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Tea catechins inhibit hepatocyte growth factor receptor (MET kinase) activity in human colon cancer cells: kinetic and molecular docking studies

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Abstract

Most cancer deaths result from spread of the primary tumor to distant sites (metastasis). MET is an important protein for metastasis in multiple tumor types. Here we report on the ability of tea catechins to suppress MET activation in human colon cancer cells, and propose a mechanism by which they might compete for the kinase domain of the MET protein.

Hepatocyte growth factor receptor, also known as MET or c-Met^a, is important during embryonic development and wound healing.¹ Its ligand is hepatocyte growth factor (HGF), also known as scatter factor. MET is deregulated in a variety of tumor types and is associated with poor prognosis.¹

Green tea (*Camellia sinensis*) and its polyphenolic compounds, the catechins, have been studied for their ability to prevent and treat chronic conditions such as cancer, cardiovascular diseases, neurodegenerative disorders, diabetes, and osteoporosis.² Green tea catechins include (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), and (-)-epigallocatechin gallate (EGCG). (-)-Catechin (CAT) is the epimer of EC, whereas gallocatechin gallate (GCG) is the epimer of EGCG (Figure 1).

Tea catechins recently were reported to inhibit MET signaling in breast³ and hypopharyngeal carcinoma cells;⁴ however, the mechanism(s) were not examined. In the present investigation, HCT116 human colon cancer cells were treated with the various compounds shown in Figure 1, and the cell lysates were subjected to immunoblotting for phospho-MET. As expected, HGF

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^aAbbreviations:

MET/c-Met	hepatocyte growth factor receptor
HGF	hepatocyte growth factor
GA	gallic acid
CAT	(-)-catechin
EC	(-)-epicatechin
EGC	(-)-epigallocatechin
ECG	(-)-epicatechin gallate
EGCG	(-)-epigallocatechin gallate
GCG	(-)-gallocatechin gallate

alone caused an increase in phospho-MET expression, but this was inhibited markedly by the gallate-containing catechins ECG, EGCG and GCG (Figure 2).

An *in vitro* MET kinase activity assay next was performed (Figure 3). Consistent with findings from the cell culture experiments, ECG, EGCG and GCG inhibited MET kinase activity, whereas the other test compounds had little or no effect. For ECG, EGCG and GCG, inhibition occurred in a dose-dependent manner, and the concentration for 50% inhibition (IC_{50}) for each compound was $\sim 10 \mu M$.

Because EGCG is the most abundant catechin in green tea, and it was an effective inhibitor in the MET kinase activity assay, EGCG was selected for further kinetic analyses (Figure 4). The Cornish-Bowden plot ($[S]/V$ versus $[I]$) produced a series of parallel lines, whereas the Dixon plot ($1/V$ versus $[I]$) had lines that intersected above the x -axis. These findings are consistent with reversible competitive enzyme inhibition.⁸⁻¹⁰ Based on linear regression analysis of the Dixon plot (Figure 4B), the inhibitor constant (K_i) was determined to be $3.3 \mu M$ EGCG.

Molecular docking was used to investigate how the tea catechins might interact with MET. The MET kinase domain first was constructed using the experimentally resolved crystal structures available in the Protein Data Bank, with access code 3CD8,⁵ and energetically minimized in the internal coordinate space.^{6,7} To validate the model, a known MET inhibitor⁵ was docked within a $4 \text{ \AA}$ sphere around its 3D-position in the crystal structure. The inhibitor docked with a score of -44 , having a low all atoms-RMSD as compared to the crystallographic orientation. The conformation of the inhibitor after docking was “U-bended” (Figure 5, in orange/blue), with hydrogen bond interactions water-mediated between N1 and the quinoline aromatic nitrogen and the backbone -NH of residues Asp1222 and Met1160, respectively, as described in the literature.⁵

Using the same iterative approach, EGCG was docked into the MET kinase domain. The tea catechin fit favorably into the MET binding site, with a score of -20.08 (Figure 5, in purple). Two of the hydroxyl groups of the gallate moiety interacted with the backbone -NH of Met1160 and Pro1158.

When the MET inhibitor⁵ and EGCG alignments were superimposed, the quinoline ring of the former molecule and the gallate phenyl ring of EGCG were orientated in a similar manner in the crystal structure (Figure 5). Thus, the hydrogen bond interaction between the ligand (through -OH groups or the aromatic quinoline nitrogen) and the backbone of Met1160, which was observed in the crystal structure⁵ and from docking studies, appears to be an important feature for binding to the kinase domain.

