Insulin-like growth factor-1 receptor expression in pediatric tumors: a comparative immunohistochemical study

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1. Introduction
Insulin-like growth factor-1 receptor (IGF-1R) is a receptor tyrosine kinase that plays a crucial role in cancer cell proliferation, migration, and prevention of apoptosis (1). IGF-1R is also involved in malignant tumor transformation and is overexpressed in a variety of human malignancies such as lung, prostate, breast, and colorectal carcinomas (2–5). Studies have revealed a pronounced expression of IGF-1R in pediatric tumors, such as Ewing sarcoma/primitive neuroectodermal tumors, peripheral neuroblastic tumors, acute lymphoblastic lymphoma, Hodgkin lymphoma, Burkitt lymphoma, retinoblastoma, pleuropulmonary blastoma, Langerhans cell histiocytosis, endodermal sinus tumors (ESTs), and myeloid sarcoma. Analysis was performed on tissue sections by immunohistochemically staining for IGF-1R expression.

Background/aim: Insulin-like growth factor-1 receptor (IGF-1R) is a pivotal receptor tyrosine kinase involved in the cell cycle and malignant tumor transformation. It is differentially expressed in various types of tumors. We aimed to determine the expression of IGF-1R in different pediatric tumors and to shed light on possible new indications of anti-IGF-1R treatment approaches.

Materials and methods: A total of 147 specimens were analyzed according to their expression of IGF-1R. Specimens included those from rhabdomyosarcomas, Wilms tumors, Ewing sarcoma/primitive neuroectodermal tumors, peripheral neuroblastic tumors, acute lymphoblastic lymphoma, Hodgkin lymphoma, Burkitt lymphoma, retinoblastoma, pleuropulmonary blastoma, Langerhans cell histiocytosis, endodermal sinus tumors (ESTs), and myeloid sarcoma. Analysis was performed on tissue sections by immunohistochemically staining for IGF-1R expression.

Results: All six specimens of EST cases showed positivity for IGF-R1. Additionally, about 56% of the Hodgkin lymphoma, 80% of the rhabdomyosarcoma, and 70% of the Wilms tumor specimens showed positivity for IGF-R1 expression.

Conclusion: All ESTs examined in our study expressed IGF-1R and to our knowledge this is the first report regarding ESTs and IGF-1R expression. IGF-1R could be included among confirmatory markers for ESTs and, from a therapeutic viewpoint, ESTs should also be examined for IGF-1R expression for beneficial regimens.

Key words: Insulin-like growth factor-1 receptor, endodermal sinus tumor, neuroblastoma, Wilms tumor, rhabdomyosarcoma
so far. We aimed to identify additional tumor types that might express IGF-1R and to shed light on possible new areas and therapeutic indications of anti-IGF-1R treatment protocols.

2. Materials and methods

2.1. Tissue specimens
This study included 147 pediatric tumor specimens sampled from patients diagnosed and/or treated at the Ankara Children's Hematology and Oncology Research and Training Hospital during a 10-year period (2006–2015). For the use of tissue specimens, informed consent was obtained from subjects’ parents or guardians and ethical approval (2014-077) was obtained from the Ethical Committee of the Ankara Children’s Hematology and Oncology Research and Training Hospital, Ankara, Turkey.

2.2. Immunohistochemistry
Immunohistochemical analysis was performed on 4-µm-thick, formalin-fixed, paraffin-embedded tissue sections. Samples were immunohistochemically stained for IGF-1R expression. For immunohistochemistry, 3-µm-thick sections were tested with a Ventana Benchmark GX immunostainer (Ventana, Tucson, AZ, USA) according to the instructions of the manufacturer. Diaminobenzidine was used as a chromogen.

IGF-1R (IGF-1R, Biocare, USA) was used at a dilution of 1:250. A human colon sample was included as the positive control. For negative controls, primary antibodies were omitted. Evaluation of IGF-1R staining was performed by a pathologist without knowledge of the medical history of the relevant specimen. Immunoreactivity was scored by determining the percentage of cells showing weak (1+), moderate (2+), or strong (3+) staining with a cut-off at 10% of cells (2). For all tumors, immunohistochemical staining frequency patterns were determined.

3. Results
The study group consisted of 147 pediatric tumor specimens, including 12 RMS, 13 Wilms tumor, eight ES/PNET, 35 pNT, 11 ALL, 39 HL, 12 BL, one Rb, one PPB, six LCH, six EST, and three MS samples. The distribution of several tumors and the staining characteristics of the tumors for IGF-1R expression are detailed in the Table.

