also helps in developing more-specifically targeted analogues with potentially less toxicity, greater preventive activity, and less variability in formulation, which is important for assuring the desired effects of an intervention.

The substantial data reported thus far on mechanisms of chemopreventive agents are just the tip of an iceberg of mostly unknown mechanisms involving about 20,000 protein-coding human genes and the epigenetic machinery that contributes to the regulation of gene expression. Most advances in understanding the mechanisms of natural agent actions have been confined, until recently, to cell culture studies. Now, however, various animal models (e.g., knock-out, knock-in, or transgenic mice) are frequently employed to establish the *in vivo* mechanistic aspects of natural agents. Furthermore, the use of "omic" approaches (genomic, proteomic, metabolomic, etc.) in chemoprevention has helped in speedily measuring altered expression of thousands of genes in response to phytochemicals (plant-derived chemical compounds) and promises to further crystallize natural-agent mechanisms.

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Silibinin, a constituent of milk thistle, can help in illustrating current mechanistic study of natural agent mechanisms. Milk thistle extracts for centuries have been used as a medicament for hepatobiliary diseases and during the last few decades in clinical testing for treating acute mushroom poisoning, hepatic cirrhosis and acute viral hepatitis (5,6). Milk thistle extract, also labeled silymarin or silibinin, is now marketed as a nutritional supplement to promote healthy liver function (5). In the 1970s we reported the first evidence of the cancer preventive activity of silymarin/silibinin in a series of *in vivo* studies employing mouse skin cancer models (7,8). Studies of the last 15 years in different *in vitro* and *in vivo* models have established the mechanistic details of silibinin cancer preventive effects in various epithelial cancers including prostate, lung, bladder, colorectal, kidney, oral, skin, renal, breast, ovarian and tongue cancer (6,7,9,10). These mechanistic studies showed that silibinin treatment inhibits unchecked cellular proliferation in cancer cells by targeting the cell cycle through modulation of various cell-cycle regulators (11). This activity includes strongly inhibiting constitutive as well as growth factor-induced receptor tyrosine signaling and inhibiting androgen receptor and signal transducer and activator of transcription (STAT) signaling (9). The growth of cancer cells is almost always accompanied by the loss of apoptotic response, and silibinin treatment has been shown to induce apoptosis in many cancer cell lines and in tumor tissues via modulation of various Bcl2 and inhibitor of apoptosis (IAP) family members' expression through inhibition of nuclear factor kappa B (NF-κB) and with or without the activation of various caspases (7, 9). Silibinin treatment has been shown to target the expression of various pro-angiogenic factors (e.g., vascular endothelial growth factor, basic fibroblast growth factor, inducible nitric oxide synthase), thereby affording strong anti-angiogenic efficacy (7,9). Overall, these studies suggest pleiotropic mechanisms for silibinin anticancer activity; more recent studies, however, showed that inhibition of epidermal growth factor receptor activation is necessary and sufficient for the anti-cancer effects of silibinin (12). This places silibinin in the class of receptor tyrosine kinase inhibitors, which have undergone extensive clinical testing for cancer control (13).

Another good example of a mechanistically evaluated natural agent with chemopreventive potential is the phytochemical deguelin. Deguelin has several relevant mechanisms including inhibition of Akt, a very prominent target for molecular-targeted drug development, *in vitro* and *in vivo* and blocked tobacco-induced lung carcinogenesis in A/J mice (14). *In vitro* study of deguelin in premalignant human bronchial epithelial cells appears to be the first work to illustrate the importance of Akt targeting in lung chemoprevention (14). In other words, mechanistic study of deguelin was used for target identification and stimulated tremendous interest in developing specific inhibitors of Akt and the PI3K/Akt pathway for lung cancer prevention and therapy. More recently, deguelin has been shown to inhibit HSP90 function leading to degradation of its various client proteins including Akt and HIF-1α (15). The caveat in developing deguelin as a cancer preventive drug, however, is that it can inhibit NADH:ubiquinone oxidoreductase activity (16), which could cause neuronal or other toxicity. Therefore, investigators are now developing deguelin analogues with greater specificity for Akt and thus potentially greater potency in lung carcinogenesis and lesser potential toxicity. These analogues are being patented and thus have enhanced potential for developmental funding support from federal and industry sources. Mechanisms have been reported not only for silibinin and deguelin but for several other well-studied natural agents, including genistein, curcumin, apigenin, indole-3-carbinol, green tea, lycopene, grape seed extract, inositol hexaphosphate (or phytic acid), garlic and cruciferous constituents, as well.

