Original Article

Combined Tumor Markers as Prognostic Factors in Osteosarcoma

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Objective: To analyze the expression of Ki-67, CD99, P53 and S100 proteins in osteosarcoma tissues by immunohistochemistry, and evaluate the feasibility of these protein expressions in prognosis of malignant bone tumor.

Materials and Methods: Paraffin embedded blocks of osteosarcoma tissues of thirty-two patients were obtained from the Department of Pathology, Faculty of Medicine, Khon Kaen University, Thailand, were analyzed for protein Ki-67, CD99, P53 and S100 expressions by immunohistochemical technique. The clinical data were collected from pathological record forms, medical record forms, and database of Tumor Registration Unit. The correlations of protein expressions and survival time were analyzed and evaluated by Kaplan-Meier analysis with log rank test and Cox's proportional hazards model.

Results: The Ki-67, CD99, P53 and S100 proteins were immunohistochemistrical expressed in osteosarcoma tissues in 93.75%, 68.75%, 37.5% and 12.5% of the cases, respectively. The Ki-67 was statistic significantly correlated with survival time (p = 0.0116). The osteosarcoma tissue with Ki-67 signal <0.4 showed better prognosis than those with Ki-67 ≥0.4 (p = 0.016, adjust HR = 0.156, 95% CI = 0.035 to 0.704), while negatively-stained P53 yielded poor prognostic value when compared to those with positively-stained P53 (p = 0.027, adjust HR = 5.332, 95% CI = 1.214 to 23.406).

Conclusion: The ki-67 and P53 proteins are likely to be feasible prognostic factors of osteosarcoma. Expression of high signal Ki-67 (signal ≥0.4) and negative P53 expressions predict poor prognosis in osteosarcoma patients. The study of protein expressions in larger cohort is suggested to affirm the feasibility of clinical use.

Keywords: Osteosarcoma, Immunohistochemistry, Ki-67, CD99, P53, S100, Tumor markers, Prognostic factor

J Med Assoc Thai 2018; 101 (Suppl. 5): S157-161
Full text. e-Journal: http://www.jmatonline.com

Osteosarcoma is the most common primary bone tumor. The osteosarcoma lesion is commonly detected in the metaphysis of the long bone[1,2]. A 5-year survival rate is approximately 68%. Osteosarcoma patients are usually diagnosed by x-ray, MRI, and tissue biopsy. After diagnosis, clinicians usually give prognostication via variety of means including age, gender, tumor staging, and percentage of tumor necrosis (% TN) from tissue biopsy. Livingston et al[3], report independent factors that predict shorter survival were male sex, >2 metastatic sites, >3 previous therapies, hemoglobin level <10.5 g/dL, platelet count >200 x10^3/L, creatinine level ≥1.3 mg/dL, and lactate dehydrogenase level >upper limit of normal. According to the Royal Marsden Hospital Scoring system that takes prognostic factors into account, patients with good prognostic scores due to less factors had longer overall survival than those with more factors[3].

Immunohistochemical investigation is not generally useful for diagnosis, its primary utility lies in the ability to exclude other diagnostic possibilities such as metastatic sarcomatid carcinoma and synovial sarcoma[4].

Osteosarcoma usually has diffuse moderate to strong intracytoplasmic staining of the transmembrane glycoprotein CD99[4]. Forced expression of CD99 suppressed osteosarcoma cell growth[5]. The calcium-binding protein S100 expression in osteosarcoma tissues was significantly higher than

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How to cite this article: Patjanasoontorn S, Sumananont C, Vaeteewoottacharn K, Chamgramol Y, Settasatian C. Combined Tumor Markers as Prognostic Factors in Osteosarcoma. J Med Assoc Thai 2018;101;Suppl. 5: S157-S161.

that in non cancerous bone tissues, and S100 positively stained case showed a significantly decreased overall survival time compared with negatively stain cases[6]. The nuclear protein Ki-67 which is associated with cell proliferation was expressed in all diagnostic protein P53-positive immunostaining tended to associate with decreased 2-year survival rates[7].

The aims of the present study were to study the expressions of Ki-67, CD99, P53 and S100 proteins in osteosarcoma tissues by immunohistochemical technique and evaluate the feasibility of expressions of those proteins in prognosis of malignant bone tumor.

