

Increased beclin 1 Levels result in autophagic degradation of full-length caspase-8 in TRAIL resistant colon cancer cells

Sheng Dai^{1,2**}, Shu Yang^{2#}, Xin Hu², Wei Sun², Gregory Tawa², Wenge Zhu³, Aaron D Schimmer⁴, Chao He¹, Bingliang Fang⁵, Hongbo Zhu^{1*}, and Wei Zheng^{2*}

¹Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou 310016, China.

²National Center for Advancing Translational Sciences (NCATS), NIH, Bethesda, MD 20892, USA.

³Department of Biochemistry and Molecular Medicine, the George Washington University Medical School, Washington, DC, USA

⁴Princess Margaret Cancer Centre, Toronto, ON M5G 2M9, Canada

⁵Department of Thoracic and Cardiovascular Surgery, The University of Texas M. D. Anderson Cancer Center, Houston, TX, USA.

*To whom correspondence should be addressed:

Wei Zheng: wzheng@mail.nih.gov, Hongbo Zhu: ykzhh@zju.edu.cn, Sheng Dai: daimd@zju.edu.cn

#These authors contributed equally to this work.

Running title: 17-HW restored TRAIL response in resistant cancer cells.

Supplementary Table S1. Summary of the antibodies used in experiments

	Name	Isotype	Vendor	Catalog#	Dilution factor
Primary	MAP1LC3B	Rabbit	Cell signaling	3868s	Western: 1:1000, IF: 1:200
	SQSTM1	Mouse	Santa Cruz Bio.	sc-28359	Western: 1:200, IF: 1:50
	GAPDH	Rabbit	Cell signaling	5174s	Western: 1:10000
	ACTB/ β -actin	Rabbit	Cell signaling	4970s	Western: 1:10000
	BECN1	Rabbit	Novus Biologicals	NB110-87318	IHC: 1:300
	CASP8	Rabbit	Novus Biologicals	NBP1-05123	IHC: 1:400
	CASP8	Mouse	Cell signaling	9746s	Western: 1:1000 IP:1:100
	Cleaved CASP8	Rabbit	Cell signaling	8592s	Western: 1:1000
	CASP3	Rabbit	Cell signaling	9668s	Western: 1:1000
	Cleaved CASP3	Rabbit	Cell signaling.	9664s	Western: 1:1000
	CASP7	Mouse	Cell signaling	9494s	Western: 1:1000
	Cleaved CASP7	Rabbit	Cell signaling	8438s	Western: 1:1000
	CASP9	Mouse	Cell signaling	9508s	Western: 1:1000
	CASP10	Rabbit	Cell signaling	9752s	Western: 1:1000
	p-MTOR Ser2448	Rabbit	Cell signaling	5536s	Western: 1:1000
	p-MTOR Ser2481	Rabbit	Cell signaling	2974s	Western: 1:1000
	MTOR	Rabbit	Cell signaling	2983s	Western: 1:1000
	p-ULK1 Ser317	Rabbit	Cell signaling	12753s	Western: 1:1000
	ULK1	Rabbit	Cell signaling	8054s	Western: 1:1000
	TNFRSF10A	Mouse	Santa Cruz Bio.	sc-8411	Western: 1:200
	TNFRSF10B	Mouse	Santa Cruz Bio.	sc-65314	Western: 1:200
	FADD	Rabbit	Cell signaling	2782s	Western: 1:1000
	CFLAR	Rabbit	Cell signaling	8510s	Western: 1:1000
	PIK3C3	Rabbit	Cell signaling	4263s	Western: 1:1000
	PARP	Rabbit	Cell signaling	9532s	Western: 1:1000
	FLAG	Rabbit	Cell signaling	14793s	Western: 1:1000, IP: 100 μ g/1mg protein
	BECN1	Mouse	Cell signaling	4122s	Western: 1:1000, IP: 100 μ g /1mg protein
p-BECN1 Ser15	Rabbit	Cell signaling	84966s	Western: 1:1000, IP: 100 μ g /1mg protein	
p-BECN1 1 Ser93	Rabbit	Cell signaling	14717s	Western: 1:1000	
Secondary	Anti-Rabbit IgG, Alexa Fluor 488	Donkey	Thermal fisher	A21206	1:200
	Anti-Mouse IgG, Alexa Fluor 594	Rabbit	Thermal fisher	A11062	1:200
	Anti-rabbit IgG, HRP	Rabbit	Cell signaling	7074s	1:5000
	Anti-mouse IgG, HRP	Mouse	Cell signaling	7076s	1:5000

Supplementary Table S2. List of compounds confirmed in the high throughput screening with DLD1-R cells

