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EGCG Induce Internalization of Epidermal Growth Factor Receptors (EGFR)

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Abstract

EGCG, the major biologically active constituent of green tea, inhibits activation of the EGFR and its downstream signaling pathways in several types of human cancer cells. We recently discovered that this inhibition is associated with internalization of the EGFR.

Although the precise mechanism of ligand-induced EGFR internalization is still unclear, some reports suggest that a src family non-receptor tyrosine kinase (SFK) may play a role in this process. Therefore, we hypothesized that a src family kinase may also play a role in EGCG-induced EGFR internalization. Using SW480 cells, we conducted a time course study and found that EGCG can activate c-src in a dose- and time-dependent manner. Activation was detected with as low as 1μM of EGCG and within 10min. This time course is consistent with our previous findings that EGCG can induce EGFR internalization within 30 min. Next, we investigated the effect of EGCG on the level of cell surface EGFR using a quantitative ELISA assay, and found that EGCG 40μM causes a significant decrease in the amount of cell surface EGFR. This reduction was partially rescued by the src kinase specific inhibitor PP1. We confirmed these findings using fluorescence microscopy by demonstrating that combined treatment with EGCG and PP1 decreased the amount of internalized vesicles containing EGFR.

We examined whether EGCG can bind to the c-Src protein by affinity chromatography using EGCG-coupled Sepharose beads. SW480 total cell lysates were applied to the EGCG affinity column and eluted fractions were analyzed by western blot analysis. c-Src was detected in the fraction containing proteins that bind to EGCG with high affinity.

Taken together, our studies provide the first evidence that EGCG can bind to and preferentially activate c-src and that this activation may play a role in EGCG-induced internalization of the EGFR in SW480 cells. However, this activation of c-src does not activate the EGFR and its downstream signaling pathways. Our findings suggest a novel mechanism by which EGCG inhibits the EGFR pathway. Further studies will be required to determine the significance of c-src activation by EGCG with respect to its anti-cancer effects.

Introduction

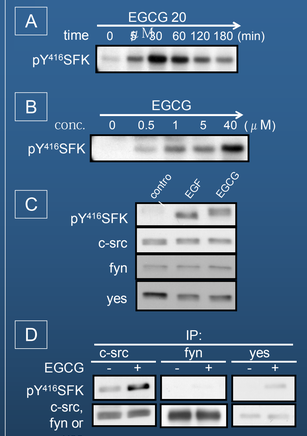
Accumulating evidence suggests that consumption of tea, especially green tea, may prevent certain types of cancer. Some epidemiological studies have revealed that there is an inverse correlation between increased green tea intake and relative risk for specific cancers. Numerous studies in rodents have also shown anticancer effects of extract of green tea. To elucidate the mechanisms of these anticancer effects, considerable green tea, effort has been devoted to investigating the anticancer effects of (-)-epigallocatechin-3-gallate (EGCG), a major component of green tea.

EGCG has been shown to inhibit the growth of several types of human cancer cell lines. This is associated with inhibition of phosphorylation of the EGFR and inhibition of several downstream signaling pathways. EGCG can also inhibit activation of other RTKs, including HER2, HER3, HER4, IGF1-R, PDGFR and FGFR.

Although it was reported that the binding of EGF to EGFR was inhibited by EGCG, the precise mechanism by which EGCG acts on the EGFR is not known. In this report, we show that EGCG causes internalization of the EGFR into vesicles and that activation of c-src plays a role in this process of internalization.

Results

Fig.1 **EGCG causes phosphorylation of src family kinases (SFK) and c-src is preferentially activated.**



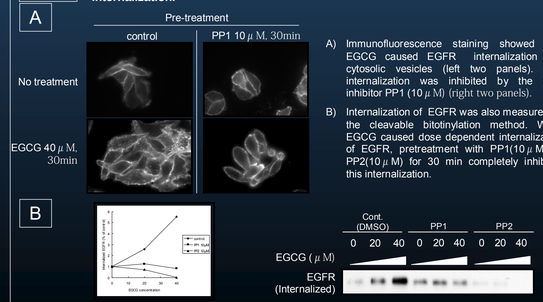
A) SW480 human colon cancer cells were treated with 20 μM EGCG for the indicated times. SFK activation (phosphorylation) was detected within 5min, and diminished after 30min.

B) SW480 cells were treated increasing concentration of EGCG for 10min. SFK activation was detected with as little as 0.5μM EGCG and increased with higher doses.

