Cancer science

Tumor suppressor miR-193a-3p enhances efficacy of BRAF/MEK inhibitors in *BRAF*-mutated colorectal cancer

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Figure S1

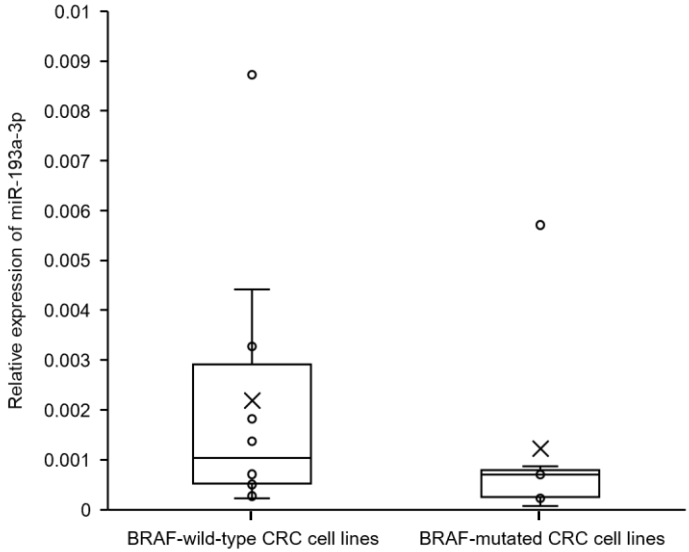

Figure S1 Comparison of miR-193a-3p expression in *BRAF*-mutated and *BRAF*-wild-type CRC cell lines. miR-193a-3p expression levels of CRC cell lines were quantified by Taqman real-time RT-PCR (qRT-PCR). qRT-PCR was performed using a CFX96 real-time PCR detection system (Bio-Rad Laboratories, Hercules, USA). The relative expression of miR-193a-3p was calculated by the delta CT value method, through the use of RNU48 as a normalizer. The results were obtained from two independent experiments. We used 4 *KRAS/BRAF*-wild-type CRC cell lines (LIM1215, COLO320, SW48 and Caco-2), 6 *KRAS*-mutated/*BRAF*-wild-type CRC cell lines (LOVO, SW480, HCT116, HCT8, GEO and HCT15) and 7 *BRAF*-mutated CRC cell lines (RKO, HT29, SW1417, COLO205, LIM2405, CO115 and WiDr) for experiments. HCT116 and Caco-2 were purchased from RIKEN (Ibaraki, Japan). WiDr was purchased from Japanese Collection of Research Bioresources Cell Bank (Tokyo, Japan). SW48, HCT8, COLO205, HCT15, LIM2405, CO115 and GEO were provided by Dr. Mariadason at the Ludwig Institute for Cancer Research (Heidelberg, Australia).

Figure S1 (continued)

Mutation status of *KRAS* and *BRAF* was analyzed by direct DNA sequencing in Table S5 and reported in previous studies [1,2]. In the boxplot, the whiskers depicted 1.5 times the interquartile range, the box signifies the upper and the lower quartiles, and the mean is represented by a cross mark. The mean and median miR-193a-3p expression values were 0.00122 and 0.000700 in *BRAF*-mutated CRC cell lines, and 0.00219 and 0.00104 in *BRAF*-wild-type CRC cell lines, respectively. The mean and median miR-193a-3p expression value tended to be lower in *BRAF*-mutated CRC cell lines compared to *BRAF*-wild-type CRC cell lines, although statistical analyses by Student's *t* test (p=0.43) and Mann-Whitney U-test (p=0.33) showed no significant difference.

References

1) Ahmed D, Eide PW, Eilertsen IA, et al. Epigenetic and genetic features of 24 colon cancer cell lines. Oncogenesis. 2013; 2: e71.

2) Mouradov D, Sloggett C, Jorissen RN, et al. Colorectal cancer cell lines are representative models of the main molecular subtypes of primary cancer. Cancer Res. 2014; 74: 3238-3247.

