CORRESPONDENCE

Gene amplified in oesophageal cancer 1 (GAEC1) amplification in colorectal cancers and its impact on patient's survival

GAEC1 (gene amplified in oesophageal cancer 1) is located at 7q22.1, first identified in oesophageal cancer.[1] Initial work indicated that *GAEC1* can act as an oncogene.[2] Our pilot study found ~80% of colorectal cancers showing amplification of *GAEC1*.[3] In this research, we will study *GAEC1* copy number in colon cancer cell lines and colorectal tissues, and its prognostic significance.

Two human colon cancer cell lines (SW480 and SW48) and one normal colonic epithelial cell line (FHC) were obtained from American Type Culture Collection. Culturing conditions for these cell lines were as published previously.[4] Tissues were collected from 283 patients (213 Australian; 70 Japanese) diagnosed with colorectal cancers. Ninety surgically removed non-cancer colorectal tissues (diverticular diseases, hyperplastic polyps and volvulus) were used as controls. H&E stained sections from each cancer were checked to select a block with sufficient cancer tissue and representative morphological features for each patient for DNA extraction.

Cancers were staged according to the Union for International Cancer Control (UICC) for TNM (tumour, node and metastasis) classification.[5] Only primary adenocarcinomas were included. Actuarial survival rates of patients were calculated from the date of surgical resection of the colorectal cancers to the date of death or last follow-up. DNA extraction and PCR reactions for *GAEC1* copy number were performed as in our previous studies.[3 4] A ΔCt of <-1 was considered as gain of *GAEC1* copies, Ct value >1 was considered as reduced *GAEC1* copies and a Ct between these ranges was defined as normal/no change in *GAEC1* copies.

GAEC1 DNA was detectable in all colon cell lines tested. The colon cancer cell lines (SW480 and SW48) showed reduced *GAEC1* copies compared with normal colonic epithelial cells (FHC) (figure 1A). At the tissue level, *GAEC1* gene copies were higher in colorectal cancer compared with non-cancer tissues ($p<0.0001$; table 1).

In Australian samples, there was no significant correlation between *GAEC1* copy number and clinical/pathological parameters (table 2). Japanese patients showed increased percentage of *GAEC1* deletion in colorectal cancers with lymph node metastasis compared with cancers without any lymph node metastasis (10% vs 0%, $p=0.046$) (table 3). *GAEC1* deletion was less common in early stage (Stages I and II) colorectal cancers compared with advanced stage (Stages III and IV) cancers in Japanese patients (0% vs 10%, $p=0.046$) (table 3). In addition, the proportion of cancers with *GAEC1* amplification was slightly higher in the Japanese compared with the Australian populations (80% vs 59%). *GAEC1* copy number distribution was significantly different, with *GAEC1* copy number higher among Japanese cancers compared with Australian ($p=0.003$).

Median follow-up period for Australian and Japanese patients was 5 years. Survival data was available in 188 Australian patients (134 survived and 54 died of cancer). Among Japanese patients, 64 patients had follow-up data (43 patients survived and 21 died of cancer). Kaplan-Meier analysis indicated that mean survival of Australian and Japanese patients with colorectal cancer depended on pathological stage of cancer ($p<0.0001$). For Australian and Japanese patients with colorectal cancer, patients with high *GAEC1* copies had longer survival compared with patients with low/

