

# **BACH1-induced ferroptosis drives lymphatic metastasis by repressing the biosynthesis of monounsaturated fatty acids**

Xiufeng Xie<sup>#</sup>, Lusong Tian<sup>#</sup>, Yan Zhao, Fang Liu, Shuyang Dai, Xinglu Gu, Yuxin Ye, Lanping Zhou, Xinmiao Liu, Yulin Sun, Xiaohang Zhao

State Key Laboratory of Molecular Oncology, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100021, P. R. China

## **Supplementary Figure:**

**Supplementary Fig. S1.** RT-qPCR assays validated the mRNA expression of SLC7A11, GCLM, FTH1 and FTL genes after overexpression or knockdown of BACH1 in KYSE150 and KYSE170 cells.

## **Supplementary Tables:**

**Supplementary Table S1** Clinical characteristics of study populations used in screening of HuProt Proteome arrays

**Supplementary Table S2** Clinical characteristics of study populations used in immunoassay

**Supplementary Table S3** Primers information

**Supplementary Table S4** Antibodies information

**Supplementary Table S5** The upregulated IgG autoantibodies detected in patients with T1-stage ESCC

**Supplementary Table S6** The differentially expressed IgG autoantibodies between T1-stage ESCC that with or without lymph node metastasis

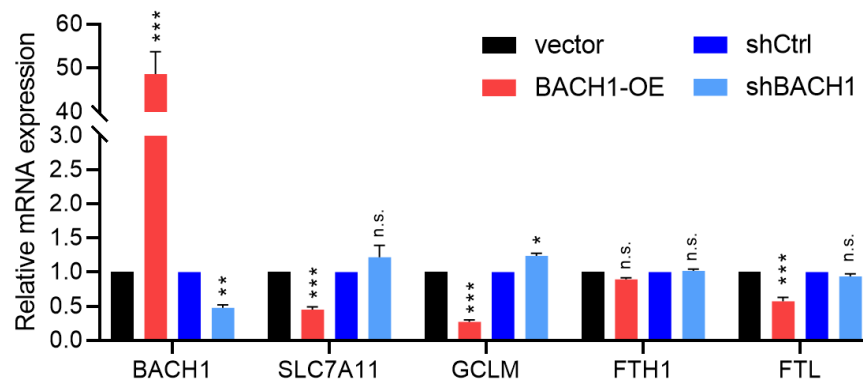
**Supplementary Table S7** The 41 overlapped tumor-related IgG autoantibodies in T1-stage ESCC with LNM compared with those without LNM

**Supplementary Table S8** P Percentage of anti-BACH1 IgG autoantibody positive cases by immunoblotting

**Supplementary Table S9** Clinical significance of anti-BACH1 autoantibody detected in patients with ESCC using immunoassay

**Supplementary Table S10** Clinicopathological characteristics of BACH1 protein expression in 574 ESCC specimens

## Supplementary Figure:



**Supplementary Figure S1.** RT-qPCR assays validated the mRNA expression of SLC7A11, GCLM, FTH1 and FTL genes after overexpression or knockdown of BACH1 in KYSE150 and KYSE170 cells. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ ; N.s., not significant.

## Supplementary Tables:

**Supplementary Table S1.** Clinical characteristics of study populations used in screening of HuProt Proteome arrays

Patient Characteristics	ESCC w/ LNM (n = 12)	ESCC w/o LNM (n = 12)	Health control (n=12)	P value
Age in years				
Mean $\pm$ SD	59.5 $\pm$ 8.93	60 $\pm$ 9.93	59.41 $\pm$ 8.60	0.98 <sup>a</sup>
Range	47-76	43-70	46-70	
Gender				
Male	10	9	10	0.838 <sup>b</sup>
Female	2	3	2	
TNM stage				
T1	12	12		1.00 <sup>b</sup>
T2	0	0		
T3	0	0		
T4	0	0		
Histological grade				
High	0	0		0.881 <sup>b</sup>
Middle	4	4		
Low	4	5		
unknow	4	3		

a: P values were calculated by one-way ANOVA

b: P values were calculated by *Chi* square test

**Supplementary Table S2.** Clinical characteristics of study populations used in immunoassay

Patient Characteristics	ESCC w/ LNM (n = 78)	ESCC w/o LNM (n = 50)	Health control (n = 122)	<i>P</i> value
Age in years				
Mean $\pm$ SD	60.58 $\pm$ 9.45	59.58 $\pm$ 8.35	62.3 $\pm$ 7.79	0.122 <sup>a</sup>
Range	37-79	41-77	43-77	
Gender				
Male	69	37	102	0.104 <sup>b</sup>
Female	9	13	20	
TNM stage				
T1	6	10		0.017 <sup>b</sup>
T2	10	13		
T3	45	22		
T4	17	5		
Histological grade				
High	6	9		0.164 <sup>b</sup>
Middle	32	22		
Low	15	10		
unknow	25	9		

