

ORIGINAL ARTICLE

Upregulation of Breast Cancer Resistance Protein Expression was Decreased in Plasma Membrane of Colon Cancer with Metastasis of Lymphatic Node

Ao Wu^{1,2}, Jiaoli Zhang¹, Liyan Zeng², Feng Li², Zhaoguang Dong², Yanling Feng²,
Xiaofang Jia², Jun-O Jin², Lijun Zhang^{1,2}

¹ School of Laboratory Medicine and Life Science, Wenzhou Medical University, Wenzhou, China
² Shanghai Public Health Clinical Center, Fudan University, Shanghai, China

SUMMARY

Background: ATP-binding cassette sub-family G member 2 (ABCG2), an ABC transport protein involved in the efflux of anticancer compounds, has been reported to have altered expression levels in several cancers including breast, colon, and gastric cancer, etc. However, its expression change (up- or down-regulated in cancer) is still contradictory.

Methods: We performed immunohistochemistry to examine ABCG2 expression in the microarray with 90 pairs of colon cancer and their adjacent normal tissues. To find the expression of ABCG2 in lymphatic node metastasis (N1) and N0, we performed immunofluorescence, analyzed by Confocal technology.

Results: Compared to adjacent normal tissues, the percentage and density of positive cells expressing ABCG2 in colon cancer were significantly increased. In addition, ABCG2 expression in plasma membrane was related to lymph node metastasis in colon cancer, which was further verified to be downregulated by 2.7-fold in N1 to N0 through immunofluorescent analysis.

Conclusions: ABCG2 expression in colon cancer was up-regulated, and its expression was decreased in the plasma membrane of colon cancer with lymphatic node metastasis (N metastasis).

(Clin. Lab. 2018;64:xx-xx. DOI: 10.7754/Clin.Lab.2017.170729)

Correspondence:

Lijun Zhang
School of Laboratory Medicine and Life Science
Wenzhou Medical University
Wenzhou
China
Shanghai Public Health Clinical Center
Fudan University
201508 Shanghai
China
Phone: +86 21-37990333-5368;
Email: zhanglijun1221@163.com

Ao Wu
Shanghai Public Health Clinical Center
Fudan University
201508 Shanghai
China
Email: 15096008235@163.com

Manuscript accepted October 16, 2017

Supplementary Table

Table S1. The clinicopathological factors of the colorectal cancer patients in the tissue microarray.