All of the foregoing evidence supports a role for phytochemicals as cancer chemopreventive agents and warrants more vigorous work to identify, and pre-clinical testing of, novel natural agents with chemopreventive activity. Such preclinical work is reflected in the kava studies by Johnson et al. (17) and Tang et al. (18) reported in this issue of the journal. Discussed in detail below, these studies portray a rational sequence of natural agent development for prevention,

with a proof of the preventive potential of kava extract *in vivo* in mice (17) and a more-detailed mechanistic analysis of a specific kava constituent both *in vitro* and in a mouse xenograft model (18). Such work is a necessary precursor to clinical development of kava or any other natural agent, notwithstanding seemingly compelling epidemiologic and general biologic evidence of its preventive potential.

Kava (*Piper methysticum*) is an ancient crop of the western Pacific islands, where it has been used as a medicine, social drink, and sacred plant in religious ceremonies (19). The traditional kava drink is prepared from the plant's roots, and its consumption causes a mildly talkative and sociable behavior, clear thinking and anxiolytic and muscle-relaxing effects (20). Kava extract consists mainly of two classes of compounds: kavalactones and flavokawains (FKs), or chalcones. Kavalactones are the major constituents (3%–20%) of kava extract and mainly include methysticin, dihydromethysticin, 7,8- dihydrokawain, kawain, desmethoxyyangonin, yangonin, and 7,8-epoxyyangonin (20). Chalcones include FKA, -B and -C and constitute less than 1% of total kava extract (20). Kava (20) attracted global (and mechanistic) attention in the 1990s as an herbal supplement for reducing anxiety, stress and insomnia (21). Strong epidemiologic evidence suggests that kava-drinking populations have an unusually low cancer incidence despite high rates of smoking (22,23). The age-standardized cancer incidences for Fiji, Vanuatu and Western Samoa, the three countries with the highest consumption of kava drink, were reported to be one-third or one-fourth of the cancer incidences in non-kava-drinking countries (23). Furthermore, the cancer incidences in these populations were lower in men compared with women, despite much higher smoking rates among men, who also consume more kava (22–24). This collection of evidence suggests that the traditional herb kava would be useful in the prevention and/or treatment of smoking-related diseases such as lung and bladder cancer. Several previous studies also have examined kava mechanisms in various preclinical carcinogenesis systems (20,25,26).

As published in this issue of the journal, Johnson et al. (17) demonstrated for the first time the *in vivo* cancer chemopreventive potential of kava against chemical carcinogen-induced lung tumorigenesis. Kava extract (10 mg/g mixed in food) significantly reduced NNK [4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone] plus B(a)P [benzo(a)pyrene]-induced lung tumor multiplicity in A/J mice. The kava regimen significantly reduced lung tumor multiplicity whether given during carcinogen treatment only, after carcinogen treatment only, or both during and after. These results suggest that kava might inhibit events of initiation as well as promotion associated with chemical carcinogenesis. Kava also reduced the proliferation marker PCNA and increased markers of apoptosis (caspase-3 and PARP cleavage) and inhibited activation of NF-κB (17). Of note, this study also showed that kava extract given in food for 30 weeks at a dose of 10 mg/g does not cause hepatotoxicity, which was measured in terms of liver weight, liver pathology, and markers of liver damage (alanine aminotransferase, aspartate aminotransferase, and gamma-glutamyltransferase).

Also in this issue of the journal, Tang et al. (18) published a report on kava and bladder carcinogenesis, that strongly complements the promising results of Johnson et al. in lung carcinogenesis. The bladder study follows up on work reported by the same group (26) showing that kava extract and FKA, -B and -C strongly induced apoptosis in human bladder cancer cells via an increase in the active form of Bax protein and a decrease in the expression of X-linked IAP (XIAP) and survivin. This earlier study also showed that FKA treatment inhibited the anchorage-independent growth and *in vivo* xenograft growth of bladder cancer EJ cells (26). Tang et al. now show that the kava chalcone FKA (50 mg/kg of body weight) strongly decreased the *in vivo* growth rate of a bladder cancer xenograft (RT4 cells) in athymic nude mice, without causing toxicity. The studies of Tang et al. and Johnson et al. clearly and convincingly suggest the strong preventive and therapeutic potential of kava against the major tobacco-related diseases lung and bladder cancer.

