

Killing glioblastoma cells with glycosylated indolocarbazole-based derivative LCS1269: a potential crosstalk between micronuclei formation and the concurrent induction of apoptosis, necroptosis, and pyroptosis

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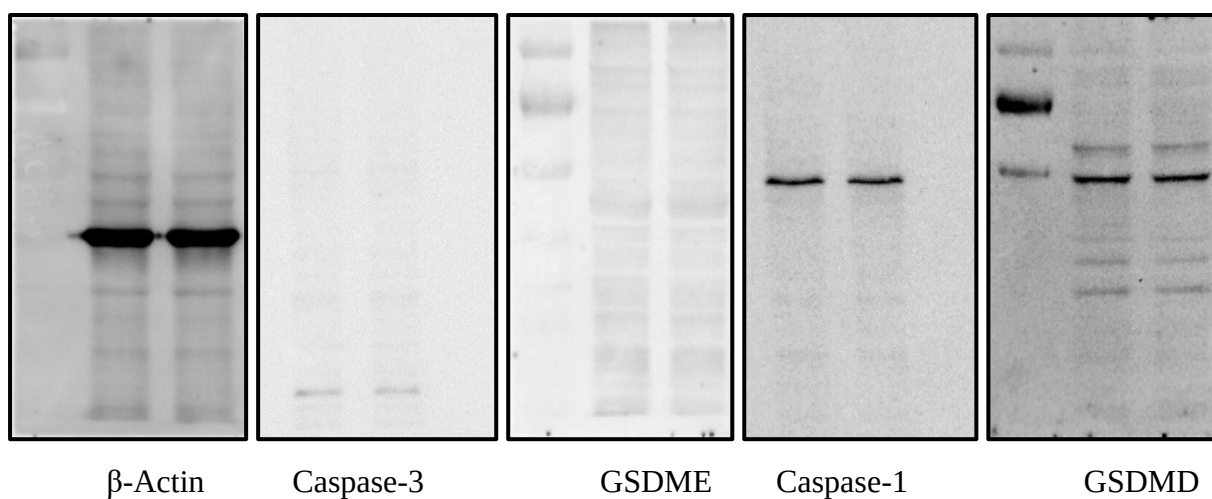
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MCF-7