Tissue code	Clinicopathological factors of patients used in tissue microarray													ABC2 expression in CRC			ABC2 expression in adjacent					
	Survival state	Survival time	Gender	Years	Distant metastasis	Primary organ (Yes or No)	Metastasis (Yes or No)	Grade	Size (cm)	Lymph node number	Positive lymph node	T	N	M	The AJCC Cancer Staging 7th Edition	Score in Cytoplasm	Score in plasma membrane	Percent of positive cells (optical density) (%)	No./mm ² (optical density)	Score in Cytoplasm	Percent of positive cells (optical density) (%)	No./mm ² (optical density)
277	Alive	97	M	62	No	Yes	No	II	4 x 3.5 x 1.5	5	0	T3	N0	M0	2A	2.5	2.5	68.43	5,432.365	1	7.19	119.873
280	Alive	97	F	81	No	Yes	No	II	9 x 7 x 3	ND	ND	T3	N0	M0	2A	1.5	0	38.62	2,722.878	1	7.21	229.267
281	Dead	31	M	70	No	Yes	No	II	6 x 5.5 x 1, 7 x 5 x 0.5	ND	ND	T3	N0	M0	2A	1	2.5	4.79	261.121	1	12.49	360.037
284	Alive	96	M	71	No	Yes	No	II	6 x 4 x 1.5	ND	ND	T3	N0	M0	2A	1	2.5	28.46	1,962.390	1	6.64	139.153
285	Dead	55	F	70	No	Yes	No	II - III	5.5 x 4.5 x 0.7	3	1	T3	N1 ^a	M0	3B	1.5	0	5.42	546.548	1	7.23	233.458
287	Alive	96	M	55	No	Yes	No	II - III	7.5 x 4.5 x 2.5	ND	ND	T3	N0	M0	2A	0	2.5	11.21	279.982	1	1.73	63.289
288	Dead	30	F	58	No	Yes	No	II - III	5 x 4 x 1.5	7	4	T3	N2 ^a	M0	3B	1.5	2.5	31.12	1,786.773	ND	5.13	63.709
289	Dead	5	M	72	No	Yes	No	I - III	4 x 2.5 x 2	ND	ND	T3	N0	M0	2A	1.5	2.5	59.8	2,916.341	1	13.27	439.253
291	Alive	95	F	62	No	Yes	No	II - III	7.5 x 5 x 2	10	0	T3	N0	M0	2A	1.5	0	9.54	783.363	0.75	2.82	89.642
292	Alive	95	M	85	No	Yes	No	II - III	4 x 4 x 2.5	9	0	ND	N0	M0	ND	1	2.5	5.34	317.283	1	3.71	87.179
293	Dead	62	F	75	No	Yes	No	II	4.5 x 3 x 1.5	2	2	ND	N1 ^b	M0	3	1.5	2.5	35.91	2,715.156	1.5	24.29	539.846
295	Dead	51	F	24	No	Yes	No	II - III	3.5 x 3 x 1	24	4	T3	N2 ^a	M0	3B	1	1.5	1.77	92.210	1	12.24	252.738
296	Dead	55	M	80	No	Yes	No	II	4 x 3 x 1.5, 2 x 1.7 x 1	ND	ND	T3	N0	M0	2	0.8	2.5	47.32	4,882.046	0.75	5	108.136
297	Dead	24	M	75	No	Yes	No	II	7 x 6 x 2	7	2	T4 ^a	N1 ^b	M0	3B	0.8	0	5.08	192.802	1.5	13.11	382.251
298	Alive	95	F	59	No	Yes	No	II - III	4.5 x 3 x 1.5	4	0	T3	N0	M0	2A	1.5	0	12.57	981.195	0.75	7.94	308.064
309	Dead	26	M	83	No	Yes	No	I - II	7.5 x 5 x 2	6	2	T4 ^b	N1 ^b	M0	3C	2	2.5	79.51	8,144.629	0.75	7.56	212.501
311	Dead	25	F	52	No	Yes	No	II	5 x 4 x 2.5	25	6	T3	N2 ^a	M0	3B	1	2.5	13.87	675.226	1.5	11.4	126.579
312	Alive	95	M	80	No	Yes	No	II - III	5 x 4.5 x 1	5	0	T3	N0	M0	2A	1.5	2.5	66.8	7422.460	0.5	2.62	93.886
313	Dead	56	M	81	No	Yes	No	II - III	3 x 2.5 x 1	1	0	T3	N0	M0	2A	2	2.5	72.21	6,050.584	0.5	6.12	181.065
314	Dead	34	M	75	No	Yes	No	II	8 x 5 x 4	10	0	T3	N0	M0	2A	0.8	2.5	26.02	1,366.380	0.75	14.81	352.492
317	Dead	13	M	75	No	Yes	No	I - III	3.5 x 3 x 1	3	2	T3	N1 ^b	M0	3B	1	2.5	39.67	3,152.733	0.5	9.02	331.117
318	Alive	94	M	Adult	No	Yes	No	III	9.5 x 5 x 2	27	0	T3	N0	M0	2A	1.5	2.5	60.59	6,500.313	0.75	3.83	164.300
319	Dead	12	F	90	No	Yes	No	II	3 x 2 x 1	4	0	T3	N0	M0	2A	1.5	2.5	27.53	962.753	0.75	8.83	174.360
322	Alive	94	M	56	No	Yes	No	I - II	8 x 7 x 2	10	0	ND	N0	M0	ND	1.5	2.5	84.62	12,374.539	0.5	2.17	70.834
324	Alive	94	M	65	No	Yes	No	II	6 x 4 x 1	14	0	T3	N0	M0	2A	1	2	8.19	506.734	1	20.7	568.347

Table S1 (continued). The clinicopathological factors of the colorectal cancer patients in the tissue microarray.

