Prognostic And Predictive Value Of c-erbB2 Overexpression In Osteogenic Sarcoma

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Citation

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Abstract

Introduction: Osteogenic sarcoma is a highly malignant tumor of the bone, seen between 10-30 years of age. The over expression of c-erbB-2 proto-oncogene has been correlated with early metastasis and poor survival rate in various human tumors. There are very few reports in the literature regarding determination of c-erb B-2 (HER-2/neu) expression in cases of osteogenic sarcomas and also the results in these reports are quite variable. The aim was to study the expression of c-erbB-2 and its correlation with the survival rate in patients of osteogenic sarcoma.

Materials and Methods: A retrospective study (1997-2003) of a total of 20 cases of osteogenic sarcoma. Monoclonal antibody against c-erbB-2/HER- 2/Neu Ab-12 (clone CB 11) was used and expression of c-erbB-2 in these 20 cases of osteogenic sarcoma was studied and correlated with the disease free survival time of patients.

Results: 3 cases showed no staining positivity, 1+ was seen in 5 cases. 3 cases showed 2+ and 3 cases had 3+ positivity. 4+ cytoplasmic positivity was noted in 6 cases. The intensity of cytoplasmic staining of c-erbB-2 did not correlate with survival rate of the patients, response to chemotherapy and metastasis.

Conclusion: c-erbB-2/HER-2 has been considered as an independent prognostic factor in osteogenic sarcoma, but in the present study, the expression was not seen to be correlating with survival rate of the patients. Therefore, further studies are needed to reach a consensus regarding the reliability of c-erbB-2 as an independent prognostic factor in osteogenic sarcoma.

INTRODUCTION

Osteogenic sarcoma is the most frequent and highly malignant bone tumor, seen during second and third decade of life[1]. Several oncogenes and tumor suppressor genes are reported to be involved in the oncogenesis of osteogenic sarcoma. Although survival rate increased up to 60-70% within the last 20 years, the problem of non-response to chemotherapy remains. There are many factors thought to have an influence on prognosis of osteosarcoma, but their exact role is still controversial. The overexpression of c-erbB-2 (also called neu or HER2) proto-oncogene has been studied and correlated with poor survival rate in various human tumors especially breast carcinomas. Little is known about the expression of c-erbB-2 in sarcomas[2], especially osteogenic sarcoma and only few reports are available in the literature, that to showing controversial results. Therefore, in the present study, we studied 20 cases of osteogenic sarcoma for expression of c-erbB-2 (HER-2/neu) and correlated the expression with the survival rate of patients.

MATERIALS AND METHODS

The present study is a retrospective study (1997-2003) in the Department of histopathology. A total of 49 cases of osteogenic sarcoma were retrieved from surgical pathology files. Clinical details of cases were reviewed for age, sex, clinical presentation & diagnosis, pathological diagnosis and treatment. Out of these 49 cases, proper follow up data was available in only 20 cases. The follow up data was collected from the radiotherapy files of these cases and their total as well as disease free survival was noted. Histological sections were obtained from formalin fixed paraffin embedded tissues. The sections were restained in these cases and all cases were reviewed by the pathologist to confirm the diagnosis of osteogenic sarcoma. For immunohistochemistry, a representative blocks with adequate viable tumor (avoiding areas of necrosis) were chosen. Monoclonal antibody against c-erbB-2/HER- 2/Neu

Ab-12 (clone CB 11) was obtained from Neomarkers. 3-4 µm thick sections were cut from tissue blocks, placed on pretreated glass slides, dried at 37°C overnight and then at 60°C for 30 minutes. Sections were dewaxed and rehydrated through graded alcohols. Inhibition of endogenous peroxidase was done by treatment for 30 minutes with 3% hydrogen peroxide in methanol. After washing, blocking was done with normal serum and 4 rinses with buffer were given. The slides were placed in microwave for 3 cycles for 4 minutes each and then these were allowed to cool at room temperature for 90 minutes. The slides were incubated with primary antibody at room temperature for 90 minutes followed by washing (3 times for 5 minutes each).Then slides were incubated with secondary antibody for 30 minutes followed by Avidin- Biotin reagent for 30 minutes and then DAB (Diaminobenzidine). The sections were again placed in running tap water, dehydrated in a graded series of alcohols, cleared in 2 changes of xylene and permanently mounted. Sections of breast carcinoma with known over expression of HER- 2/neu oncogene served as the positive control. The positivity was scored independently by two histopathologists. The immunohistochemical positivity was scored as:

0 = no staining
1+ = 1-25% positive cells
2+ = 26-50% positive cells
3+ = 51-75% positive cells
4+ = 76-100% positive cells.

