The hSEP1 gene is a novel candidate tumor suppressor gene in osteogenic sarcoma

*Sarkar, G; *Zhang, K; *Rock, M; ++Bolander, ME
++Mayo Clinic, MN

Introduction:
The prevalence of osteogenic sarcoma (OGS) among the children and young adults presents a remarkable distinction from high-incidence cancers (such as cancers of the breast, prostate, lung, or skin) that typically affect people of advanced age (1). An age-restricted prevalence of OGS presents a persuasive rationale to hypothesize that OGS has a genetic predisposition since children experience significantly less environmental exposure than individuals of advanced age. To date, only a few genes such as the p53 and Rb (which are associated with cancers of diverse types) have been shown to be also associated with OGS (2,3). However, the value of these markers for diagnosis and/or prognosis of OGS remains poor. Evidently, more OGS-specific genetic markers need to be identified and characterized for improving management of patients with the disease.

In the course of our work to identify potential markers for OGS, we identified loss or subnormal expression of hSEP1 mRNA in several OGS-derived cell lines. hSEP1 is the human homolog of yeast sepl which regulates important cellular functions such as meiosis, RNA metabolism and homologous recombination (4). In this communication we show loss or reduced level of hSEP1 mRNA expression in OGS biopsy specimens. Additionally, we identify a heterozygous missence mutation and a homozygous missence mutation in the gene in two different OGS-derived cell lines, U2OS and TE85 respectively. Our data coupled with the report that the chromosomal locus immediately adjacent to the hSEP1 locus undergoes loss of heterozygosity (LOH) at a >70% frequency (5) provide evidence supporting hSEP1 as a novel tumor suppressor gene in osteosarcoma.

Materials and Methods:
Extraction of total RNA from OGS-derived cell lines and OGS biopsy specimen, cDNA synthesis, and RT-PCR were carried out by standard methods. Screening for mutations in hSEP1 mRNA was achieved by RT-PCR amplification of overlapping segments of the mRNA followed by direct automated sequencing.

Results:
Co-amplification by RT-PCR of segments of glyceraldehyde acid dehydrogenase (GAP) and hSEP1 mRNAs were performed on biopsy specimen obtained from nine patients with OGS. Results of a representative experiment demonstrating relative level of expression of hSEP1 mRNA is presented in Figure 1. To quantify level of hSEP1 mRNA expression, RT-PCR for each sample was carried out in duplicate and repeated three times. Radioactivity in amplified DNA bands representing GAP and hSEP1 mRNAs were quantified by a Phosphorimager. Results obtained from these experiments are presented in Table 1.

Mutations were found in two of four OGS-derived cell lines examined. The mutations were not observed in sixteen normal samples. Results confirming these mutations are presented in Figure 2.

Discussion:
Our quantification of hSEP1 mRNA expression show that the mRNA is absent in three of nine samples and the expression is <10% in another three OGS biopsy specimen. Only one of the nine biopsy specimen showed hSEP1 mRNA expression comparable to FOB cells. These data indicate that hSEP1 mRNA expression is either lost or reduced in OGS. The identification of a homozygous mutation suggest that the hSEP1 chromosomal locus itself undergoes LOH. These data coupled with other published reports strongly indicate that hSEP1 is a tumor suppressor gene in OGS. Currently we are developing a screening method to identify mutations in the hSEP1 gene in OGS tumor before the administration of chemotherapy.

Table 1: Level of hSEP1 mRNA expression in OGS biopsy specimens.

<table>
<thead>
<tr>
<th>Sample identity</th>
<th>Mean (% FOB)</th>
<th>STD</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOB</td>
<td>100.0%</td>
<td>0.0</td>
</tr>
<tr>
<td>GS 53</td>
<td>15.6%</td>
<td>3.9</td>
</tr>
<tr>
<td>CB47</td>
<td>4.2%</td>
<td>7.9</td>
</tr>
<tr>
<td>AM13</td>
<td>111.9%</td>
<td>30.8</td>
</tr>
</tbody>
</table>

Legend to Table 1: An experiment consisted of duplicate PCR for a sample. Duplicate values were first used to obtain a mean. The ratio of the mean value for hSEP1 to GAP mRNAs in FOB cells was considered 100%. Values for biopsy specimens were expressed as %FOB. Four such % values for each sample were obtained from separate experiments which were used to calculate mean and standard deviation (STD).

Figure 1: Relative level of hSEP1 mRNA expression in OGS biopsy specimens.

Legend to Figure 1. Relative expression of hSEP1 mRNA in OGS biopsy specimens (except FOB which is an osteoblast cell line used as a reference) by RT-PCR. Desired segments of hSEP1 and GAP (glyceraldehyde acid phosphate dehydrogenase) mRNAs were co-amplified by PCR. Duplicate tubes for PCR were prepared for each cDNA sample. 5 uL of the PCR-products were electrophoresed on a 2% agarose gel which was dried and subjected to autoradiography. Patient samples are indicated. M-100 bp DNA ladder.

Figure 2: Homozygous mutation in the hSEP1 mRNA in TE-85 cell line

References:

Acknowledgement:
This work has been supported by an award from the Aircast Foundation to GS and from the OREF to MR.