Clinicopathological Implications of *GNAS* in Ewing Sarcoma

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ABSTRACT

The objective of the present study: To determine whether guanine nucleotide-binding protein α stimulating (GNAS) gene expression correlates with pathognomonic signs by analyzing the mutations, methylation status and G-protein α subunit (Gsα) expression of GNAS in Ewing sarcoma (ES).

Materials and Methods: Formalin-fixed paraffin-embedded tissue samples from 77 patients with primary ES were obtained in South Korea, Argentina and Brazil, and were studied via methylation chip assay and direct sequencing of the GNAS gene and immunohistochemical analysis of Gsα. The mutation and methylation statuses of the GNAS gene were examined. Immunohistochemical results were measured with respect to proportion and staining intensity.

Results: GNAS genes in ES tumor samples were less methylated compared with normal controls. No mutations were detected at exons 8 or 9 of the GNAS locus complex on chromosome 20q13.3, indicating that the pathogenesis of ES was not associated with GNAS mutation. Gsα expression correlated well with the methylation status of the GNAS gene. Notably, high Gsα expression was detected more frequently in samples from living patients than from decedents, although this was not statistically significant (P=0.055).

Conclusion: GNAS mutation is not associated with the pathogenesis of ES tumors. This finding may be used to differentiate ES tumors from metastatic bone lesions with morphological similarity to ES tumors. Analysis of the methylation status of the GNAS gene and immunohistochemical Gsα expression suggests that hypermethylated GNAS (low Gsα expression) in ES may be associated with unfavorable progression with a non-significant trend.

INTRODUCTION

Ewing sarcoma (ES) is the second most common primary bone malignancy, and typically develops in **children and adolescents**, predominantly in white males. It is also referred to as Ewing sarcoma family tumor (ESFT), which includes extraskeletal ES and primitive neuroectodermal tumors. ESFT is a highly aggressive malignancy, with a rate of metastasis of 27% at the time of diagnosis**. Chemotherapy with intercalated locoregional managements**, such as surgery and radiation, is the generally recommended treatment. It has been reported that **the survival rate** of patients suffering from ES tends to **increase with better elucidation of pathogenesis and the application thereof to the development of management strategies.**

However, the exact pathogenesis of ES remains to be elucidated due to the complicated interaction of diverse causative factors. for this reason, it is important to determine and act based upon the pathognomonic signs of ES.

Herein, we focus on the validation of the novel pathogenetic signaling, based on various hypotheses that expression of the guanosine nucleotide-binding protein α stimulating (GNAS) gene, which encodes the G-protein α subunit (Gsα), is associated with the pathogenesis of ES (Figure 1).

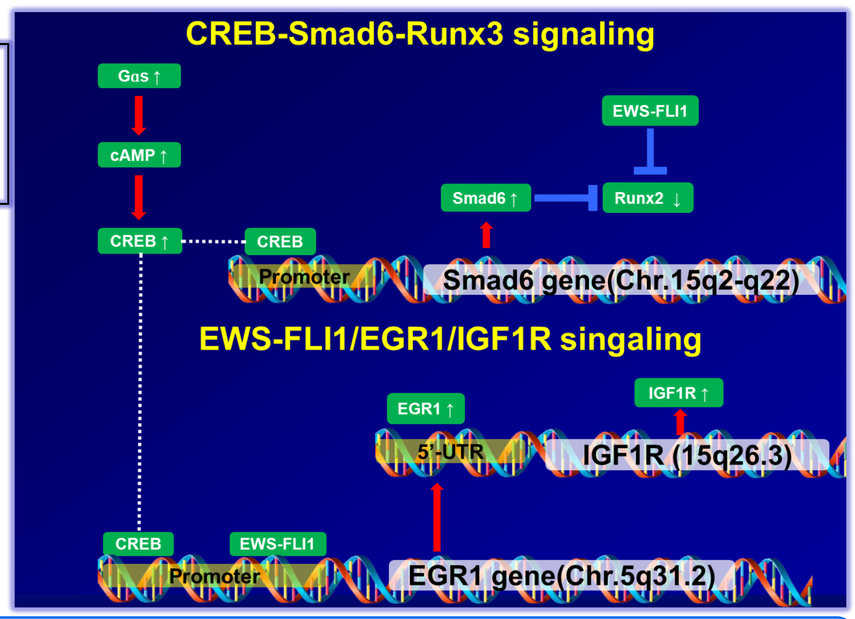

Figure 1. GNAS-associated hypothetical pathogenetic signaling in Ewing sarcoma

MATERIALS AND METHODS

1. Clinical tumor samples:

Formalin-fixed paraffin-embedded (FFPE) tissue samples from 77 patients with primary ES were obtained in South Korea, Argentina, and Brazil (**Table 1**).

2. Bisulfite conversion and methylation chip assay:

We used the GoldenGate Methylation Cancer Panel I product to process 1,505 CpG sites from a panel of 807 cancer-related genes which included oncogenes and genes related to DNA repair, tumor suppression, cell cycle, differentiation and apoptosis.

3. Direct sequencing:

Direct sequencing was performed to detect the mutational status of GNAS exons 8 and 9.

4. Immunohistochemistry:

Sections (5 μm thick) from FFPE tissues were cut and stained with Leica Auto-stainer Bond Max using the Bond Polymer Refine Detection System for an anti-human rabbit monoclonal anti-G protein α S antibody.

