RESEARCH ARTICLE

Role of GSTM1 Copy Number Variant in the Prognosis of Thai Colorectal Cancer Patients Treated with 5-FU-based Chemotherapy

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Abstract

Background: Glutathione S-transferase M1 (GSTM1) is involved in the detoxification of carcinogenic agents. DNA copy number variants of GSTM1 may be associated with cancer progression and may result in reduced survival time of various cancers. Determination of DNA copy number variants was here used to assess the association between GSTM1 copy number variant and pathological status and survival time of colorectal-cancer patients treated with 5-fluorouracil-based chemotherapy. **Methods:** One hundred thirteen Thai colorectal-cancer patients were investigated for GSTM1 copy number variant by real-time PCR. Relationships between gene copy number variants and clinico-pathological parameters were determined. **Result:** Associations were evident between GSTM1 copy number and stage of tumor (P = 0.026) and metastasis at diagnosis (P = 0.049), with odds ratio values of 0.2 and 0.3 respectively. **Conclusions:** GSTM1 copy number variant was here not related with reduced overall survival for the colorectal-cancer patients receiving 5-FU-based chemotherapy**.**

Keywords: Glutathione S-transferase M1- DNA copy number variant- Colorectal cancer- 5-FU-based Chemotherapy

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Introduction

In Thailand, Colorectal cancer is the third most common cancer in male behind liver cancer and lung cancer. The incidence in males is 14.4/100,000 and females 11.2/100,000 people with the incidence of colorectal cancer increasing with age, it is widely accepted that the accumulation of genetic alteration and environmental factors involves to the development colorectal malignancies (Imsamran et al., 2015).

The best approach to treat metastatic colorectal carcinoma is chemotherapy treatment after resection of the tumor. Fluoropyrimidines are the chemotherapeutic agents that remain widely used in colorectal cancer. With a response rate of approximately 20%, 5-fluorouracil (5-FU) is a fluoropyrimidines agent that has been most commonly used. When combined with folinic acid, the response rates vary from 20 - 30%. The combination of irinotecan with 5-fluorouracil/folinic acid (FOLFIRI) or oxaliplatin with 5-fluorouracil/folinic acid (FOLFOX) resulted in increased response rates and also prolonged survival (Mohelnikova-Duchonova et al., 2014; Funke et al., 2008).

Glutathione S-transferases (GSTs) are a superfamily of enzymes that play a major role in phase II detoxification by catalyzing the conjugation of glutathione with electrophiles including carcinogens. The glutathione S-transferase Ml (GSTM1) is a member of Glutathione S-transferases superfamily (Rebbeck, 1997). In addition, the previous data indicated that the DNA copy number variant of GSTM1 has an effect on enzyme activity and increased risk of various cancers (He et al., 2011; Mccarroll and Altshuler, 2007; Sprenger el al., 2000).

A previous report explained that, the GSTM1 copy number variant was related to progression factor of colorectal cancer and low copy numbers of GSTM1 are related to better survival of the colorectal-cancer patients (Pongtheerat et al., 2013). In a previous study, the effect of GSTM1 copy number variant to chemotherapy treatment with colorectal cancer patients was reported that the copy number variant was inversely associated with survival in colorectal cancer patients treated with oxaliplatin and mortality was significantly decreased with one GSTM1 copy (Funke et al., 2008). Further, it has been shown that over expression of the antimetabolite 5-fluorouracil gene lead to resistance of fluoropyrimidines and poor prognosis in advanced colorectal cancer patients (Aschele et al., 2002).

This study investigated the effects of DNA copy number variant in chemical detoxifying gene, especially glutathione S-transferase M1 (GSTM1) in the responsibility for chemotherapy treatment with Thai colorectal-cancer

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patients. Real-time PCR was used to determine DNA copy number variant of glutathione S-transferase M1 (GSTM1). The correlation between GSTM1 gene copy number variant and the clinic-pathological features of colorectal cancer was analyzed by Chi-square test. The prognostic analysis of colorectal cancer depended on the association between GSTM1 copy number variant and survival status, determined by Kaplan-Meier survival curve. Statistical significance was set at P-value ≤ 0.05. The conclusions of this study may be used to correlate the GSTM1 copy number to 5-fluorouracil resistance in Thai colorectal-cancer patients.

Materials and Methods

Sample collection and DNA isolation

DNA samples were extracted from formalin-fixed, paraffin-embedded, colorectal cancer samples obtained from 113 Thai colorectal-cancer patients. The samples were collected from the National Cancer Institute of Thailand. This study has been approved by the Ethics Committee, National Cancer Institute, Thailand.

