CRIP-1 OVEREXPRESSION-PROGNOSTIC FACTOR IN CHILDREN’S OSTEOSARCOMA?

A. Ivan1, N. Forna1, Luminita Ivan3, Cristina Morariu3, Mioara Calipsoana Matei2,3*, P. D. Sirbu1
“Grigore T. Popa” University of Medicine and Pharmacy Iasi, Romania
Faculty of Medicine
1. Department of Surgery (II)
2. Department of Preventive Medicine and Interdisciplinarity
3. “Dr. Iacob Czihac” Military Emergency Clinical Hospital from Iasi, Romania
*Corresponding author. E-mail: mioara.matei@umfiasi.ro

CRIP-1 OVEREXPRESSION - PROGNOSTIC FACTOR IN CHILDREN’S OSTEOSARCOMA? (Abstract): Predicting the clinical evolution of patients with osteosarcoma is an essential condition for personalized treatment. In researching new and reliable biomarkers we identified CRIP-1 (Cysteine-Rich Intestinal Protein 1). Its overexpression has been documented to have a significant prognostic impact on gastric and colorectal cancer. Therefore, we aimed to investigate the overexpression of CRIP-1 in pediatric osteosarcoma and assess its potential as a prognostic biomarker. 

Materials and methods: We analyzed 65 samples from patients diagnosed with osteosarcoma at “Sf. Maria” Emergency Clinical Hospital for Children from Iasi, between 2017 and 2021. Two staining kits were used: Mouse and Rabbit Specific HRP/DAB (ABC) Detection IHC Kit from Abcam and Novolink Poly HRP Kit.

Results: CRIP-1 was over expressed in 92.6% of the cases. The lower the degree of differentiation, the less CRIP-1 is over expressed and the more frequently it is found in the nucleus.

Conclusions: Could this mean that CRIP-1 overexpression is associated with a better prognosis? The study highlights the clinical implications of CRIP-1 in osteosarcoma, emphasizing its importance for prognosis, and personalized treatment strategies. However, it calls for further investigation into CRIP-1 role in cancer biology (molecular pathways and interactions with other prognostic factors).

Keywords: OSTEOSARCOMA, CRIP-1 OVEREXPRESSION, CANCER, PROGNOSIS.

INTRODUCTION
Osteosarcoma is the most common malignant bone tumor in children and adolescents, characterized by aggressive growth (1, 2). One of the significant challenges in managing osteosarcoma is the high rate of metastasis observed at the time of diagnosis (3). This underscores the critical need for early detection and the identification of biomarkers that can predict therapeutic outcomes and disease progression.

Although advancements in treatment protocols have increased the 5-year survival rate to 50–70% (4, 5), a considerable number of patients still face recurrent or refractory metastatic disease with limited therapeutic options (6). To address this, there is an urgent need to identify reliable biomarkers that can forecast the prognosis of osteosarcoma patients, ultimately facili-
Identifying tumor markers and elucidating their interrelationships could pave the way for targeted and individualized therapies, thereby improving the survival rates and quality of life for cancer patients.

Studying the osteosarcoma microenvironment, both in vitro and in vivo, can enhance our understanding of the tumor's evolutionary behavior. Combining the expression profiles of specific biomarkers with responses to monotherapy or combined therapies in pediatric osteosarcoma could explain various clinical and evolutionary characteristics, leading to improved clinical and therapeutic management (8).

Cysteine-Rich Intestinal Protein 1 (CRIP-1) is a member of the LIM/ double zinc-finger protein family, involved in regulating cellular processes such as growth, differentiation, and apoptosis (9, 10, 11). CRIP was first identified as a developmentally regulated intestinal gene and has since been found in a variety of different tissues and cells (11). CRIP-1 has emerged as a potential biomarker of interest in various cancers. Studies have indicated that CRIP-1 expression is altered in several malignancies, including breast, colorectal, gastric, cervical, ovarian, thyroid, pancreatic, and prostatic cancers, suggesting its role in tumor genesis and cancer progression (9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20).