The docking score of EGCG was ~ 2 -fold lower than for the triazolopyridazine inhibitor,⁵ which predicts a lower affinity of the tea catechin for MET.

Additional tea catechins (Figure 1) were docked into the MET kinase domain. Two docking orientation clusters were identified: GCG, EGCG, ECG (Figure 6) and CAT, EC, EGC (Figure 7), with docking scores of approximately -20 and -10 , respectively. Thus, the gallate group was identified as a key structural feature for binding of tea polyphenols to the MET kinase domain. The gallate-containing catechins were observed to allocate the 3-gallate phenyl ring in the same region of the binding pocket. In this position, hydrogen bonding was established between the aromatic hydroxyl groups of the gallate moiety and the backbone -NH of Met1160 and Pro1158 (with the exception of GCG with no hydrogen bond to Pro1158). In contrast, catechin derivatives lacking the gallate group did not exhibit affinity for Met1160, but interacted with the backbone -NH of Asp1222. This was associated with a lower overall docking score. Thus, from modeling studies we predict the binding affinity towards MET will

be in the relative order: triazolopyridazine inhibitor⁵ > gallate-containing catechins > non-gallate-containing compounds.

It is noteworthy that the computational predictions were in general agreement with the immunoblotting data for phospho-MET (Figure 2) and the MET kinase enzyme activity data *in vitro* (Figure 3), which revealed little if any inhibition by CAT, EC, and EGC, and intermediate inhibition by ECG, EGCG, and GCG, compared with the strong inhibition reported previously for the triazolopyridazine compound.⁵ One apparent limitation of the modeling approach is that a 2-fold difference in docking score can exist in the face of a larger range of IC₅₀ values.

In conclusion, gallate-containing tea catechins were effective at suppressing MET activation in human colon cancer cells. Enzyme kinetic studies and computational analyses supported a model in which the gallate moiety was bound to the kinase domain of the MET receptor. Recent work has shown that the 90 kDa heat shock proteins (Hsp90) contain a C-terminal nucleotide binding pocket that not only binds ATP, but also cisplatin, novobiocin, taxol, and EGCG.¹² Tea polyphenols and other flavonoids interact with the ATP-binding site of several kinases, possibly as a mimetic of the adenine moiety of ATP.¹³ The ATP binding site of MET also has been used in high-throughput virtual screening for potential new enzyme inhibitors, and some of the candidates identified had IC₅₀ values in the μ M range.¹⁴ The present investigation has revealed that several tea catechins also inhibit MET, and are effective in the 1–10 μ M range. With pharmacologic oral dosing of EGCG, peak plasma concentrations of 7.5 and 9 μ M EGCG were detected in humans and mice, respectively.¹⁵ These concentrations also may be achievable in the gastrointestinal tract following more typical green tea intake, despite extensive methylation, glucuronidation and sulfation, or conversion to valerolactone breakdown products by the gut microflora.^{15,16} It should be noted that despite the promising mechanistic data obtained here and elsewhere using *in vitro* or preclinical models,¹⁵ in humans the evidence is less compelling for chemoprevention by tea.² In the therapeutic setting there is a clear need for further investigation, including effects of tea on MET and other pathways implicated in metastasis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Structure and nomenclature of tea catechins.

Figure 2.

Tea catechins inhibit MET activation in human colon cancer cells. A, immunoblots of whole cell lysates; B, corresponding densitometry data, with phospho-MET normalized to total MET; loading control, β -actin.

Figure 3. Tea catechins inhibit MET kinase activity *in vitro*. Data = mean $\pm$ SD, $n = 3$.

Figure 4. Kinetic analyses of MET kinase inhibition by EGCG. A, Cornish-Bowden plot; B, Dixon plot ($K_i = 3.3\mu\text{M}$).

Figure 5. Modeling of the MET kinase active site with bound inhibitors. Triazolopyridazine inhibitor (orange), listed as compound [4] previously,⁵ and EGCG (purple).

Figure 6. Modeling of the MET kinase active site with bound EGCG (purple), GCG (green) and ECG (blue).

Figure 7.
Modeling of the MET kinase active site with bound CAT (purple), EC (green) and EGC (blue).