Detection of GSTM1 copy number variation

GSTM1copy number variants was determine by real-time PCR using SsoFast™ EvaGreen® Supermix (Bio-Rad Laboratories. Inc) and β-globin gene as the reference gene. PCR primers for the GSTM1 were 5'-ACC CCA GGG CTC TAT GGG AA-3' and 5'-TGA GGG CAC AAG AAG CCC CT-3' (Nørskov et al., 2009) and the primers for β-globin gene were 5'-AAC TTC ATC CAC GTT CAC C-3' and 5'-GAA GAG CCA AGG ACA GGT AC-3'.

The master mix (20µl) consisted of SsoFast EvaGreen Supermix reagent 10µl, 25 ng of DNA sample and 500 nM of primers. The real-time PCR was performed using a BioRad CFX-96 qPCR (Real Time PCR) System with the following steps: initial denaturation at 98°C for 2 minutes, 50 cycles under denaturation conditions at 94°C for 10 seconds, primer annealing at 54.4°C for 10 seconds, polymerization at 60°C for 20 seconds. Gene copy number was determined by Δ Ct method = Ct, target gene – Ct, reference gene (Covault et al., 2003).

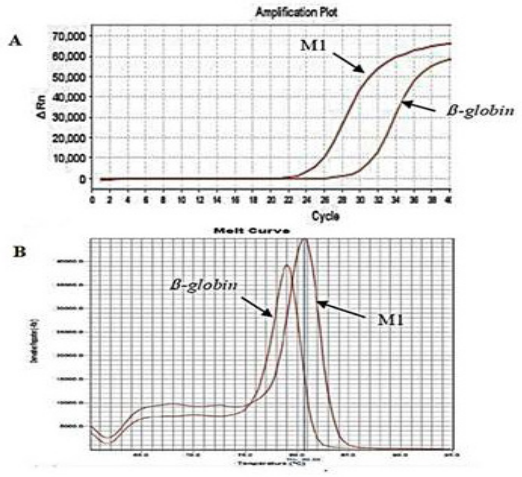

Figure 1. The Representative Fluorescent Data of Ct Value (A) and Melting Curve (B) of GSTM1 and β-Globin

Statistical analysis

The correlation between clinico-pathological parameters [stage, lymph-node metastasis, size of tumor, differentiation, metastasis at diagnosis and age at diagnosis] of the patients with GSTM1copy number variant was examined by Chi-square test.

The associations between the responsibility to chemotherapy and GSTM1 copy number, was described as odds ratios (ORs) and 95% confidence intervals (CIs) using unconditional logistic regression analysis.

Survival was calculated by Kaplan-Meier survival method and log-rank test, with patients followed up for a period of 1-300 weeks. A P-value ≤ 0.05 was considered statistically significant.

Results

Detection of GSTM1 copy number variation

The GSTM1 copy number was determined by real-time PCR technique for 113 DNA samples of colorectal-cancer patients, the representative fluorescent data of Ct value and melting curve are shown in Figure 1. The results showed the GSTM1 for genotypes 0/0 and 1/0 15.1%, 1/1 38.9% and >1/1 46% of cases.

Statistical analysis of GSTM1 copy number variation and patients' clinico-pathological parameters

Table 1. GSTM1 Copy Number Variant Status and Clinico-Pathological Parameters of Colorectal Cancer Patients

	CNV status		
	0/0+1/0 +1/1 n(%)	>1/1 n(%)	P-value *significant
Parameter			
Stage			0.059
III	14(22)	22(35)	
IV	17(27)	10(16)	
Lymph node			0.692
+	30(27)	24(22)	
-	29(26)	27(25)	
Size			0.800
≤3	7(6)	7(6)	
>3	52(47)	45(41)	
Differentiation			0.853
WD + MD	55(49)	48(43)	
PD	4(4)	4(4)	
Metastasis at diagnosis			0.042*
-	15(23)	23(36)	
+	17(27)	9(14)	
Age			0.775
<50	8(7)	6(5)	
≥50	52(47)	46(41)	

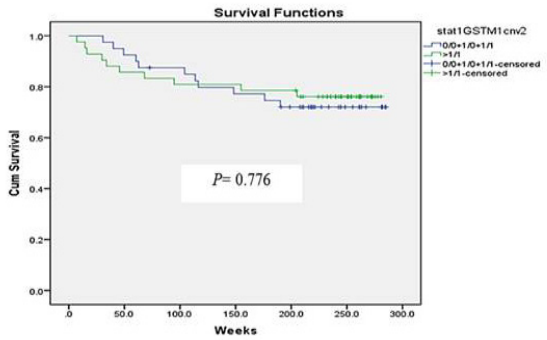

Figure 2. Kaplan-Meier Survival Curves for the Patients with Colorectal Cancer According to GSTM1Copy Number

The relations between GSTM1 copy number and patients' clinico-pathological parameters are summarized in Tables 1. GSTM1 copy number variation was associated with metastasis at diagnosis (P = 0.042) and no correlations were found with stage, size of tumor, differentiation lymph-node metastasis and age at diagnosis (P > 0.05).

