

## Turmeric and Green Tea: A Recipe for the Treatment of B-Chronic Lymphocytic Leukemia

□□ Commentary on Ghosh et al., p. 1250

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Two naturally occurring compounds, curcumin, the active ingredient in the spice turmeric, and the green tea extract epigallocatechin-3-gallate, have marked effects on the apoptotic machinery in chronic lymphocytic leukemia. These results provide a preclinical foundation for future clinical use of these compounds in this disease.

In this issue of *Clinical Cancer Research*, Ghosh and coworkers (1) investigate, in the preclinical setting, a novel approach to treating B-chronic lymphocytic leukemia (B-CLL) that combines two naturally occurring compounds, curcumin and epigallocatechin-3-gallate (EGCG). Current treatments for B-CLL include purine analogues (fludarabine) and monoclonal antibodies (rituximab; ref. 2). Although people can live for years with B-CLL, there is no effective cure. Hence, new targeted therapies are vital to improving clinical outcomes for patients with B-CLL.

Using natural compounds for the treatment of cancer is not new. Sixty-two percent of cancer drugs approved by the U.S. Food and Drug Administration between 1981 and 2002 were of natural origin (3). For example, all-*trans* retinoic acid, which is a vitamin A derivative, is a very effective treatment for acute promyelocytic leukemia. A wealth of data supports a role for curcumin, the active ingredient in the spice turmeric, as an antineoplastic agent (4, 5). This work extends previous observations by examining the molecular pathways involved in curcumin-induced apoptosis of B-CLL cells. In particular, in primary CLL cultures, curcumin inhibited expression of pro-survival molecules, including signal transducer and activator of transcription 3 (STAT3), Akt, and nuclear factor- $\kappa$ B (NF- $\kappa$ B), as well as the antiapoptotic proteins myeloid cell leukemia-1 (Mcl-1) and X-linked inhibitor of apoptosis protein (XIAP). Curcumin also up-regulated the proapoptotic protein Bcl-2-interacting mediator of apoptosis (BIM). Sequential administration of EGCG followed by curcumin resulted in increased CLL cell death and neutralized stromal protection.

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Received 11/24/08; accepted 12/10/08.

**Grant support:** National Center for Research Resources (a component of the NIH) grant RR024148 and NIH Roadmap for Medical Research (<http://www.ncrr.nih.gov/>).

**Note:** The contents of this article are solely the responsibility of the authors and do not necessarily represent the official view of the National Center for Research Resources or NIH.

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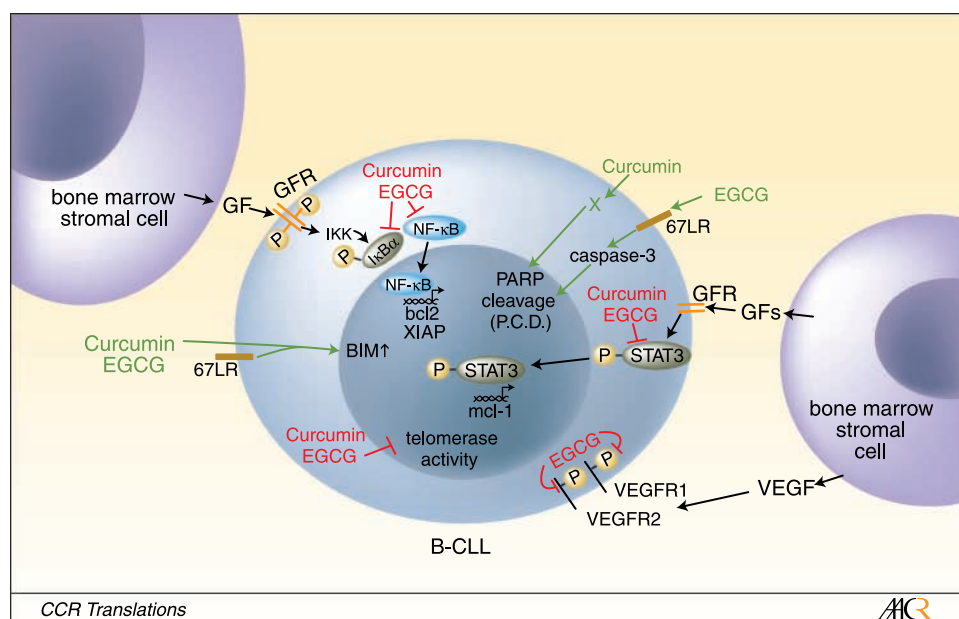
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doi:10.1158/1078-0432.CCR-08-2791

