# Metabolic Profiling-based Data-mining for an Effective Chemical Combination to Induce Apoptosis of Cancer Cells

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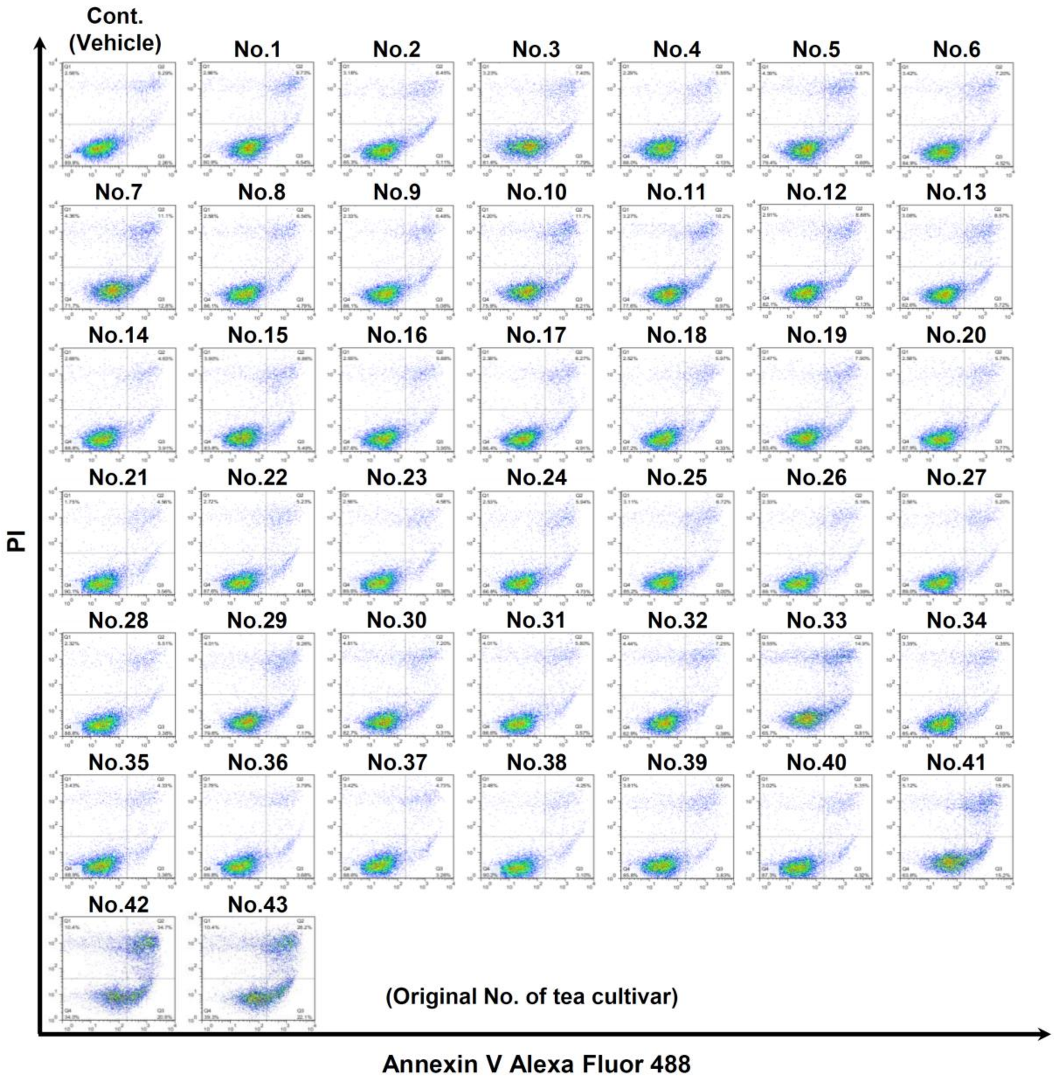
| Original No. | Cultivar | Apoptosis-inducing activity (%) | Rank | Total Polyphenol (mg/mL) |
|---|---|---|---|---|
| 1 | Seishin-oolong | 14.6 | 12 | 2.33 |
| 2 | Fukumidori | 13.5 | 14 | 1.51 |
| 3 | Benifuji | 18.7 | 8 | 2.00 |
| 4 | Minekaori | 9.7 | 29 | 1.53 |
| 5 | Benihikari | 15.9 | 10 | 2.22 |
| 6 | Minamikaori | 10.4 | 26 | 1.66 |
| 7 | Benihomare | 23.1 | 5 | 2.60 |
| 8 | Izumi | 11.9 | 19 | 1.82 |
| 9 | Fuusyun | 11.3 | 22 | 1.58 |
| 10 | Tamamidori | 19.4 | 7 | 1.59 |
| 11 | Ohba-oolong | 20.0 | 6 | 1.81 |
| 12 | Seishintaipan | 15.1 | 11 | 3.34 |
| 13 | Kuritawase | 12.4 | 16 | 1.88 |
| 14 | Syunmei | 8.5 | 37 | 2.59 |
| 15 | Sayamamidori | 13.8 | 13 | 2.03 |
| 16 | Asagiri | 10.0 | 28 | 1.82 |
| 17 | Hokumei | 10.2 | 27 | 1.79 |
