論文の内容の要旨

Dissertation Abstract

論文題目

Dissertation Title

EGCG suppressed the expression of FLT3 by disruption of FLT3/Hsp90 protein complex in AML cells

（変異 FLT3 を有する急性白血病細胞に対する EGCG の作用機序の解析）

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Background and Objectives

Acute myeloid leukemia (AML) is a heterogeneous disease characterized by a block in differentiation and uncontrolled proliferation. FLT3 is a commonly mutated gene found in AML patients. In clinical, the presence of a FLT3-ITD mutation significantly correlates with an increased risk of relapse and dismal overall survival. Therefore, activated FLT3 is a promising molecular target for AML therapies. Currently, several small molecule FLT3-tyrosine kinase inhibitors have been developed and examined in AML patients as single agents or in combination with chemotherapy. However, approval of these agents for FLT3-associated diseases is still challenging, which was suspected to be due to the failure to fully inhibit FLT3 in tumors and undesirable drug properties.

It is well documented that polyphenols of green tea, used as a beverage for over 5,000 years, have anti-cancer effects on many types of human malignancies, but not to their normal counterpart. My data so far has shown that green tea polyphenols (-)-epigallocatechin-3-gallate (EGCG) suppressed the cell proliferation, induced apoptosis in AMLs cell with FLT3 mutations. Interestingly, I found that EGCG has the ability to down-regulate the expression of FLT3 protein and suppress the activity of its downstream signaling molecules including AKT, MAPK and STAT5 [2]. However the exactly mechanism of FLT3 regulation by EGCG was not completely clarified.

In 2009, Yin et al. demonstrated that EGCG is a potential Hsp90 inhibitor [4]. Moreover, mutant FLT3 has been found to be a bona fide client protein for Hsp90 in cell models and primary AML cells [1, 3]. However, the chaperoning role of Hsp90 to wild type (WT) FLT3 is still in controversial. Thus, aims of my research are: (1) investigating the involvement of Hsp90 in the down-regulation of FLT3 protein level by EGCG, (2) investigating the influence of EGCG on FLT3 promoter activity and gene expression and (3) evaluating the combination effect of EGCG and PKC412 (FLT3 inhibitor) on AML cells with mutant FLT3.

Results and discussion

To investigate the involvement of Hsp90 in down-regulation of FLT3 protein level by EGCG, I first clarified whether FLT3-WT require Hsp90 as a molecular chaperone. The AML cells carrying FLT3-WT (THP-1), FLT3-ITD (MOLM-13; MV4-11) and FLT3-D835E (KOCL-48 cells) as well as 293FT cells-transiently overexpressing FLT3-WT or mutants were co-immunoprecipitated (co-IP) with Hsp90 antibody, then immunoblotted (IB) with FLT3 antibody. Interestingly, the endogenous FLT3-WT was not co-IP with Hsp90 in THP-1 cells but ectopic FLT3-WT expressed in 293FT was able to form a complex with Hsp90. Moreover, I found that the endogenous FLT3-WT in

THP-1 was not phosphorylated whereas ectopically expressed FLT3-WT in 293FT was phosphorylated. Using FLT3-WT/ITD/D835V-K644R mutants which are constitutively de-phosphorylated were not co-IP with Hsp90 in 293FT cells. These data indicated that Hsp90 selectively interacts with phosphorylated FLT3. Thus, it is highly possible that the phosphorylation status of FLT3 is critical for interaction with Hsp90 and FLT3-WT could bind to Hsp90 if it was phosphorylated.

Upon confirming that Hsp90 selectively binds to phosphorylated FLT3, I postulated that the suppression of cellular FLT3 protein level by EGCG could be mediated through inhibition of Hsp90 function by EGCG. Indeed, EGCG disrupted the interaction between Hsp90 and FLT3 protein in 293FT and MOLM-13 cells. As a result, EGCG could inhibit FLT3-WT expression as effective as FLT3-ITD and FLT3-D835V did in transient 293FT- and 32D- stable expression FLT3-WT cells. Interestingly, the stability of endogenous FLT3-WT in THP-1 cells was not significantly influenced by EGCG treatment, I assume that this is mainly because that FLT3-WT in THP-1 is not phosphorylated, thus not binding to Hsp90.

Moreover, I at the first time suggest that others polyphenols including epigallocatechin (EGC), epicatechin-3-gallate (ECG) might also suppressed the expression of FLT3 protein through inhibition of Hsp90 function by the same mechanism as EGCG did.

I also found that EGCG reduced the transcription level of *FLT3* by suppressing its promoter activity. Recently, others groups reported MYB, HoxA9, Meis1 and C/EBPα were regulated the transcription of FLT3. By doing gene expression profile analysis in MOLM-13 cells in time dependent manner, I have found that the expression of mRNA of *FLT3* was first observed to down-regulate at 6 hours together with the decreasing of *Hoxa9* after EGCG treatment. The changing of *C/EBPα* expression was found at 8 hours. Interestingly, the reducing mRNA of *c-Myb* by EGCG was observed at 4 hours, earlier than *FLT3* was dow-regulated. There is no changing in *Meis1* by EGCG was observed. I also found the protein level c-Myb was inhibited by EGCG in MOLM-13 and MOLM-14 cells after treated these cells with 60μM of EGCG for 8 hours. These data indicated the involvement of transcription factors in controling the expression of FLT3 by EGCG.

The particularly important issue that prevents the clinical use of EGCG is that the levels of EGCG are super-physiological (from 20 to 200 μM) and such concentrations cause cytotoxic effects to normal cells, and potentially cause unwanted side effects. In my study, by combination with PKC412, a physiological serum concentration of EGCG (<10 μM) can be achieved. For examples, when combined with PKC412, the concentration of EGCG will reduce to 10 μM (with 5nM PKC412) and even 5 μM (with 7nM PKC412) in MOLM-13 cells, suggesting a suitable strategy for using EGCG in clinical.

Conclusions

Taken together, I clarified that EGCG destabilized FLT3 protein by disrupting its interaction with the molecular chaperone, Hsp90 (figure 1). I have shown evidence that EGCG suppressed *FLT3* promoter activity and its transcription. Thus, EGCG could be an attractive reagent for regulation of FLT3 expression at transcription and protein levels. In addition, I propose that EGCG treatment could be a useful therapeutic approach for AML patients, especially when it is used in combination with other drugs, such as a FLT3 inhibitor, PKC412.

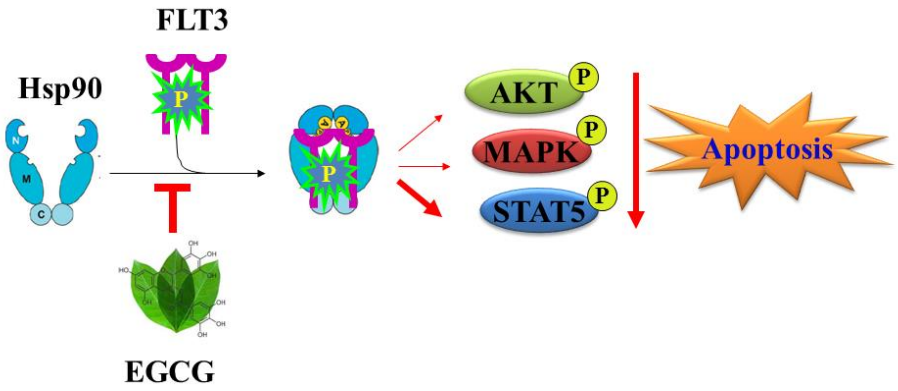

Figure 1: EGCG destabilized FLT3 protein by disrupting its interaction with Hsp90

References:

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