MUTATION ANALYSIS IN PRIMARY OSTEOSARCOMA AND OSTEOSARCOMA CELL LINES

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Introduction:
Aptosis or programmed cell-death is an important physiological process of the organism. It qualifies the organism to regulate embryogenesis and development, to maintain homeostasis and to remove senescent or mutated cells, which are potentially hazardous for the organism1-3. In regard to these biological effects, which role takes apoptosis over in the development and in the sequel of diseases if the apoptosis pathway is dysregulated? Some diseases are already contributed to an apoptosis dysregulation such as cancer, neurodegenerative and hematological disorders and it seems to be crucial if there is an inhibition or a blocking of the apoptotic pathway4-6. Thus, the aim of our study was to investigate the apoptotic signal pathway in osteosarcoma to find gene alterations such as point mutations, which can give us a clue and an understanding of the development and process of the osteosarcoma.

Methods:
DNA and RNA was isolated from 15 osteosarcoma tumor samples, 3 osteosarcoma cell lines (SAOS-2, HOS, MG63) and 20 control persons. PCR conditions were established for the amplification of the "death-domain" of the six known apoptotic death receptors and the complete coding sequence of the apoptotic adapter molecules FADD and TRADD (Figure 1). That was followed by automatic DNA sequencing. In addition it was possible to carry out mutational analysis using the RLFP method.

Figure 1:

Table 1:

<table>
<thead>
<tr>
<th>Probes</th>
<th>No.</th>
<th>Exon3</th>
<th>Exon4</th>
<th>Exon5</th>
<th>Exon10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell line</td>
<td>3</td>
<td>1/1</td>
<td>0/2</td>
<td>0/1</td>
<td>1/0</td>
</tr>
<tr>
<td>Tumor</td>
<td>15</td>
<td>9/0</td>
<td>8/0</td>
<td>3/1</td>
<td>3/1</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>14/0</td>
<td>11/7</td>
<td>7/0</td>
<td>7/0</td>
</tr>
</tbody>
</table>

he = heterozygous ho = homozygous

Discussion:
The origin of our investigation was to find indications for the development and process of the osteosarcoma. On the basis of our results we can formulate the following hypotheses:

• Our study includes all apoptotic death receptors known so far and the two most important adapter molecules for initiating the apoptotic cell-death. It seems that genetic alterations of the investigated genes and exons, with exception of DR4, are probably not responsible for the development of osteosarcoma.

• Interestingly, we could find a variety of mutations in the DR4-gene. Three out of four mutations have also been reported in other tumors such as head and neck cancer, lung cancer, ovarian cancer and bladder cancer7-9. So it could be supposed that the mutations we have identified may contribute to the development and process of the osteosarcoma. This hypothesis is supported by functional analysis of the "death-domain"-mutation of the DR4-gene, which shows an inhibition of the apoptotic cell-death10.

• Table 1 shows, that all mutations in exons 3,5 and 10 of the DR4-gene we have found in the control group were heterozygous. On the other side 33,3% of the mutations were homozygous in the tumor samples and cell lines investigated. That could be an indication for the necessity of a homozygous allele alteration regarding the mutations in exons 3,5 and 10 of the DR4-gene. The frequency of the homozygous mutation in exon 5 of the control group indicates a polymorphism.

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Reference List

**University Children’s Hospital, Ulm, Germany