| 18 | Asahi | 10.4 | 25 | 1.34 |
| 19 | Sayamakaori | 13.5 | 15 | 1.79 |
| 20 | Meiryoku | 9.3 | 30 | 1.54 |
| 21 | Kanayamidori | 8.8 | 36 | 2.09 |
| 22 | Yamatomidori | 8.9 | 32 | 1.63 |
| 23 | Asatsuyu | 7.9 | 42 | 1.82 |
| 24 | Toyoka | 11.5 | 21 | 2.34 |
| 25 | Yaeho | 11.3 | 23 | 1.79 |
| 26 | Ujihikari | 8.3 | 39 | 1.25 |
| 27 | Ooiwase | 8.3 | 40 | 1.29 |
| 28 | Gokou | 8.9 | 33 | 1.94 |
| 29 | Inzatsu131 | 16.1 | 9 | 1.41 |
| 30 | Surugawase | 11.8 | 20 | 1.23 |
| 31 | Samidori | 9.1 | 31 | 1.71 |
| 32 | Komakage | 11.9 | 18 | 1.55 |
| 33 | Hatsumomiji | 25.2 | 4 | 1.54 |
| 34 | Ryoufuu | 12.0 | 17 | 1.58 |
| 35 | Minamisayaka | 8.3 | 41 | 2.38 |
| 36 | Saemidori | 7.6 | 43 | 1.37 |
| 37 | Okuyutaka | 8.3 | 38 | 1.54 |
| 38 | Okumidori | 7.0 | 44 | 1.51 |
| 39 | Yutakamidori | 10.8 | 24 | 1.44 |
| 40 | Yabukita | 8.8 | 34 | 1.50 |
| 41 | Benifuuki | 29.3 | 3 | 1.81 |
| 42 | Nou6 | 55.3 | 1 | 1.42 |
| 43 | Sunrouge | 50.1 | 2 | 1.90 |

