EGCG, the major biologically active constituent of green tea, inhibits activation of the EGFR and its downstream signaling pathways in several types of 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. We conducted a time course study and found that EGCG can activate c-src in a dose- and time-dependent manner. Activation was detected as early as 15 min after treatment of SW480 cells with 1 M EGCG and within 10 min. This time course is consistent with our previous findings that EGCG can induce EGFR internalization within 30 min. Our results indicate that the reduction was partially reversed by an src kinase specific inhibitor PPI. We confirmed these findings using fluorescence microscopy by demonstrating that combined treatment with EGCG and PPI decreased the amount of internalized vesicles containing EGFR.

We examined whether EGCG can bind to the Src protein by affinity chromatography using EGCG-coated nickel beads. SP480 total cell lysates were eluted to the EGCG affinity column and eluted fractions were analyzed by Western analysis. C-src was detected in the fractions that bound 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. Our data suggest that c-src may play a novel role in the internalization pathway of the EGFR and that this effect can be reversed by the specific SFK inhibitor PP1.

Materials and Methods

Accumulating evidence suggests that consumption of tea, especially green tea, may prevent certain types of cancer. Some epidemiological studies have shown that there is an inverse correlation between increased green tea intake and relative risk for specific cancers. To study the possible anti-cancer effects of extract of green tea, we have conducted an in vitro study of EGCG on human colon cancer cell line SW480. This line is associated with phosphorylation (activation) of c-src. This occurred with as little as 0.5 μg/ml EGCG and increased with higher concentrations of EGCG.

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Results

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Summary

1. Treatment of SW480 with EGCG causes internalization of the EGFR and this is associated with inhibition of phosphorylation of the EGFR. This occurred with as little as 1 μM EGCG and within 30 min of exposure to EGCG.

2. Src family kinases, c-src and fyn, are expressed in SW480 cells. We show that EGCG preferentially activates c-src in these cells.

3. Affinity chromatography showed that EGCG can bind directly to c-src.

4. A hypothetical model that may explain these results is shown in Fig. 5.