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Green Tea Extract EGCG Induces Apoptosis in CLL Cells and Overcomes the Supportive Effect of Primary Bone Marrow Stromal Cells Through the Regulation of PI3K/Akt Cascade and Proteasome Activity

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Abstract

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There is accumulating evidence that green tea extract EGCG [(-)-epigallocatechin-3-gallate] may exert a preventive or a direct anti-tumor effect in several tumor types including chronic lymphocytic leukemia (CLL) and clinical trials with EGCG are already on-going. However, EGCG has a broad spectrum of activities and downstream targets. Therefore, it would be necessary to precisely characterize the key targets of this compound and identify the CLL patients who would most likely profit from EGCG. Therefore, the aim of this study was to evaluate the effect of EGCG on the viability of CLL cells in a well characterized cohort of patients and to get insight into its mechanism of action in CLL.

Peripheral blood mononuclear cells (PBMC) of 27 CLL patients were included in this study. Patients were characterized according to the Rai/Binet stage, IgVH mutation status and cytogenetics (13q-del, 11q-del, 17p-del, trisomy-12). The percentage of the leukemic cells (CD19+/CD5+) ranged between 60–98%. CLL cells were exposed to a wide range of concentrations of EGCG (0.1 – 200µM) and cell viability was evaluated by cell titer blue (CTB) assays and FACS analysis after 4 hours, 1, 2 and 3 days. Treatment with EGCG was performed in suspension cultures and under co-culture with primary human bone marrow stromal cells (BMSC).

Cell viability assays demonstrated a dose and time dependent decrease in the cell viability after the exposure to EGCG with an IC_{50} ranging between 50–80μM (25–50μg/ml). A moderate variation in the response to EGCG was observed between patients demonstrating the heterogeneity of the disease. No clear correlation between the in vitro response to EGCG and the clinical background and prognostic markers could be observed in this cohort of patients. Annexin V/propidium iodide (Anx/PI) staining showed that EGCG increased the percentage of early apoptotic (Anx+/PI-) and late apoptotic/necrotic cells (Anx+/PI+). These data suggest that EGCG exerts a pro-apoptotic effect and activates other cell killing mechanisms in CLL cells. The leukemic cells (CD19/CD5) were relatively more sensitive to the compound compared to T cells and monocytes. Co-culture experiments showed that EGCG effectively overcomes the supportive effect of BMSC and induces apoptosis/cell killing in CLL cells. BMSC were less sensitive to the compound and a toxic effect was observed at a concentration of 200 μM or higher. RT-PCR showed a downregulation of the catalytic domain p110a and the regulatory domain p85 of phosphoinositide 3-kinases (PI3K) as well as Bcl-2 and Mcl-1 mRNA expression after exposure to EGCG. Western blotting analysis demonstrated a decrease in the phosphorylation of Akt particularly at pThr308 residue and de-phosphorylation of the tumor suppressor PTEN at pSer380 residue in parallel to the induction of PARP cleavage. In addition, EGCG induced a decrease in the protein expression of the activation marker CD23 and the adhesion molecule CD44. Furthermore, proteasome assays showed that EGCG inhibits the chymotrypsin-like activity within 4 hours of incubation in parallel to induction of early apoptosis. This effect was more remarkable after 24 hours. However, EGCG was less effective in proteasome inhibition compared to Bortezomib.

In conclusion, these data demonstrate that EGCG induces cell death in CLL cells through a complex mechanism which may involve the inactivation of PI3K/Akt signaling cascade and inhibition of proteasome activity. The results also point to a potential therapeutic effect of EGCG in CLL which warrants further evaluation.

Disclosures:

No relevant conflicts of interest to declare.

Author notes

* Asterisk with author names denotes non-ASH members.