Supplementary Information

Caspase 2 Detection

Immunocytochemistry was performed for normal and metastasized B16 4A5 cells at a density of 10,000 cells/cm² in 4-well glass chamber slides (Nalgene Nunc International, New York, NY, USA) and expanded for 24 h in culture medium. After 24 h, new medium containing 10 mM BA and 10 mM BA:GCDG, respectively, were added. Control well contained untreated cells. Cells were maintained in culture for 72 h after addition of substances, and then prepared for immunocytochemical staining. After removing the culture medium, cells were washed, fixed with 4% paraformaldehyde (Sigma-Aldrich Company, Taufkirchen, Germany), permeabilized with 0.1% Triton X-100 (Sigma-Aldrich Company) and then investigated for the expression of the proteins of interest, using for labeling anti-h/m Caspase 2 (mCaspase 2 affinity purified rabbit IgG) (R&D Systems, Abingdon, UK). Staining protocol continued with secondary biotinylated antibody binding and substrate addition (AEC) (Dako EnVision™ + System-HRP, Dako, Carpinteria, CA, USA) following the manufacturer's protocol. After counterstaining with hematoxylin solution (Hematoxylin, Mayer's Lillie's Modification, Dako) for 30 s and washing with tap water the slides were mounted in an aqueous mounting media (Crystal/Mount™, Biomeda, CA, USA). Microscopy analysis was performed on a Nikon Eclipse E800 microscope (Tokyo, Japan).

Figure S1. Caspase 2 staining after a period of incubation of 72 h for normal B164A5 cells: (a) Control; (b) BA 10 mM; (c) BA:GCDG 10 mM; and (d) GCDG 10 mM.

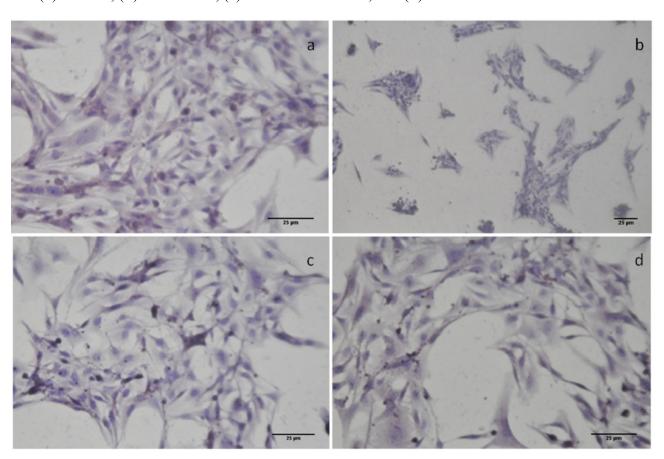
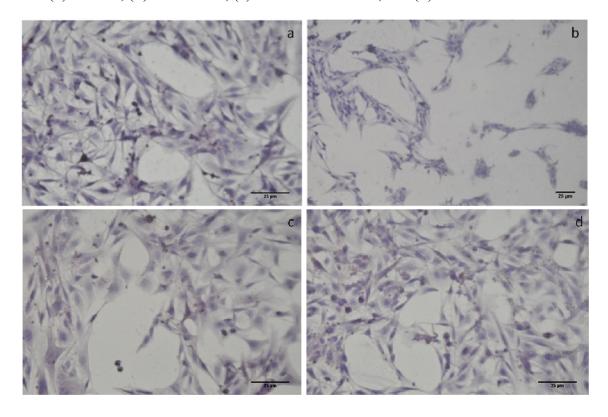


Figure S2. Caspase 2 staining after a period of incubation of 72 h for metastatic B164A5 cells: (a) Control; (b) BA 10 mM; (c) BA:GCDG 10 mM; and (d) GCDG 10 mM.



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