a: *P* values were calculated by one-way ANOVA

b: *P* values were calculated by *Chi* square test

### Supplementary Table S3. Primers information

Primer sequences and oligonucleotides

BACH1 qPCR primer, Forward: AGACGACTCTGAGACGGACA

BACH1 qPCR primer, Reverse: CGCTGTGCAGCAATTCTGTT

SREBF1 qPCR primer, Forward: ACAGTGACTTCCCTGGCCTAT

SREBF1 qPCR primer, Reverse: GCATGGACGGGTACATCTTCAA

SCD1 qPCR primer, Forward: TTCCTACCTGCAAGTTCTACACC

SCD1 qPCR primer, Reverse: CCGAGCTTTGTAAGAGCGGT

ATP5J qPCR primer, Forward: GTTCTCCTCTGTCATTCGGTCA

ATP5J qPCR primer, Reverse: TCACTCCAGATGTCTGTCGCTTAGAT

ATP5G qPCR primer, Forward: CCAGAGTTGCATACAGACCAAT

ATP5G qPCR primer, Reverse: CCCATTAAATACCGTAGAGCCCT

UCP3 qPCR primer, Forward: TGTTTTGCTGACCTCGTTACC

UCP3 qPCR primer, Reverse: GACGGAGTCATAGAGGCCGAT

TIMM8B qPCR primer, Forward: TCACTTCATGGAGTTATGTTGGG

TIMM8B qPCR primer, Reverse: AGACAATTTTCAGTGCAGAGATC

SLC25A22 qPCR primer, Forward: GCCAGCCAAGCTCATCAATG

SLC25A22 qPCR primer, Reverse: GAGGCAGTCGGACATGCTC

PDHX qPCR primer, Forward: TTGGGAGGTTCCGACCTGT

PDHX qPCR primer, Reverse: CAACCACTCGACTGTCACCTG

OCT1 qPCR primer, Forward: GTGTGTAGACCCCCTGGCTA

OCT1 qPCR primer, Reverse: GTGTAGCCAGCCATCCAGTT

NDUFB6 qPCR primer, Forward: CCACAGAAGATGGGGCCTATG

NDUFB6 qPCR primer, Reverse: TCCAGACAGGTACAAGTACATGA

SLC7A11 qPCR primer, Forward: TCTCCAAAGGAGGTTACCTGC

SLC7A11 qPCR primer, Reverse: AGACTCCCCTCAGTAAAGTGAC

GPX4 qPCR primer, Forward: GAGGCAAGACCGAAGTAAACTAC

GPX4 qPCR primer, Reverse: CCGAACTGGTTACACGGGAA

ChIP-PCR primer 1, Forward: CCTTGTGCTTGTGGGCTTTG

ChIP-PCR primer 1, Reverse: TCCCAGGTAGAGGGGTTTGA

ChIP-PCR primer 2, Forward: TAGCAACTGGTCGTGATTCCAG

ChIP-PCR primer 2, Reverse: GAGGCATTCTCCTAAGGGCT

ChIP-PCR primer 3, Forward: AATGCCTCTGGCAACTGA

ChIP-PCR primer 3, Reverse: AGCAAGGGAGCTTCTAGGGT

ACTB qPCR primer, Forward: CTCACCATGGATGATGATATCGC

ACTB qPCR primer, Reverse: TAGGAATCCTTCTGACCCATGC

siRNA negative control sense (5'-3'):UUCUCCGAAGGUGUCACGUTT

siRNA negative control antisense (5'-3'):ACGUGACACGUUCGGAGAATT

---

BACH1 silencing siRNA -1 sense (5'-3'): GGACCAGAGGGAUCUAGAATT

---

BACH1 silencing siRNA -1 antisense (5'-3'): UUCUAGAUGCCUCUGGUCCTT

---

SCD1 silencing siRNA -1 sense (5'-3'): GGAGAAACAUCAUCCUUAUTT

---

SCD1 silencing siRNA -1 antisense (5'-3'): AUAAGGAUGAUGUUUCUCCTT

---

SCD1 silencing siRNA -2 sense (5'-3'): GCUUGCUGAUGAUGUGCUUTT

---

SCD1 silencing siRNA -2 antisense (5'-3'): AAGCACAUCAUCAGCAAGCTT

---

SCD1 silencing siRNA -3 sense (5'-3'): GCGAUAUGCUGUGGUGCUUTT

---

SCD1 silencing siRNA -3 antisense (5'-3'): AAGCACACAGCAUAUCGCTT

---

**Supplementary Table S4. Antibodies information**

Antibodies	Source	Catalog number/ identifier
BACH1 for WB, IHC & IF	Abclonal	A5393/ RRID: AB_2766202
BACH1 for IP	R&D Systems	AF5776/ RRID: AB_2061974
SLC7A11	Proteintech	26864-1-AP, RRID: AB_2880661
GPX4	Abcam	ab41787, RRID: AB_941790
ACSL4	Abcam	ab205197
AMPK	CST	5831/ RRID: AB_10622186
p-AMPK	CST	2535/ RRID: AB_331250
SREBF1	Proteintech	14088-1-AP/ RRID: AB_2255217
SCD1	Abcam	ab236868
$\beta$ -Actin	Sigma-Aldrich, St. Louis, MO	A5316, RRID: AB_476743
Flag	MBL, Sakae Naka-ku Nagoya, Japan	M185-3L/ RRID:AB_11123930
HA	CST, Danvers, MA	3724s/ RRID:AB_1549585
HRP-conjugated mouse secondary antibody	CST	58802/ RRID:AB_2799549
HRP-conjugated rabbit secondary antibody	CST	93702/ RRID:AB_2800208

