Tumor invasion: molecular shears blunted by green tea

In the same time, the inhibition of angiogenesis is a very appealing result for humans: the indication is that plasma concentrations of EGGC after the consumption of two or three cups of tea (0.1-0.3 μM) are sufficient to exert such a promising biological activity.

In our search for the molecular mechanisms influenced by EGGC, we first examined by zymography the direct effect of EGGC on gelatinolytic activity. EGGC exerts dose-dependent inhibition of both MMP-2 and MMP-9 (Fig. 1a). The concentrations giving 50% inhibition (IC50) were 20 and 50 μM, respectively (lowest registered values 8 and 25 μM), considerably lower than values reported to inhibit uronidase (4 mM). The molar ratio is too low to account for inhibition through chelation of calcium, essential for enzymatic activity.

Using a modified Boyden chamber assay, we then tested the invasive behavior of MMP-2- and MMP-9-expressing cancer cells in the presence of increasing concentration of EGGC. Migration through gelatin-coated filters to a chemoattractant (chemotaxis) remained unaffected, excluding cytoskeleton or cell motility impairment. In the same conditions, migration through a reconstituted basement membrane matrix, which mimics the normal anatomical barrier, was inhibited (chemoinvasion) with an IC50 less than 0.1 μM EGGC (Fig. 1b). These results may help resolve an apparent contradiction: certain MMPs, such as MMP-3, -7, -9 and -12, in addition to their essential role in tumor and endothelial cell invasion, can also contribute to the generation of angiostatin, a potent angiogenesis inhibitor derived by cleavage of plasminogen. Our results indicate that EGGC, as direct inhibitor of MMPs, inhibits angiogenetic and metastasis upstream of the action of angiostatins inhibitors derived from the action of MMPs.

Our data show that the EGGC concentration effective in inhibiting MMP-2 and MMP-9 is of orders of magnitude lower than that reported for uronidase (down to 1/500), and that even lower concentration (equivalent to that in plasma of drinkers of moderate amounts of green tea) is effective in reducing tumor cell invasion by 50%. We suggest that green tea exerts its beneficial effects against cancer by impairing tumor invasion and nourishment through direct inhibition of two gelatinases that open up pathways for cell migration. Furthermore, in addition to having a preventive role, EGGC may be effective in combination with angiostatin (inhibitor of endothelial cell proliferation) in a dual-action clinical treatment.

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Fig. 1. a, Zymographic evidence of direct suppression of MMP-2 activity. Human cultured endothelial cells were separated by electrophoresis in 0.1% gelatin-containing polyacrylamide. After removal of SDS, separate lanes were incubated overnight at 37 °C in Trit-Car gel buffer. After polymerization of gelatin, the gelatinolytic activity of the buffer was unaltered at highest concentration. Inset, Coomassie blue staining. Densitometric values of the gelatin bands were plotted for gelatin (a) and activated (b) forms of gelatinase. b, HT1080 cells (expressed as % of control) cultured for 24 h on the bottom surface of the Boyden chamber filter after rinsing gelatin (chemoinvasion) and Matrigel (chemoinvasion) coatings, towards a chemoattractant, a.s.Int of triplicate experiments.