RESULTS

Archival formalin- fixed, paraffin embedded material and medical records showing follow up data were available in 20 cases of osteogenic sarcoma, which formed basis of the present study. Only pretreatment tissues from the primary tumor were used, except for one case, which had recurrence. No metastases were evaluated. In a total of 20 cases, the age ranged from 8 years to 38 years with male to female ratio of 3.2: 1. 13 cases were amputation specimens and 7 cases were biopsies from the tumors. All cases were high grade conventional osteosarcoma (Fig 1), except one case of telangiectatic variant of osteosarcoma. Six cases on histopathology were rich in osteoclastic type of giant cells (Fig 2). The primary site of involvement was lower end of femur in 11 cases and proximal end of tibia in 5 cases. Other sites involved were maxilla, fibula, chest wall and sacrum (one case each). One case had lung metastasis at the time of presentation. Disease free survival in these 20 cases varied from one month to $3^{1/2}$ years.

Figure 1

Figure 1: showing osteoid production by tumor cells in a case of osteogenic sarcoma (H&E, 250X).

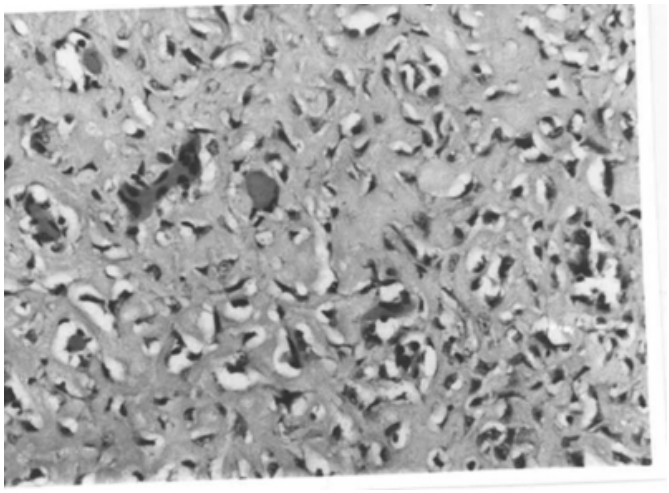
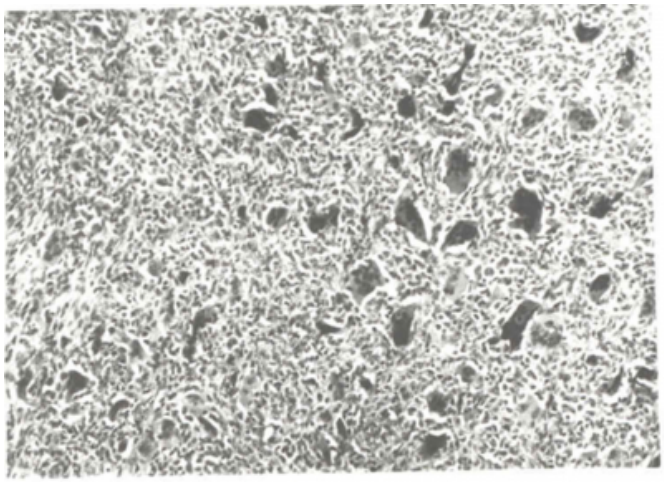


Figure 2

Figure 2: showing a case of giant cell rich variant of osteogenic sarcoma (H&E, 125X).