WB, western blot analysis; IHC, immunohistochemistry; IF, immunofluorescence taining; IP, immunoprecipitation; MBL, Medical & Biological Laboratories; CST, Cell Signaling Technology; RRID, Research Resource Identifier (<https://scicrunch.org/resources>)

**Supplementary Table S8.** Percentage of anti-BACH1 IgG autoantibody positive cases by immunoblotting

	Negative	Positive	<i>P</i> value	AUC	Sensitivity%	Specificity%
Healthy controls	119 (97.5%)	3 (2.5%)	<0.001	0.767	32.8	95
ESCC	98 (76.6%)	30 (23.4%)				
Healthy controls	119 (97.5%)	3 (2.5%)	0.0095	0.695	20.5	95
Early ESCC	34 (87.2%)	5 (12.8%)				
ESCC w/o LNM	44 (88%)	6 (12%)	0.0145	0.672	14.1	95
ESCC w/ LNM	54 (69.2%)	24 (30.8%)				

LNM, lymph node metastasis.

The cut-off value was determined as the mean+3SDs of healthy controls.

*P* values were calculated by *Chi* square test.



**Supplementary Table S9.** Clinical significance of anti-BACH1 autoantibody detected in patients with ESCC using immunoassay

	Negative, n (%)	Positive, n (%)	<i>P</i> value
Age			0.880
>=60	54 (76.1%)	17 (23.9%)	
<60	44 (77.2%)	13 (22.8%)	
Gender			0.058
Male	78 (73.6%)	28 (26.4%)	
Female	20 (90.9%)	2 (9.1%)	
Histological grade			0.568
High	12 (80%)	3 (20%)	
Middle	44 (81.5%)	10 (18.5%)	
Low	17 (68%)	8 (32%)	
Unknown	25 (73.5%)	9 (26.5%)	
Tumor location			0.1848
Upper	7 (87.5%)	1 (12.5%)	
Middle	19 (63.3%)	11 (36.7%)	
Lower	29 (82.9%)	6 (17.1%)	
Unknown	43 (78.2%)	12 (21.8%)	
Tumor size			0.0924
<5 cm	41 (87.2%)	6 (12.8%)	
>= 5cm	37 (71.2%)	15 (28.8%)	
Unknown	20 (69%)	9 (31%)	
T stages			0.290
T1	14 (87.5%)	2 (12.5%)	
T2	20 (87%)	3 (13%)	
T3	49 (73.1%)	18 (26.9%)	
T4	15 (68.2%)	7 (31.8%)	
N staging			0.0145
Negative	44 (88%)	6 (12%)	
Positive	54 (69.2%)	24 (30.8%)	
M staging			0.99
Yes	7 (77.8%)	2 (22.2%)	
No	91 (76.5%)	28 (23.5%)	
AJCC			0.0012
Stage 0-II	45 (91.8%)	4 (8.2%)	
Stage III-IV	53 (67.1%)	26 (32.9%)	

*P* values were calculated by *Chi* square test.

**Supplementary Table S10.** Clinicopathological characteristics of BACH1 protein expression in 574 ESCC specimens

	BACH1 expression		<i>P</i> value
	Negative, n (%)	Positive, n (%)	
Gender			0.14119
Male	360 (86.33%)	57 (13.67%)	
Female	140 (90.91%)	14 (9.09%)	
Age			0.624601
<65	269 (88.20%)	36 (11.80%)	
>= 65	231 (86.84%)	35 (13.16%)	
Histological grade			0.000079
I	35 (25.74%)	101 (74.26%)	
I-II+II	175 (55.03%)	143 (44.97%)	
II-III+III	82 (71.93%)	32 (28.07%)	
Tumor size			0.65449
<5cm	120 (78.95%)	32 (21.05%)	
>= 5cm	103 (81.10%)	24 (18.90%)	
T staging			0.901433
T1	27 (90.00%)	3 (10.00%)	
T2	95 (86.36%)	15 (13.64%)	
T3	349 (87.47%)	50 (12.53%)	
T4	13 (86.67%)	2 (13.33%)	
N staging			0.03646
N0-2	481 (88.10%)	65 (11.90%)	
N3	15 (71.43%)	6 (28.57%)	
M staging			1
M0	493 (87.57%)	70 (12.43%)	
M1	7 (87.50%)	1 (12.50%)	
TNM staging			0.251737
I	54 (93.10%)	4 (6.90%)	
II	208 (86.67%)	32 (13.33%)	
III	196 (87.89%)	27 (12.11%)	
IV	26 (78.79%)	7 (21.21%)	