Abstract

Green tea and its major polyphenolic flavonoid, epigallocatechin gallate (EGCG), have been credited with cancer chemopreventive activity for many years; the mechanism for this activity, however, has remained obscure. Now, as reported in this issue of the journal (beginning on page 1366), Urusova and colleagues showed direct binding of EGCG to the peptidyl prolyl cis/trans isomerase Pin1, which inhibited Pin1 enzymatic activity. They showed that Pin1 expression is required for EGCG effects on cell growth, c-Jun activation, and transcription regulation mediated by NF-κB and activator protein-1. The data provide a glimpse of the mechanism of action of EGCG and set a new bar for the future study of natural products with chemopreventive activity. Cancer Prev Res; 4(9); 1343-5. ©2011 AACR.

Among the general public and many scientists and clinicians, it has become widely accepted that green tea consumption is associated with numerous health benefits. The putative benefits include cancer prevention, a conclusion that arose from the lower prevalence of some forms of cancer among populations that consume large quantities of the beverage. Many animal studies have confirmed protective effects of green tea and/or its components against lung, breast, prostate, skin, intestinal, pancreatic, liver, and colorectal cancers; attempts to establish clinical protective effects in humans, however, have yielded equivocal results (1–3).

The beneficial effects of green tea have been attributed primarily to its polyphenolic flavonoids, including epigallocatechin gallate (EGCG, Fig. 1A), which is the flavonoid present in the highest concentration (4). Therefore, most efforts to define the mechanism of anticancer activity of green tea have focused on this compound. Phenolic hydroxyl groups in EGCG suggest the potential for antioxidant activity, but the poor correlation between antioxidant activity and antitumor activity for many antioxidants indicates that other mechanisms are operative. Indeed, EGCG and other naturally occurring polyphenols have been found to alter many signal transduction pathways. Numerous studies, mostly carried out in tumor cell lines in culture, have identified a wide array of biochemical processes modulated by micromolar concentrations of EGCG that may explain its cancer chemopreventive activity. These processes include modulation of growth factor receptor signaling (including the epidermal growth factor receptor, hepatocyte growth factor, and insulin-like growth factor receptor), angiogenesis, mitogen-activated protein kinases, cyclin-dependent kinases, matrix metalloproteinases, proteasomes, DNA methyl transferase, apoptosis, and oxidative stress (5–7).

The pleiotropic effects of EGCG suggest that it interacts with a master regulator that controls many different pathways or that it interacts with multiple targets in parallel. Insufficient data have been available to allow a choice between these two options, or others. Therefore, the article by Urusova and colleagues appearing in this issue of the journal (8) represents a major breakthrough in understanding how EGCG inhibits tumorigenesis and provides a framework for studying the effects of other naturally occurring polyphenols.

These investigators hypothesized that many actions of EGCG could be explained by inhibition of the peptidyl prolyl cis/trans isomerase Pin1. As its name implies, Pin1 catalyzes the cis/trans isomerization of the peptidyl proline bond of proteins. There are many peptidyl prolyl isomerases (PPIases), and some of them (e.g., cyclophilin and FK506-binding protein) have well-recognized roles in molecular signaling and cancer. Pin1 is unique, however, in that it specifically binds proteins containing a phosphoserine- or phosphothreonine–proline sequence (Fig. 1B). The Pin1-induced alteration of the geometry of the proline-containing peptide bond leads to significant changes in the conformation of the substrate protein, which can result in altered function, stability, etc. Thus, Pin1-mediated isomerization of a phosphorylated target protein is an important mechanism for modulating kinase-dependent signaling in pathways such as those leading to activation of activator protein 1 (AP-1), NF-κB, nuclear factor of activated T cells (NFAT), and β-catenin (9). All of these pathways have been implicated in the cancer chemopreventive action of EGCG. Thus, Pin1 may be the master regulator that mediates these many, seemingly disparate effects.
Urusova and colleagues used isothermal titration calorimetry to confirm that EGCG binds to Pin1. They determined a dissociation constant ($K_d$) of $21 \, \mu\text{mol/L}$, which is comparable with the concentrations of EGCG that alter the properties of tumor cells. This finding confirmed that EGCG binding to Pin1 likely occurs at chemopreventive concentrations of EGCG. EGCG also directly inhibited Pin1 enzymatic activity in vitro. Not satisfied to simply show a binding interaction, however, the investigators crystallized the Pin1–EGCG complex and determined its structure at 1.9 Å resolution by X-ray diffraction. This accomplishment was significant because the $K_d$ of $21 \, \mu\text{mol/L}$ indicates that EGCG binding by Pin1 is not strong, and such complexes are frequently difficult to crystallize. The structure displayed the WW domain (amino acids 1–39) of Pin1, through which it interacts with the phosphopeptide substrate, and its PPIase domain (amino acids 45–168), which catalyzes the Pin1-mediated isomerization reaction. A flexible linker (amino acids 40–44) connecting the two domains was not visualized. Surprisingly, the crystal structure revealed a molecule of EGCG bound to each domain (Fig. 2). Interactions between one EGCG molecule and the WW domain occurred through hydrogen bonds to the side chain and amide group of Arg-17. The other molecule of EGCG bound to the PPIase domain through interactions with the main chain carbonyl of Asp-112, the side chain and amide of Ser-114, and the amide of Trp-73. The importance of Arg-17 in the EGCG–Pin1 interaction was confirmed by showing that wild-type (WT), but not the R17A mutant Pin1, could bind to EGCG-Sepharose affinity chromatography beads.

