**Clinicopathological Implications of GNAS in Ewing Sarcoma**

BYEONG-JOO NOH1, J-HOUNG SUNG2, YOUNG WHAKIM1, EDUARDO SANTINI ARAUJO3, RICARDO KARIM-KALIS2, WOON-WON JUNG4, HYUN-SOOK KIM5 and YONG-KOO PARK2

1Department of Pathology, School of Medicine, Kyung Hee University, Seoul 02447, Korea (*Presenter & corresponding author)
2Laboratory of Orthopedic Pathology, Central Army Hospital, Buenos Aires C1426BOR, Argentina
3Molecular Pathology Division, Sarah Network of Rehabilitation Hospitals, Brasilia 70335-901, Brazil
4Department of Biomedical Laboratory Science, College of Health Sciences, Cheongju University, Cheongju-si, South Korea

**ABSTRACT**

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

**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 product to process 1,505 CpG sites from a panel of 807 cancer-related genes which included oncogenes and genes.

3. Direct sequencing: Direct sequencing was performed to detect the methylation status of GNAS exons 8 and 9.

4. Immunohistochemistry:

   - Sections (3 μm thick) from FFPE tissues were cut and DNA extracted from any of the FFPE tumor samples from the ES patients (Figure 3).
   - DNA methylation levels as β-values ranging from 0 (no DNA methylation detected) to 1 (complete DNA methylation).
   - Gsa expression correlated well with the methylation status of the GNAS gene. Analysis of the methylation status of the GNAS gene and immunohistochemical Gsa expression suggests that hypermethylated GNAS (low Gsa expression) in ES may be associated with unfavorable progression with a non-significant trend.

**RESULTS**

1. Immunohistochemical analysis of Gsa expression:

   - The correlation of Gsa 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 Gsa expression compared with 18/52 samples (34.6%) with low Gsa expression.

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.

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).

**DISCUSSION**

In summary, 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 Gsa expression suggests that hypermethylated GNAS (low Gsa expression) in ES may be associated with unfavorable progression with a non-significant trend.

**REFERENCES**