

# Markers CD40, VEGF, AKT, PI3K, and S100 Correlate with Tumor Stage in Gastric Cancer

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**Supplemental Table 1.** Associations between differentiation and lymph node metastasis compared with expression of CD40, PI3K, AKT, VEGF, and S100 in cancer tissues

	Differentiation, n (%)		p value	Lymph node metastasis, n (%)		p value
	no (n = 49)	yes (n = 79)		no (n = 33)	yes (n = 95)	
CD40	-	16 (32.7)	0.944	14 (42.4)	31 (32.6)	0.403
	+	15 (30.6)		9 (27.3)	31 (32.6)	
	++	14 (28.6)		6 (18.2)	27 (28.4)	
	+++	4 (8.2)		4 (12.1)	6 (6.3)	
VEGF	-	9 (18.4)	0.372	9 (27.3)	24 (25.3)	0.352
	+	0 (0.0)		1 (3.0)	0 (0.0)	
	++	13 (26.5)		6 (18.2)	25 (26.3)	
	+++	27 (55.1)		17 (51.5)	46 (48.4)	
AKT	-	7 (14.3)	0.863	4 (12.1)	11 (11.6)	0.349
	+	4 (8.2)		5 (15.2)	5 (5.3)	
	++	9 (18.4)		6 (18.2)	21 (22.1)	
	+++	29 (59.2)		18 (54.5)	58 (61.1)	
PI3K	-	8 (16.3)	0.809	5 (15.2)	15 (15.8)	0.387
	+	3 (6.1)		5 (15.2)	7 (7.4)	
	++	12 (24.5)		9 (27.3)	20 (21.1)	
	+++	26 (53.1)		14 (42.4)	53 (55.8)	
S100 in DC	-	36 (73.5)	0.367	16 (48.5)	67 (70.5)	0.020 <sup>a</sup>
	+	11 (22.4)		13 (39.4)	25 (26.3)	
	++	2 (4.1)		2 (6.1)	3 (3.2)	
	+++	0 (0.0)		2 (6.1)	0 (0.0)	

<sup>a</sup>Indicates a significant association was observed.

DC = Dendritic cells.

**Supplemental Table 2.** Associations between clinical stages and expression levels of CD40, PI3K, AKT, VEGF, and S100

	Stage, n (%)				p value
	I (n = 15)	II (n = 25)	III (n = 49)	IV (n = 39)	
CD40+	-	8 (53.3)	11 (44.0)	16 (32.7)	10 (25.6) 0.173
	+	5 (33.3)	6 (24.0)	16 (32.7)	13 (33.3)
	++	1 (6.7)	5 (20.0)	16 (32.7)	11 (28.2)
	+++	1 (6.7)	3 (12.0)	1 (2.0)	5 (12.8)
VEGF	-	7 (46.7)	7 (28.0)	11 (22.4)	8 (20.5) 0.076
	+	1 (6.7)	0 (0.0)	0 (0.0)	0 (0.0)
	++	4 (26.7)	4 (16.0)	13 (26.5)	10 (25.6)
	+++	3 (20.0)	14 (56.0)	25 (51.0)	21 (53.8)
AKT	-	3 (20.0)	6 (24.0)	4 (8.2)	2 (5.1) 0.154
	+	4 (26.7)	0 (0.0)	2 (4.1)	4 (10.3)
	++	2 (13.3)	3 (12.0)	10 (20.4)	12 (30.8)
	+++	6 (40.0)	16 (64.0)	33 (67.3)	21 (53.8)
PI3K	-	4 (26.7)	6 (24.0)	6 (12.2)	4 (10.3) 0.004 <sup>a</sup>
	+	4 (26.7)	1 (4.0)	4 (8.2)	3 (7.7)
	++	6 (40.0)	4 (16.0)	11 (22.4)	8 (20.5)
	+++	1 (6.7)	14 (56.0)	28 (57.1)	24 (61.5)
S100 in DC	-	6 (40.0)	14 (56.0)	33 (67.3)	30 (76.9) 0.026 <sup>a</sup>
	+	6 (40.0)	9 (36.0)	15 (30.6)	8 (20.5)
	++	1 (6.7)	2 (8.0)	1 (2.0)	1 (2.6)
	+++	2 (13.3)	0 (0.0)	0 (0.0)	0 (0.0)

<sup>a</sup>Indicates a significant association was observed.

DC = Dendritic cells.