Smoking exposure (among other factors) results in cancer initiation via a number of molecular changes including mutations that inactivate tumor suppressor genes (such as *p53*, retinoblastoma [*Rb*] and *INK4*) and/or mutations that activate various oncogenes (such as epidermal growth factor receptor, *Ras* and cyclin D1; ref. (27). Through these changes, cancer cells acquire the capability of uncontrolled multiplication, apoptosis evasion, and constitutive activation of survival signaling pathways such as NF-κB and Akt, which is followed by neo-angiogenesis and metastatic spread (28). Johnson et al. (17) and Tang et al. (18) showed that kava or its constituent FKA could inhibit proliferation and NF-κB activation and induce apoptosis in tobacco-related lung and bladder cancer cells. The antiproliferative effect of FKA was more prominent in bladder cancer cell lines harboring mutations in both *p53* and *Rb*, which are frequent in tobacco-related human cancers (17,18,27).

Targeting the deregulated cell cycle has emerged as an ideal prevention strategy for checking the development and uncontrolled growth of cancer cells (29). Tang et al. (18) showed that FKA treatment differentially induced G1 cell-cycle arrest in wild-type *p53* and G2M cell-cycle arrest in mutant-*p53* bladder cancer cells. Furthermore, in low-grade bladder cancer cells carrying wild-type *p53*, FKA treatment increased the levels of cyclin-dependent kinase (CDK) inhibitors (p21 and p27) and decreased CDK2 kinase activity (18). However, in *p53*-mutant, high-grade bladder cancer cells, FKA treatment reduced the expression of CDK1 inhibitory kinases, Myt1 and Wee1, and increased cyclin B1 levels leading to CDK1 activation (18). These results suggest that FKA potentially is a G2 checkpoint abrogator in cancer cells carrying mutant *p53*, which is consistent with the induction by FKA of M-phase arrest in cancer cells. Of interest, FKA induced M-phase arrest through signaling events downstream of widely known cellular checkpoints (Chk1 and Chk2). Whether this unscheduled entry into M-phase in response to FKA treatment leads to activation of spindle check point and results in mitotic catastrophe remains to be examined. The effect of FKA treatment on the expression of key mitotic kinesins and kinases (Plk and Aurora kinases) also must be examined in order to understand the mechanistic details underlying M-phase arrest. FKA treatment also promoted mitotic slippage in bladder cancer cells, which needs to be studied more closely since mitotic slippage could lead not only to cell-cycle arrest and apoptosis but also could promote genetic instability and cancer progression (30). All of the *in vitro* and *in vivo* evidence, along with the epidemiological data from Pacific island populations, support the cancer chemopreventive potential of kava and its constituents. Other issues, however, remain to be addressed. Little or no literature is available regarding the bioavailability of kava constituents in plasma and other organs of interest. Of particular importance to prevention, kava has potential toxicity. The use of kava as an herbal supplement was banned by many Western countries in 2002 after reports of its severe hepatotoxicity. The numerous studies of kava toxicity (21,25,31–33) have shown, for example, that this toxicity is linked to kava formulation/extraction (acetone/ethanol extraction or extraction from the stem or leaves of the kava plant), to genetic background (e.g., CYP2D6 deficiency is prevalent in 7%–9% of Europeans but is rare in Polynesians and Asians), and strongly to the interaction of kava with other drugs (21,31–33).

The papers in this issue of the journal raise the question of whether it is better to screen and develop natural products for their cancer preventive or therapeutic activity or to take a targeted, mechanistic approach in developing specific inhibitors of known critical molecules in cancer cells. Either approach has the potential to identify effective cancer preventive or therapeutic agents. Many scientists or pharmaceutical houses, however, prefer one or the other of these strategies. The benefits of screening specific targets (for example performing a screen of all known kinases to develop specific kinase inhibitor drugs) are that (a) a target is known once a lead compound is selected, and (b) extensive mechanistic data on the particular target in relation to cancer cell growth may be available. However, if multiple signaling molecules are required to be inhibited to effectively prevent or treat cancer, this single-target screen may ultimately fail, and screening a natural compound with the potential to affect many signaling pathways

at once may be more productive. Advantages, disadvantages and technical issues involved in specific molecular-targeted versus natural-agent development would be a worthy topic for a future perspective or commentary.

In conclusion, although the ultimate success of kava will depend on the outcomes of further preclinical and clinical studies, this herb exemplifies the principle of “nature to bench to bedside” and supports the identification and pre-clinical and clinical testing of natural agents for cancer chemoprevention. Kava presents as well a venue for examining the value of robust mechanistic studies in advancing rational natural-agent development.

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