Tissue code	Clinicopathological factors of patients used in tissue microarray													ABCG2 expression in CRC			ABCG2 expression in adjacent					
	Survival state	Survival time	Gender	Years	Distant metastasis	Primary organ (Yes or No)	Metastasis (Yes or No)	Grade	Size (cm)	Lymph node number	Positive lymph node	T	N	M	The AJCC Cancer Staging 7th Edition	Score in Cytoplasm	Score in plasma membrane	Percent of positive cells (optical density) (%)	No./mm ² (optical density)	Score in Cytoplasm	Percent of positive cells (optical density) (%)	No./mm ² (optical density)
325	Alive	94	F	65	No	Yes	No	III	7.5 x 6 x 3.5	6	2	ND	N1 ^b	M0	3	1.5	2.5	66.74	9,650.581	0.75	9.99	200.346
327	Dead	8	M	71	Liver, Spleen	Yes	No	II - III	4.5 x 3.7 x 0.8	2	2	T3	N1 ^b	M1 ^b	4B	1	0	5.24	379.317	0.75	2.02	49.877
334	Dead	28	F	76	No	Yes	No	I - III	6.5 x 5.5 x 2	14	1	T4 ^b	N1 ^a	M0	3C	1.5	2.5	86.66	10,164.780	0.5	9.83	397.340
335	Alive	93	M	72	No	Yes	No	II	5.5 x 3 x 0.7	ND	ND	T4 ^a	N0	M0	2B	1.5	2.5	28.24	3,204.705	0.5	15.92	258.187
336	Dead	52	M	76	No	Yes	No	II - III	3.5 x 3 x 1	4	0	T3	N0	M0	2A	1.5	0	15.71	921.258	0.75	17.1	724.684
337	Dead	13	ND	72	Epiploon	Yes	No	II - III	6 x 4.5 x 3, 1 x 1 x 0.5	9	4	T3	N2 ^a	M1 ^b	4B	0.8	1.5	4.81	174.359	0.75	8.6	557.449
340	Alive	93	M	Adult	No	Yes	No	II	4.5 x 4 x 1	5	0	T3	N0	M0	2A	0.8	2.5	46.84	5,356.544	0.5	11.22	409.076
341	Dead	79	F	65	No	Yes	No	II - III	7 x 4.5 x 1.5	5	0	T4 ^a	N0	M0	2B	0.8	1.5	9.42	733.067	0.75	7.77	279.144
343	Alive	93	M	79	No	Yes	No	II	7 x 4 x 1.5	4	0	T3	N0	M0	2A	2	2.5	88.51	8,788.840	0.75	22.21	641.276
344	Dead	6	F	61	No	Yes	No	I - III	4 x 3 x 1	6	0	T3	N0	M0	2A	0.8	2.5	34.8	2,992.624	0.75	5.75	216.692
345	Dead	20	M	75	No	Yes	No	II	1.5 x 1 x 0.5	7	1	ND	N1 ^a	M0	3	1.5	0	27.09	1,391.947	0.75	5.83	191.964
346	Dead	1	F	74	No	Yes	No	II	6 x 6 x 2	12	4	T4 ^b	N2 ^a	M0	3C	0.8	0	6.68	459.787	1.5	18.91	1,145.496
348	Dead	93	F	79	No	Yes	No	II - III	8 x 7.5 x 2	12	1	T4 ^a	N1 ^a	M0	3B	0.5	0	5.27	337.823	0.75	11.4	297.167
352	Dead	40	M	50	No	Yes	No	I	7 x 3.7 x 1	3	0	T3	N0	M0	2A	1.5	2.5	52.9	5,497.373	0.75	14.92	434.643
353	Alive	92	F	82	No	Yes	No	II	4 x 3 x 0.8	5	2	T3	N1 ^b	M0	3B	1.5	0	28.17	2,618.336	0.5	5.94	158.433
356	Dead	87	F	56	No	Yes	No	I - II	5 x 3.5 x 1	13	0	T4 ^a	N0	M0	2B	2	2.5	66.13	5,774.421	0.75	14.79	659.299
395	Alive	92	F	83	No	Yes	No	I	4 x 3.3 x 2.5	ND	ND	T2	N0	M0	1	0.8	0	10.54	437.158	0.5	4	121.130
398	Alive	92	M	72	No	Yes	No	III	6.5 x 5 x 1	12	0	T3	N0	M0	2A	1.5	2.5	33.35	2,461.580	0.5	6.63	121.549
400	Dead	28	F	Adult	No	Yes	No	I - III	3 x 2.5 x 1.5, 5 x 3 x 2.5	13	0	T3	N0	M0	2A	2	2.5	71.23	5,601.319	0.5	4.06	73.768
402	Dead	23	M	60	No	Yes	No	II - III	6 x 5 x 1.5	20	0	T3	N0	M0	2A	0.5	0	1.45	25.148	0.75	12.63	343.272
403	Dead	72	M	78	No	Yes	No	I - III	3.5 x 3 x 2	2	1	T3	N1 ^a	M0	3B	0.8	1.5	8.29	430.029	0.5	1.18	35.626
404	Dead	6	M	80	No	Yes	No	II - III	9 x 8 x 7	10	0	T4 ^b	N0	M0	2C	0.8	0	2.94	95.144	0.75	7.28	200.346
405	Dead	12	F	62	No	Yes	No	III	10 x 10 x 9	19	10	T3	N2 ^b	M0	3C	1.5	0	17.14	1,472.421	0.5	6.25	107.718
431	Dead	50	M	62	No	Yes	No	II	1 - 4 x 3 x 3	6	0	T3	N0	M0	2A	0.8	0	1.42	24.729	0.75	15.05	457.695

