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Screening of synergistic interactions of epigallocatechin-3-gallate with antiangiogenic and antitumor compounds

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ABSTRACT

Purpose: To screen for possible synergistic interactions of epigallocatechin-3-gallate (EGCG) with a selection of 10 anti-angiogenic or anti-tumor compounds on the survival of endothelial and tumor cells.
Methods: Human HMEC endothelial and MDA-MB231 breast cancer cells were treated with different concentrations of EGCG and the 10 tested compounds either as single agents or in paired combinations with EGCG for 3 days and final survival of cells was determined by the MTT assay. IC_{50} values, sensitization factors and combination indexes were calculated.
Results: IC_{50} values of 140 ± 2 and 45 ± 6 μM were determined for EGCG-treated endothelial and tumor cells, respectively. IC_{50} values for all tested compounds were within the micromolar and the submillimolar range. The values of the sensitization factor increased and those of the combination index decreased for paired combinations of EGCG with 4-methylumbelliferone. The opposite was true for the combination of EGCG with vitamin D_3. Other tested combinations did not exhibit a clear monotonic effect but rather a biphasic behaviour.
Conclusion: Combinations of EGCG and 4-methylumbelliferone synergistically decrease endothelial and tumor cell survival. In contrast, the presence of EGCG antagonizes with the antiproliferative effect exerted by vitamin D_3 on endothelial and tumor cells.

1. Introduction

Solid cancers have several common characteristics that Hanahan & Weinberg named as the hallmarks of cancer [1]. Angiogenesis is an essential hallmark of cancer because tumor cells need oxygen and nutrients delivered by the vascular system [2]. In fact, tumor growth and metastasis are angiogenesis dependent processes, and microvascular endothelial cells recruited by tumors have become an important target in cancer therapy [1–3].

Drug discovery efforts have identified several potential therapeutic targets in endothelial cells and selective inhibitors capable of slowing tumor growth or producing tumor regression by blocking angiogenesis in in vivo tumor models [4]. However, the currently available therapies have limited success in patients, due to complex mechanisms of resistance of the tumor cells [5,6]. One approach to overcoming these problems is to use combinations of drugs with different modes of action that may lead to enhanced antitumor and antiangiogenic effects without injuring the host [5–7]. The combined use of two drugs may sometimes produce enhanced, unchanged or diminished effects in comparison with their individual effects. These three different types of behaviour of the interacting drugs are called synergy, additive/indifferent and antagonistic effects [8].

Our research group is devoted to the screening, identification and characterization of new modulators of angiogenesis. In the last years we have identified a number of natural compounds as new inhibitors of angiogenesis [see for instance [9–14]]. We have also claimed for the need of combinatorial approaches to manage angiogenesis [5].

Epigallocatechin-3-gallate (EGCG) is an abundant polyphenol in green tea leaves with a number of biological activities, including antiangiogenesis [5], anticarcinogenesis [16–18], antimetastasis [19], as well as cancer chemoprevention, among others [20]. Furthermore, our group has previously shown that EGCG is a potent antiinflammatory compound [21,22] targeting histidine decarboxylase [23–25].

In the present work, we analyze paired combinations of EGCG with other 10 bioactive compounds (see Table 1), some described

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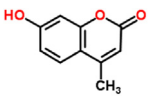
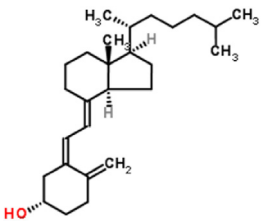
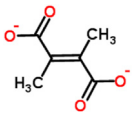
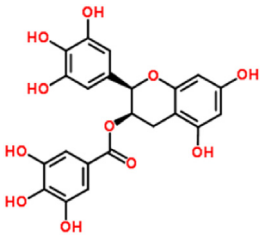
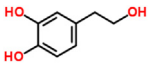
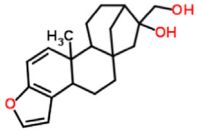
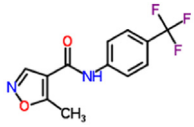
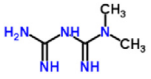
Table 1
Names, structures and molecular targets of the compounds tested in this study.

Compound	Molecular structure	Describe as . . .	Molecular target
4-methylumbelliferone (4-MU)		Anti-angiogenic	Inhibitor of hyaluronic acid synthesis
Cholecalciferol (Vit D_3)		Anti-tumoral	Inhibitor signalling of Wnt pathway
Dimethylfumarate (DMF)		Anti-angiogenic	Inhibitor of VEGFR-2
Epigallocatechin gallate (EGCG)		Anti-angiogenic	Inhibitor of topoisomerases
Hydroxytyrosol (HT)		Anti-angiogenic	Inhibitor of MMP-2 activity
Kahweol		Anti-angiogenic	Inhibitor of MMP-2, uPA activity and VEGF
Leflunomide		Anti-angiogenic	Inhibitor of polymerization of β-tubulin
Metformin		Anti-tumoral	Inhibitor of TOR pathway