Lung metastasis was seen in four cases with pleural effusion in three cases. Cervical and retroperitoneal lymphadenopathy was seen in three cases and two cases showed bone metastasis in the scapula and infraorbital region. Complete membranous positivity was not seen in any of our osteosarcoma cases. 3 cases showed no staining positivity, focal cytoplasmic positivity in 1-25% cells (1+) was seen in 5 cases.3 cases showed 2+ cytoplasmic positivity and 3 cases had 3+ positivity. 4+ cytoplasmic positivity was noted in 6 cases (Fig 3). Osteoclast- type giant cells also showed strong cytoplasmic positivity in all six cases. The expression of c-erbB-2 was correlated with the disease free survival time of patients of osteogenic sarcoma. The intensity of cytoplasmic staining of monoclonal

antibody against c-erbB-2/HER- 2/Neu Ab-12 (clone CB 11) did not correlate with survival rate of the patients, response to chemotherapy and metastasis as shown in table no 1. Spearman rank correlation test was applied and p value calculated was 0.692, which was not significant.

Figure 3

Figure 3: showing 4+ cytoplasmic positivity of c-erbB-2 in tumor cells of a case of osteogenic sarcoma (Immunostain, 250X).

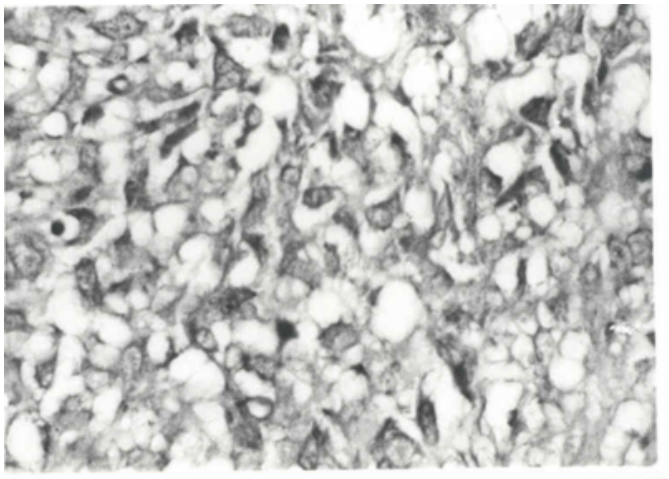


Figure 4

Table 1: Showing poor correlation between cytoplasmic positivity of C-erbB-2 and disease free survival time of patients of osteogenic sarcoma.

Positivity score	Total no. of cases	Disease free survival in months
0+	3	9, 2, 8
1+	5	8, 6, 24, 26, 9
2+	3	7, 6, 11
3+	3	10, 16, 42
4+	6	8, 11, 30, 1, 4, 6

DISCUSSION

Osteogenic sarcoma is the most frequent primary malignant bone tumor, exclusive of hematopoietic malignancy. It usually occurs in patients between 10 and 25 years of age and another peak age incidence occurs after 40, in association with other disorders. There is a slight male preponderance. Most osteogenic sarcomas arising denovo are located in the metaphyseal area of the long bones, particularly, lower end of femur, the upper end of tibia and the upper end of the humerus. Metastasis occurs most commonly to lungs (98%), other bones (37%), pleura (33%), and heart (20%).[3] 5- year survival is approximately 50-70%.[4]

Various prognostic factors are tumor size, response to chemotherapy, post chemotherapy tumor necrosis, serum elevation of alkaline phosphatase levels. However, very few prognostic factors have been found to be truly independent predictors of outcome. Several oncogenes and tumor suppressor genes are reported to be involved in the oncogenesis of osteogenic sarcomas as c-myc, c-raf, c-myb, c-met, c-sis, c-gli, RB and p53.[5,6,7,8,9,10] The amplification of mdm-2 gene has also been correlated with poor prognosis in osteogenic sarcomas.[11]

c-erb B-2 (HER-2/neu) proto-oncogene is located on human chromosome 17 at q21.[12] It encodes a 185- kilo dalton trans-membrane glycoprotein that shows significant structural similarity to epidermal growth factor receptor.[13] The neu differentiation factor (NDF) stimulates the kinase activity of erb B-2. It, however does not directly interact with erbB-2. Instead it interacts with a heterodimer of erb b-2 and the related receptor such as erbB3 or erbB4.[14] the authentic ligand for erbB-2 is unknown.