**Supplementary Table 1**

**Rank order of apoptosis induction potency of the leaf extracts from 43 Japanese green tea cultivars on human MM cells.**

Apoptosis-inducing effects of GTEs were measured by annexin/PI double staining as described in Figure 1. The percentages of annexin-$V^+$ cells were calculated by combining annexin $V^+/PI^-$ (early annexin V-positive) and annexin $V^+/PI^+$ (late annexin V-positive). Apoptotic cells were Original No shows the sample No in Figure1.




**Supplementary Figure 1**

**Apoptosis induction potency of the leaf extracts from 43 Japanese green tea cultivars on human MM cells.**

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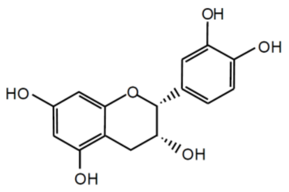
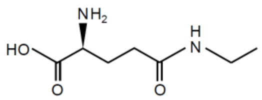
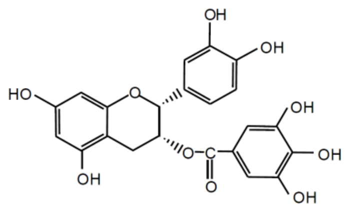


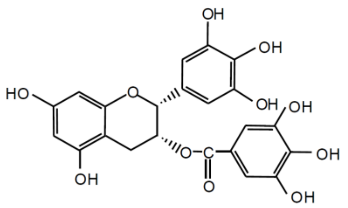
(-)-Epicatechin (EC)



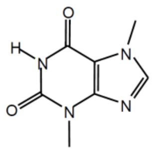
Theanin



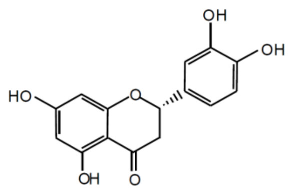
(-)-Epicatechin-3-*O*-gallate (ECG)



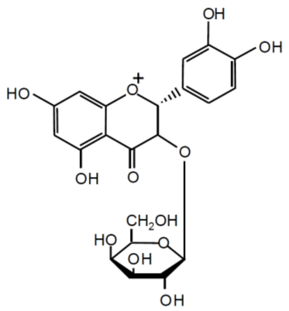
(-)-Epigallocatechin-3-*O*-gallate (EGCG)



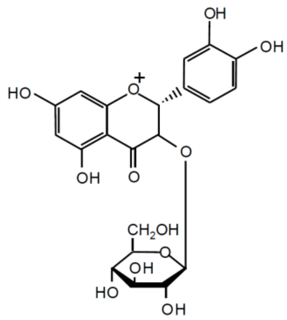
Theobromine



Eriodictyol



Cyanidin-3-*O*-galactoside
(Cya-gal)



Cyanidin-3-*O*-glucoside
(Cya-glu)

**Supplementary Figure 2**

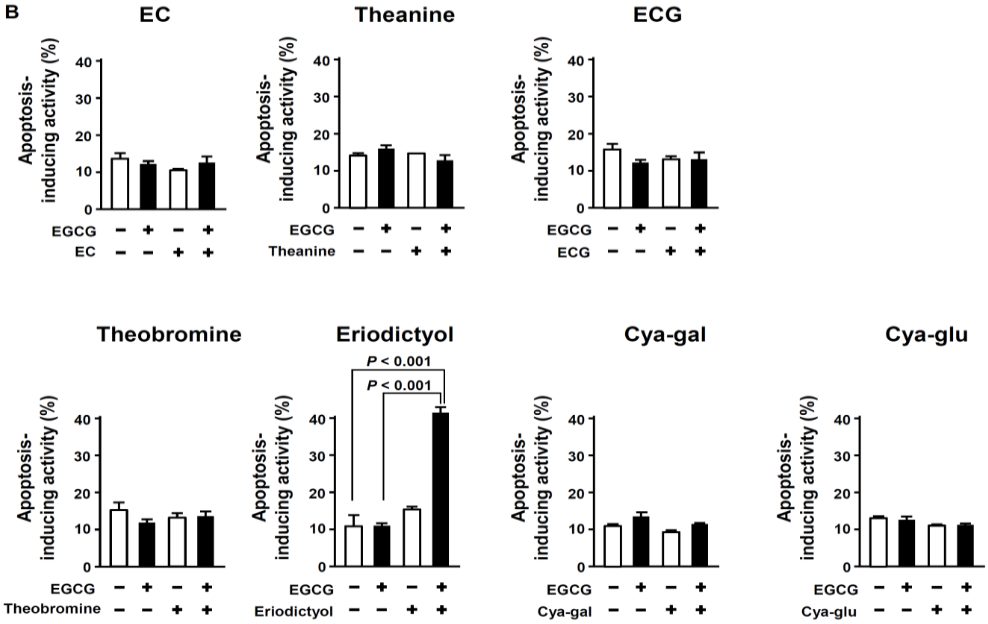
**Chemical structure of metabolites with high VIP values.**

Chemical structure of the candidate potentiator of the EGCG anti-MM effect.