Table S1 (continued). The clinicopathological factors of the colorectal cancer patients in the tissue microarray.

Clinicopathological factors of patients used in tissue microarray													ABC2 expression in CRC			ABC2 expression in adjacent						
Tissue code	Survival state	Survival time	Gender	Years	Distant metastasis	Primary organ (Yes or No)	Metastasis (Yes or No)	Grade	Size (cm)	Lymph node number	Positive lymph node	T	N	M	The AJCC Cancer Staging 7th Edition	Score in Cytoplasm	Score in plasma membrane	Percent of positive cells (optical density) (%)	No./mm ² (optical density)	Score in Cytoplasm	Percent of positive cells (optical density) (%)	No./mm ² (optical density)
434	Alive	91	F	75	No	Yes	No	II	5 x 4 x 3	5	0	T2	N0	M0	1	1.5	2.5	64.52	5,490,667	0.5	11.75	419,135
435	Dead	16	M	Adult	No	Yes	No	II - III	5.5 x 4.5 x 3.5	10	0	T3	N0	M0	2A	2.5	0	32.48	1,359,674	0.5	2.17	83,408
436	Alive	91	F	Adult	No	Yes	No	II - III	3.5 x 2 x 2	5	0	T3	N0	M0	2A	0.8	2.5	19.15	1,788,029	0.5	2.75	59,098
437	Dead	18	F	71	No	Yes	No	II - III	7 x 7 x 4	10	0	T2	N0	M0	1	0.8	2.5	16.17	1,241,059	0.5	7.56	277,048
438	Alive	91	F	70	No	Yes	No	II	7 x 7 x 4	6	2	T3	N1 ^b	M0	3B	0.8	1.5	6.81	598,105	0.5	3.23	89,695
439	Alive	90	M	63	No	Yes	No	II	9 x 8 x 8	12	0	T3	N0	M0	2A	ND	ND	26.31	153,821	ND	7.81	25,567
440	Dead	20	M	74	No	Yes	No	II	15 x 13 x 11	6	1	ND	N1 ^a	M0	3	0.8	1.5	1.96	97,239	0.75	2.12	129,094
441	Dead	16	F	79	No	Yes	No	II	6 x 6 x 5	5	2	T4 ^b	N1 ^b	M0	3C	0.5	1.5	1.16	37,303	0.75	16.34	560,802
476	Dead	5	M	48	No	Yes	No	III	8 x 7 x 3	5	0	T4 ^b	N0	M0	2C	0.8	0	0.36	4,266	0.75	9.1	282,497
442	Dead	70	M	89	No	Yes	No	II - III	15 x 13 x 11	6	0	T3	N0	M0	2A	2	2.5	27.68	1,067,537	0.5	25.08	563,736