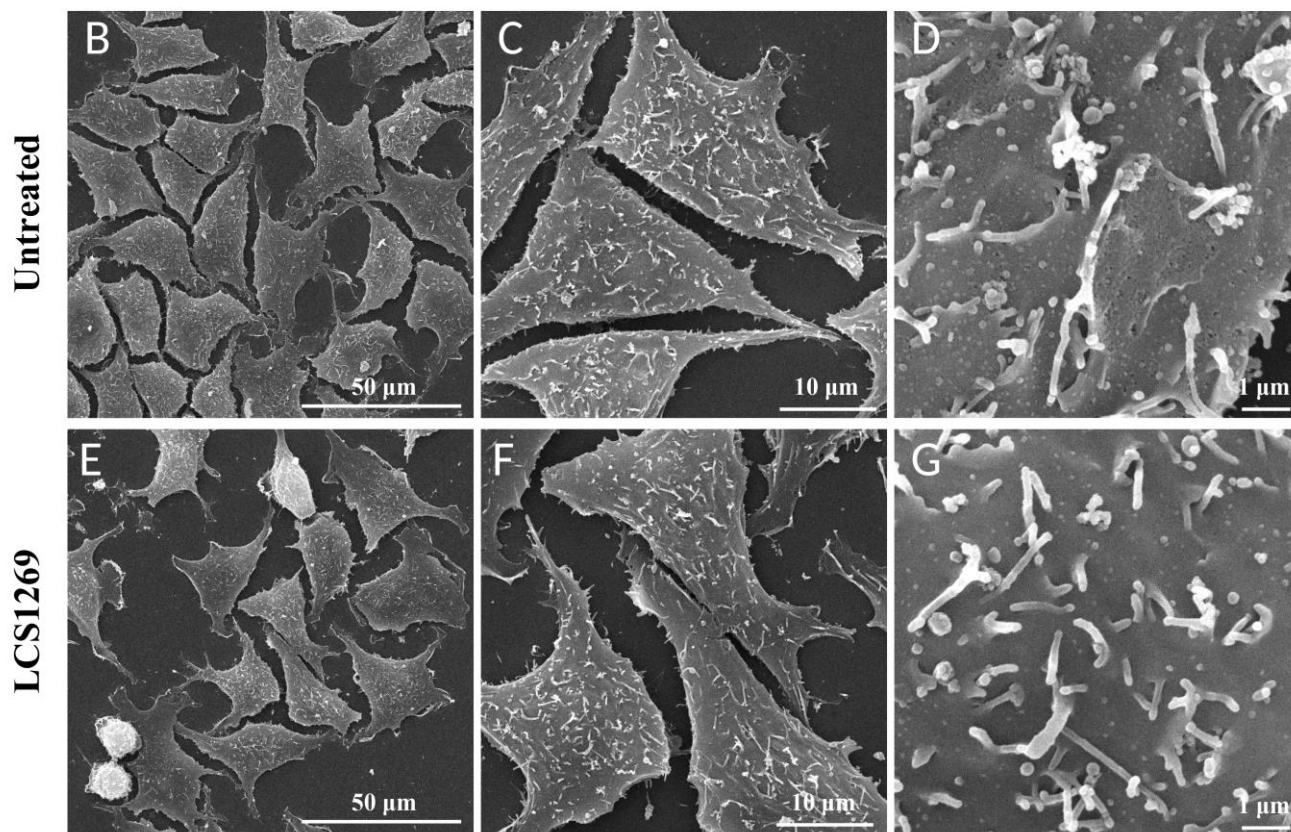


Figure S1. Effect of LCS1269 on morphological features of pyroptosis in the constitutive caspase-3/GSDME double mutated MCF-7 cells.

(A) Representative images showing baseline content of caspase-3, GSDME, caspase-1, and GSDMD proteins expressed in MCF-7 cells. (B–D) Scanning electron micrographs of untreated MCF-7 cells

captured at the different magnification. (E–G) Cell surface of MCF-7 cells exposed to LCS1269 (25 μ M) for 48h. . β -Actin was used as a loading control.

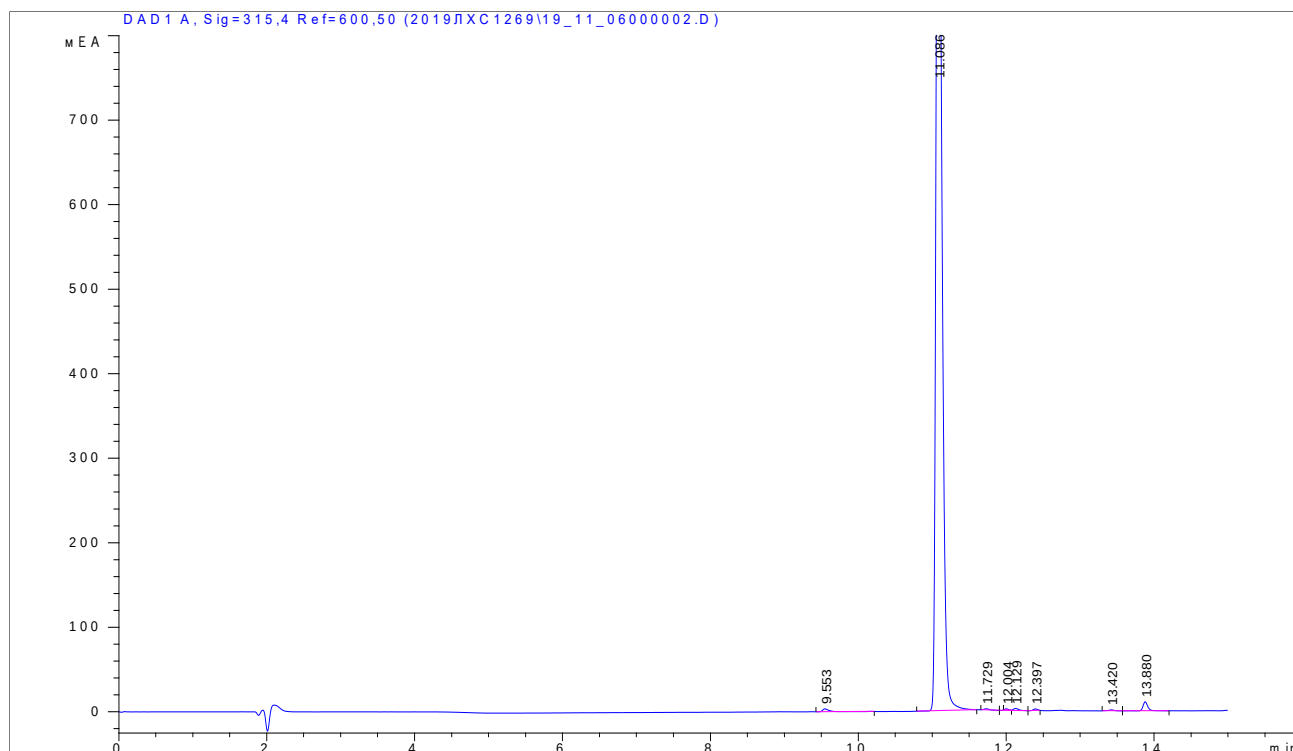


Figure S2. HPLC analysis of LCS1269. LCS1269 purity was 98 – 99%. The gradient elution at 40°C was carried out. The gradient started at water/acetonitrile (9:1) + trifluoroacetic acid 0.1% (mobile phase A) and ended at water/acetonitrile (0.5:9.5) + trifluoroacetic acid 0.1% (mobile phase B) after 15 min. The retention time was 10.9 ± 0.2 min, λ 315 nm.