A

| VIP Rank | VIP Value | Observed *m/z* | Theoretical *m/z* | Compound |
|---|---|---|---|---|
| 1 | 6.13 | 289.071 − | 289.071 [M – H]$^-$ | EC |
| 2 | 5.68 | 175.106 + | 175.108 [M + H]$^+$ | Theanin |
| 3 | 5.01 | 441.083 − | 441.082 [M – H]$^-$ | ECG |
| 4 | 4.31 | 345.076 + | — | unknown |
| 5 | 4.20 | 392.033 − | — | unknown |
| 6 | 3.89 | 479.083 − | — | unknown |
| 7 | 3.80 | 459.085 + | 459.093 [M + H]$^+$ | EGCG |
| 8 | 3.31 | 337.091 − | — | unknown |
| 9 | 3.09 | 457.167 − | — | unknown |
| 10 | 3.09 | 457.080 − | 457.077 [M – H]$^-$ | EGCG |
| 11 | 3.03 | 337.155 + | — | unknown |
| 12 | 3.01 | 181.070 + | 181.073 [M + H]$^+$ | Theobromine |
| 13 | 2.90 | 357.102 − | — | unknown |
| 14 | 2.87 | 289.066 + | 289.071 [M + H]$^+$ | Eriodictyol |
| 15 | 2.74 | 449.102 + | 448.100 [M + H]$^+$ | Cya-gal/-glu |