475	Dead	36	M	90	No	Yes	No	II - III	5 x 4 x 1	6	0	T3	N0	M0	2A	2	2.5	78.06	3,643,120	ND	7.51	77,121
444	Dead	21	F	62	No	Yes	No	II	4 x 3 x 2.5, 2.5 x 2 x 1.5	16	12	ND	N2 ^b	M0	3	0.8	1.5	11.67	912,876	0.75	11.13	278,306
447	Dead	52	F	80	No	Yes	No	II	4 x 4 x 1.5	8	0	T3	N0	M0	2A	2	2.5	90.54	9,832,905	0.5	18.56	1,119,090
452	Dead	10	F	67	No	Yes	No	II	15 x 10 x 10, 2 x 2 x 1.5	4	1	T4 ^a	N1 ^a	M0	3B	1.5	0	17.1	1,154,297	0.75	7.66	188,610
455	Dead	49	F	78	No	Yes	No	II	3.5 x 3 x 1	5	1	T3	N1 ^a	M0	3B	ND	ND	1.05	15,927	0.5	3.66	122,387
458	Alive	89	F	57	No	Yes	No	II - III	3.5 x 3 x 0.8	7	0	T2	N0	M0	1	0.5	1.5	3.75	294,652	0.5	3.71	176,037
459	Alive	89	M	65	No	Yes	No	II	5.5 x 3 x 0.8	3	0	T3	N0	M0	2A	2	2.5	67.48	6,157,094	0.5	7.03	226,752
460	Dead	62	M	80	No	Yes	No	I - II	8 x 5 x 5	8	0	T3	N0	M0	2A	0.5	0	1.9	28,082	0.75	6.55	211,663
463	Dead	44	M	67	No	Yes	No	II - II	3.5 x 3 x 2	8	3	T3	N1 ^b	M0	3B	0.8	2.5	50.53	5,166,257	0.5	23.29	644,210
465	Dead	37	M	72	No	Yes	No	II	10 x 10 x 8.2	14	1	T3	N1 ^a	M0	3B	0.8	1.5	6.07	262,379	0.75	26.76	561,222
466	Alive	89	M	62	No	Yes	No	I - II	6 x 6 x 3	9	0	T3	N0	M0	2A	0.8	1.5	5.25	229,267	0.75	7.13	163,882
524	Dead	83	F	82	No	Yes	No	I	9 x 5.5 x 3	2	0	T2	N0	M0	1	0.8	0	1.06	28,501	0.75	9.69	284,593
525	Alive	88	F	57	No	Yes	No	II - II	4.5 x 3 x 1	12	1	T3	N1 ^a	M0	3B	2	0	6.73	481,167	0.5	2.08	36,046
528	Alive	88	M	78	No	Yes	No	I - II	2.7 x 2.5 x 2	4	0	T1	N0	M0	1	1.5	2.5	71.06	8,532,682	1.5	20.16	448,471
529	Alive	88	F	77	No	Yes	No	II	ND	4	0	T3	N0	M0	2A	0.8	0	5.03	319,800	0.75	34.61	839,108

Table S1 (continued). The clinicopathological factors of the colorectal cancer patients in the tissue microarray.

