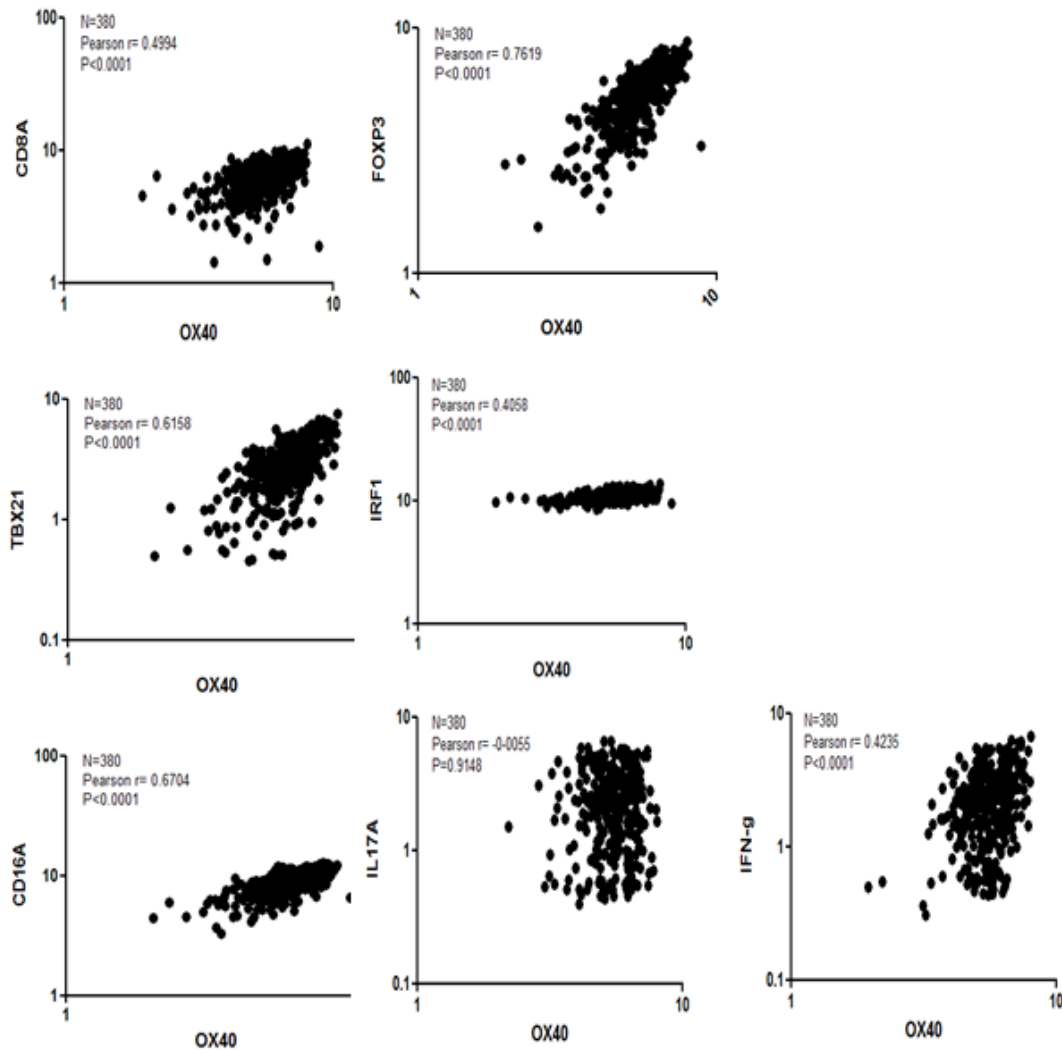


OX40 expression enhances the prognostic significance of CD8 positive lymphocyte infiltration in colorectal cancer

Supplementary Material



Supplementary figure 1. CRC gene expression profiles from TCGA public database. The expression of the indicated genes was evaluated by next generation sequencing (NGS) in 380 CRC specimens.

Table 1s Comparison of clinical-pathological characteristics of the training and validation subsets (n=328 and n=329)*