B

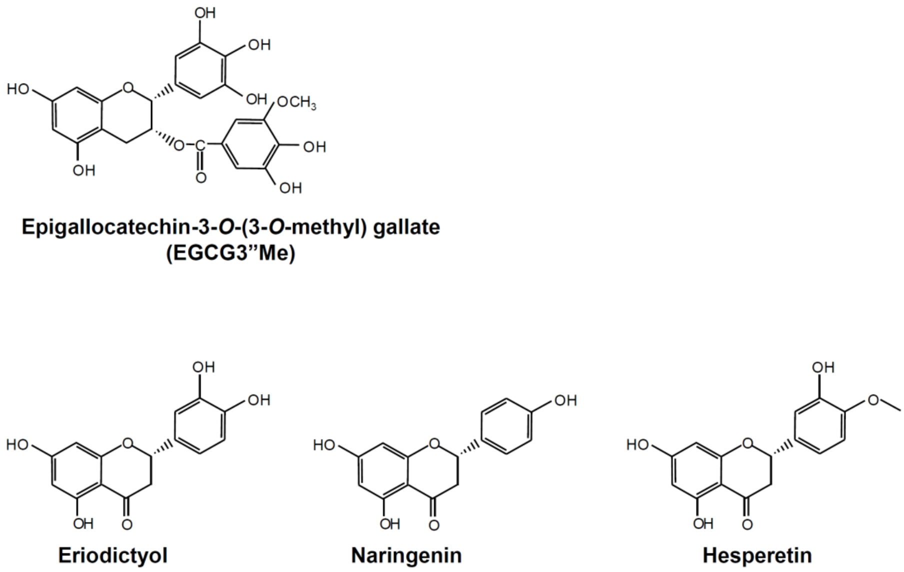



**Supplementary Figure 3**

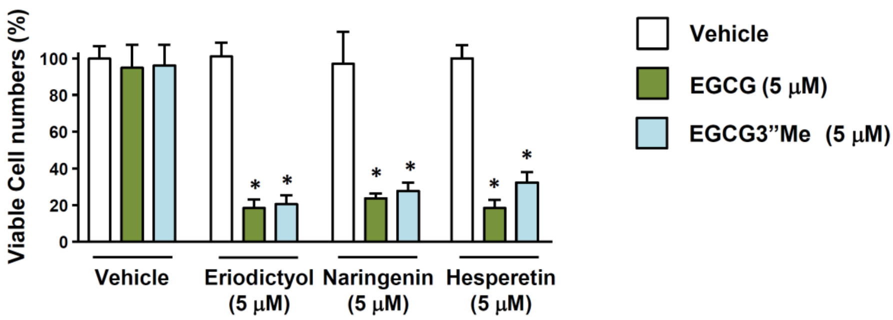
**The evaluation of the effects of compounds with high VIP values on the anti-MM effect of EGCG.**

(A) List of MS peaks focused by successive OPLS regression model. (B) Effect of compounds with high VIP values and EGCG in combination on myeloma cells. Cells were inoculated and then treated with the indicated compounds for 96 h. Cells were then double-stained with annexin V-Alexa Fluor 488 and PI. All data are expressed as mean ± SEM.