Materials and Methods

Preserved osteosarcoma tissues from 32 patients in paraffin blocks from the Department of Pathology, Faculty of Medicine, Khon Kaen University, Thailand were selected. Protein Ki-67, CD99, P53 and S100 were performed via immunohistochemical analysis using rabbit monoclonal antibody (SP6), monoclonal mouse anti human CD99, monoclonal mouse anti-human P53 protein and polyclonal rabbit anti-S100. The medical data of patients were selected from pathological record forms, medical record forms, and database of tumor registration unit. The correlations of protein expressions and survival time were analyzed and evaluated by Kaplan-Meier analysis with log rank test and Cox's proportional hazards model using Graphpad prism and IBM SPSS Statistics 23. The data were presented in percentage, adjusted hazard ratio [HR], 95% confident interval (95%CI) and *p*-value, $p<0.05$ was considered statistically significant.

Results

Clinical data of osteosarcoma patients

Medical data of patients including characteristics of tumors were summarized in Table 1. The average patient's age at time of tissue preparation was 17.7 years. There were predominantly male (68.8%). The average survival time was 1,648 days. At the time of study, 16 cases had already passed away.

Immunohistochemistry analysis of four proteins

The immunohisochemical analysis result of Ki-67, CD99, P53 and S100 are shown in Table 2. Ki-67 was expressed in all level of signals in 30 cases (93.75%). The median score of Ki-67 signal was 0.4. The number of cases with positive high signal Ki-67 (signal ≥0.4) was equal to positive low signal Ki-67 (signal <0.4). CD99, P53, and S100 were positive in 68.75%, 37.5%, and 12.5% of the cases, respectively.

Table 1. Clinical data of osteosarcoma patients and characteristics of tumors

	n (%)
Male	22 (68.75)
Female	10 (31.25)
Mean age	17.7 (range 8 to 48)
Mean survival time	1,648
Living status:	
Alive	16 (50)
Dead	16 (50)
Subtypes of osteosarcoma	
Osteoblastic	17 (51.13)
Non-osteoblastic	15 (46.88)
Conventional	9 (28.13)
Chondroblastic	3 (9.38)
Other subtypes	3 (9.38)
Tumor necrosis	
90%	7 (21.88)
<90%	25 (78.12)
Vascular invasive	
Negative	27 (84.38)
Positive	5 (15.63)
Local invasion	
Negative	29 (90.63)
Positive	3 (9.38)
Surgical margin	
Negative	30 (93.75)
Positive	2 (6.25)
Distant metastasis	
Negative	18 (56.25)
Positive	14 (43.75)
Pathological fracture	
Negative	28 (87.50)
Positive	4 (12.50)
Tumor size (mean = 9.2)	
≤9.2	17 (53.13)
>9.2	15 (46.88)

Correlation between survival time and predicting factors

By using Kaplan-Meier analysis with log rank test the survival time was statistically insignificant correlated with age, sex, tumor necrosis, clinical stage, CD99, S100 and P53 ($p = 0.39, 0.1298, 0.1878, 0.1197, 0.97, 0.8$ and 0.05 respectively), but significant correlated with tumor size, and Ki-67 ($p = 0.0448$ and 0.0116, respectively).

Proportional hazards model analysis for prognosis

Tumor size of less than 9.2 cm and Ki-67 of signal <0.4 implied good prognosis of osteosarcoma ($p = 0.019$, adjust HR = 0.169, 95% CI = 0.038 to 0.742 for

Table 2. Immunohistochemical analysis of osteosarcoma tissues biopsy

Protein expression	n (%)
CD99	
Positive	22 (68.75)
Negative	10 (31.25)
S100	
Positive	4 (12.5)
Negative	28 (87.5)
P53	
Positive	12 (37.5)
Negative	20 (62.5)
Ki-67	
Positive	
≥0.4	15 (46.88)
<0.4	15 (46.88)
Negative	2 (6.25)

Table 3. Proportional hazard analysis of factors predicted prognosis

Variables	n	Adjust HR	95% CI	*p*-value
Age				
≤16	17	1.755	0.368 to 8.364	0.480
>16	15			
Sex				
Male	22	1.220	0.192 to 7.731	0.833
Female	10			
Tumor size				
≤9.2 cm	17	0.169	0.038 to 0.742	0.019*
>9.2 cm	15			
Clinical Stage				
II	22	0.771	0.124 to 4.810	0.781
III	10			
Tumor Necrosis				
<90%	25	1.108	0.181 to 6.786	0.912
≥90%	7			
Ki-67				
<0.4	15	0.156	0.035 to 0.704	0.016*
≥0.4	15			
P53				
Negative	20	5.332	1.214 to 23.406	0.027*
Positive	12			

* Statistical significant, $p<0.05$

tumor size and $p = 0.016$ adjust HR = 0.156, 95% CI = 0.035 to 0.704 for Ki-67 of signal <0.4) negative P53 on the other hand, indicated poor prognosis ($p = 0.027$, adjust HR = 5.332, 95% CI = 1.214 to 23.406).