Figure S2

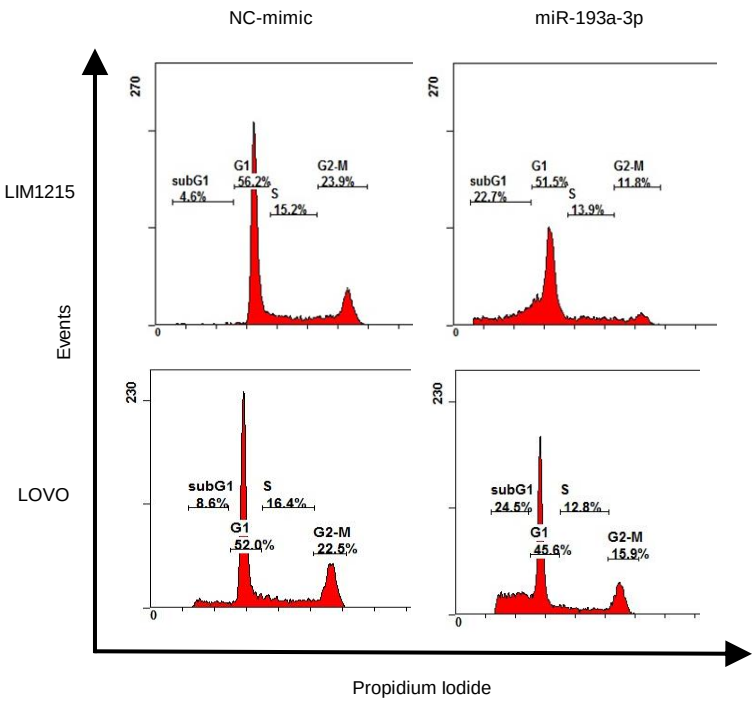

Figure S2 Overexpression of miR-193a-3p increased cells in the subG1 phase in LIM1215 and LOVO cells. Cell cycle analysis was performed 48 h after transfection. Images of flow cytometry show representative data from three independent experiments.