A



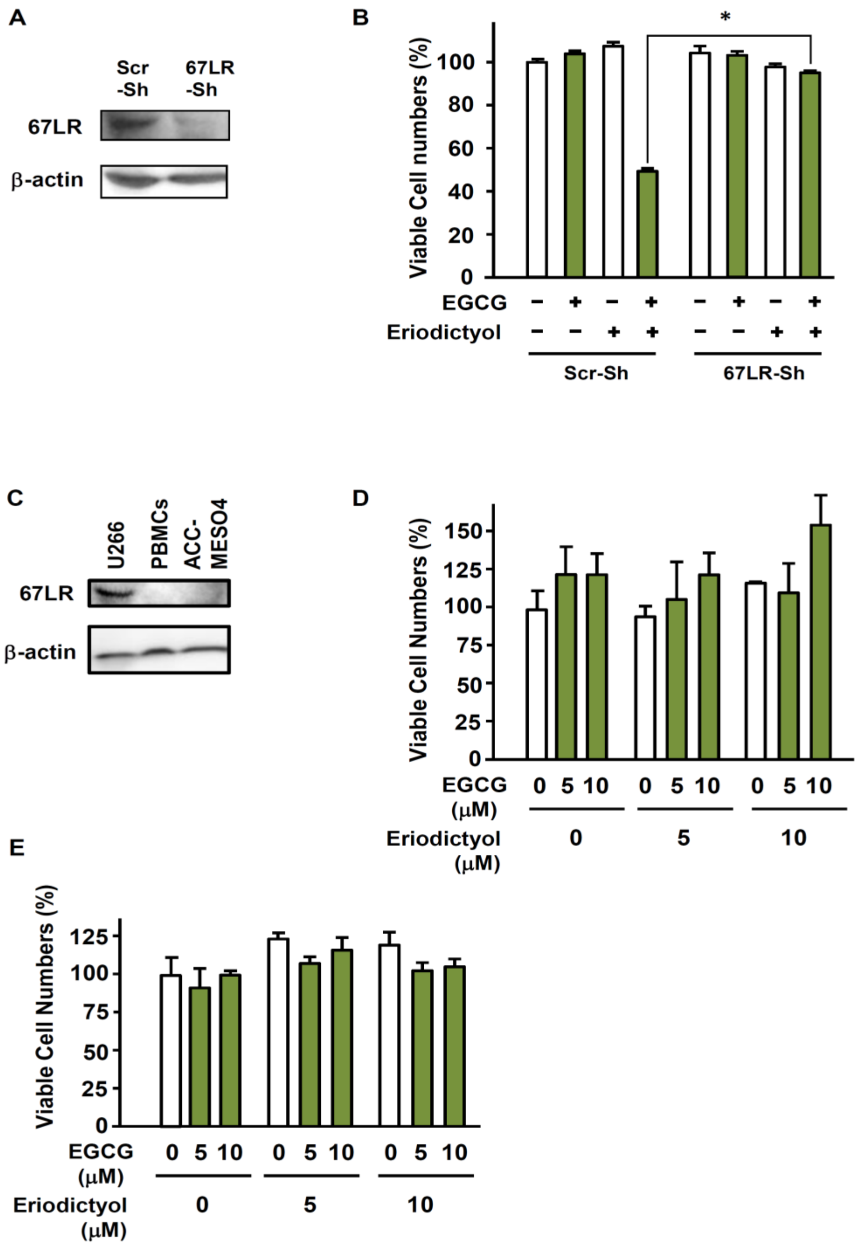

B




**Supplementary Figure 4**

**Eriodictyol and its analogues potentiate the anti-MM effect of EGCG and its *O*-methylated EGCG analogue.**

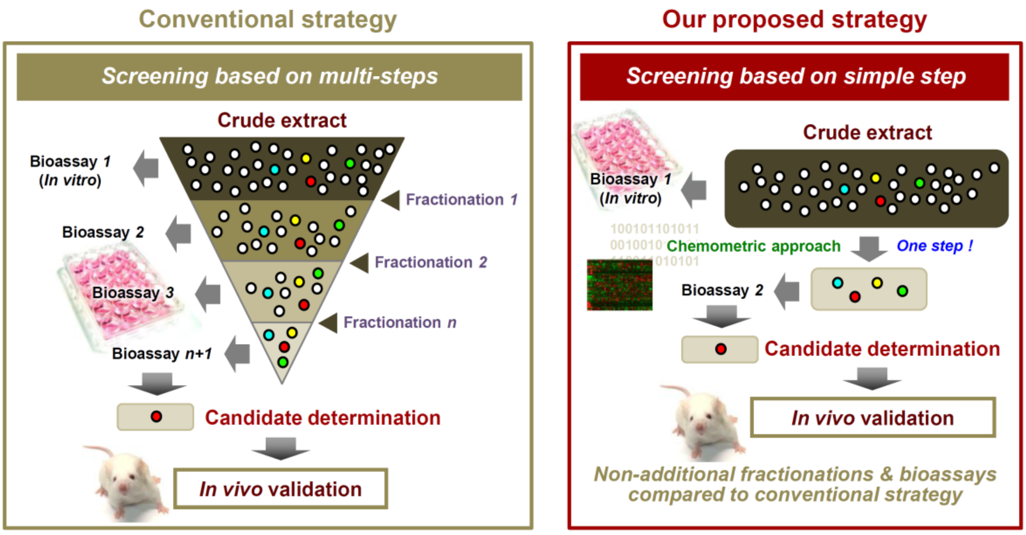
(A) Structure of the *O*-methylated EGCG derivative and eriodictyol analogues. (B) U266 cells were inoculated into 24-well plates and then treated with the indicated compounds for 96 h. After a 96 h treatment, the ATPlite OneStep assay was performed. All data are expressed as mean ± SEM.




**Supplementary Figure 5**

**67LR mediates anti-cancer effect of EGCG/Eriodictyol in combination.**

(A) Immunoblot analyses of 67LR-knockdown in U266 cells. (B) Sensitivity of U266 cells to EGCG (5 μM) and eriodictyol (5 μM) for 96 h after 67LR knock-down. (C) Immunoblot analyses of 67LR. (D) Sensitivity of normal PBMC cells to EGCG and eriodictyol for 96 h. (E) Sensitivity of ACC-MESO4 cells to EGCG and eriodictyol for 96 h.




**Supplementary Figure 6**

**Comparison of metabolic profiling-based screening with conventional screening of bioactive compounds.** All photographs were taken by Motofumi Kumazoe, Yoshinori Fujimura and Shiori Hidaka.