Tissue code	Clinicopathological factors of patients used in tissue microarray												ABCG2 expression in CRC			ABCG2 expression in adjacent						
		The AJCC Cancer Staging 7th Edition	M	N	T	Positive lymph node	Lymph node number	Size (cm)	Grade	Metastasis (Yes or No)	Primary organ (Yes or No)	Distant metastasis	Years	Gender	Survival time	Survival state	No./mm ² (optical density)	Percent of positive cells (optical density) (%)	Score in Cytoplasm	No./mm ² (optical density)	Percent of positive cells (optical density) (%)	Score in plasma membrane
530	1	M0	N0	T2	0	5	2 x 2 x 1.5	II	No	Yes	No	84	F	88	Alive	1,064.183	30.19	0.75	5,940.399	53.36	2.5	0.8
531	3B	M0	N1 ^b	T3	2	9	10 x 6 x 1	III	No	Yes	No	80	M	88	Alive	557.445	17.44	0.5	87.180	2.33	0	0.5
532	2A	M0	N0	T3	0	11	5.5 x 4 x 1	II	No	Yes	No	65	F	88	Alive	186.096	7.49	0.5	92.628	1.82	0	0.5
533	2A	M0	N0	T3	0	9	8 x 4.5 x 2	II	No	Yes	No	76	F	34	Dead	181.905	8.3	0.5	4,378.704	54.03	0	1.5
535	2A	M0	N0	T3	0	6	5 x 4 x 1.5	II	No	Yes	No	74	M	82	Dead	356.684	9.55	0.5	16.765	0.44	0	0.5
537	3B	M0	N2 ^a	T3	4	9	4.5 x 4 x 1	II	No	Yes	No	65	F	88	Alive	585.531	32.85	0.5	5,865.793	74.44	2.5	1.5
539	3B	M0	N2 ^a	T3	4	6	5 x 4 x 1.3, 5 x 3.5 x 1	I - III	No	Yes	No	71	F	15	Dead	289.203	12.51	0.5	3,389.963	41.91	2.5	0.8
540	3B	M0	N1 ^a	T4 ^a	1	10	4 x 3.5 x 1	II - III	No	Yes	No	54	F	88	Alive	330.989	8.38	0.5	67.061	2.92	0	0.5
541	3B	M0	N1 ^b	T3	2	7	4 x 3 x 1.5	II - III	No	Yes	No	67	F	24	Dead	1,468.649	33.87	0.5	156.337	3.11	0	0.5
544	2A	M0	N0	T3	0	3	5 x 5 x 1.5	I - II	No	Yes	No	58	M	88	Alive	147.535	4.55	0.5	2,644.741	33.16	2.5	0.8
575	1	M0	N0	T2	0	4	2.3 x 2 x 0.5	II	No	Yes	No	62	M	80	Dead	586.784	14.56	0.5	3,174.528	44.81	0	2.5
576	2A	M0	N0	T3	0	7	4 x 4 x 1	II	No	Yes	No	54	F	87	Alive	176.875	12.94	0.5	7,069.493	76.16	2.5	2
577	2A	M0	N0	T3	0	4	3.5 x 3 x 1, 7 x 6 x 3	II	No	Yes	No	76	M	11	Dead	178.132	5.82	0.5	3,047.949	31.9	2.5	0.8
579	2A	M0	N0	T3	ND	ND	5 x 4 x 1.3	II	No	Yes	No	73	F	6	Dead	1,047.418	29.95	0.75	917.905	13.5	2.5	0.8
580	2A	M0	N0	T3	0	7	4.5 x 3.5 x 1.5	II - III	No	Yes	No	53	M	87	Alive	35.626	8.51	ND	105.203	1.64	0	0.5
583	2A	M0	N0	T3	0	12	5.5 x 4 x 2.5	II - III	No	Yes	No	64	M	28	Dead	134.961	4.42	0.5	1,684.503	42.64	2.5	0.8

The tissue microarray is a commercial product of Shanghai Outdo Biotech, China (catalog no. HCol- Ade180Sur-06). ^a- ND indicates that there were no data.

