ω-3 free fatty acids and all-trans retinoic acid synergistically induce growth inhibition of three subtypes of breast cancer cell lines

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Supplementary Figure S1. Cell growth. MCF7, SK-BR-3, HCC1806 and MDA-MB-231 cells were treated with different concentrations ω-3 FFAs and ATRA for 72 h. Cell morphology (a).
Supplementary Figure S1. Cell growth. SK-BR-3 and HCC1806 cells were treated with different concentrations ω-3 FFAs and ATRA for 72 h. CCK8 assays (b) and Cell counting assays (c). The inhibitory effects of combined treatment with 80 μM ω3-FFAs and 20 μM ATRA on SK-BR-3 and HCC1806. Cell morphology (d), CCK8 assays and cell counting assays (e). Values represent the mean ± SD (n = 3). *P< 0.05; **P< 0.01; ***P< 0.001.
Supplementary Figure S2. Cell cycle. SK-BR-3 and HCC1806 cells were treated with 80 μM ω3-FFAs and 20 μM ATRA alone or in combination for 24 h. (a) Cell cycle was analyzed by flow cytometry (upper panel) and percentages of the total cell population in the different phases of cell cycle. (b) The expression of p21, p27 and cyclin D1 protein and quantification of p21, p27 and cyclin D1 (relative to β-Actin).
Supplementary Figure S3. Cell apoptosis. SK-BR-3, HCC1806 and MDA-MB-231 cells were treated with 80 μM ω3-FFAs and 20 μM ATRA. (a) Cell apoptosis was analyzed by flow cytometry with PI and Annexin V-FITC staining and percentage of apoptotic cells at two different stages. (b) PARP and Bcl-2 protein level after treatment from 12 h to 48 h.
Supplementary Figure S4. p53 and Cell apoptosis. SK-BR-3 and HCC1806 cells were treated with 80 μM ω3-FFAs, 20 μM ATRA alone or in combination for 48 h. (a) Relative expression of p53 was determined by Q-PCR. (b) The expression of p53 protein. (c) Quantification of p53 protein level (relative to β-Actin). SK-BR-3 and HCC1806 cells were treated with 80 μM ω3-FFAs and, 20 μM ATRA alone or in combination in the absence or presence of proteasome inhibitor MG132 or lysosome inhibitor CQ. (d) Cell counting assays. (e) The expression of PARP and p53 protein. Values represent the mean ± SD (n = 3). *P< 0.05; **P< 0.01; ***P< 0.001.
Supplementary Figure S5. Caspase signaling pathway. SK-BR-3 and HCC1806 cells were pretreated with 10 µM Z-VAD-FMK and BOC-D-FMK for 1 h and then exposed to 80 µM ω3-FFAs and 20 µM ATRA for 48 h. (a) Cell counting assays. (b) Cell apoptosis was analyzed by flow cytometry with PI and Annexin V-FITC staining. (c) The expression of PARP protein. (d) The expression of Caspase-3 protein. (e) The expression of Caspase-6, -7 and -9 protein. β-Actin was used as an internal control. Values represent the mean ± SD (n = 3). *P< 0.05; **P< 0.01; ***P< 0.001.
Supplementary Figure S6. Scanned images of immunoblotting. Originals for cropped bands in: (a) Figure 2 panel b and Supplementary Figure S2 panel b. (b) Figure 3 panel b and Supplementary Figure S3 panel b. (c) Figure 4 panel e and Supplementary Figure S4 panel e. (d) Figure 5 panel c, d, e and Supplementary Figure S5 panel c, d, e.
Supplementary FigureS6. Scanned images of immunoblotting. Originals for cropped bands in: (a) Figure 2 panel b and Supplementary FigureS2 panel b. (b) Figure 3 panel b and Supplementary FigureS3 panel b. (c) Figure 4 panel e and Supplementary FigureS4 panel e. (d) Figure 5 panel c, d, e and Supplementary FigureS5 panel c, d, e.