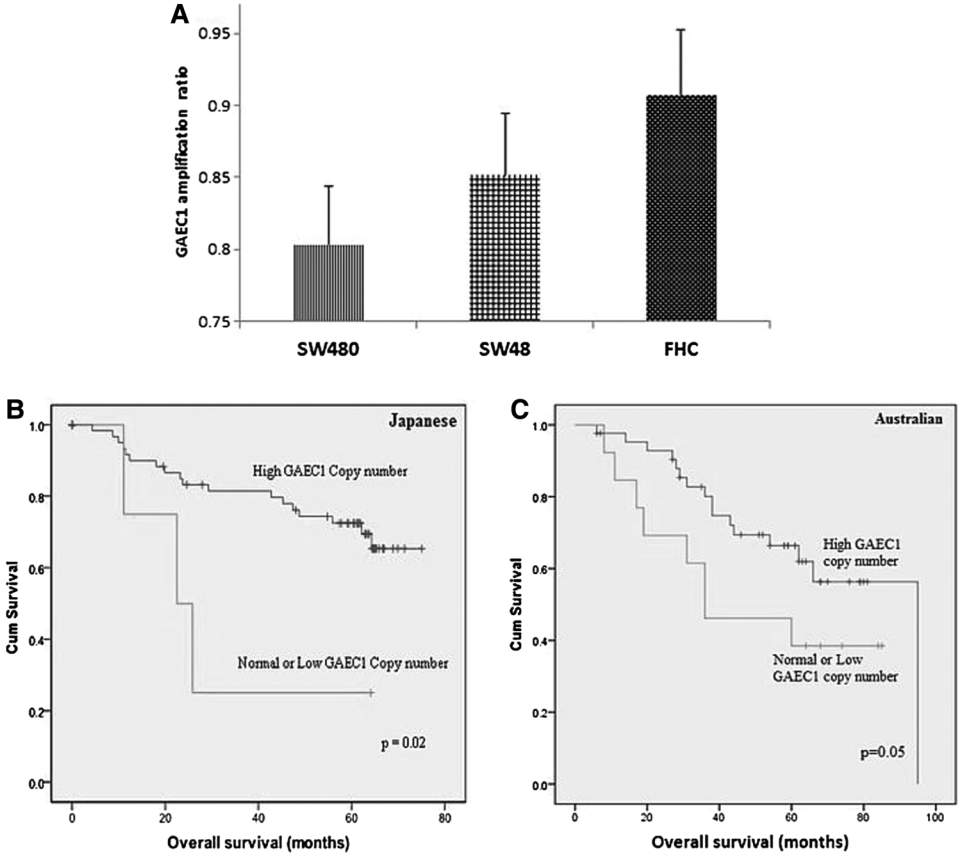

Figure 1 (A) Changes in *GAEC1* copy number variation in colon cancer cell lines. Inverse ratio of *GAEC1* versus *HBD* (control) (ct amplification ratio) was used to represent *GAEC1* DNA levels in different cell lines. Cancer cell lines (SW480 & SW48) showed lower copies of *GAEC1* DNA compared with normal epithelial cell line (FHC). (B and C) Correlation of *GAEC1* copy number changes and patients' survival. Australian (B) and Japanese (C) patients diagnosed with colorectal cancers showed better prognosis when they had high *GAEC1* copy numbers at the tissue level. On the contrary, patients with cancer having low *GAEC1* copy number showed poorer prognosis than other patients. These findings were statistically significant in Australian ($p=0.05$) and Japanese populations ($p=0.02$).

Table 1 DNA copy number variations of *GAEC1* in patients with colorectal cancers and non-neoplastic lesions

	Total	High GAEC1	Low GAEC1	No change	p Value
Australian cancer	213	125 (59%)	34 (16%)	54 (25%)	**<0.0001**
Japanese cancer	70	56 (80%)	3 (4%)	11 (16%)	
Non-neoplastic	90	18 (20%)	34 (38%)	38 (42%)	

All the statistical tests were done by χ^2 test.

normal *GAEC1* copies (Australians: mean survival time—71 months vs 49 months, p=0.05; Japanese: mean survival time—60 months vs 30 months, p=0.02; figure 1B,C).

In this study, Japanese patients showed a higher percentage of *GAEC1* amplification and fewer low copy number samples than Australian patients with colorectal cancer. This difference may reflect a real variance in characteristics or causes of colorectal cancer in the two populations. Also, these changes in *GAEC1* could be related to different environmental and genetic factors in these populations. Further studies on these parameters in conjunction with *GAEC1* may be helpful in identifying exactly what forces are behind these differences. Japanese patients with colorectal cancer showed correlations between *GAEC1* copy number with pathological stages and presence of lymph node metastasis, with high *GAEC1* copies in cancers with earlier pathological stages or no lymph node metastasis. The findings imply that *GAEC1* may function more in early stages of colorectal cancer progression. At later pathological stages, *GAEC1's* oncogenic roles may be carried on to potential interactors in molecular carcinogenesis. Further experiments including matched primary and lymph node metastases in the same patient would help in understanding this phenomenon.

Table 2 *GAEC1* copy number variations and the relationships between clinical and pathological features of Australian patients with colorectal adenocarcinoma