Curcumin has impressive antioxidant, chemopreventive, chemotherapeutic, and chemosensitizing activities (4, 5). The apoptosis of B-CLL cells induced by curcumin is dose dependent, and normal B lymphocytes are less sensitive to its cytotoxic effects than B-CLL cells (1, 6). Curcumin induces poly(ADP-ribose) polymerase (PARP) cleavage in primary B-CLL cells (1), reflecting the activation of programmed cell death. PARP cleavage is induced by curcumin in other tumor types, including pancreatic and colorectal cancer (7–9). Interestingly, the authors did not observe activation of upstream caspases following curcumin treatment. This differs from several other studies that show specific caspase-3 activation by curcumin in HL-60 and other tumor cell lines (4, 5, 10). In primary B-CLL, the mechanism of PARP cleavage remains unclear. Other cellular events that contribute to curcumin-induced apoptosis include the release of cytochrome *c* from the mitochondrial membrane. This occurs in several different cell lines following treatment with curcumin, as do changes in mitochondrial membrane potential (4, 5). The role of mitochondrial events in curcumin-induced apoptosis in B-CLL cells was not examined in this study.

Curcumin also inhibits the constitutive activation of pro-survival pathways, some of which are preferentially active in primary B-CLL cells, including STAT3, Akt, and NF- $\kappa$ B. Constitutive activation of STAT3 has been reported in several cancers, including breast, prostate, head and neck squamous cell carcinoma, multiple myeloma, and pancreatic cancer (4, 8, 11). STAT3 plays an important role in the induction of antiapoptotic genes and angiogenic factors and is vital to various cytokine signaling pathways (4, 5, 11). Curcumin effectively inhibits constitutive STAT3 phosphorylation in B-CLL (1), agreeing with other studies that show that curcumin is an effective inhibitor of STAT3 function (4, 5, 11). *Mcl-1*, a pro-survival gene downstream of STAT3, is also down-regulated by curcumin in primary B-CLL (1). Constitutive phosphorylation of Akt is down-regulated by curcumin in primary B-CLL cells (1) and other tumors, including non-Hodgkin's B-cell lymphoma, prostate cancer, and renal cell carcinoma (4). However, curcumin can also induce its proapoptotic and antiproliferative effects without perturbing Akt phosphorylation status (5, 7, 12).

A pro-survival molecule that seems to be universally inhibited by curcumin is the NF- $\kappa$ B transcription factor. NF- $\kappa$ B is down-regulated by curcumin in many different cancers (4–8, 11).



**Fig. 1.** Bone marrow stromal cells provide prosurvival and antiapoptotic signals to B-CLL cells. Curcumin and EGCG inhibit prosurvival pathways (red) and induce proapoptotic pathways (green). GF, growth factor; GFR, growth factor receptor; 67LR, 67-kDa laminin receptor; P.C.D., programmed cell death; IKK, IκB kinase.

Constitutive phosphorylation of IκBα in primary B-CLL cells is inhibited by curcumin, indicating that genes downstream of NF-κB should be inhibited in these cells. Indeed, XIAP, a downstream target of NF-κB, is down-regulated in primary B-CLL following curcumin treatment (1). The lack of down-regulation of Bcl-2 by curcumin is an interesting finding considering previous work showing that Bcl-2 is a direct transcriptional target of NF-κB and is down-regulated by curcumin (4, 5). BIM, a proapoptotic protein, is up-regulated by curcumin in primary B-CLL. Hence, curcumin can down-regulate survival pathways and up-regulate apoptotic pathways in B-CLL.

