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Molecular mechanisms of anticancer activity of natural dietetic products

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Abstract. The efficiency of dietetic supplements in cancer prevention and treatment is a popular and controversial subject of research. New in vitro and in vivo research results indicate that some dietetic supplements do indeed show anticancer activity. The strongest anticancer action has been demonstrated by natural compounds with multifunctional activity. For instance, antioxidants, which also bind to and modulate the activity of protein kinases involved in signal transduction cascades show both cytostatic and cytotoxic activity towards cancer cells. Other activities such as angiogenesis inhibition, nitric oxide synthase inhibition, and pro-oxidants production have also been observed. Catechins and polyphenols from plant extracts such as green tea show the strongest anticancer activity. The initial clinical trials with some flavonoid molecules are already underway.

Keywords. Dietetic supplements - Mechanism of anticancer activity

Abbreviations. *CDK*: Cyclin-dependent kinase *ECGC*: Epigallocatechin 3-gallate *JNK*: c-Jun N-terminal kinase *PDGF*: Platelet-derived growth factor *VSMC*: Vascular smooth muscle cell

There is no doubt that dietary supplements are becoming increasingly popular in Western society. More than US$ 12 billion were paid for such supplements in 1998 in the United States alone [*1*]. The need for alternative solutions in medicine is growing, especially regarding cancer patients. Last year the United States Congress enhanced financing to the National Institutes of Health to probe unconventional therapies, with $50 million for research on alternative and complementary medicine [*2*]. It now appears that worldwide investments in research on the mechanisms of action of dietetic supplements, one of the more promising alternative medical products, has started to deliver the first results.

When devastating diseases such as cancer strike, alternative therapies are often sought which employ less toxic and unpleasant treatments than current chemotherapy and radiation treatments. While alternative therapies are often the result merely of wishful thinking and unprofessional, commercial behavior, it seems that some dietetic products can indeed aid in

treating cancer. Anecdotal evidence indicates that some vegetables and fruits, polyphenol sources such as green tea, and even powdered natural zeolites may be helpful in enhancing the efficacy of cancer treatment. Oncologists are still very skeptical towards such products, partly due to lack of in vivo animal studies and in vitro molecular studies which would outline the mechanism of anticancer action of dietetic products. This perceived lack of data is rapidly changing.

It is indeed difficult to imagine the possible biochemical mechanism of the anticancer action of such a diverse group of products that includes soybean extract, green tea extract, thiol antioxidants, and zeolite powder. Many researchers have recently tested the activity of such products and the possible mechanisms of their anticancer action [3, 4, 5, 6, 7, 8]. A common activity noted for most of such dietetic products is that they act as potent antioxidants and free radical scavengers.

Reports by several research groups in recent issues of *Methods of Enzymology* (volumes 299, 300, and 301) have clearly shown that some dietetic products outperform vitamins C and E and other classical antioxidants by more than an order of magnitude in their ability to scavenge free radicals and produce a more reducing intracellular environment. The question now is how can potent antioxidants influence gene expression regulation, cell proliferation, differentiation, survival and death? Scientists have only begun to understand underlying mechanisms. It was recently reported in *Nature Medicine* that strong antioxidants such as pyrrolidine dithiocarbamate and *N*-acetyl cysteine causes partial growth inhibition in vitro and in vivo when added to human colorectal adenocarcinoma cells grown in cell culture, and when fed to mice with implanted tumors [3]. Moreover, when used with chemotherapy agents such as 5-fluorouracil and doxorubicin, antioxidants enhance the cytotoxicity of chemotherapy agents and cause complete remissions, where only partial remission is possible with chemotherapy agents only [3].

Chinery and coworkers [3] went one step further and asked: how does this happen? Recent studies indicate that some of the most potent molecules which control cell growth and possible tumorigenesis are tumor suppressor molecules [4]. Such molecules modify gene expression and activity of proteins involved in the initiation of cell division. Cyclins have been identified as molecules which directly stimulate cell division. On the other hand, cyclin-dependent kinases (CDKs) are needed to activate cyclin molecules by phosphorylation, a common signal transduction strategy. Some of the most potent tumor suppressor molecules are actually inhibitors of CDK-2 and CDK-4 [4]. Two such molecules are known as $p21^{WAF1/CIP1}$ and $p27^{KIP1}$. Another common tumor suppressor molecule, p53, is needed to activate $p21^{WAF1/CIP1}$ [4].

It has also been shown that antioxidants induce transcription of $p21^{WAF1/CIP1}$ without a need for p53, which is inactivated in almost one-half of human tumors [3]. Further research indicated that the transcription factor which activates transcription of the $p21^{WAF1/CIP1}$ gene is C/EBP^{β} (also known as NF-IL6) [3]. The researchers went even further and showed that C/EBP^{β} in its activated form moves from cytoplasm to the nucleus, where it stimulates transcription of $p21^{WAF1/CIP1}$ by binding to the CCAAT enhancer sequence of DNA [5]. It was also shown that the possible first step in the activation of $p21^{WAF1/CIP1}$ is antioxidant-induced activation of protein kinase A. The reduced form of protein kinase A binds to plasma membrane, becomes activated, and then phosphorylates C/EBP^{β}. This causes its translocation to the nucleus and induction of transcription of $p21^{WAF1/CIP1}$ [5].

