Role of osteopontin in breast cancer patients

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ABSTRACT

Aim and background. In breast cancer, as in almost all neoplastic diseases, the prognosis is strictly related to the invasive capacity, local and distant, that characterizes the growth of all tumors. Since the mechanisms that regulate replication of the neoplastic cells, with consequent capacity to metastasize, are not completely known, identification of new markers represents the gold standard of research in the stratification of patients with such a pathology. Osteopontin, a specific phosphoglycoprotein originally isolated from extracellular bone matrix and actively involved in mechanisms of bone reabsorption, appears to play a key role in osteoclastogenesis at the level of the skeleton in some pathologic situations. It has been found that patients with metastatic bone lesions from breast or prostate cancer present, with respect to subjects without repetitive bone lesions, elevated serum levels of the protein, indicating that osteopontin could play an important role in the development and progression of the neoplastic disease at the bone level.

Methods and study design. The authors studied a group of patients with breast cancer, evaluating as a marker also serum osteopontin levels.

Results and conclusions. The results, although obtained on a small number of patients, showed that osteopontin evaluation in breast cancer patients can be a particularly interesting method of research in staging of the disease as well as in the prognosis, thereby attributing a role of a biotumor marker also in the follow-up of the therapy.

Introduction

Breast cancer is the main cause of death of women in the western world. However, owing to the possibility of an early diagnosis, in a high percentage of cases it is possible to obtain good results in terms of cure and long-term survival. Unfortunately, when metastatic lesions are present the prognosis is very poor. Extensive research throughout the last century has emphasized the prognostic significance of a number of pathological factors, which include the size of the primary tumor, the histological grade, and the appearance of tumor deposits in the draining lymph nodes of the primary breast carcinoma. In spite of the progress observed in the study of tumor growth, one of the main limitations of modern oncology is the lack of knowledge on the mechanisms that rule the kinetics of replication of neoplastic cells and particularly of its metasatasizing capacity.

Among the many studies carried out to identify the mechanisms underlying the basis of tumor growth and secondary replication, interesting are those that have demonstrated that osteopontin – a 44-kDa phosphotylated glycoprotein with the amino acid sequence Arg-Gly-Asp that elicits binding of integrin and is secreted by the autocrine system of the cells of some aggressive multiple myeloma and invasive cancer (breast, ovarian, lung, prostate and bladder) – is actively involved in the mechanisms of bone metastasis during progression of the neoplastic disease. It is

Key words: bone metastases, breast cancer, osteopontin.

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upregulated upon transformation of cells and shows high affinity binding to hydroxyapatite, playing a role in modulating mineralization of calcifying tissues. Osteopontin, having an important adhesive function, may therefore play a role in tumor cell invasion.

As the clinical and biological significance of increased serum levels are not yet clear, the aim of our study was to make a clinical contribution to this fascinating issue.

Material and methods

Twenty-six women with breast cancer were enrolled in the study. Their ages ranged from 42 to 82 years. In 15 patients (57.6%), the tumor was localized at the level of the upper-quadrant, in 5 (19.2%) at retroareolar level, and in 6 (23%) at the lower-quadrant. Preoperative staging, carried out with mammography, breast and hepatic ultrasound, chest X-ray, total body bone scan and serum markers, classified 11 patients as T1N0M0, 11 as T2N2aM0, and 4 as T2N2aM1. Preoperative scintiscan staging made it possible to identify 4 patients with metastatic bone lesions and 5 with suspect osteolytic lesions that radiologic examination had classified as benign. No parenchymatous repetitive lesion was documented. No patient was submitted to neoadjuvant therapy.

All patients underwent surgery: quadrantectomy in 14, Patey mastectomy in 7, and a simple mastectomy in 5. All cases were completed by lymphadenectomy of the ipsilateral axilla. All patients were submitted to adjuvant therapy. Three patients at stage I (T1N0M0), with positive hormone receptors and c-erb2 negative, were treated with radiotherapy (60 Gy) and hormone therapy. The patients at stage IIA (T1N1M0) (T1N1M0) were treated with chemotherapy and radiotherapy; the patient with positive hormone receptors was submitted also to hormone therapy. The patients at stage IIB (T2N2aM0) were treated with chemotherapy and radiotherapy; the patient with positive hormone receptors was submitted also to hormone therapy. The patients at stage IIIC (T2N3M0) were treated with chemotherapy, followed, in the cases with positive hormone receptors, by hormonal therapy. In 3 patients submitted to quadrantectomy, radiotherapy was also performed. The 4 patients at stage IV (T2N3M1), with negative hormonal receptors and negative Her2, were treated only with chemotherapy.

The serum value of osteopontin was measured before surgery and after 6, 12, 18 and 24 months. The quantitative determination of serum levels of osteopontin was performed using an enzyme-linked immunosorbent assay (ELISA) kit from R&B Systems (Minneapolis, MN, USA) according to the manufacturer's instructions. The kit is a 4.5 hour solid-phase ELISA designed to measure human osteopontin in serum and plasma.

The data are expressed as mean and standard deviations (SD). Assumption of normal distribution for continuous variables was tested by the Kolmogorov-Smirnov test. Variables not normally distributed were compared by the Mann-Whitney U test. Categorical variables were compared by the chi-squared test. Spearman's rank correlation coefficient was used to correlate all variables. P values of less than 0.05 were considered to indicate statistical significance and P values were based on a two-sided test.