Table 1. Demographics of ES patients

Clinicopathologic parameters	N (%)
Age at diagnosis (years)	
Range	1-57
Median	17
Gender	
Male	45 (58.4)
Female	32 (41.6)
Tumor site	
Peripheral	48 (62.3)
Central	29 (37.7)
Follow-up (months)	
Range	6-96
Median	30.5
Lung metastasis	
Occurrence	6 (7.8)
Absent	39 (50.6)
Not available	32 (41.6)
Patient outcome	
Alive	25 (32.5)
Dead	20 (26.0)
Not available	32 (41.6)

RESULTS

1. Immunohistochemical analysis of Gsα expression:

The correlation of Gsα expression with clinicopathological parameters was analyzed using a binary system approach, grouping low expression (grades 0-1) vs. high expression (grades 2-3). ES tumor samples were found in 34/52 samples (65.4%) with high Gsα expression, compared with 18/52 samples (34.6%) with low Gsα expression.Ewing's sarcoma samples and controls (**Figure 2**).

2. Mutation analysis of the GNAS gene:

No mutations were detected in exons 8 or 9 of the GNAS locus complex on chromosome 20q13.3 in DNA extracted from any of the FFPE tumor samples from the ES patients (**Figure 3**)

3. Methylation analysis of the GNAS gene:

The degree of methylation of the GNAS gene was assessed using the Illumina GoldenGate Methylation Cancer Panel I microarray. The GoldenGate DNA methylation method measures DNA methylation levels as β-values ranging from 0 (no DNA methylation detected) to 1 (complete DNA methylation). Gsα expression correlated well with the methylation status of the GNAS gene. high Gsα expression was detected more frequently in samples from living patients than decedents, although this was not statistically significant (**Table 2**)

Figure 1. Immunohistochemistry of Gsα

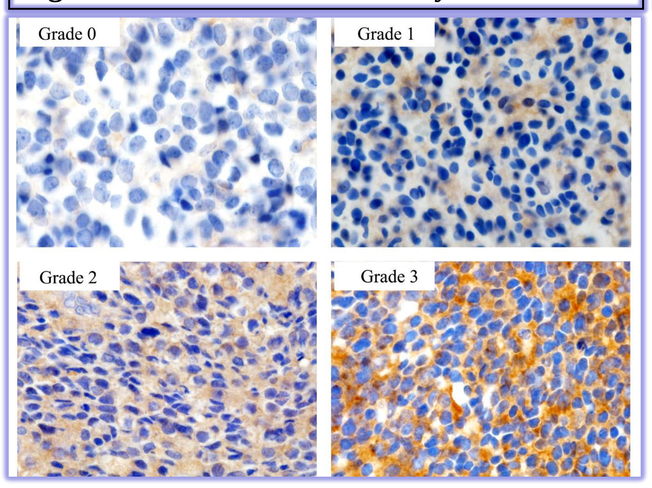

Figure 3. Direct sequencing of GNAS

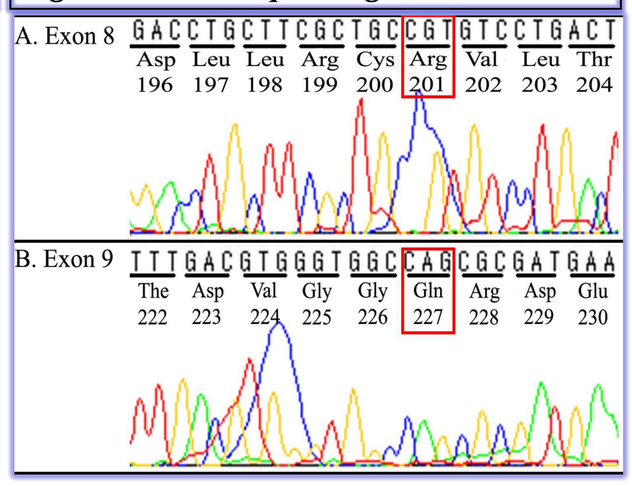

Table 2. Association of clinicopathological parameters with Gsα expression

Clinicopathological parameters[a]	$G_{s\alpha}$ Expression		Total	P-value
	Low	High		
Age, Mean ± SD	21.17 ± 11.03	18.62 ± 10.60	19.16 ± 10.50	0.420[b]
Sex, n (%)				
Male	12 (36.4)	21 (63.6)	33	0.727[c]
Female	6 (31.6)	13 (68.4)	19	
Site involved, n (%)				0.451[c]
Peripheral	13 (38.2)	21 (61.8)	34	
Central	5 (27.8)	13 (72.2)	18	
β-value, Mean ± SD	0.681 ± 0.304	0.245 ± 0.229	0.478 ± 0.345	0.001[b]
Degree of Methylation, n (%)				
Hypomethylation	3 (17.6)	14 (82.4)	17	0.009[c]
Hypermethylation	4 (80.0)	1 (20.0)	5	
Dead or Alive, n (%)				
Alive	2 (13.3)	13 (86.7)	15	0.055[c]
Dead	6 (46.2)	7 (53.8)	13	

[a]Clinicopathological cases involving missing values or without available clinicopathological values were removed for statistical analyses.; [b]Student's t-test ;[c]Chi-square test ; $G_{s\alpha}$, G-protein α subunit; SD, standard deviation

DISCUSSION

In summary, GNAS mutation is not associated with the pathogenesis of ES tumors. This finding may be used to distinguish metastatic bone lesions with GNAS mutations that have morphological similarities to ES tumors. Analysis of the methylation status of the GNAS gene and immunohistochemical Gsα expression suggests that hypermethylated GNAS gene (low Gsα expression) in ES may be associated with unfavorable progression with a non-significant trend. Further studies with a larger sample of patients are required to verify these results.

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