Figure S3

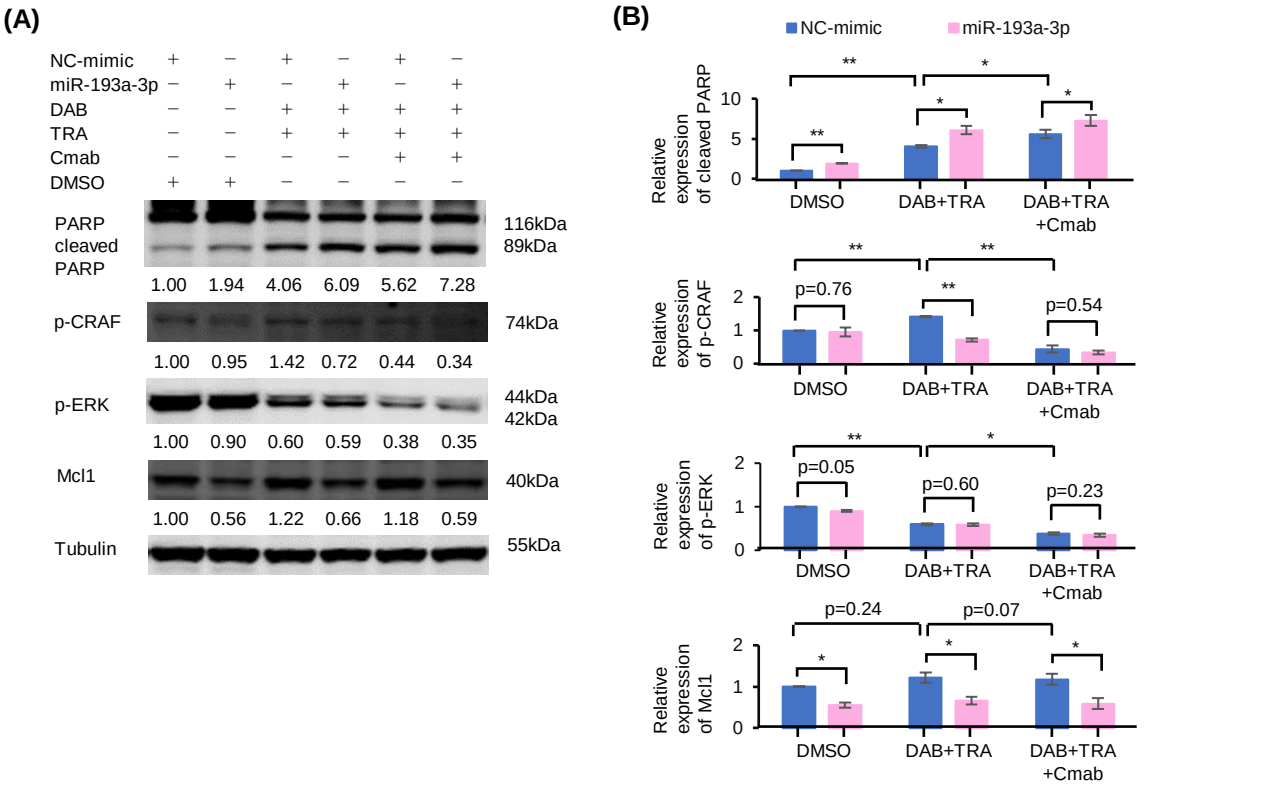

Figure S3 miR-193a-3p potentiates the efficacy of combination therapy with DAB and TRA, and DAB and TRA plus Cmab in SW1417 cells. (A) (B) In SW1417 cells treated with DAB/TRA and DAB/TRA plus Cmab combination therapies, transfection of miR-193a-3p increased cleaved PARP and decreased Mcl1 more potently than transfection of negative control of miRNA mimic (NC-mimic). Reactivation of p-CRAF by DAB/TRA was inhibited by addition of miR-193a-3p overexpression, as well as addition of Cmab to DAB/TRA. Quantification of protein expression is shown in (B). Numbers below each band represent the levels of each protein relative to that of tubulin, as determined by densitometry. Images show representative data. Relative protein expression was calculated by normalization to NC-mimic with DMSO treatment. Data were obtained from three or more independent experiments. Data represent mean ± SEM; p values were determined by Student's t test. *p < 0.05 and **p < 0.01.

Figure S4

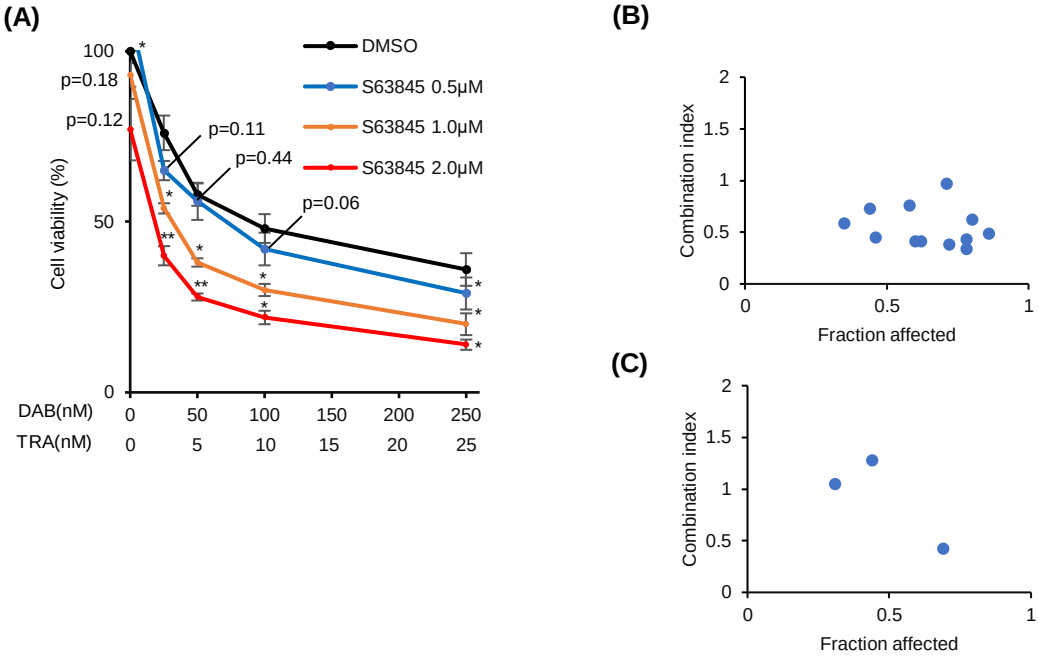

Figure S4 Chou and Talalay plots to assess drug synergy between DAB/TRA and S63845. (A) Cell proliferation was assessed after treatment with S63845 and DAB/TRA for 72 h in RKO cells. Cell viability was compared between cells treated with DMSO and cells treated with indicated doses of DAB/TRA and S63845 using Student's *t* test. Data represent mean ± SEM. *p < 0.05 and **p < 0.01. (B) The Chou and Talalay plot of results of Figure S4A is shown. The combination indices at all doses examined were lower than 1 in RKO cells. (C) The Chou and Talalay plot for S63845 and DAB/TRA in SW1417 cells is shown. SW1417 cells were treated with DAB/TRA and S63845 for 72 h. The combination index at one dose was lower than 1 in SW1417 cells. Fraction affected represents the fraction of cells whose proliferation were inhibited after the drug exposure. Chou and Talalay plots were obtained using the CompuSyn software. Data were obtained from three or more independent experiments. The interaction between S63845 and DAB/TRA was determined to be synergistic when the combination indices were lower than 1.