Compound Name	NCGC Number	Category	EC ₅₀ (μ M)		Fold of EC ₅₀ change
			- TRAIL	+ TRAIL	
17-hydroxy Wortmannin	NCGC00345783-05	Class III PI3K inhibitor	10.2	0.2	51.0
MG-115	NCGC00345815-01	Proteasome inhibitor	161.6	5.3	30.5
Ixazomib citrate	NCGC00249612-01	Proteasome inhibitor	5.3	0.3	17.7
ISOX	NCGC00250390-01	HDAC6 inhibitor	1.6	0.1	16.0
CHIR-265	NCGC00250407-01	Raf inhibitor	111.2	7.1	15.7
Carfilzomib	NCGC00249613-01	Proteasome inhibitor	10.6	0.7	15.1
Marizomib	NCGC00344630-01	Proteasome inhibitor	1.1	0.08	13.8
GDC-0941	NCGC00187482-01	PI3K inhibitor	8.6	0.8	10.8
Resistomycin	NCGC00160275-03	Antibiotic/Anti-HIV	7.5	1.6	4.7
Mocetinostat	NCGC00263182-05	HDAC inhibitor	2.67	0.8	3.3
IMD-0354	NCGC00165811-03	IkappaB inhibitor	9.7	3.8	2.6
CTK4B5975	NCGC00263220-05	HDAC inhibitor	2.5	1.0	2.5

Supplementary Table S3. BECN1 knockdown re-sensitize DLD1-R cells to TRAIL

	Caspase-8 Activity	Caspase-3/7 Activity	Cell viability
	EC₅₀(ng/mL)	EC₅₀(ng/mL)	IC₅₀(ng/mL)
DLD1	5.2	1.6	3.0
DLD1_R/BECN shRNA	14.8	3.2	9.3
DLD1_R/CTL shRNA	NA	NA	NA

Supplementary Table S4. IC₅₀ of TRAIL treatment in each cell line

Cell line	TRAIL IC ₅₀ (ng/ml)		
	TRAIL Only	TRAIL+0.5μM 17-HW	TRAIL+1.0μM 17-HW
Caco-2	2269.0	11.2	4.9
LS174T	1945.0	16.5	9.5
LS180	677.6	18.0	9.7
ROK	>2000	>2000	>2000
HCT-116	5.1	1.2	0.5
HT-29	>2000	>2000	>2000
T84	>2000	>2000	>2000
SW1417	>2000	>2000	>2000
SW620	1906.0	1667.0	1457.0
HCT-15	11.2	2.4	1.0
COLO320	>2000	>2000	>2000
NCI-H508	220.4	46.0	25.3
SNU-C2B	7.9	4.8	3.4
LS123	>2000	>2000	>2000
SW403	17.6	3.5	2.1

Supplementary Table S5. 17-HW re-sensitizes multiple colon cancer cell lines to TRAIL

	Vehicle CTL	0.5 μM 17-HW	1.0 μM 17-HW
Caco-2 IC ₅₀ (ng/mL)	2269	11.2	4.9
LS174T IC ₅₀ (ng/mL)	1945	16.5	9.5
LS-180 IC ₅₀ (ng/mL)	677.6	18	9.7

Supplementary Table S6. BECN1 knockdown re-sensitize multiple colon cancer cell lines to TRAIL

	Caco-2	LS174T	LS180
	IC₅₀(ng/mL)	IC₅₀(ng/mL)	IC₅₀(ng/mL)
CTL shRNA	2658.0	1552.0	769.4
BECN shRNA	6.4	5.8	16.5

Supplementary Table S7. Patient demographics and baseline characteristics

Characteristic	Patients, n (%)
Total	N = 120
Median age (range), years	65 (35-90)
Sex, n (%)	
Female	58 (48.3)
Male	62 (51.7)
Pathologic T stage	
pT1	1 (0.8)
pT2	5 (4.2)
pT3	95 (79.2)
pT4	19 (15.8)
Pathologic N stage	
pN0	62 (51.7)
pN1	39 (32.5)
pN2	19 (15.8)
TNM stage	
I	6 (5.0)
II	56 (46.7)
III	51 (42.5)
IV	7 (5.8)
Histological differentiation	
Well	27 (22.5)
Moderately	79 (65.8)
Poorly	14 (11.7)

Supplementary Table S8. Clinicopathological factors and their relationship to the expression of beclin1 and caspase-8

	Beclin1		Caspase-8		Beclin 1 (H)/ Caspase-8 (L)	Exclude Beclin 1 (H) /Caspase-8 (L)
	Low	High	Low	High		
No. patients (%)	59 (49.2)	61 (50.8)	69 (57.5)	51 (42.5)	37 (30.8)	83 (69.2)
Pathologic T stage						
pT1-T2	50 (84.7)	51 (83.6)	56 (81.2)	45 (88.2)	26 (70.3)	75 (90.4)
pT3-T4	9 (15.3)	10 (16.4)	13 (18.8)	6 (11.8)	11 (29.7)	8 (9.6)
p value		0.864		0.294		0.005
Pathologic N stage						
pN0	28 (47.5)	34 (55.7)	37 (53.6)	25 (49.0)	21 (56.8)	41 (49.4)
pN1-2	31 (52.5)	27 (44.3)	32 (46.4)	26 (51.0)	16 (43.2)	42 (50.6)
p value		0.364		0.618		0.456
Histological grade						
Well/Moderately	52 (88.1)	54 (88.5)	60 (87.0)	46 (90.2)	29 (78.4)	77 (92.8)
Poorly	7 (11.9)	7 (11.5)	9 (13.0)	5 (9.8)	8 (21.6)	6 (7.2)
p value		0.947		0.585		0.023