Characteristics	Training		Validation		P
	N or median, mean	(% or range)	N or median, mean	(% or range)	
Age, years (median, mean)	70, 68.9	(36-90)	72, 70.8	(40-96)	0.041
Tumor size in mm (median, mean)	50, 50.4	(5-170)	50, 51.7	(6-150)	0.593
Gender					0.326
Female (%)	186	(56.7)	173	(52.6)	
Male (%)	142	(43.3)	156	(47.4)	
Anatomic site of the tumor					0.563
Left-sided (%)	217	(66.2)	208	(63.2)	
Right-sided (%)	110	(33.5)	118	(35.9)	
T stage					0.452
T1 (%)	16	(4.8)	13	(4.0)	
T2 (%)	55	(16.8)	48	(14.6)	
T3 (%)	208	(63.4)	208	(63.2)	
T4 (%)	39	(11.9)	52	(15.8)	
N stage					0.9324
N0 (%)	165	(50.3)	168	(51.1)	
N1 (%)	86	(26.2)	88	(26.7)	
N2 (%)	61	(18.6)	67	(20.4)	
Tumor grade					<0.001
G1 (%)	8	(2.4)	7	(2.1)	
G2 (%)	277	(84.5)	285	(86.6)	
G3 (%)	3	(1.0)	30	(9.1)	
UICC					0.053
Stage IA (%) T1N0	12	(3.6)	9	(2.7)	
Stage IB (%) T2N0	39	(11.9)	34	(10.3)	
Stage IIA (%) T3N0	103	(31.4)	99	(30.1)	
Stage IIB-C (%) T4N0	7	(2.1)	23	(7.0)	
Stage III (%) N+	145	(44.2)	151	(45.9)	
Tumor border configuration					0.157
Infiltrative (%)	216	(65.9)	202	(61.4)	
Pushing (%)	99	(30.2)	119	(36.2)	
Vascular invasion					0.717
No (%)	231	(70.4)	231	(70.2)	

Yes (%)	84	(25.6)	91	(27.7)	
Microsatellite Stability					0.929
Proficient (%)	276	(84.1)	276	(83.9)	
Deficient (%)	52	(15.9)	53	(16.1)	
Localisation					0.379
Rectal cancers (%)	105	(32.0)	114	(34.7)	
Rectosigmoid cancers (%)	16	(4.9)	25	(7.6)	
Median overall survival time (months)	91.5, 83	0-152	93, 85.8	0-151	0.469
5-year overall survival % (95%CI)	58.9	53.6 – 64.8	52.1	46.7 – 58.0	0.071

*Percentages may not add to 100% due to missing values of defined variables. Age and tumor size were evaluated using the Kruskal-Wallis test. Gender, anatomical site, T stage, N stage, grade, vascular invasion, and tumor border configuration were analyzed using the χ^2 test. Survival analysis was performed by using the Kaplan-Meier method.

Supplementary table 2s. Association of OX40+ and CD8+ low and high density infiltration and clinical-pathological features in CRC

		OX40 ^{high} / CD8 ^{high}		OX40 ^{low} / CD8 ^{high}		OX40 ^{high} / CD8 ^{low}		OX40 ^{low} / CD8 ^{low}		p-value
		N=48	(%)	N=73	(%)	N=158	(%)	N=354	(%)	
Age	years, mean	68 ± 10		70 ± 10		69 ± 11		71±11		0.10
Tumor diameter	mm, mean ± SD	56,7 ± 17,1		55,3 ± 29.2		47,7 ±23.1		51,5 ± 20.8		0.26
Gender	Female Male	30 18	63 37	49 24	67 33	75 82	47 52	191 162	54 46	0.14
Tumor location	Left- sided Right- sided	32 15	63 31	35 38	48 52	112 45	71 28	225 125	64 35	0.007
Histologic subtype	Mucinous Non- mucinous	1 47	2 98	6 67	8 92	7 151	4 96	33 321	9 91	0.11
pT stage	pT1-2 pT3-4	12 33	25 69	22 48	30 66	43 108	27 68	47 300	13 85	0.00005
pN stage	pN0 pN1-2	32 14	67 29	46 27	63 37	93 58	59 37	151 190	43 54	0.00004
Tumour grade	G1-2 G3	39 6	81 13	59 10	81 14	141 11	89 7	314 31	89 9	0.29
Vascular invasion	Absent Present	35 10	73 21	52 17	71 23	120 31	76 21	234 112	66 32	0.034
Tumor border	Pushing Infiltrating	19 26	40 54	34 35	47 48	50 101	32 64	103 242	29 68	0.01
PTL inflammation	Absent Present	29 16	60 33	47 22	64 30	121 31	77 20	283 63	80 18	0.007
Microsatellite stability	Deficient Proficient	10 38	21 79	14 59	19 81	17 140	11 89	60 293	17 83	0.189
5-year overall survival rate	(95%CI) nr. of deaths	0.82	0.72- 0.94	0.60	0.49-0.73	0.59	0.53-0.68	0.48	0.43- 0.54	0.0001

*Percentages may not add to 100% due to missing values of defined variables. Variables are indicated as absolute numbers, %, median or range. Age and tumor size were evaluated using the Kruskal-Wallis test. Gender, anatomical site, T stage, N stage, grade, vascular invasion, and tumour border configuration were analyzed using the χ^2 test. Survival analysis was evaluated using the Kaplan-Meier method.