However, the role of CRIP-1 in osteosarcoma, particularly in the pediatric population, remains underexplored. Given its involvement in other cancer types, CRIP-1 may serve as a prognostic factor in osteosarcoma, potentially influencing tumor behavior and patient outcomes. Investigating CRIP-1 expression in osteosarcoma could provide new insights into the disease's molecular mechanisms and identify targets for novel therapeutic interventions.

This study aims to evaluate CRIP-1 overexpression as a prognostic biomarker in pediatric osteosarcoma. By analyzing CRIP-1 expression levels in osteosarcoma tissue samples and correlating these levels with clinical outcomes and pathological features, we seek to determine its potential utility in predicting disease progression and guiding treatment decisions. Our findings could contribute to the development of more effective, individualized treatment strategies for young osteosarcoma patients, ultimately improving survival rates and quality of life.

MATERIALS AND METHODS

A total of 65 samples from patients diagnosed with osteosarcoma at “Sf. Maria” Emergency Clinical Hospital for Children from Iasi, between 2017 and 2021, were analyzed. The tissue samples obtained from the cases in this study were processed using a standardized protocol. They were fixed in 10% buffered formalin, decalcified with EDTA, and embedded in paraffin. Sections of 5/4 µm were then prepared and transferred to electrostatically charged slides for subsequent immunohistochemistry analysis.

Two staining kits were used: Novolink Poly HRP and Mouse and Rabbit Specific HRP/DAB (ABC) Detection IHC Kit and CRIP-1 antibody from Abcam.

Initially, we used the Novolink Poly HRP kit, but it proved incompatible with the antibodies. Consequently, we switched to the Abcam Mouse and Rabbit Specific HRP/DAB (ABC) Detection IHC kit, which yielded positive results. Each staining process included both positive and negative controls to ensure that the staining
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system functioned correctly, the positive and negative staining procedures were specific, and the specific protocol was properly followed.

CRIP-1 shows cytoplasmic and nuclear staining. The expression of CRIP-1 was assessed using the scores reported by Yu et al (21). The H-scores were calculated by determining the intensity of the staining and the percentage of the staining region with that intensity. Only stained cancerous cells were examined. The samples were divided into four different categories based on the intensity of nuclear or cytoplasmic staining: none = negative (0), weak (1), medium (2), and strong (3). The indexed total was calculated by multiplying the intensity grade by the percentage of stained region.

Specificity and sensitivity points can help maximize the positive rate. A score was assigned based on the number of stained cells and ranges from 0-4% to 80–100%. The score was judged poor from 1 to 5 (1 = 0-4%; 2 = 5-19%; 3 = 20-39%; 4 = 40-59%; 5 = 60-79%) and high from 6 (= 80-100%) to 18. We have multiplied 0 - 18.

The research was approved by the Ethics Committee of the “Sf. Maria” Emergency Clinical Hospital for Children from Iasi

RESULTS

In our research, CRIP-1 expression was assessed in 65 osteosarcoma tissue samples that met histopathological criteria. Patient observation sheets provided clinical and pathological information such as age, sex, tumor size, tumor grading, and the presence or absence of metastases. In the majority of cases, a diagnostic bone biopsy was the primary surgical procedure. By comparing the anatomopathological reports to the clinical data and immunohistochemistry findings, we discovered that the majority of patients were male, with ages ranging from 8 to 19 years; the highest incidence occurred at age 16. The most prevalent tumor location was the femur, namely the distal femur. The most common histological diagnosis was osteoblastic osteosarcoma (figs. 1, 2), followed by chondroblastic osteosarcoma, pleomorphic osteosarcoma, and clear-cell osteosarcoma.

Fig. 1. Osteoblastic osteosarcoma, staining HEx10

Fig. 2. Osteoblastic osteosarcoma, staining HEx2
From the perspective of tumor grading, our findings indicated that over 70% of the osteosarcoma cases were classified as high-grade, characterized by poorly differentiated and undifferentiated tumors (tab. I).

