The Correlation of Malignant Potential with NELL-1 Expression in Benign and Malignant Bone Tumors

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Introduction: NELL-1 is a secreted osteoinductive protein that binds Integrinβ1 to activate the Wnt/β-catenin signaling pathway. Recent interest has grown in using recombinant NELL-1 as both a local or systemic agent to induce bone formation. However, the role and expression of NELL-1 in benign and malignant bone tumors is entirely unknown. Our purpose was to examine the expression patterns of NELL-1 in a comprehensive analysis of bone-forming skeletal tumors, accompanied by an examination of the effects of NELL-1 in osteosarcoma (OS) cells.

Methods: First, the expression of NELL-1 was assessed across a diverse set of human bone-forming skeletal tumors using immunohistochemistry. This included benign tumors (e.g. osteoid osteoma, osteoblastoma), low grade malignant tumors (e.g. parosteal osteosarcoma), and high grade malignant tumors (e.g. osteosarcoma and its variants, including osteoblastic OS, chondroblastic OS, fibroblastic OS, and telangiectatic OS). Semi-quantitation was performed to describe the distribution and intensity of immunostaining. Using in vitro studies, osteosarcoma cell lines, Saos-2, MG-63, and 143B, were treated with NELL-1, and the effects on osteogenic differentiation, proliferation, and migration were assessed via standard methods.

Results: Results showed that NELL-1 was expressed to some degree in all types of bone-forming tumors (Fig. 1). Diffuse, intense staining for NELL-1 was observed in all benign bone tumors, and was localized to bone-lining osteoblasts, osteocytes, and bone matrix (Table 1) - areas correlating with osteogenic differentiation. While expression of NELL-1 was present in malignant tumors, benign bone tumors had a stronger correlation with more widespread staining pattern. A relative loss of NELL-1 expression was observed in osteosarcoma specimens, with predominant weak, patchy NELL-1 immunostaining. Among OS subtypes, fibroblastic OS demonstrated the highest NELL-1 immunostaining. In vitro, NELL-1 significantly induced the osteogenic differentiation of Saos-2 OS cells by all markers examined (*p<0.01). Additional results showed that NELL-1 negatively influenced proliferation as well as cell migration.

Discussion: In summary, NELL-1 is an osteoinductive cytokine that is widely expressed in both benign and malignant bone-forming skeletal tumors. NELL-1 is diffusely and robustly expressed in benign tumors, and its expression is much reduced in malignant bone tumors. Results from in vitro studies
suggest that NELL-1 negatively influences osteosarcoma cell proliferation and migration. These in vitro results prompt further investigation into the role of NELL-1 in vivo and with evidence that NELL-1 affects certain critical factors of malignancy and metastasis, knowledge of its role in tumorigenesis and tumor invasion will be vital. Since NELL-1 induces osteodifferentiation of OS cells, manipulation of its expression may represent a novel approach to induce the differentiation of sarcoma stem cells in combination with traditional chemotherapeutic regimens. Future areas of investigation include the specificity of NELL-1 in bone-forming tumors, and the in vivo effects of NELL-1 on osteosarcoma disease progression.

Significance: With the ability of NELL-1 to induce osteodifferentiation of OS cells potentially we can harness its function and induce the differentiation of sarcoma stem cells and other bone-forming cell lines. Using NELL-1 in this fashion may represent a novel approach to traditional chemotherapeutic regimens for treatment of tumors.

![Figure 1: H&E and NELL-1 expression in multiple bone-forming tumors.](image)

(A) H&E images of different tumor types next to their corresponding (B) NELL-1 expression images through immunohistochemistry. Images show expression of NELL-1 localized to bone-lining osteoblasts, osteocytes, and bone matrix. Benign tumors (Osteoid Osteoma) displayed a high correlation of NELL-1 expression near bone forming regions which diffuse expression and strong intensity. Low grade malignant tumor (Periosteal OS) showing diminished staining intensity and significantly decreased distribution. High grade malignant tumor (Telangiectatic OS) showing moderate distribution and staining intensity.

<table>
<thead>
<tr>
<th>Tumor Grade</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
<th>Mean % of cells stained (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>-</td>
<td>-</td>
<td>2/10 (20%)</td>
<td>9/10 (90%) (±10%)</td>
</tr>
<tr>
<td>Low Grade OS</td>
<td>-</td>
<td>2/4 (50%)</td>
<td>2/4 (50%)</td>
<td>21% (±7%)</td>
</tr>
<tr>
<td>High Grade OS</td>
<td>-</td>
<td>2/11 (19%)</td>
<td>6/11 (54%)</td>
<td>5/11 (27%) (±27%)</td>
</tr>
</tbody>
</table>

\*p < 0.05 in comparison to benign tumor staining intensity; \*p < 0.01 in comparison to benign tumor staining intensity; \*p < 0.01 in comparison to benign tumor staining distribution.

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