

Table S1. Correlation between HAND1 expression and clinicopathological parameters of GC patients

Clinicopathological parameters	N (%)	HAND1 expression		χ^2	p-value
		Low (%)	High (%)		
Total	165	111 (67.3)	54 (32.7)		
Gender					
Male	120(72.7)	87 (72.5)	33 (27.5)	5.461	0.019*
Female	45(27.3)	24 (53.3)	21(46.7)		
Age					
≥62	89(53.9)	65 (73)	24 (27)	2.913	0.088
<62	76(46.1)	46(60.5)	30 (39.5)		
Histopathological grading					
Well / moderately	103(62.4)	75(72.8)	28 (27.2)	3.825	0.050*
Poorly	62(37.6)	36 (58.1)	26(41.9)		
Depth of invasion					
pT1/T2	53(32.1)	29(54.7)	24(45.3)	5.591	0.018*
pT3/T4	112(67.9)	82 (73.2)	30(26.8)		
Lymph nodal status					
N0	50(30.3)	27(54)	23 (46)	5.740	0.017*
N1/2/3	115(69.7)	84(73)	31(27)		
Distant metastasis					
M0	135(81.8)	87 (64.4)	48(35.6)	2.698	0.100
M1	30(18.2)	24(80)	6 (20)		
TNM stage					
I	35(21.2)	15 (42.9)	20(57.1)	13.033	0.005*
II	34(20.6)	23(67.6)	11(32.4)		
III	67(40.6)	50(74.6)	17(25.4)		
IV	29(17.6)	23(79.3)	6 (20.7)		

* $p < 0.05$.

Table S2. Univariate survival analyses (Cox regression model) of various factors in GC patients

Characteristics	Categories	HR	95% CI	<i>p</i> -value
Gender	Male/Female	0.945	0.588-1.517	0.813
Age	≥62/<62	0.571	0.368-0.885	0.012*
Histopathological grading	Well/moderately/poorly	0.63	0.399-0.993	0.047*
Depth of invasion	T1/T2/T3/T4	5.848	3.01-11.361	< 0.001*
Lymph node metastasis	N0/N1/N2/N3	3.761	2.039-6.939	< 0.001*
Distant metastasis	M0/M1	4.734	2.921-7.673	< 0.001*
TNM stages	I/II/III/IV	2.613	1.996-3.422	< 0.001*
HAND1 expression	Low/High	2.632	2.01-3.448	< 0.001*

HR, hazard ratio; CI, confidence interval. **p*< 0.05.

Table S3. Multivariate survival analyses (Cox regression model) of prognostic factors in GC patients

Characteristics	Categories	HR	95% CI	<i>p</i> -value
Age	≥62/<62	0.744	0.47-1.178	0.207
Histopathological grading	Well/moderately/poorly	0.818	0.505-1.324	0.413
Depth of invasion	T1/T2/T3/T4	3.859	1.833-8.122	^{<} 0.001*
Lymph node metastasis	N0/N1/N2/N3	1.998	0.942-4.241	0.071
Distant metastasis	M0/M1	2.465	1.018-5.97	0.046*
TNM stages	I/II/III/IV	1.139	0.643-2.019	0.656
HAND1 expression	Low/High	0.216	0.105-0.445	^{<} 0.001*

HR, hazard ratio; CI, confidence interval. **p*< 0.05.

Table S4. PCR primers used in the study

Primers	Sequences (5'-3')
For HAND1 RT-PCR	
HAND1-F	CCAAGCTCTCCAAGATCAAG
HAND1-R	GTCCTTTAATCCTCTTCTCG
Control for RT-PCR	
GAPDH-F	GGCTCTCCAGAACATCATCCCTGC
GAPDH-R	GGGTGTCGCTGTTGAAGTCAGAGG
For detecting methylation of HAND1 promoter by MSP	
HAND1-M-F	TTTTTTTATCGGTTTTTGTCGC
HAND1-M-R	CTAACGAACTAAAATAACGCG
For detecting unmethylation of HAND1 promoter by MSP	
HAND1-U-F	GTTTTTTTATTGGTTTTTGTTGT
HAND1-U-R	TACTAACAACTAAAATAACACA
For BGS analysis of HAND1 methylation	
HAND1-BGS-F	TATTTTATAGAGTTGGTTGTTGAGTT
HAND1-BGS-R	CTCCTTCTTAAATCCTAAACCTTTC
For CHIP-PCR analysis of CHOP promotor region that HAND1 bound to	
CHOP-CHIP-F	CTCACACAGGGAGTCACAGG
CHOP-CHIP-R	GTGGACCCGATTCTGTTTGG
For CHIP-PCR analysis of BAK promotor region that HAND1 bound to	
BAK-CHIP-F	CCACCCAGATCACCTACAG
BAK-CHIP-R	GTTTCCAGGAATGGGCGTC
For quantitative real time RT-PCR	
GAPDH-F	TGCACCACCAACTGCTTAGC
GAPDH-R	GGCATGGACTGTGGTCATGAG
HSPA5-F	CGTCCTATGTCGCCTTCACT
HSPA5-R	TGTCTTTGTTTGCCACCTC
ATF6-F	GCTCTCTTTGCTGAACTCGG
ATF6-R	TGAGGAGGCTGGAGAAAGTG
PERK-F	CGGAACCAGACGATGAGACA
PERK-R	TCCCACATCCAAATCCCACT
ATF4-F	GCCAAGCACTTCAAACCTCA
ATF4-R	GGTCATCTGGCATGGTTTCC
IRE1a-F	GTGTCAACGCTGGATGGAAG
IRE1a-R	ACAGGCTGCCATCATTAGGA
XBP-1-F	TGGTGCCAGCCCAGAGAG
XBP-1-R	CTGTTCCAGCTCACTCATTCCG
Ero1-La-F	GAGACAGCGGCACAGAGGT
Ero1-La-R	CAGCACAGTCCCTTCTTCCA
CHOP-F	CATTGCCTTTCTCCTTCGGG

CHOP-R	CCAGAGAAGCAGGGTCAAGA
BAK-F	TAGCCCAGGACACAGAGGAG
BAK-R	ATAGCGTCGGTTGATGTCGT