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XB130 Expression in Human Osteosarcoma: A Clinical and Experimental Study

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Identifying prognostic factors for osteosarcoma (OS) aids in the selection of patients who require more aggressive management. XB130 is a newly characterized adaptor protein that was reported to be to be a prognostic factor of certain tumor types. However, the association between XB130 expression and the prognosis of OS remains unknown. In the present study, we investigated the association between XB130 expression and clinicopathologic features and prognosis in patients suffering osteosarcoma, and further investigated its potential role on OS cells in vitro and vivo. A retrospective immunohistochemical study of XB130 was performed on archival formalin-fixed paraffin-embedded specimens from 60 pairs of osteosarcoma and noncancerous bone tissues, and compared the expression of XB130 with clinicopathological parameters. We then investigate the effect of XB130 silencing on invasion in vitro and lung metastasis in vivo of the human osteosarcoma cell line. Immunohistochemical assays revealed that XB130 expression in osteosarcoma tissues was significantly higher than that in corresponding noncancerous bone tissues (P=0.001). In addition, high XB130 expression more frequently occurred in osteosarcoma tissues with advanced clinical stage (P=0.002) and positive distant metastasis (P=0.001). Moreover, osteosarcoma patients with high XB130 expression had significantly shorter overall survival and disease-free survival (both P<0.001) when compared with patients with the low expression of XB130. The univariate analysis and multivariate analysis shown that high XB130 expression and distant metastasis were the independent poor prognostic factor. We showed that XB130 depletion by RNA interference inhibited invasion of XB130-rich U2OS cells in vitro and lung metastasis in vivo. This is the first study to reveal that XB130 overexpression may be related to the prediction of metastasis potency and poor prognosis for osteosarcoma patients, suggesting that XB130 may serve as a prognostic marker for the optimization of clinical treatments. Furthermore, XB130 is the potential molecular target for osteosarcoma therapy.

Osteosarcoma; XB130; Immunohistochemistry; Prognosis; RNA interference

Introduction

Osteosarcoma is a type of aggressive bone cancer of mesenchymal origin generally found in youths aged between 10 and 25 years. Any bone of the human body can be affected by this neoplasia and the general survival at five years is approximately 65 to 75% [1]. The main causes of death are pulmonary metastases diagnosed by computed tomography (CT) in 35 to 45% of the patients [2]. Even though effective chemotherapy for patients with OS has indeed led to a significant improvement of clinical outcome, between 30 and 50% of patients with non-metastatic disease of extremities still die from this neoplasia, in spite of having had a complete surgical removal of the tumor and intensive chemotherapy [3,4]. Currently, many immunohistochemical markers and genetic proteins have been studied, but with prognostic and therapeutic relevance doubtful [5-9]. Thus, it is crucial to identify new and efficient biomarkers which can provide insight into tumor progression and outcome, especially in screening poor prognosis patients who should be offered more aggressive therapy at an early time point in the clinical treatment.

XB130 is a newly discovered adaptor protein for intracellular signal transduction; it is involved in gene regulation, cell proliferation, cell survival, cell migration, and tumorigenesis [10]. XB130 has been detected in human esophageal squamous cell carcinoma (ESCC) [11], follicular and papillary thyroid carcinoma, as well as in human lung carcinoma cell lines [12]. In ESCC cells, expression of XB130 may affect cell cycle progression and impact prognosis of patients with ESCC [11]. In thyroid and lung cancer cells, XB130 has been implicated as a substrate and regulator of tyrosine kinase-mediated signaling and in controlling cell proliferation and apoptosis [12]. In the gastric cancer, reduced XB130 protein expression is a prognostic biomarker for shorter survival and a higher recurrence rate in patients with GC, as well as for the response to chemotherapy [13]. However, in patients with hepatocellular carcinoma (HCC), protein expression of XB130 is not associated with the postoperative prognosis of patients with HCC [14]. However, clinical evidence is not yet well established between expression of XB130 and clinical significance in osteosarcoma patients.

To date, the association between XB130 expression and prognosis of OS remains unknown. In this study, we analyzed the association of XB130 expression in OS with clinical factors and overall survival, and further investigated its potential role on cell proliferation, invasion and lung metastasis *in vitro* and vivo.

Materials and Methods

Cell lines and culture conditions

OS cell lines (U2OS) were purchased from the American Tissue Culture Collection (ATCC). The cells were cultured in DMEM (Life Technologies, Inc., San Diego, CA) supplemented with 10% FCS, 2 mM glutamine, and 1% nonessential amino acids (complete medium).