Metastasis was identified in two cases: one with lung metastasis and the other with skin metastasis.

### TABLE I.

**Anatomopathological characteristics of the cases**

<table>
<thead>
<tr>
<th>Variables</th>
<th>No. of cases</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tumor grade</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 1 - well differentiated</td>
<td>8</td>
<td>12.3</td>
</tr>
<tr>
<td>Grade 2 - moderate differentiated</td>
<td>11</td>
<td>16.9</td>
</tr>
<tr>
<td>Grade 3 - poorly differentiated</td>
<td>38</td>
<td>58.5</td>
</tr>
<tr>
<td>Grade 4 - undifferentiated</td>
<td>8</td>
<td>12.3</td>
</tr>
<tr>
<td><strong>H-score (CRIP-1% of nuclear/cytoplasmic staining)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 = between 20-39</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>4 = between 40-59</td>
<td>2</td>
<td>3.1</td>
</tr>
<tr>
<td>5 = between 60-79</td>
<td>25</td>
<td>38.5</td>
</tr>
<tr>
<td>6 = between 80-100</td>
<td>37</td>
<td>56.9</td>
</tr>
<tr>
<td><strong>CRIP-1 intensity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>negative</td>
<td>3</td>
<td>4.6</td>
</tr>
<tr>
<td>weak</td>
<td>19</td>
<td>29.2</td>
</tr>
<tr>
<td>medium</td>
<td>29</td>
<td>44.6</td>
</tr>
<tr>
<td>strong</td>
<td>14</td>
<td>21.6</td>
</tr>
</tbody>
</table>

Regarding CRIP-1 antibody staining, one case could not be assessed due to a technical defect. However, in the remaining cases, cytoplasmic and/or nuclear staining was observed, with over 60% of tumor cells showing positive staining (scoring 5 and 6) (tab. I).

Medium CRIP-1 staining was observed in 88.88% of osteoblastic osteosarcomas (figs. 3, 4, 5) and 85.71% of chondroblastic osteosarcomas (fig. 6). In pleomorphic osteosarcomas, 50% of cases exhibited strong CRIP-1 staining.

In the majority of examined cases, the staining intensity was medium and localized either cytoplasmic, nuclear, or both (Table I). Strong staining was noted in all cases of well-differentiated osteosarcoma. Among poorly differentiated osteosarcoma, 50% demonstrated intense staining. Also, high-score nuclear staining was identified in 50% of the undifferentiated osteosarcoma forms.

CRIP-1 was over expressed in 92.59% of cases. In the 2 metastatic blocks, staining was absent in the case with frontal skin metastasis and weak in the case with pulmonary metastasis, despite the overexpression (strong staining intensity) observed in the primary tumor. A correlation between histopathological type and staining intensity for CRIP-1 could not be established.

The lower the degree of differentiation, the less CRIP-1 is over expressed and the more frequently it is found in the nucleus.
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**DISCUSSION**

Even though several studies have linked CRIP-1 expression to a poor prognosis in colorectal, breast, gastric, prostate, cervical, ovarian, thyroid, pancreatic, and endometrial cancer (10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25), there are very few published papers on the role of CRIP-1 in osteosarcoma (9, 22).

The data reported on the topic revealed contradictory results regarding the impact of CRIP-1 on osteosarcoma. A study published in 2011 demonstrating that CRIP-1 expression is strongly associated with a
favorable outcome and as significant negative predictors of systemic spread in osteosarcoma (9). A more recent work reported that overexpression of CRIP-1 was associated with a poor survival of osteosarcoma patients (22). Probably CRIP-1 has oncogenic and tumor suppressor properties specific to the tumor type, which require further clarification of the pathogenesis.

