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The prognostic value of *DAPK1* hypermethylation in gliomas: A site-specific analysis

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ABSTRACT

Background and aims: The gene of death associated protein kinase 1 (*DAPK1*) has been reported to be methylated in various cancers including gliomas. However, its prognostic value for gliomas is still controversy, and the methylation at specific CpG sites of *DAPK1* has not been investigated. The aim of this study was to prognostically evaluate the methylation level of different CpG sites within *DAPK1* promoter region in gliomas.

Methods: Based on sodium bisulfite treated DNA products, we made use of DNA pyrosequencing method to evaluate overall and site-specific methylation of *DAPK1* in 143 gliomas and 26 benign tumors (meningeomas) or normal brain tissues. We both statistically analyzed the association between methylation levels of each CpG site and the clinicopathological characteristics, and estimated the prognosis predictive value of site-specific methylation for glioma patients.

Results: Methylation status of *DAPK1* site −1527, −1543, and the overall five sites concerned was higher in gliomas than controlled subjects ($p < 0.001$). Hypermethylation at site −1527 or together with site −1543 associated with better survival in patients taken postoperative therapies (−1527: $p = 0.002$; −1527 & −1543: $p = 0.023$), as well as in patients just underwent radiotherapy after surgery (-1527: $p = 0.015$; −1527 & −1543: $p = 0.030$). However, Cox regression analysis indicated the site-specific methylation was not independent contributor for gliomas prognosis.

Conclusion: Analysis of *DAPK1* gene promoter by quantitative pyrosequencing provided more detailed information of methylation status of CpG sites. *DAPK1* methylation level is associated with gliomas clinical features and outcomes. Interestingly, the hypermethylation at site −1527 or together with site −1543 indicated good sensitivity of postoperative therapies, especially radiotherapy. Thus, site specifically analysis of *DAPK1* methylation may be a valuable diagnostic and prognostic estimation for gliomas.

1. Introduction

Gliomas account for 40%–50% of primary central nervous system tumors and lead the most prevalent malignancy of brain tumors [1]. The World Health Organization (WHO) classified these tumors into grade I–IV according to their cellular origin and the degree of malignancy [2,3]. The most malignant gliomas, grade IV gliomas, also called glioblastomas (GBMs), are only with a 5-year survival rate of 9.8% at best. Low grade gliomas (LGGs) exhibit benign tendencies and portend a favorable prognosis for the patients. However, they have a unique rate of recurrence and increase in grade over time. Local radiotherapy, along with surgical resection, comprises the main treatment of gliomas, as systemic chemotherapy is commonly impaired by the blood-brain barrier. Although large body of studies have shown that median survival can actually be doubled if an early, multi-stage and personalized therapy is applied, lack of response to radiotherapy is another implication for poor prognosis of gliomas [4]. As known, genetic and epigenetic alterations are major culprits of cellular transformation and therapy resistance. Thus, more effort should be spent to better understand the molecular basis of gliomas.

Global genomic hypomethylation along with promoter hypermethylation of specific genes is a hall mark of various cancers. The genome-wide hypomethylation has been believed to activate transcription of the commonly silenced transposons like repetitive sequences (e.g., the Alu and LINE repeats in mammals), which contributes to the genomic instability [5–7]. The hypermethylation of gene promoter region is associated with repression of a series of tumor suppressor genes which always involve in vital biological processes,