However, in the case of colorectal cancer patients who received 5-FU-based treatment, there were statistically significant correlations. The results found significantly associations between GSTM1 copy number with reduced stage of tumor (P = 0.026) and metastasis at diagnosis (P = 0.049). Odds Ratio values for stage of tumor and metastasis at diagnosis were 0.2 and 0.3 respectively (data are summarized in Tables 2).

Survival of patients with GSTM1 copy number variation

Survival analysis was explained by Kaplan-Meier survival curve. In summary, there was no association between GSTM1 gene copy number and patient survival (Figure 2, P=0.776).

Discussion

A previous study has indicated that GSTM1 copy number variant was associated with resistance to chemotherapeutic agents of colorectal cancers (Funke et al., 2008). In the present study, we investigated gene copy number for GSTM1 from 113 colorectal cancer samples by real-time PCR technique and found the GSTM1 for genotypes 0/0 and 1/0 15.1%, 1/1 38.9% and >1/1 46%. The result showed that GSTM1 copy number variant was associated with metastasis at diagnosis (P = 0.042) and in the case of colorectal cancer patients who received 5-FU-based treatment, the GSTM1 copy number variant related to reduced stage of tumor (P = 0.026) and metastasis at diagnosis (P = 0.049). Odds Ratio values were 0.2 and 0.3 respectively. These observations indicated that the patient with GSTM1 high copy number who received 5-FU-based chemotherapy did not progress to higher stages of tumor and the GSTM1 copy number variant was not associated with decreased survival of the patients.

5-FU belongs to the fluoropyrimidine family and is widely used in the treatment of colorectal cancer. Earlier studies indicate that the treatment with 5-FU based chemotherapy in colorectal-cancer patient leads to prolonged survival and a decreased stage of tumor and metastasis at diagnosis (Mohelnikova-Duchonova et al., 2014).

Table 2. GSTM1 Copy Number Variant Status and Clinico-Pathological Parameters of Colorectal Cancer Patients who Received 5-FU/ folfox4+ LV

	CNV status			
Parameter	0/0+1/0 +1/1 n(%)	>1/1 n(%)	OR	P-value *significant
Stage			0.22	0.026*
III	6(16)	15(39)		
IV	11(29)	6(16)		
Lymph node			0.38	0.094
+	8(16)	18(36)		
-	13(26)	11(22)		
Size			1.19	0.771
≤3	7(14)	9(18)		
>3	13(27)	20(41)		
Differentiation			0.74	0.835
WD + MD	20(41)	27(55)		
PD	1(2.0)	1.0(2)		
Metastasis at diagnosis			0.26	0.049*
-	8(21)	14(37)		
+	11(29)	5(13)		

Previous reports demonstrated that GSTM1 copy number variant was related to progression factor of colorectal-cancer patients (Pongtheerat et al., 2013) and many experiments found that high GSTM1 copy numbers and high GSTM1 expression level in many cancers, result in resisting chemotherapeutic agents (Cheng et al., 1997; Depeille et al., 2004; Satta et al., 1992). However this does not correlate with all cancers. Seo et al, showed that a GSTM1 positive genotype was associated with progression in advanced gastric cancer patients treated with FOLFOX (Seo et al., 2009). It is unknown whether this was the result of the type of cancer or the treatment chosen, or both.

We hypothesized that GSTM1 polymorphism may affect the survival status in colorectal cancer patients treated with 5-FU based chemotherapy. Mortality might be significantly increased in patients with high GSTM1 copy number. However our experiments showed inverse results and that the GSTM1 copy number variant was not associated with reduced overall survival or clinical response for the colorectal-cancer patients receiving 5-Fluorouracil-Based Chemotherapy. Our studies are in accordance to previous experiments that specified that the GSTM1 copy number variant and GSTM1 polymorphism were not related to survival of the rectal cancer and colon cancer patients treated with 5-Fluorouracil-Based Chemotherapy (Lai et al., 2013; Stoehlmacher et al., 2002). Previous studies of colorectal cancers patients with defective function of the DNA mismatch repair (MMR) system also receiving 5-FU-based therapy were

assessed by Sinicrope (2011)Their result showed reduction in recurrence rates in higher stage cancers (Sinicrope et al., 2011).

5-Fluorouracil-Based Chemotherapy is still an effective chemotherapeutic agent used to treat colorectal-cancer patients who have high copy number of GSTM1, because the patients still see prolonged survival and the stage of cancer does not progress.

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