3.1. RMSs
Ten of 12 (83%) RMSs showed positivity for IGF-1R with membranous, cytoplasmic, and nuclear staining patterns. Eight of 12 (about 67%) showed moderate to strong nuclear and membranous staining. Immunoreactivity was regarded as positive when staining was localized to the nuclei or cytoplasm. Among the histological parameters, a higher frequency of nuclear IGF-1R expression was observed in spindle cell morphology.

3.2. Wilms tumors
Nine of 13 (69%) Wilms tumors were regarded as positive for IGF-1R with membranous, cytoplasmic, and nuclear staining patterns. Eight of 12 (about 67%) showed moderate to strong nuclear and membranous staining. Immunoreactivity was regarded as positive when staining was localized to the nuclei or cytoplasm. Among the histological parameters, a higher frequency of nuclear IGF-1R expression was observed in spindle cell morphology.

Table. Immunohistochemical staining intensity frequencies for insulin-like growth factor-1 receptor expression in pediatric tumors.

<table>
<thead>
<tr>
<th>Tumor types</th>
<th>Specimen, n</th>
<th>Staining intensity of IGF-1R</th>
<th>Overall staining</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Weak</td>
<td>Moderate</td>
</tr>
<tr>
<td>EST</td>
<td>6</td>
<td>2</td>
<td>33.3</td>
</tr>
<tr>
<td>RMSs</td>
<td>12</td>
<td>2</td>
<td>16.7</td>
</tr>
<tr>
<td>Wilms tumors</td>
<td>13</td>
<td>4</td>
<td>30.8</td>
</tr>
<tr>
<td>pNTs</td>
<td>35</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td>HL</td>
<td>39</td>
<td>12</td>
<td>30.8</td>
</tr>
<tr>
<td>ES/PNET</td>
<td>8</td>
<td>1</td>
<td>12.5</td>
</tr>
<tr>
<td>Rb</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PPB</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

RMSs = Rhabdomyosarcomas, pNTs = peripheral neuroblastic tumors, HL = Hodgkin lymphoma, Rb = retinoblastoma, PPB = pleuropulmonary blastoma, EST = endodermal sinus tumor, ES/PNET= Ewing sarcoma/primitive neuroectodermal tumors. Positivity for IGF-1R is categorized as weak (+1), moderate (+2), and strong (+3). Percentages are given according to the total number of specimens. For tumor types consisting of single specimens, percentages are not given.
blastemal cells, there was no staining observed. Five of 13 (38%) showed moderate membranous staining.

3.3. ES/PNETs
Three of eight (37%) ES/PNETs showed positivity for IGF-1R, all with membranous staining patterns, of which two showed moderate positivity.

3.4. pNTs
Twenty-one of 35 (60%) pNT specimens were evaluated as positive for IGF-1R, either membranous with a cytoplasmic or a nuclear staining pattern. Fourteen of 35 (40%) showed moderate to strong membranous, cytoplasmic, and nuclear staining pattern. The frequency of IGF-1R positivity in GNBs and GNs was significantly higher than in NBs (data not shown). IGF-1R was mainly stained in ganglion cells and Schwannian stroma.

3.5. HL
Twenty-two of 39 (56%) HL specimens showed positivity for IGF-1R with membranous and cytoplasmic staining patterns. Expression of IGF-1R was observed in the vast majority of Hodgkin and Reed–Sternberg (HRS) cells (Figure 1). Ten of 39 (25%) specimens showed moderate to strong membranous and cytoplasmic staining.

3.6. ESTs
All of the six (100%) EST specimens were evaluated as positive for IGF-1R, all with membranous staining patterns. Four of the specimens showed moderate to strong IGF-1R positivity (Figure 2).

3.7. Rb, PPB
Rb and PPB cases showed positivity for IGF-1R.

3.8. LCH, ALL, BL, and MS
None of the 6 LCH, 11 ALL, 12 BL, or 3 MS samples were evaluated as positive for IGF-1R expression.

4. Discussion
Increased activation of IGF-IR is known to lead to dysregulated protein synthesis via certain kinase signaling pathways, a fact that has already been described for certain metabolic conditions and for several tumors (8). The expression pattern of IGF-IR has been investigated for several tumors but, to our knowledge, the current study is the first addressing the expression of IGF-1R in ESTs. To our knowledge, there are also only three studies in which IGF-1R protein expression was analyzed directly in tumor tissue of HL patients, as referred to in the following paragraphs.

It is known that IGF-1R is overexpressed in RMSs, Wilms tumors, ES/PNETs, and pNTs. However, studies investigating IGF-1R expression in these groups are rare. In our study, ten of 12 (83%) specimens tested for IGF-1R expression stained positive and in spindle cell morphology a higher frequency of nuclear IGF-1R expression was observed. A nationwide study comprising 112 cases, performed by van Gaal et al. in the Netherlands, reported a slightly lower frequency for IGF-1R expression in RMS cases (72% for alveolar and 61% for embryonal RMSs) (9). The slightly higher frequency in our study may be due to our sample size but still can be regarded in parallel with van Gaal et al.’s nationwide study. Although we did not perform clinical correlation or survival analysis, expression of nuclear IGF-1R has been reported as a negative prognostic factor for RMS regarding 5-year survival rates (9).