Importantly, a series of recent papers on the in vitro and in vivo anticancer activities of dietetic products report a similar mechanism of action. For instance, it has been shown that plant flavonoids induce $p21^{WAF1/CIP1}$ in A549 human lung adenocarcinoma cells [6]. This results in growth arrest and apoptosis. The growth arrest is independent of p53. It has also been found that genistein from soybeans induces $p21^{WAF1/CIP1}$ and blocks the G_1 to S phase transition in mouse fibroblast and melanoma cells [7]. Other researchers have showed that green tea extract enhances the chemotherapy activity of doxorubicin (in vitro and in vivo) towards human ovarian cancer cells with low sensitivity to doxorubicin [8]. Also, epigallocatechin 3-gallate (ECGC) is reported to induce the CDK inhibitors $p21^{WAF1/CIP1}$ and $p27^{KIP1}$ in a p53-independent manner and to inhibit CDK-2 and CDK-4, with subsequent induction of cell cycle arrest at G_1 phase in MCF-7 breast cancer cells [9].

A flavonoid has been identified which binds directly to CDK-2 and CDK-4 and inhibits both of these CDKs directly [10]. This flavonoid, termed flavopiridol, has been shown to cause growth arrest in a human breast carcinoma cell line. The three-dimensional structure of the complex between CDK-2 and this particular flavonoid has been determined. These data will be used for intelligent design of more potent CDK inhibitors [11].

Finely ground natural clinoptilolite zeolite powder has also been shown to induce activation of $p21^{WAF1/CIP1}$. However, tissue culture experiments demonstrated that activated zeolite particles inhibit protein kinase B/akt, another kinase involved in antiapoptotic processes and cancer promotion (K. Pavelic et al., manuscript submitted to the *Journal of Molecular Medicine*) This happens only when growth of cells in tissue cultures is stimulated by the addition of growth factors. Zeolite particles might adsorb growth factors or prevent interaction of protein kinase B with membranes, where it is phosphorylated by phosphatidylinositol 3-kinase. It has recently been shown that inactivation of protein kinase B by, for example, the novel tumor suppressor molecule PTEN also results in induction of the tumor suppressor-CDK inhibitor $p27^{KIP1}$ [12].

There also seems to be a relationship between $p21^{WAF1/CIP1}$ and another protein kinase involved in cell "decisions" about proliferation, arrest or apoptosis, namely c-Jun N-terminal kinase (JNK) 1. JNK-1 is a member of the recently discovered stress-activated protein kinases [4]. Interestingly, while in reaction to stress such kinase activation results in apoptosis, its activation in some cancer cells actually promotes uncontrolled proliferation. This is particularly obvious in the human lung adenocarcinoma cell line A549 [13]. Research has shown that $p21^{WAF1/CIP1}$ inhibits JNK-1 [14], and recent work indicates that those two molecules form a tight complex [15]. Inactivation of JNK-1 may be part of the reason why antioxidants enhance cytotoxicity of chemotherapeutic agents towards cancer cells, while, on the other hand, they protect neurons from apoptosis caused by free radical damage.

Other possible mechanisms for anticancer activity of herbal dietetic products have also been observed. It has been demonstrated that EGCG from green tea extract selectively inhibits the platelet-derived growth factor (PDGF) BB induced intracellular signaling transduction pathway in vascular smooth muscle cells (VSMCs). It also inhibits transformation of *sis*-transfected NIH 3T3 fibroblasts and human glioblastoma cells [16]. Treatment with the EGCG-inhibits $p42^{MAPK}$ and $p44^{MAPK}$ kinase isoforms in VSMCs after PDGF-BB activation.

Quantification of the immunoprecipitated tyrosine-phosphorylated PDGF-R^{β},

phosphatidylinositol 3 kinase, and phospholipase C-γ1 by enhanced western blotting has revealed that EGCG treatment effectively inhibits tyrosine phosphorylation of these kinases in VSMCs. The Japanese herbal medicine sho-saiko-to also upregulates the Fas-mediated apoptosis of melanoma cells [17]. Ingredient analysis has identified baicalin as the main active constituent. EGCG has also recently been reported to suppress angiogenesis induced by one of the most potent angiogenic factors, vascular endothelial growth factor [18].

It seems that most dietetic products with anticancer activity act as strong antioxidants and/or modify the activity of one or more protein kinases involved in cell cycle control. Kinases such as protein kinase A, protein kinase B, protein kinase C, JNK-1, CDK-2, and CDK-4 are either activated or deactivated by these antioxidants, as shown in Fig. 1. This can happen directly or indirectly through activation of some transcription factors such as NF-IL6 or tumor suppressor genes such as $p21^{WAF1/CIP1}$ and $p27^{KIP1}$.