Elevated expression of erbB-2 family members have been reported in a variety of human tumors, particularly breast carcinomas. In the majority of studies, erb B-2 (HER-2/neu) over expression was found to be correlated with decreased survival.[15] Most recent series failed to find any prognostic relation between HER-2/neu overexpression and disease outcome. Such conflicting results can be explained by the low patient number evaluated in some studies and different methods used to determine c-erbB-2 (HER-2/neu) status, including solid matrix- blotting, Immunohistochemistry (IHC), fluorescent in situ hybridization (FISH) and enzyme linked immunoadsorbent assay (ELISA). Immunohistochemistry (IHC) is a preferred method, as it can be performed easily on a routine basis and it is used to identify proteins expressed by fixed cells in paraffin embedded tissue sections.[16]

Normal bone shows very little expression of c-erbB-2.[17] There are very few reports in the literature regarding determination of c-erbB-2 (HER-2/neu) expression in cases of osteogenic sarcoma and in these few reports also the results are quite variable. Onda[4] et al in 1996 studied expression of c-erbB-2 in the tumors of 26 patients with conventional osteosarcoma by immunoblot analysis and confirmed by immunohistochemical studies (CB11 antibody). They documented c-erbB-2 expression in 11 of 26 osteosarcoma cases (42%) and expression of c-erbB-2 was strongly correlated with early pulmonary metastasis and poor survival rate for the patients. They suggested that c-erbB-2 plays a significant role in aggressive tumor growth and in the promotion of metastatic potential in osteogenic

sarcomas. Gorlick[18] et al in 1999 analyzed expression of c-erbB-2 (HER-2/neu) in 47 patients of osteosarcoma by immunohistochemical methods. They documented high levels of HER-2/neu expression in 20/47 osteosarcoma samples (42.6%). High levels of c-erbB-2 were seen in patients with metastatic disease at presentation and at the time of relapse. They suggested that HER-2/neu should be evaluated prospectively as a prognostic indicator. Zhou and colleagues[19] reported a correlation between cytoplasmic staining of HER-2/neu and increased risk of pulmonary metastasis. Maitra et al[20] in 2001, studied 18 high grade osteosarcomas by Immunohistochemistry and FISH analysis. Overexpression of erb B-2 was not observed in their study.

Kilpatrick et al in 2001[21] also studied expression of HER-2/neu oncogene in 41 cases of osteosarcoma by immunohistochemical method. Complete membranous positivity was not seen in any of these cases and cytoplasmic positivity was observed in most of the cases irrespective of their subtype/grade. The expression was not associated with response to preoperative chemotherapy or disease progression.

Although immunohistochemical staining appears to be the predominant method for determining HER-2/neu oncogene overexpression, the results may be substantially affected by technical issues, including fixation, duration and prolonged storage. Also, type and sensitivity of the antibodies also differ. In the present study, membranous positivity of the expression of osteosarcoma was not seen. Cytoplasmic positivity also was not seen to correlate with response to chemotherapy, metastasis and disease free survival. Therefore, we recommend more prospective studies with larger number of cases to establish the role of c-erbB-2, as an independent prognostic factor in human osteosarcoma.