Having defined the nature of the interaction between EGCG and Pin1, the investigators next explored the functional implications of that interaction. They used mouse embryonal fibroblasts (MEF) from PIN1 knockout (KO) mice and from isogenic WT mice (10) to show that the absence of the Pin1 protein leads to a reduced growth rate in culture. The growth rate differential between PIN1 WT and PIN1 KO MEFs was more striking in the case of MEFs overexpressing the Neu oncogene. More importantly, EGCG inhibited the growth of PIN1 WT MEFs and had no effect on PIN1 KO MEFs, regardless of Neu expression status in either cell type. Transfecting PIN1 KO cells with WT PIN1, but not with R17A mutant PIN1, restored their sensitivity to EGCG.

Further studies in the PIN1 WT and KO MEFs showed that Pin1 plays a role in c-Jun activation and in the transcription of genes regulated by both AP-1 and NF-κB. EGCG inhibited all of these processes, but only in cells expressing WT PIN1. EGCG induced cell-cycle arrest and apoptosis in Neu-overexpressing MEFs that also expressed WT PIN1, but not in PIN1 KO cells. Using an anchorage-independent soft agar cell transformation assay, Urusova and colleagues showed that EGCG inhibited colony...
formation in Neu-overexpressing MEFs with WT PIN1. The potency of EGCG was similar to that of juglone and exceeded that of PiB, two known Pin1 inhibitors. In this assay, PIN1 KO reduced colony formation in the absence of any Pin1 inhibitor and completely protected cells from the suppressive effects of all such inhibitors. Transfecting PIN1 KO cells with WT, but not R17A mutant, PIN1 restored sensitivity to EGCG suppression of colony formation.

Finally, Urusova and colleagues tested the effects of EGCG in vivo using a tumor xenograft model in athymic nude mice. Neu-overexpressing PIN1 WT MEFs readily established tumors in this model, and EGCG significantly inhibited the growth of these tumors. Xenografts of PIN1 KO Neu-overexpressing cells were dramatically less tumorigenic than were Neu-overexpressing PIN1 WT xenografts, confirming the role of PIN1 as an oncogene in this model.

The study by Urusova and colleagues is a beautiful example of the use of chemical biology to validate a novel molecular target for cancer prevention. Their structure–function analysis led them to construct a Pin1 mutant protein (R17A) that was unable to bind EGCG, and they used this mutant protein to validate the importance of the EGCG–Pin1 interaction in the control of cell signaling, anchorage-independent growth, and tumorigenesis. Their ability to drill down on an important signaling node raises the bar for future research of novel chemopreventive agents: Molecular targets can be identified, and the nature of their interactions can be defined.

This work raises many exciting questions. Is Pin1 the single master regulator of all of the activities of EGCG? Because micromolar concentrations of EGCG are usually required for cellular effects, it seems likely that other binding partners may also act in parallel for EGCG to exert its pleiotropic effects. What are the structural consequences of the R17A mutation of Pin1? Does the mutation have a discrete effect or does it induce global structural changes in Pin1? Can derivatives of EGCG be synthesized or novel compounds be discovered that are more selective for or potent against Pin1 or that have improved pharmacokinetic properties? The availability of a molecular target enables application of the tools of drug discovery to accelerate the development of novel chemopreventive agents targeted to Pin1.

Even as this work opens new avenues of research, specifically with regard to EGCG and Pin1, it also challenges all of us in the field of cancer prevention. There is an abundance of foods, natural products, and lifestyle changes that have been proposed as “natural” cancer chemopreventive agents. A depressingly small number of these interventions, however, is understood mechanistically at the molecular level. Urusova and colleagues have provided the information necessary to pursue a meaningful evaluation of the benefits of EGCG and the green tea containing it. Data of comparable quality for other proposed “natural” preventive agents are sorely needed.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

Green Tea Gets Molecular

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