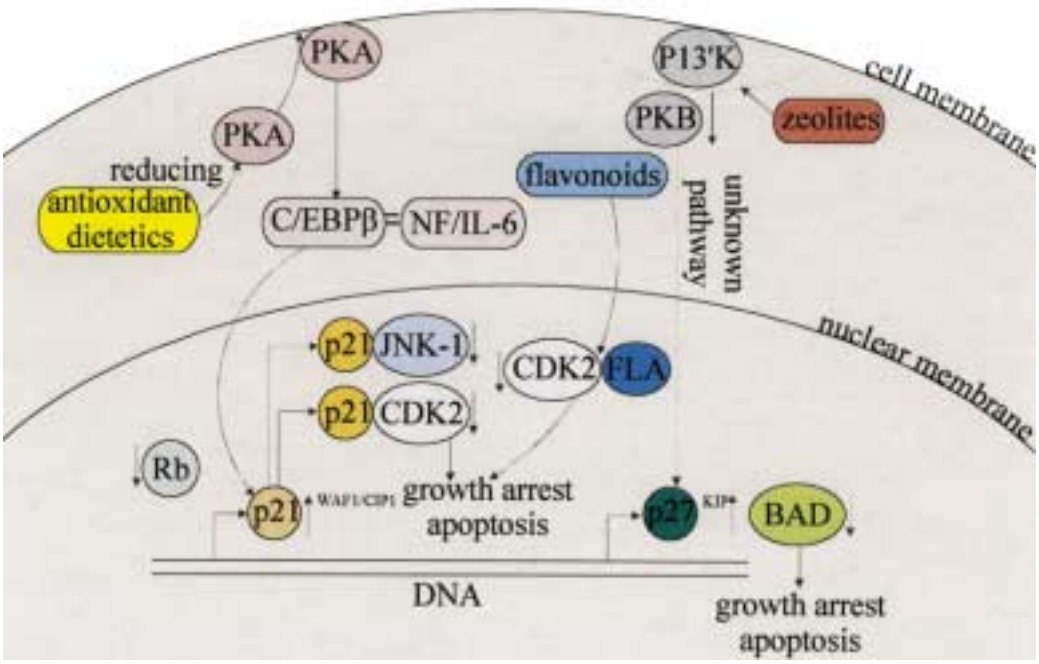
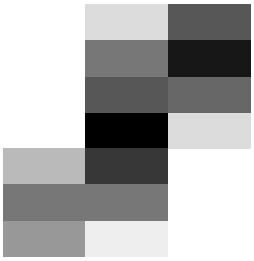


Fig. 1. Schematic presentation of signal transduction cascades modified by some dietetic products with anticancer activity. Products with antioxidants activity reduce protein kinase A (*PKA*). The

reduced form of PKA translocates to the membrane where it phosphorylates transcription factor C/EBP$^{\beta}$. C/EBP$^{\beta}$ then translocates to the nucleus where it induces the transcription of $p21^{WAF1/CIP1}$ in a p53-independent way. Induction of $p21^{WAF1/CIP1}$ results in inhibition of CDK-2 and growth arrest of cells in G_1 phase. $p21^{WAF1/CIP1}$ also binds to JNK-1 and inactivates this stress-activated protein kinase (*SAPK*). Some flavonoids such as flavopiridol directly bind to CDKs and modulate their activity. Polyanions such as silicates, zeolites, heparin sulfates, and suramin interfere with the binding of growth factors with receptors and activation of protein kinase B (*PKB*). This results in the induction of tumor suppressor $p27^{KIP1}$. Some reagents such as green tea catechins and dietary fibers butyrate



induce both $p21^{WAF1/CIP1}$ and $p27^{KIP}1$. *PI3* *K* Phosphatidylinositol 3-kinase

What we now need is a better understanding of the way in which active substances from orally administered dietetic products come to regulate the activity of enzymes and regulatory molecules such as transcription factors that regulate gene expression, which in turn affects cell proliferation, survival, and death. We should also try to isolate active components from dietetic supplements in their pure form and use them at a range of higher concentrations. This also may improve therapy by removing constituents with opposing activities that may be in the parent extract. We should also study the bioavailability of such substances and try to improve their adsorption into the body and their residence time in tumor tissues. More in vivo studies should also be performed. Synergistic interactions of such substances with chemotherapy agents should be studied. Synergistic effects should be used in better treatment of resistant tumors [19]. This will hopefully lead to well-controlled studies with human volunteers. Fortunately, such efforts are currently underway [20, 21]. Better cancer treatment therapies with milder side effects are desperately needed.

Finally, we should also mention that modulation of activity of protein kinases by antioxidants is only one attractive way to improve current cancer therapies and prevention strategies. Studies on the role of diet in preventing colon cancer have recently shown that antioxidants are less beneficial than had been expected. Instead it seems that agents which modulate acetylation of histones, such as butyrate, are more important in colon cancer prevention [22]. Such agents are also capable of inducing $p21^{WAF1/CIP1}$ and $p27^{KIP1}$. Modulation of the activity of some other nuclear regulatory proteins such as heat shock related proteins and growth arrest proteins such as gadd45, mdm2, and p53 can also be used to improve current cancer

therapies. An information-intensive approach to the molecular pharmacology of cancer is currently being used to select natural and synthetic compounds with anticancer activity [*23*].

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