Our study aimed to investigate the expression of CRIP-1 in osteosarcoma tissue samples and its correlation with various clinical and pathological features, exploring the potential of CRIP-1 as a prognostic biomarker. The results revealed significant patterns in the demographic distribution, tumor characteristics, and CRIP-1 staining intensity, providing insights that contribute to the existing body of knowledge on osteosarcoma.

The demographic data showed that the majority of osteosarcoma patients were male, with ages ranging from 8 to 19 years, and the highest incidence occurring at age 16. These findings are consistent with previous research, which indicates a higher prevalence of osteosarcoma in males during adolescence—a period marked by rapid bone growth (22, 23). The predominant tumor location was the distal femur, aligning with literature that highlights the long bones around the knee as common sites for osteosarcoma (9, 22).

Osteoblastic osteosarcoma was the most frequent histological subtype, followed by chondroblastic, pleomorphic, and clear-cell osteosarcomas. This distribution mirrors established patterns, where osteoblastic osteosarcoma is commonly the most diagnosed subtype (22, 24). In the current research, over 70% of cases were classified as high-grade, characterized by poorly differentiated and undifferentiated tumors, which are known to be associated with aggressive disease and poorer prognosis (23). The presence of metastasis in two cases further underscores the aggressive nature of high-grade osteosarcomas.

Our findings on CRIP-1 expression revealed that over 60% of tumor cells exhibited positive staining for CRIP-1, with varying intensities and localization patterns, results that were in line with the literature (9). Baumhoer and collaborators reported 45% of cases with CRIP-1 expression (9). In the current research, CRIP-1 was overexpressed in 92.59% of cases, predominantly showing medium staining intensity. Notably, strong CRIP-1 staining was observed in all cases of well-differentiated osteosarcoma, and high-score nuclear staining was identified in 50% of undifferentiated osteosarcomas. This suggests that CRIP-1 expression may be associated with tumor differentiation status, where better-differentiated tumors show stronger CRIP-1 expression.

Interestingly, the correlation between CRIP-1 staining and metastatic potential revealed that metastatic tumors exhibited weaker CRIP-1 staining compared to their primary counterparts. Specifically, the case with pulmonary metastasis showed weak staining, and no staining was observed in the case with frontal skin metastasis. This disparity suggests that CRIP-1 expression might be reduced in metastatic lesions, indicating a possible role of CRIP-1 in the primary tumor environment but not in metastatic sites.

The study is limited by the small sample size and the retrospective nature of data collection, which may affect the general applicability of the findings. Additionally, technical issues in assessing one sample highlight the need for robust methodologies.
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in future studies. Prospective studies with larger cohorts are necessary to validate our findings and further elucidate the role of CRIP-1 in osteosarcoma pathogenesis. Functional studies exploring the molecular mechanisms of CRIP-1 could provide deeper insights into its role as a potential biomarker and therapeutic target.

CONCLUSIONS

Our study offers significant insights into the demographic and clinical characteristics of osteosarcoma, with a particular focus on the expression of CRIP-1. The data indicate that CRIP-1 is markedly overexpressed in osteosarcoma tissues and its expression appears to be correlated with the differentiation status of the tumor. Specifically, well-differentiated tumors tend to exhibit stronger CRIP-1 staining. Furthermore, the observed disparity in CRIP-1 expression between primary and metastatic tumors suggests that CRIP-1 may play a distinct role in the primary tumor environment compared to metastatic sites. These findings highlight the potential of CRIP-1 as a biomarker for osteosarcoma and suggest avenues for future research into its mechanistic roles and therapeutic potential. Prospective studies with larger patient cohorts and functional analyses of CRIP-1 are essential to validate these observations and to fully elucidate the implications of CRIP-1 expression in osteosarcoma. Understanding the role of CRIP-1 could lead to the development of novel diagnostic and therapeutic strategies, ultimately improving clinical outcomes for patients with osteosarcoma. We propose to expand this study by investigating the correlation between CRIP-1 overexpression and survival rate in patients with osteosarcoma.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest in their scientific research. This research received no funding.

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