A detailed expression profile of IGF-1R at the genome and protein level was investigated by Natrajan et al. and it was reported that IGF-1R protein expression was restricted to the epithelial cells of fetal kidney and Wilms tumors in most cases (10). About 70% (nine of 13) of the specimens investigated in our study showed positivity for IGF-1R expression.
expression in the epithelial component but not perinuclear localization in blastemal cells. IGF-1R expression in Wilms tumors has been associated with tumor relapse and is inversely correlated with the Wilms tumor suppressor gene (10,11).

In Ewing sarcoma, the aberrant EWS/FLI-1 transcription factor product has been shown to upregulate the expression of IGF-1R (12). R1507 is an antibody directed against the human IGF-1R and the Sarcoma Alliance for Research through Collaboration conducted a phase two, multiarm study with this agent in various subtypes of recurrent or refractory sarcomas including Ewing sarcomas and concluded that IGF-1R inhibitors could play a role in the treatment of selected sarcomas (7). Three of eight (37%) ES/PNET specimens were defined as positive for IGF-1R in our study. Although studies with IGF-1R antibodies in ES/PNETs are limited or disappointing, there are assertions in the literature that IGF-1R inhibitors may play a role in the treatment of ES/PNETs since it is regarded as a key mechanism in proliferation (7,12,13).

Neuroblastoma is considered the most common malignant tumor in the first year of life and IGF-1 signaling via IGF-1R is thought to contribute to the growth of the tumor (14). Additionally, IGF-1R is reported as being a major determinant of the metastatic potential of neuroblastoma (15). Hence, IGF-1R seems to be a promising therapeutic target when conventional therapies fail. Sixty percent (21 of 35) of the pNT specimens we evaluated stained positive for IGF-1R. The antineoplastic therapy of advanced pNTs is still unsatisfactory and innovative therapeutic approaches are needed. Anti-IGF-1R therapies are reported to show beneficial effects in some preclinical studies (15).

HL is one of the most frequent nodal lymphomas and about 40% of patients are reported to be refractory to initial treatment or relapse after remission (16). Recently, Eppler et al. and Liang et al. reported that IGF-1R of variable frequency is found in cHL patients (16,17). In our study, about 56% (22 of 39) of the specimens were found to be expressing IGF-1R, in line with the 55% expression rate reported by Liang et al. However, in the case of HL, the expression of IGF-1R with regard to inhibitory options is somewhat arguable. Liang et al. reported that IGF-1R is strongly expressed in mitotic cHL cells and inhibition of IGF-1R decreases proliferation and induces cell-cycle arrest in cell lines, but in clinical correlation analysis the expression of IGF-1R was found to be related with better prognosis (17). Koh et al. recently reported a much lower expression of IGF-1R (about 14%) in HRS cells and found a similar prognostic value as reported by Liang et al. (18). Our study, to our knowledge, is the fourth study addressing IGF-1R expression in HL cases. In the case of HL, it is plausible to suggest that studies with larger samples and using certain antibody clones would clarify such discrepancies between different studies.

The EST specimens we evaluated in our study showed 100% (6 of 6 specimens) positivity for IGF-1R, all with membranous staining patterns. Four of them showed moderate to strong IGF-1R positivity. These findings suggest a possible role for IGF-1R in ESTs. The current study is the first to evaluate IGF-1R expression using IHC on EST specimens. Although histologic characteristics and frequent AFP expression allow for an accurate diagnosis in the majority of EST cases, a wide histologic spectrum and an occasional unexpected immunophenotype may pose diagnostic challenges. We propose that IGF-1R could be included among a panel of confirmatory markers for EST.

Our results suggest that IGF-1R may have potential value as a therapeutic target in pediatric tumors including RMSs, Wilms tumors, ES/PNET, pNTs, and ESTs. IGF-1R has a role in malignant transformation and progression; thus, anti-IGF-1R drugs may be an option for the treatment of refractory pediatric tumors after validation in future prospective studies with larger sample size. We report for the first time that EST expresses IGF-1R, but we are aware that this result has to be validated with larger sample sizes. Our results can be enriched by using different antibody clones for verification and/or by verifying expression patterns also at the mRNA level. The findings of our study highlight a potential and possible role of targeting IGF-1R in pediatric solid tumors by using IGF-1R immunohistochemistry.

References