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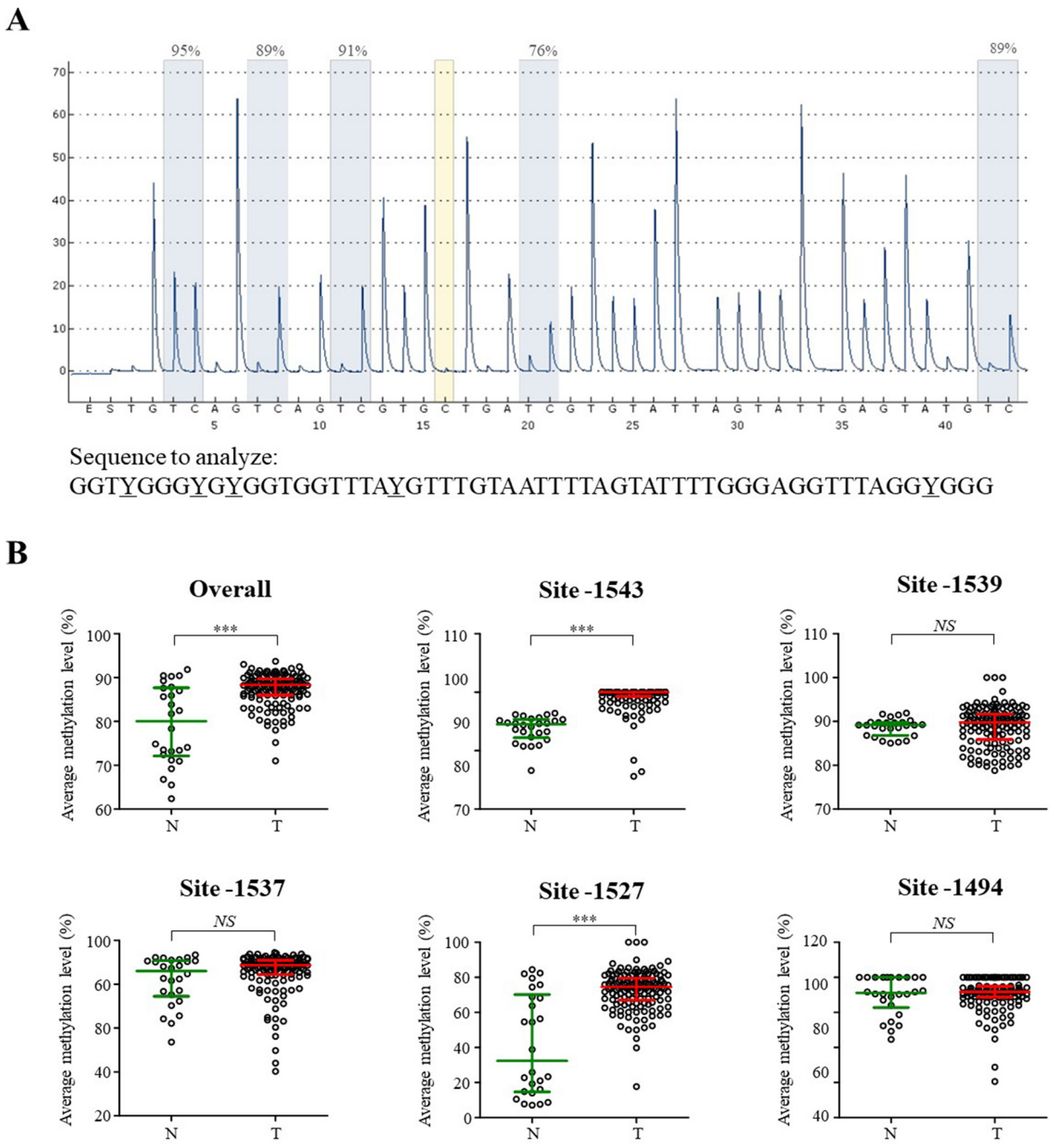

Fig. 1. A bisulfite pyrosequencing assay was performed to analyze overall and site-specific methylation levels of *DAPK1* in a cohort of gliomas and control subjects. (A) Representative pyrogram showed methylation levels of the indicated CpG sites within *DAPK1* gene promoter. (B) Analysis of overall and site-specific methylation levels of *DAPK1* in glioma samples using pyrosequencing assay. Horizontal lines represented the median ± interquartile range. T, glioma tissues; N, control subjects; ***, $p < 0.001$; *NS*, not significant.

including DNA repairment, and cell cycle controlling [2,8]. The aberrant promoter methylation of certain tumor suppressor genes may serve as a biomarker for early diagnosis and predictor of prognosis, even significant to disease management [9]. For example, hypermethylation of the *MGMT* promoter has been proved to be a powerful predictor for the sensitivity of alkylating agents for glioma patients [10].

Death associated protein kinase 1 (*DAPK1*) is a vital serine/theoreine kinase involved in multiple cellular processes such as autophagy, apoptosis and inflammation. The dysfunction of *DAPK1* in turn have been linked with various diseases such as cancer, stroke, inflammation and atherosclerosis [11]. Hypermethylaion of *DAPK1* and loss of its expression have been reported in many types of cancers including lymphoma [12], oral squamous cell carcinoma [13], and cervical cancer [14]. In gliomas, several groups have reported hypermethylation of *DAPK1*, with incidence rates varied in a wide range, from 14.6% to 66% [15,16]. Nevertheless, they all found high-grade tumor patients posses significantly higher levels of *DAPK1* promoter methylation, as compared to lower grade patients, suggesting that the promoter methylation status positively correlates with tumor grade [9,15,16]. However, there's still lack of concordant evidence on prognostic value of *DAPK1* promoter methylation in glioma patients.

To better evaluate the predictive power of *DAPK1* methylation in gliomas outcomes, we aim to use pyrosequencing method to detect the site-specific methylation information in a cohort of 143 glioma patients