Patients and tissue samples

This study was approved by the Research Ethics Committee of Yishui central hospital, China. All specimens were handled and made anonymous according to the ethical and legal standards. Paraffin-embedded OS sections from 60 patients who were diagnosed with primary OS and had undergone initial surgery at the Yishui central Hospital, Yishui, China, between January 2008 and December 2012 were obtained from

measured by adding 400 ul of Routine Medium containing 10% (v v^{-1}) FCS to the lower compartment, and 2×10^5 cells (U2OS, U2OS / XB130shRNA and U2OS / shRNA)in 200 ml of Routine Medium, but with 2% (v v^{-1}) FCS to the upper compartment of the Boyden chamber. The cells were incubated for 24 h, the upper side of the filter was wiped with a cotton swab to remove any non-motile cells, and the motile cells on the lower side of the filter were fixed and stained using the Diffquik histochemical stain (Dade Behring, Du¨dingen, Switzerland) according to the manufacturer's instructions. The lower compartment of the Boyden chamber was checked for cells and the number of stained cells/field of $0.50mm^2$on the lower side of the filter was counted using a Dynascope with a ×20 objective (Vision Engineering, Working, Surrey, UK). For each cell line, four experiments were carried out, each experiment consisting of three filters and 10 fields per filter were counted. In controls, fixed

significant difference was observed between the expression of XB130 and patient's age, gender, tumor size, location and response to chemotherapy.

		XB130 expression	
Tissue			
Normal tissue	60	0(100%)	60(100%)
Osteosarcoma tissue	60	24(40%)	36(60%)
Age(year)			
>20	19	7(36.8%)	12(63.2%)
≤20	41	17(41.4%)	24(58.6%)
Gender			
Female	22	8(36.4%)	14(63.6%)
Male			

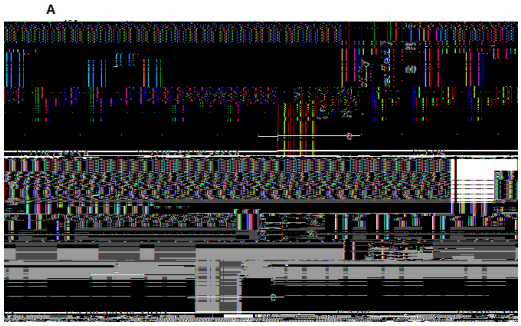

Figure 4: Down-regulation of XB130 decreased cancer cell motility and invasion. A: Cell motility, average cells/field for 4 experiments ± S.E. (Materials and Methods). Each experiment consisted of three filters per cell line and 10 fields per filter were counted. B: Cell invasion through Matrigel, average cells per field for three experiments ± S.E. (Materials and Methods). Each experiment consisted of three filters per cell line and 10 fields per filter were counted. Significantly lower for the XB130 shRNA-transfected cell line compared to its parental cell line

HCC was 75.0%, but XB130 is not associated with the postoperative prognosis of patients with HCC. In human esophageal squamous cell carcinoma (ESCC) [11], 71.2% of the patients expressed XB130 in the nuclei and/or cytoplasm of the ESCC cells. Further, nuclear expression of XB130 was an independent prognostic factor of postoperative survival. Shi et al. [13] has reported that both XB130 mRNA and protein expression were detectable in normal gastric tissues. The reduced XB130 protein expression is a prognostic biomarker for shorter survival and a higher recurrence rate in patients with GC, as well as for the response to chemotherapy. The studies above indicated XB130 expression was related with the tumor type.

In the current study, we examined a cohort of 60 OS specimens and report the evidence that XB130 correlated with distant metastasis and high clinical stage. No significant difference was observed between the expression of XB130 and patients' age, gender, tumor size, location and response to chemotherapy. The patients with high XB130 expression had a significantly worse 5-year overall and disease-free survival rate than those with low XB130 staining. The multivariate analysis showed that high XB130 expression was found to be statistically significant independent poor prognostic factors. Our data suggest that XB130 may serve as a marker for poor prognoses. To our knowledge, this is the first IHC study to investigate the potential utility of XB130 as a biomarker of OS among Chinese patients.

In thyroid tumor cells, knockdown of XB130 using small interfering RNA inhibited G (1)-S phase progression, induced spontaneous apoptosis, and enhanced intrinsic and extrinsic apoptotic stimulus-induced cell death. Growth of tumors in nude mice formed from XB130 shRNA stably transfected WRO cells were significantly reduced, with decreased cell proliferation and increased apoptosis [12]. In the present study, we found that knockdown of XB130 inhibited invasion and mobility in OS cells in vitro, but the molecular contribution of XB130 in lung metastasis of OS cancer is still unknown. Particularly, it is unclear whether XB130 gene is relevant to the progression of OS cancers in lung tissue. Here, we found XB130 silencing resulted in a significant reduction in the number of lung metastases in mice injected with XB130 shRNA-transfected U2OS cells.

In summary, our present study has provided first evidence that XB130 overexpression may be related to the prediction of metastasis potency