What happens to the effectiveness of curcumin treatment when B-CLL cells are cocultured in the context of the stromal environment? Stromal cells typically maintain an antiapoptotic environment through direct contact and through soluble mediators. Coculture of B-CLL cells with stromal cells provided substantial protection from curcumin-induced apoptosis at lower curcumin doses, but not at higher doses (20 μmol/L; ref. 1). These results lend insight into the effect of the host environment on curcumin-induced apoptosis and also indicate that higher therapeutic doses of curcumin may be required to achieve the level of apoptosis seen *in vitro*.

It is unlikely that curcumin alone will be an effective treatment for B-CLL due to incomplete apoptotic responses. Curcumin itself enhances the effectiveness of a variety of compounds, including vincristine, in B-CLL *in vitro* (6). Natural agents, such as curcumin, are desirable candidates for adjuncts to chemotherapy because of their negligible toxicities. EGCG, the principal polyphenol in green tea, induces apoptosis in B-CLL cells *in vitro* through partial inhibition of vascular endothelial cell growth factor receptor (VEGFR) 1 and VEGFR2 phosphorylation and also by caspase-3 activation and PARP cleavage. Bcl-2 is down-regulated by EGCG, as is Mcl-1 and XIAP (1). Treatment of primary B-CLL cells with the combination of EGCG and curcumin was investigated. The sequential administration of EGCG followed by curcumin was the most effective treatment combination, whereas simultaneous administration resulted in antagonistic effects.

EGCG and curcumin target many of the same molecular pathways, including induction of PARP cleavage and the inhibition of telomerase activity (1, 4, 5, 10). Although EGCG and curcumin down-regulate many common survival pathways, including STAT3, Akt, NF-κB, and the antiapoptotic genes *Mcl-1* and *XIAP*, not all of their targets overlap (Fig. 1). For example, although both compounds induce PARP cleavage and apoptosis in B-CLL, EGCG accomplishes this through activation of caspase-3, whereas curcumin does not induce caspase-3 activation in these cells (1). Thus, these two natural agents have many pathways in common but can diverge from each other as well and can potentially exhibit opposing effects. Hence, further study of the molecular mechanisms involved in the antiproliferative and proapoptotic effects of these agents is warranted.

Clinical trials with curcumin and EGCG as individual agents, or in combination with standard chemotherapy, are already underway in several cancers (4, 5, 11).<sup>1</sup> A phase II clinical trial of curcumin in advanced pancreatic cancer was recently published by our group and showed a patient with a 73% tumor regression and another patient who was stable on curcumin for more than 2.5 years (11).<sup>2</sup> No side effects were observed. However, overall response rates were low perhaps because curcumin absorption after oral administration is poor (11).

Oral green tea extracts have been taken voluntarily by patients with B-CLL, and EGCG has already begun phase I clinical trials in Rai stage 0 to II patients with B-CLL (1). The current study provides rationale for the combination of curcumin plus EGCG in the clinical setting and provides information about the dose and administration of these two compounds. A constant ratio of 10:1 (EGCG/curcumin) was established as effective at inducing apoptosis in B-CLL. Sufficiently high doses of EGCG and of circulating curcumin

<sup>1</sup> <http://clinicaltrials.gov>

<sup>2</sup> R. Kurzrock, unpublished data.

will be necessary to overcome stromal protection *in vivo*. The data also indicate that sequential treatment of B-CLL with EGCG followed by curcumin is preferable to simultaneous treatment and is crucial to the efficacy of the combination of these two compounds. Simultaneous treatment of B-CLL cells with EGCG and curcumin resulted in antagonistic effects. Ultimately, however, the optimal studies will probably require the use of a curcumin compound modified to enhance its bioavailability. Encapsulating curcumin in liposomes allows it to be administered systemically, and several groups are

working on altering curcumin to increase its absorption when taken orally (4, 5, 9, 13). Based on the biological properties of curcumin alone or in combination with EGCG or other compounds, these second-generation curcumin moieties should be explored for the treatment of CLL and other cancers.

### Disclosure of Potential Conflicts of Interest

R. Kurzrock, ownership interest, patent filed for liposomal curcumin, SignPath.

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*Clin Cancer Res* 2009;15:1123-1125.

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