Discussion

Osteosarcoma is the most common, non-haemopoietic, primary malignant bone tumor. Any bone can be affected by this aggressive cancer. There does not appear to be significant association with ethnic group or race. This disease most frequently occurs in the second decade with some 60% of patients under the age of 25 years, and affects males more frequently than females in a ratio of 3: 2[4]. Osteosarcoma patients in the present study are likewise common in young males than females (2.2: 1) with mean age of 17.7 years.

The clinical features of osteosarcoma are non-specific. Most arise in long bones without recognizable precursor lesion; therefore a long period of time may elapse until the tumor is diagnosed. All osteosarcoma produce osteoid or bone and represent different entities based on clinical, roentgenographic, or histological features. Combining both radiological and histological criteria is most appropriate diagnosis. In general, conventional x-ray radiography is the starting point. CT is the examination of choice in diagnosis of the site of osteoid osteoma in dense bone. Focal extent and staging is based on MRI. The absence of reproducible evidence of specific findings minimizes the use of both electron microscopy and immunohistochemistry in osteosarcoma. Recently, many laboratories are evaluating the usefulness of the determination of combined tumor markers using immunohistochemical techniques as an adjunctive procedure in diagnosis and prognosis of bone tumor.

Gorlick et al[9] concluded that P53 and p-glycoprotien expression did not correlate with histologic response or patient event-free survival. A study by Becker et al[10] revealed low prevalence of human epidermal growth factor receptor 2 (HER2/erbB-2) and vascular endothelial growth factor in osteosarcoma biopsies. Mardanpour et al[11] found a strong evidence that coexistence of HER2 and Ki-67 overexpression and P53 accumulation could predict the development of lymph node involvement and metastases in patients with high-grade osteosarcoma. They found positive Ki-67 in 96.5% of 56 osteosarcoma tissues.

The present study demonstrated that there was positive Ki-67 expression in most of osteosarcoma tissues (93.75%) and its correlation with survival time was statistically significant. Ki-67 implied an effective indicator of cell division and growth[8] and correlates

with aggressiveness of the tumor[12].

We found positive expression of P53 in much lesser quantity than the previous report[11]. The role of P53 as a covariate of survival was controversial. A study by Yao et al[8] demonstrated that positive P53 predicted a decreased short-term survival. Gorlick et al[9] found that positive P53 did not correlate with survival. Chen et al[13] found that P53 mutations had an unfavorable impact on 2-year overall survival. Fu et al[14] inferred that P53 was an effective biomarker of survival in patients with osteosarcoma. Wunder et al[15] found no evidence that TP53 mutations predicted for development of metastases in patients with high-grade osteosarcoma. Patient age was the only factor that varied with P53 gene status. This present study revealed that about 60 percent of cases were negatively-stained P53 which showed poor prognostic value when compared with positively-stained P53.

Cao et al[6] reported that the expression of the S100A4, a promoter of metastasis, was significantly higher than that in corresponding noncancerous bone tissues. S100A4 positively stained cases showed a significantly decreased overall survival time and disease-free survival compared with negatively stained cases. The present study revealed that positive S100 and CD99 did not imply statistically significant correlation with survival time.

Conclusion and suggestions

Analyses of combined tumor markers using immunohistochemistry revealed that expression of high signal Ki-67 (signale”0.4) was considerable factors of poor prognosis in most osteosarcoma tissues. Negative P53 expression was another feasible predictor, but in a lesser number of cases. Both CD99 and S100 could not be applied to prognostic prediction. Limitation of this retrospective study is rather a small sample size. Therefore, the study of protein expressions in larger cohort is suggested to affirm the feasibility of clinical use.

Ethical considerations

The present was approved by the KKU ethical committee, HE 581291.

What is already known on this topic?

Protein expressions of immunohistochemical investigation those found to be common in osteo-sarcoma were S100, CD99, Ki-67, and P53. There were different conclusions about the prognostic values of expression of some proteins.

What this study adds?

This study revealed that immunehitochemical markers in osteosarcoma which are likely to predict prognosis are positive high signal Ki-67 and negative P53. Both S100 and CD99 expression did not imply statistically significant correlation with survival time.

Acknowledgements

The study made up part of the first author’s thesis in the studying of a master of science in pathology at the Faculty of Medicine, Khon Kaen University. Furthermore, the authors thoroughly appreciate the support from all mentors up to study fulfillment. This, in addition to fund support from the research unit at the Faculty of Medicine, Khon Kaen University is also deeply appreciated (grant number: IN59332). The study was also gratefully supported by The Center of Cleft Lip-Cleft Palate and Craniofacial Deformities, Khon Kaen University under Tawanchai Royal Grant Project (Tawanchai Center).

Potential conflicts of interest

The authors declare no conflicts of interest.

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