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Research Paper

Serum YB-1 (Y-box binding protein 1) as a biomarker of bone disease progression in patients with breast cancer and bone metastases

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ABSTRACT

YB-1 (Y-box binding protein 1) is a multifunctional cold-shock protein that has been implicated in all hallmarks of cancer. Elevated YB-1 protein level was associated with poor prognosis in several types of cancers, including breast cancer (BC), where it is a marker of decreased overall survival (OS) and distant metastasis-free survival across all subtypes. YB-1 is also secreted by different cell types and may act as an extracellular mitogen; however the pathological implications of the secreted form of YB-1 (sYB-1) are unknown. Our purpose was to retrospectively evaluate the association between YB-1 measured by ELISA in serum and disease characteristics and outcomes in patients with BC and bone metastases (BM). In our cohort, sYB-1 was detected in the serum of 22 (50%) patients, and was associated with the presence of extra-bone metastases (p=0.044). Positive sYB-1 was also associated with faster bone disease progression (HR 3.1, 95% CI 1.09–8.95, P=0.033), but no significant differences were observed concerning OS, and time to development of skeletal-related events. Moreover, patients with positive sYB-1 also had higher levels of IL-6, a known osteoclastogenic inducer. Therefore, detection of sYB-1 in patients with BC and BM may indicate a higher tumor burden, in bone and extra-bone locations, and is a biomarker of faster bone disease progression.

1. Introduction

Y box binding protein 1 (YBX1 or YB-1) is one of the three members of the YBX family of transcription factors that contain a conserved cold shock domain (CSD) [1], and its features and functions were deeply reviewed by Eliseeva et al. [2].

YB-1 is a recognized oncoprotein, overexpressed in different types of cancers including breast cancer (BC). YB-1 plays an important role in tumor cell proliferation and progression, and is not only a marker of poor prognosis, but is also emerging as a putative molecular target for the development of new therapeutic strategies, as recently reviewed by Kosnopfel et al. [3]. In BC, YB-1 is a prognostic marker of disease aggressiveness and tumor resistance to chemotherapy, across all tumor sub-types, although an association with clinicopathological characteristics was not always found [4–13]. A seminal paper has established that YB-1 expression is related to epithelial-to-mesenchymal transition (EMT) induction and enhanced metastatic potential and reduced proliferation rates of mammary epithelial cells [14]. This effect is due to ability of YB-1 to directly activate cap-independent translation of messenger RNAs encoding transcription factors implicated in EMT.

Beside its intracellular role, YB-1 can also be secreted through a non-classical vesicle-mediated pathway, from mesangial and monocytic cells under lipopolysaccharide (LPS)-induced inflammatory stress [15]. In this case, secreted YB-1 showed mitogenic as well as promigratory effects in cell line models. However, despite the correlation of sYB-1 with infectious response and lysosomal metabolism [16], its role in the setting of cancer is still largely unknown. In this setting, using an *in vitro* model of BC it was demonstrated that addition of recombinant YB-1 increased the proliferation of MCF-7 cells [17]. In addition, in the clinical arena, it was shown that YB-1/p18 is detectable in plasma samples by immunoblotting, and that it was present in 78% of cases from a small cohort of patients with different malignancies.

Abbreviations: BC, breast cancer; BM, bone metastases; BPs, bisphosphonates; CSD, cold shock domain; CT, computed tomography; CTCs, circulating tumor cells; CV, coefficient of variation; EMT, epithelial-to-mesenchymal transition; HCC, hepatocellular carcinoma; IL-6, interleukin 6; IQR, interquartile range; LPS, lipopolysaccharide; NTX, N-terminal telopeptide; OS, overall survival; SREs, skeletal related events; sYB-1, secreted/serum YB-1; TAMs, tumor-associated macrophages; TTBP, time to bone progression; TTSRE, time to first skeletal-related event;; YB-1, Y-box binding protein 1

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Furthermore, in this cohort that included ten patients with BC, the detection of sYB-1 was independent of the tumor origin. Of note, sYB-1 concentrations altered during therapeutic interventions, but sYB-1 did not predicted prognosis when combining all tumor types. At the same time, other groups are exploring the role of sYB-1 in the diagnosis of hepatocellular carcinoma (HCC) [18], and epithelial ovarian cancer [19].

Interestingly, it was also found in *in vitro* studies of BC, that YB-1 interacts with other mediators, in specific interleukin 6 (IL-6) creating a positive feed-forward loop driving epithelial to mesenchymal (EMT)-like metastatic features during cancer progression [20]. IL-6 is known for its strong pro-tumorigenic activity due to its multiple effects on bone metabolism, tumor cell proliferation and survival, angiogenesis, and inflammation. In addition, IL-6 is known to promote BC metastasis, and to be associated with poor prognosis in BC patients [21]. Finally, IL-6 has an important role in the development of bone metastases (BM), as reviewed by Ara and DeClerck [22]. Therefore, we hypothesize that levels of sYB-1 in patients with BM may be associated with disease characteristics and/or patients' outcomes.

In the present study we performed a retrospective analysis of the prognostic value of sYB-1 in patients with BC and BM, and assessed the correlation between sYB-1 and serum IL-6 (sIL-6).

2. Material and methods

2.1. Study population and design

In this retrospective cohort study we included 44 consecutive female patients followed at the Oncology Division of Santa Maria Hospital (HSM), Lisbon, Portugal. All the eligible patients had the diagnosis of BC with BM (either *de novo* or after recurrence) between 1998 and 2014, were started on bisphosphonates (BPs; zoledronic acid), agreed to participate in a prospective collection of biological specimens, and had available peripheral blood collected at the time of first treatment with BPs (following BM diagnosis).