Results

Histologic staging classified 3 patients at stage I (T1N0M0), 8 at IIA (T1N1M0), 11 at IIIC (T2N3M0), and 4 at IV (T2N3M1).

In the 22 patients who did not have metastatic bone lesions (group A), the preoperative serum levels of osteopontin documented a very low concentration of the glycoprotein (average value, 0.04 µg/ml ± 0.01), whereas in the 4 patients with bone metastases (group B), osteopontin measurement showed higher serum levels (average value 35.75 µg/ml ± 8.66).

The control carried out at 6 months showed no variations in serum levels of osteopontin in group A. In group B, adjuvant treatment determined a significant reduction in serum osteopontin levels (average value, 18 µg/ml ± 11.6) in 2 patients and moderate in the other 2, with partial remission and stabilization of the size of the bone metastases, respectively. At the 12-month control in these latter 2 patients, we observed an increase to 35 µg/ml of the serum levels of osteopontin; they both died for progression of the disease respectively after 3 and 5 months.

Three patients of group A developed bone metastases and showed an increase in serum levels of osteopontin. At 18 months of follow-up in these 3 patients after a second-line chemotherapy, serum levels of osteopontin had decreased to 15 µg/ml, with stabilization in the size of the bone metastases. An increase in the serum levels of the marker was observed in the 2 alive patients of group B (average value, 11 µg/ml ± 1.41).

At the 24-month control, one patient previously staged as IIIC and the 3 patients of group A with increased osteopontin serum levels had died. In the 2 patients of second group, a slight increase (15.5 µg/ml ± 2.12) of osteopontin serum levels was documented. The 24-month overall survival for the two groups was respectively 81.8% and 50%. For the first group, in stage I, IIA and III C, 24-month overall survival was 100%, 87% and 72.7%, respectively (Table 1).

Statistical evaluation of the results demonstrated a strong correlation between the serum levels of osteopontin and the occurrence of bone metastases (r = 0.865; P < 0.001), between the serum levels of osteopontin, the occurrence of bone metastases and overall survival (r = 0.963; P < 0.001), and an inversely proportional relationship between the serum levels of osteopontin and overall survival (r = -0.866; P < 0.001).
**Table 1 – Patient characteristics**

<table>
<thead>
<tr>
<th>Patient N°</th>
<th>GROUP A</th>
<th>GROUP B</th>
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<tbody>
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<td>1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22</td>
<td>23 24 25 26</td>
</tr>
<tr>
<td>Stage</td>
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<tr>
<td>Bone metastasis</td>
<td>Pre-op</td>
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<td></td>
<td>6 mo</td>
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<td></td>
<td>24 mo</td>
<td>t t t t</td>
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<tr>
<td>Serum levels of osteopontin (mean values)</td>
<td>Pre-op</td>
<td>0,04</td>
</tr>
<tr>
<td></td>
<td>6 mo</td>
<td>0,04</td>
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<td></td>
<td>12 mo</td>
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<td>24 mo</td>
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<tr>
<td>24 months overall survival</td>
<td>100% 87% 81,8%</td>
<td>72,7% 50%</td>
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Group A, patients without bone metastasis; Group B, patients with bone metastasis. X, presence of bone metastasis; t, death; pre-op, pre-operative.
Discussion

In the last 20 years, the interest of many investigators has been directed to the activity of osteopontin, a specific phosphoglycoprotein present in biologic fluids including blood and urine, which seems to play a key role in various physiopathologic processes such as bone remodeling, angiogenesis, cicatrization of wounds and cell growth. In particular, osteopontin, which represents one of the most abundant non-collagenous proteins of the bone matrix, acts directly by influencing the activity of osteoblasts and osteoclasts in the process of bone formation. Regulation of its activity is complex and appears to be influenced by numerous hormones and growth factors. In fact, osteopontin inhibits bone mineralization and favors the differentiation of osteoclasts. Whereas its activity does not seem important during normal bone development, it assumes a key role in osteoclastogenesis in many pathologic conditions of the bone or in sites where new bone matrix is present.

Numerous studies have shown that osteopontin mediates the adhesion of osteoclasts to bone by the expression of integrin receptors.

Elevated serum levels of the protein in patients with metastatic bone lesions from breast or prostate cancer suggest the hypothesis that osteopontin plays an important role in the development and progression of the neoplastic disease at bone level. The osteolytic lesions are mediated by an increased expression of osteopontin in osteoblasts. Moreover, it appears that osteopontin selectively activates the receptors of the epidermoid growth factor and of interleukin-6, thereby favoring the process of tumorigenesis in prostate cancer and multiple myeloma. The capacity to stimulate tumor growth appears related to an inhibitory activity on apoptosis and to an abnormal proliferation of tumor cells. In fact, by stimulating migration and cell adhesion, osteopontin potentiates the invasive capacity of tumor cells in patients with breast cancer.

Many reports have shown that in patients with bone metastases from breast cancer, increased osteopontin values were correlated with a shorter long-term survival. Instead, experimental studies on murine models showed that low serum levels of osteopontin were associated with a lower incidence of metastases at the level of the bone and soft tissues. In light of the results obtained and while waiting for further scientific evidence, it is possible to hypothesize a direct involvement of the protein in mechanisms of chemotaxis and adhesion of the neoplastic cells in sites of bone metastasis in subjects with breast cancer. Consequently, one could assign to the glycoprotein a role of biotumor marker, particularly in those patients who present suspect bone lesions. Its measurement, used in the clinical staging of the neoplastic disease, could have a prognostic value as an indicator of response to adjuvant therapy.

References