**Supplemental Table 3.** Univariable and multivariable Cox proportional hazard model for the risk of recurrence

	Univariable		Multivariable	
	HR (95% CI)	p value	HR (95% CI)	p value
Age, years	1.011 (0.992, 1.030)	0.267		
Tumor size, cm	1.125 (1.058, 1.195)	< 0.001 <sup>b</sup>	1.099 (1.020, 1.183)	0.013 <sup>b</sup>
Gender (female vs. male)	1.184 (0.741, 1.891)	0.481		
Differentiation	1.574 (0.995, 2.492)	0.053	3.074 (1.845, 5.125)	< 0.001 <sup>b</sup>
Lymph nodes metastasis	4.288 (2.204, 8.343)	< 0.001 <sup>b</sup>		
T stage				
T1	—			
T2	2.940 (0.609, 14.194)	0.179		
T3	7.717 (1.882, 31.644)	0.005 <sup>b</sup>		
T4	12.615 (2.420, 65.760)	0.003 <sup>b</sup>		
N stage <sup>a</sup>				
N0	—			
N1	4.176 (2.013, 8.661)	< 0.001 <sup>b</sup>		
N2	6.271 (2.917, 13.481)	< 0.001 <sup>b</sup>		
N3	4.358 (1.723, 11.025)	0.002 <sup>b</sup>		
M stage				
M0	—		—	
M1	4.736 (2.914, 7.696)	< 0.001 <sup>b</sup>	4.507 (1.793, 11.324)	0.001 <sup>b</sup>
Clinical stage				
I	—		—	
II	3.462 (0.747, 16.043)	0.112	3.300 (0.698, 15.601)	0.132
III	10.527 (2.527, 43.847)	0.001 <sup>b</sup>	14.655 (3.421, 62.778)	< 0.001 <sup>b</sup>
IV	19.585 (4.671, 82.113)	< 0.001 <sup>b</sup>	12.210 (2.382, 62.583)	0.003 <sup>b</sup>
CD40 (++/+++ vs. -/+)	1.287 (0.828, 2.000)	0.262		
VEGF (++/+++ vs. -/+)	1.250 (0.762, 2.051)	0.377		
PI3K (++/+++ vs. -/+)	1.503 (0.894, 2.527)	0.124		
AKT (++/+++ vs. -/+)	2.100 (1.155, 3.819)	0.015 <sup>b</sup>		
S100 in DC (++/+++ vs. -/+)	0.533 (0.168, 1.691)	0.285		

<sup>a</sup>1 missing value in N stage (Nx).<sup>b</sup>Indicates a significant impact on the risk of recurrence was observed.

— Indicates the reference group.

HR = Hazard ratio; CI = confidence interval; DC = dendritic cells.

**Supplemental Table 4.** Univariable and multivariable Cox proportional hazard model for the risk of death

	Univariable		Multivariable	
	HR (95% CI)	p value	HR (95% CI)	p value
Age, years	1.012 (0.992, 1.033)	0.243		
Tumor size, cm	1.140 (1.069, 1.215)	< 0.001 <sup>b</sup>	1.128 (1.040, 1.224)	0.004 <sup>b</sup>
Gender (female vs. male)	1.129 (0.692, 1.842)	0.628		
Differentiation	1.629 (1.005, 2.642)	0.048 <sup>b</sup>	3.283 (1.902, 5.668)	< 0.001 <sup>b</sup>
Lymph nodes metastasis	3.987 (1.982, 8.019)	< 0.001 <sup>b</sup>	2.180 (0.906, 5.247)	0.082
T stage				
T1	—			
T2	3.117 (0.646, 15.042)	0.157		
T3	6.735 (1.639, 27.675)	0.008 <sup>b</sup>		
T4	13.110 (2.501, 68.732)	0.002 <sup>b</sup>		
N <sup>a</sup>				
N0	—			
N1	3.692 (1.780, 7.661)	< 0.001 <sup>b</sup>		
N2	4.495 (2.050, 9.860)	< 0.001 <sup>b</sup>		
N3	3.610 (1.385, 9.405)	0.009 <sup>b</sup>		
M stage				
M0	—	—		
M1	4.280 (2.608, 7.025)	< 0.001 <sup>b</sup>	3.377 (1.430, 7.974)	0.005 <sup>b</sup>
Clinical stage				
I	—	—		
II	3.099 (0.658, 14.605)	0.153	2.145 (0.436, 10.553)	0.348
III	8.663 (2.075, 36.169)	0.003 <sup>b</sup>	5.707 (1.138, 28.627)	0.034 <sup>b</sup>
IV	16.575 (3.943, 69.676)	< 0.001 <sup>b</sup>	6.617 (1.171, 37.377)	0.032 <sup>b</sup>
CD40 (++/+++ vs. -/+)	1.209 (0.762, 1.918)	0.421		
VEGF (++/+++ vs. -/+)	1.352 (0.796, 2.296)	0.264		
PI3K (++/+++ vs. -/+)	1.324 (0.782, 2.242)	0.296		
AKT (++/+++ vs. -/+)	2.082 (1.116, 3.883)	0.021 <sup>b</sup>		
S100 in DC (++/+++ vs. -/+)	0.371 (0.091, 1.516)	0.167		

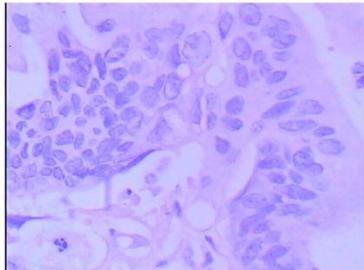
<sup>a</sup>1 missing value in N stage (Nx).<sup>b</sup>Indicates a significant impact on the risk of recurrence was observed.

— Indicates the reference group.

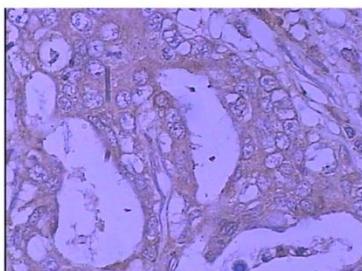
HR = Hazard ratio; CI = confidence interval; DC = dendritic cells.

**Supplemental fig. 1.** Adjacent normal (**A** and **C**) and gastric cancer cells (**B** and **D**) stained for CD40 (**A** and **B**) and S100 (**C** and **D**) (magnification  $\times 400$ ).

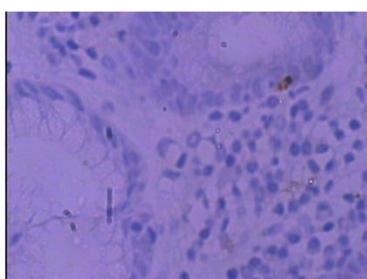
**A**



**B**



**C**



**D**