C) Western blot of total cell lysate indicated that both EGF and EGCG caused SFK activation by EGCG or EGF. Although both EGF and EGCG activated SFK, the activated band with EGCG had a slower mobility than that with EGF.

D) Western blot analysis of immunoprecipitates obtained with anti- c-src, anti-fyn or anti-yes antibodies showed that these three members were expressed in SW480 and that EGCG preferentially activated c-src (Fig.1D). Yes was also slightly activated. Activation of fyn was not detected in SW480.

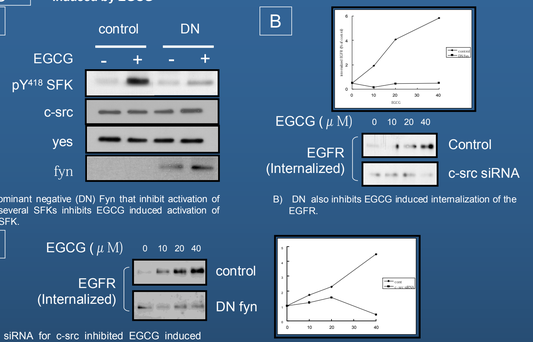
Fig.2 **SFK specific tyrosine kinase inhibitors inhibited EGCG induced EGFR internalization.**



A) Immunofluorescence staining showed that EGCG caused EGFR internalization into cytosolic vesicles (left two panels). This internalization was inhibited by the SFK inhibitor PP1 (10 μM) (right two panels).

B) Internalization of EGFR was also measured by the cleavable bitotinylation method. While EGCG caused dose dependent internalization of EGFR, pretreatment with PP1(10 μM) or PP2(10 μM) for 30 min completely inhibited this internalization.

Fig.3 **Dominant Negative for SFK and siRNA for c-src inhibit EGFR internalization induced by EGCG**

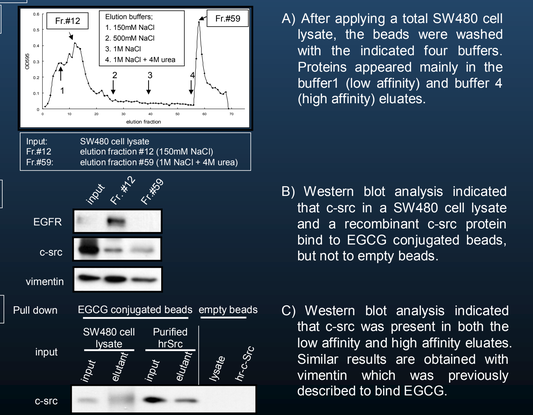


A) Dominant negative (DN) Fyn that inhibit activation of several SFKs inhibits EGCG induced activation of SFK.

B) DN also inhibits EGCG induced internalization of the EGFR.

C) siRNA for c-src inhibited EGCG induced EGFR internalization.

Fig.4 **EGCG binds to c-src**



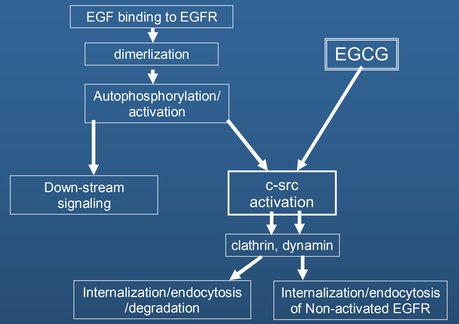
A) After applying a total SW480 cell lysate, the beads were washed with the indicated four buffers. Proteins appeared mainly in the buffer1 (low affinity) and buffer 4 (high affinity) eluates.

B) Western blot analysis indicated that c-src in a SW480 cell lysate and a recombinant c-src protein bind to EGCG conjugated beads, but not to empty beads.

C) Western blot analysis indicated that c-src was present in both the low affinity and high affinity eluates. Similar results are obtained with vimentin which was previously described to bind EGCG.

Summary

Fig.5 **Hypothetical Model of the Role of Src in Causing Internalization of the EGFR**



1. Treatment of SW480 with EGCG causes internalization of the EGFR and this is associated with phosphorylation (activation) of c-src. This occurred with as little as 1 μM EGCG and within 10min of exposure to EGCG.
2. The SFK inhibitors, pp1 and pp2, a dominant negative fyn and siRNA for c-src inhibit EGCG induced internalization of the EGFR.
3. Affinity chromatography indicated that EGCG can bind directly to c-src.
4. A hypothetical model that may explain these results is shown in Fig. 5.

References

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