Tissue code only used the last three numbers. For example, No. 277 - 298 represents RDgCol0609A0277 to RDgCol0609A0298, 309 - 327: RDgCol0611A0309 to 327, 334 - 356: RDgCol0612A0334 to 356, 395 - 405: RDgCol0701A0395 to 405, 431 - 466: RDgCol0704A0431 to 466, 524 - 544: RDgCol0705A0524 to 544, 575 - 583: RDgCol0706A0575 to 544.

Table S2. The correlation analysis between the expression of ABCG2 and the characteristics of patients.

	Correlation coefficient	Sig (2-tailed)	N*	Correlation coefficient	Sig (2-tailed)	N*	Correlation coefficient	Sig (2-tailed)	N*	Correlation coefficient	Sig (2-tailed)	N*
Intensity in cytoplasm of CRC	1.000	-0.043	88	-0.043	0.306**	88	0.004	0.004	88	ND	ND	85
Intensity in cytoplasm of control	0.699	0.699	85	1.000	0.627	86	0.627	0.627	85	ND	ND	86
Intensity in PM of CRC	0.306**	0.306**	88	0.306**	1.000	85	1.000	1.000	85	ND	ND	85
Intensity in PM of control	0.699	0.699	85	0.627	0.627	86	0.627	0.627	85	ND	ND	86
Gender	0.018	-0.021	83	0.009	0.031	81	0.083	0.031	81	0.145	0.031	81
Years	0.851	0.851	83	0.851	0.778	83	0.851	0.778	83	0.778	0.778	83
Tumor size	0.284	0.284	87	0.158	0.123	87	0.158	0.123	87	0.123	0.123	87
Pathological grading	0.555	0.555	88	-0.148	0.843	86	-0.148	0.843	86	-0.021	0.843	86
T	0.654	0.654	81	0.174	0.243	79	0.174	0.243	79	-0.131	0.243	79
N	0.313	-0.109	88	0.020	0.037	86	0.020	0.037	86	-0.223*	0.037	86
M	0.636	-0.051	88	0.077	0.268	86	0.077	0.268	86	-0.119	0.268	86
TNM	0.499	-0.074	86	0.063	0.093	84	0.063	0.093	84	-0.182	0.093	84
Spearman's rho												

* - The mucosa in some specimens was lost during the experiments. Sig. (2-tailed) (p) < 0.05 represents significant difference, correlation coefficient > 0, positive correlation. ND represents no data. T - Tumor (Topography), N - Lymph Node Metastasis, M - Metastasis, TNM - Total grading.

Table S3. The clinicopathological data of 8 patients used for confocal analysis.

Years	Gender	Pathological type	Tumor size	Tumor location	T	N	M	TNM	Survive state	Operation time
54	Male	Adenocarcinoma	8*7*6cm	Sigmoid colon	T4	N0	M0	II	Alive	2012.10.12
83	Male	Adenocarcinoma	7*5.3*4.5cm	Transverse colon		N0	M0	ND	Alive	2013.12.31
63	male	Adenocarcinoma	3.3*2.7*1.2cm	Transverse colon	T3	N0	M0	ND	Alive	2013.12.30
68	Male	Adenocarcinoma	5*4.5*3.5cm	Caecum	T4	N0	M1	IV	Alive	2012.11.30
76	Female	Signet ring cell carcinoma	6*5*5cm	Ileocecus	T4	N2	M1	ND	Alive	2012.12.17
45	Female	Adenocarcinoma	5*4*3cm	Ascending colon	Duckes C	N1	M0	ND	Alive	2012.12.03
66	Male	Adenocarcinoma	9.5*5*3cm	Transverse colon	T3	N1	M0	III	Alive	2012.09.21
59	Female	Adenocarcinoma	5.5*4.5*1cm	Rectum	T3	N1	M0	III	Alive	2014.04.10