Table S1. Primers used for quantitative real-time PCR (qRT-PCR)

	Gene	Primer sequences
1	<i>TNF-α</i>	5'-GAGGCCAAGCCCTGGTATG-3' (forward)
		5'-CCGGCCGATTGATCTCAGC-3' (reverse)
2	<i>FasL</i>	5'-TGCCTTGGTAGGATTGGGC-3' (forward)
		5'-GCTGGTAGACTCTCGGAGTTC-3' (reverse)
3	<i>TRAIL</i>	5'-TGCCTGCTGATCGTGATCTTC-3' (forward)
		5'-GCTCGTTGGTAAAGTACACGTA-3' (reverse)
4	<i>Fas (CD95)</i>	5'-AGATTGTGTGATGAAGGACATGG-3' (forward)
		5'-TGTTGCTGGTGAGTGTGCATT-3' (reverse)
5	<i>TNFR1</i>	5'-TCACCGCTTCAGAAAACCACC-3' (forward)
		5'-GGTCCACTGTGCAAGAAGAGA-3' (reverse)
6	<i>ZBP1</i>	5'-AACATGCAGCTACAATTCCAGA-3' (forward)
		5'-AGTCTCGGTTACATCTTTTGC-3' (reverse)
7	<i>AIM2</i>	5'-TGGCAAAACGTCTTCAGGAGG-3' (forward)
		5'- AGCTTGACTTAGTGGCTTTGG-3' (reverse)
8	<i>RIPK1</i>	5'-GGGAAGGTGTCTCTGTGTTTC-3' (forward)
		5'- CCTCGTTGTGCTCAATGCAG-3' (reverse)
9	<i>YWHAZ</i>	5'-ACTTTTGGTACATTGTGGCTTCAA-3' (forward)
		5'-CCGCCAGGACAAACCAGTAT-3' (reverse)

Table S2. Primary antibodies used in Western blot analysis

Antibody name	Manufacturer	Catalogue number	Dilution rate
Bcl-2 (D55G8) Rabbit mAb	Cell Signaling Technology	4223	1:1000
Phospho-Bcl-2 (Ser70) (5H2) Rabbit mAb	Cell Signaling Technology	2827	1:1000
Phospho-Bcl-2 (Thr56) Antibody	Cell Signaling Technology	2875	1:1000
Bcl-xL (54H6) Rabbit mAb	Cell Signaling Technology	2764	1:1000
Mcl-1 (D35A5) Rabbit mAb	Cell Signaling Technology	5453	1:1000
PARP Antibody	Cell Signaling Technology	9542	1:1000
Caspase-3 (D3R6Y) Rabbit Monoclonal Antibody	Cell Signaling Technology	14220	1:1000
Caspase-7 (D2Q3L) Rabbit Monoclonal Antibody	Cell Signaling Technology	12827	1:1000
Anti-NF-kB p65 antibody [E379]	Abcam	ab32536	1:1000
Phospho-NF- kappaB p65 (Ser536) (93H1) Rabbit Monoclonal Antibody	Cell Signaling Technology	3033	1:1000
MLKL Antibody	MedChemExpress	HY-P80227	1:1000

Phospho-MLKL (Ser358) Antibody (YA16231)	MedChemExpress	HY-P81878	1:500
NLRP3 Antibody	Affinity Biosciences	DF7438	1:500
Polyclonal Antibody to Caspase 1 (CASP1)	Cloud-Clone	PAB592Hu01	1:400
Polyclonal Antibody to Interleukin 1 Beta (IL1b)	Cloud-Clone	PAA563Hu01	1:1000
GSDMD Antibody	Affinity Biosciences	AF4012	1:1000
GSDME Antibody	MedChemExpress	HY-P80697	1:500
RIP (D94C12) XP [®] Rabbit mAb	Cell Signaling Technology	3493	1:1000
Phospho-RIP (Ser166) (D1L3S) Rabbit mAb	Cell Signaling Technology	65746	1:1000
beta-Actin (C4) Mouse mAb HRP	Santa Cruz Biotechnology	sc-47778 HRP	1:500