References

1. Trieb K, Lehner R, Stuling T, Sulzbacher I, Shroyer KR. Survivin expression in human osteosarcoma is a marker for survival. Eur J Surg Oncol 29: 379-82, 2003.
2. Dujin Z, Hector B, Yokota J, Yamammoto T, Martin JC. Association of multiple copies of the c-erbB-2 oncogene with spread of breast cancer. Cancer Res 47: 6123-5, 1987.
3. Uribe-Botero G, Russell WO, Sutow WW, Martin RG. Primary osteosarcoma of bone. A clinicopathologic investigation of 243 cases, with necropsy studies in 54. Am J Clin Pathol 67: 427-35, 1977.
4. Onda M, Matsuda S, Higaki S, Iijima T, Fukushima J, Yokokura A, Kojima T, Horiuchi H, Kurokawa T, Yamamoto T. ErbB-2 expression is correlated with poor prognosis for patients with osteosarcoma. Cancer 7:71-8, 1996.
5. Yokota J, Yokota YT, Battifora H, Fevre CL, Cline MJ. Alteration of myc,
myb and H-ras proto-oncogenes in cancers are frequent and show clinical correlation. Science 231: 261-5, 1986.
6. Bogenmann E, Moghadam H, DeClerck YA, Mock A. c-myc amplification and expression in newly established human osteosarcoma cell lines. Cancer Res 47: 3808-14, 1987.
7. Roberts WM, Douglass EC, Peiper SC, Houghton PJ, Look AT. Amplification of the gli gene in childhood sarcoma. Cancer Res 49: 5407-13, 1989.
8. Graves DT, Owen AJ, Barth RK, Tempst P, Winoto A, Fors L et al. Detection of c-sis transcripts and synthesis of PDGF like proteins by human osteosarcoma cells. Science 226: 972-4, 1984.
9. Friend SH, Bernards R, Rogelj S, Weinberg RA, Rapaport JM, Albert DM et al. A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. Nature 323: 643-6, 1986.
10. Miller C, Aslo A, Tsay C, Slamon D, Ishizaki K, Toguchida J et al. Frequency and structure of p53 rearrangements in human osteosarcoma. Cancer Res 50: 7950-4, 1990.
11. Ladanyi M, Cha C, Lewis R, Jhanwar SC, huvos AG, Healey JH. MDM2 gene amplification in metastatic osteosarcoma. Cancer Res 53: 16-8, 1993.
12. Fukushige S, Matsubara K, Yoshida M, Sasaki M, Suzuki T, Semba K et al. Localisation of a novel v-erbB-related gene, c-erbB-2 on human chromosome- 17 and its amplification in a gastric cancer cell line. Mol cell Biol 6: 955-8, 1986.
13. Yamamoto T, Ikawa S, Akiyama T, Semba K, Nomura N, Miyajima N et al. Similarity of protein encoded by the human c-erbB-2 gene to epidermal growth factor receptor. Nature 319: 230-4, 1986.
14. Peles E, Ben-Levy R, Tzahar E, Liu N, Wen D, Yarder Y. Cell- type specific interaction of neu differentiation factor (NDF/ heregulin) with neu/HER-2 suggests complex ligand-receptor relationships. EMBOJ 12: 961-71, 1993.
15. Wright C,Angus B, Nicholson S, Sainsbury RC, Cairns J, Gullick WJ et al. Expression of c- erbB-2 oncoprotein: a prognostic indicator in human breast cancer. Cancer Res 49: 2087-90, 1989.
16. Selvaggi G, Scagliotti GV, Torri V, Novello S, Leonardo E, Cappia S, Mossetti C, Ardissone F, Lausi P, Borasio P. HER-2/neu overexpression in patients with radically resected nonsmall cell lung carcinoma. Cancer 94: 2669-74, 2002.
17. Press MF, Cordon- Cardo C, Slamon DJ. Expression of the HER-2/neu protooncogene in normal human adult and fetal tissues. Oncogene 5: 953-62, 1990.
18. Gorlick R, Huvos AG, Aledo A, Beardsley GP, Healey JH, Meyers PA. Expression of HER2/erbB-2 correlates with survival in osteosarcoma. J Clin Oncol 17: 2781-8, 1999.
19. Zhou H, Randall LR, Goldsby R, Smith L, Coffin CM. HER-2/neu staining in osteosarcoma: association with increased risk of metastasis. Mod Pathol 14: 19A, 2001.
20. Maitra A, Wanzer DM, Saboorian H, Weinberg AG, Ashfaq R. Amplification of the Her-2/neu oncogene is uncommon in pediatric osteosarcomas. Mod Pathol 2001; 14: 4p.
21. Kilpatrick SE, Geisinger KR, King TS, Sciarrotta J, Ward WG, Gold SH, Bos GD. Clinicopathologic analysis of HER-2/neu immunoexpression among various histologic subtypes and grades of osteosarcoma. Mod Pathol 2001; 14: 1277-83.

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