Cancer treatment was provided according to patient-physician description. Demographic and clinicopathological information was retrospectively collected, namely: age at diagnosis of primary BC and BM; primary BC histology, hormone receptors and HER2 status; TNM staging; sites of metastatic disease at BC presentation, if metastatic, or at time of recurrence; radiographic pattern of BM; date and site of disease recurrence; date of bone progression; date of skeletal related events (SREs); and survival status. Progression in bone was defined as date of new or increased bone lesions on imaging (CT scan or bone scan), or attending physician indication on medical records. SREs were defined as any of the following events: pathological bone fracture, need for radiation therapy or surgery to the bone (due to pain or impending fracture), or spinal cord compression.

This study was ethically approved by local Institutional Review Board, and complies with all national regulations. All patients provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki and good clinical practice guidelines.

2.2. YB-1 and IL-6 quantification

Peripheral blood was collected in serum separation tubes. Serum was separated by centrifugation at 2000*g* for 10 min at 4 °C, aliquoted and stored at −80 °C until usage. Freeze and thaw cycles were avoided. YB-1 was quantified in serum using Human YBX1/YB1 Sandwich ELISA kit (LSBio, LifeSpan BioSciences, Inc.), and IL-6 using Human IL-6 Quantikine ELISA kit (R & D Systems), according to manufacturer's instructions. Absorbance at 450 nm was measured in an InfiniteM200 Plate Reader (Tecan) and protein concentration was calculated based on standard curve. All determinations were performed in duplicate. YB-1 assay detection range: 0.156–10 ng/ml; assay sensitivity: 0.064 ng/ml; estimated intra-assay coefficient of variation (CV) < 10%. IL-6 assay minimum detectable dose 0.70 pg/ml; estimated intra-assay coefficient of variation (CV) < 5%. For subsequent analysis and patient stratification sYB-1 was considered to be negative (undetectable) or positive, except for correlation analysis with IL-6, where it was analyzed as a continuous variable. IL-6 was always analyzed as a continuous variable.

2.3. Statistical analysis

Demographic and clinicopathological characteristics were tabulated according to the full cohort and to sYB-1 status. Frequencies for categorical variables and central tendency, dispersion and range for continuous variables were calculated. Univariate association with sYB-1 status is presented. Survival and cumulative incidence plots were built using Kaplan–Meier methods. Univariate and multivariate differences between survival rates were tested using the log-rank test or Cox proportional hazards models, respectively. For time dependent variables, study follow-up was performed until August 2014. Overall survival (OS), if not otherwise specified, was defined as time from bone-disease recurrence to death from any cause. Time to first skeletal-related event (TTSRE) and time to bone progression (TTBP) were defined as time from bone-disease recurrence to first SRE or bone-disease progression, respectively (as reported by assistant physician). All patients with missing data in relevant variables were excluded from the multivariate analysis. Analyses were performed using Stata 13.1 software (StataCorp LP).

3. Results

3.1. Patient characteristics

Patients' demographic and clinicopathological characteristics are presented in Table 1. On the whole cohort, median age at diagnosis of BC was 54.3 (interquartile range [IQR] 44.0–62.3) years. The majority of patients had locorregional disease at BC diagnosis (n=33, 80.5%). In these patients, disease relapsed at distant sites after a median interval of 66 months (IQR 38.5–126.1), with bone-specific recurrence after a median interval of 75.9 months (IQR 45.4–130.8). As expected, the radiographic pattern of bone disease was mostly lytic (n=24, 60.0%). Finally, the majority of tumors were hormone receptor-positive (n=32, 76.2%) and HER2-negative (n=29, 80.6%).

3.2. sYB-1 levels and association with clinical features and outcomes

In the whole cohort, median sYB-1 was 4.94 ± 7.34 pg/ml (range 0.0–43.61 pg/ml), while median sYB-1 was 9.25 ± 7.23 pg/ml for the group of patients with positive sYB-1 (range 3.49–43.61 pg/ml) and 0 for the negative sYB-1 group. When comparing YB-1 levels to relevant clinicopathological characteristics, patients with positive sYB-1 presented more frequently with metastases outside the bone (n=18, 81.8% vs. n=10, 47.6% in patients with negative sYB-1; p=0.019). Moreover, despite not significantly different, patients with positive sYB-1 tended to be slightly younger, a fact mostly dependent on the strata 35–49 and 50–69 (n=11, 50% of sYB-1 positive had 35–49 at date of bone disease diagnosis, while n=12, 54.6% of sYB-1 patients of the sYB-1 group were 50–69 at date of bone disease), a cut-off closely related to menopausal status. Finally, we further observed that patients with positive sYB-1 tended more frequently to present bone lesions with a lytic radiography pattern, while those with negative sYB-1 had a more balanced distribution of radiographic patterns (lytic lesions n=15, 71.4% vs. n=9, 47.4% in patients with negative sYB-1; p=0.064).

After a median follow-up of 34.0 (IQR 21.1–74.5) months 30 patients (68.2%) died: 17 (77.3%) vs. 13 (59.1%) in the positive and negative sYB-1 groups, respectively. Patients with negative sYB-1 tended to live longer, with a median survival of 58.9 months, compared to a median survival of 32.8 months of those patients with positive sYB-