Characteristics	Number	Amplification	Deletion	No change	p-Value
Age					
≤60	36 (17%)	26 (72%)	7 (19%)	3 (8%)	0.814
>60	177 (73%)	130 (73%)	28 (16%)	19 (11%)	
Gender					
Male	122 (57%)	89 (73%)	20 (16%)	13 (11%)	0.984
Female	91 (43%)	67 (74%)	15 (16%)	9 (10%)	
Size (mm)					
≤50	160 (75%)	121 (75%)	22 (14%)	17 (11%)	0.186
>50	53 (25%)	35 (66%)	13 (25%)	5 (9%)	
Histological subtypes					
Conventional	195 (91%)	140 (72%)	33 (17%)	22 (11%)	0.087
Mucinous	18 (9%)	16 (89%)	2 (11%)	0 (0%)	
Pathological grade					
Well	64 (30%)	44 (69%)	14 (22%)	6 (9%)	0.560
Mod	116 (54%)	86 (74%)	16 (14%)	14 (12%)	
Poor	33 (16%)	26 (79%)	5 (15%)	2 (6%)	
T-stage					
T1 and T2	52 (24%)	36 (69%)	10 (19%)	6 (12%)	0.750
T3 and T4	161 (76%)	120 (75%)	25 (15%)	16 (10%)	
Lymph node metastasis					
Present	84 (39%)	62 (74%)	16 (19%)	6 (7%)	0.378
Absent	129 (61%)	94 (73%)	19 (15%)	16 (12%)	
Distant metastasis					
Present	34 (16%)	25 (74%)	4 (12%)	5 (15%)	0.525
Absent	179 (84%)	131 (73%)	31 (17%)	17 (10%)	
Stage					
Stages I and II	119 (56%)	86 (72%)	19 (16%)	14 (12%)	0.738
Stages III and IV	94 (44%)	70 (75%)	16 (17%)	8 (8%)	

All the statistical tests were done by χ^2 test and Fisher's exact test.

This study is the first to investigate the prognostic role of *GAEC1* in patients with colorectal cancer. In Australian and Japanese patients with colorectal cancer, increased *GAEC1* copy number was correlated with a longer survival time. This may indicate that patients with low *GAEC1* copies have more aggressive cancer, perhaps related to the association to pathological stage and lymph node metastasis seen in the Japanese patients. This finding supports the notion that *GAEC1* plays a role in cancer initiation rather than cancer progression.

In contrast with tissues, *GAEC1* showed loss of DNA copies in the colon cancer cell lines compared with normal colon cell lines. In cell lines, genes are less tightly regulated and they are genetically more complex than those in tissues.[6] Studies have also proven that ex vivo culture conditions can result in the attainment of genomic modification that may not show in the in vivo situation.[7] Thus, the difference in *GAEC1* copies could be due to environmental alterations in cell lines compared with solid cancer tissues. This phenomenon may indicate that *GAEC1* loses copies when cancer cells are more viable. This can be attributed to the factors influencing *GAEC1* deletion in lymph node metastases and cancer with poor patient prognosis.

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Table 3 *GAEC1* copy number variations and correlations between clinical and pathological features of Japanese patients with colorectal adenocarcinoma

Characteristics	Number	Amplification	Deletion	No change	p Value
Age					
≤60	18 (26%)	18 (95%)	1 (5%)	0 (0%)	0.708
>60	51 (73%)	48 (94%)	2 (4%)	1 (2%)	
Gender					
Male	41 (59%)	38 (93%)	3 (7%)	0 (0%)	0.082
Female	29 (41%)	28 (96%)	0 (0%)	1 (4%)	
Size (mm)					
≤50	51 (73%)	49 (96%)	2 (4%)	0 (0%)	0.256
>50	19 (27%)	17 (90%)	1 (5%)	1 (5%)	
Histological subtypes					
Conventional	66 (94%)	63 (95%)	2 (3%)	1 (2%)	0.296
Mucinous	4 (6%)	3 (75%)	1 (25%)	0 (0%)	
Pathological grade					
Well	15 (21%)	15 (100%)	0 (0%)	0 (0%)	0.499
Mod	49 (70%)	46 (94%)	2 (4%)	1 (2%)	
Poor	6 (9%)	5 (83%)	1 (17%)	0 (0%)	
T-stage					
T1 and T2	20 (29%)	19 (95%)	0 (0%)	1 (5%)	0.104
T3 and T4	50 (71%)	47 (94%)	3 (6%)	0 (0%)	
Lymph node metastasis					
Present	31 (44%)	28 (90%)	3 (10%)	0 (0%)	**0.046**
Absent	39 (56%)	38 (97%)	0 (0%)	1 (3%)	
Distant metastasis					
Present	2 (3%)	2 (100%)	0 (0%)	0 (0%)	0.887
Absent	68 (97%)	64 (94%)	3 (4%)	1 (2%)	
Pathological stage					
Stages I and II	39 (56%)	38 (97%)	0 (0%)	1 (3%)	**0.046**
Stages III and IV	31 (44%)	28 (90%)	3 (10%)	0 (0%)	

All the statistical tests were done by χ^2 test and Fisher's exact test.

Take-home messages

- GAEC1 copy number altered significantly between patients with cancer and the normal population.
- GAEC1 amplification was noticed in the cancer population and amplification of GAEC1 was different in patients with cancer from distinctive ethnic backgrounds.
- GAEC1 amplification was associated with negative lymph node metastasis and earlier pathological stages in the Japanese population.
- GAEC1 amplification in colorectal cancer was associated with better prognosis.

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