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Original Article

Expression of NY-ESO1 Protein in Multiple Myeloma

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ABSTRACT

Objective: To evaluate the proportion of New York esophageal squamous cell carcinoma 1 (NY-ESO1) protein expression, via immunohistochemistry staining in bone marrow of multiple myeloma patients. Additionally, to correlate the expression with clinical data and survival outcomes.

Material and Methods: The study population were multiple myeloma patients, diagnosed by bone marrow biopsy in Songklanagarind hospital; from 2012 and 2015. The clinical data and survival outcomes were collected. All cases were stained with NY-ESO1 (E978) antibody and interpreted by one pathologist and a resident.

Results: There were 61 patients that reached the criteria for this study. NY-ESO1 expression was detected in 4 of 61 cases (6.6%). The expression of NY-ESO1 was associated with serum albumin levels (p-value<0.01). However, no association between NY-ESO1 expression nor survival outcome was identified by survival analysis.

Conclusion: NY-ESO1 immunostaining showed low percentages of expression in multiple myeloma neoplastic cells, and was not associated with survival outcome.

Keywords: immunohistochemistry; multiple myeloma; NY-ESO1; survival

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INTRODUCTION

Multiple myeloma (MM) is a malignant disease characterized by proliferation of clonal plasma cells in bone marrow having secreted monoclonal immunoglobulins in serum and/or urine. From 1990 to 2016, the incidence of multiple myeloma has increased by 126%, with deaths increasing by 94%[1,2].

The diagnostic criteria for multiple myeloma is based on the revised international staging system for multiple myeloma[2]. Treatments for multiple myeloma are a combination of alkylating agents with corticosteroids, and bone marrow transplantation[3]; wherein, Radiation Therapy (RT) plays a role for palliative treatment of plasma cell myeloma[4,5]. Recently, newly immunotherapeutic drugs have been invented, after discovery of tumor antigens and their immune response[6]. These drugs significantly improve the survival outcome of patients[7-10].

Cancer testis antigens (CTA) are one such candidate target for immunotherapy, due to being tumor-specific and immunogenicity. These antigens express in many tumors; however, they only express in some normal tissues; such as testis and placenta[11]. The expressional control of CTA is associated with epigenetic alteration, DNA demethylation, histone modification and is miRNA-mediated[12-17].

New York esophageal squamous cell carcinoma (NY-ESO1) is one such CTA antigen[18-20]. The NY-ESO1 gene is on chromosome Xq28[21]; additionally,this protein expression is found in normal testicular tissue as well as many tumor cells[22]. The expression of NY-ESO1 in MM is found in about 9.7-40.0% of cases[23-26]. NY-ESO 1 plays a role in cell proliferation in both stem cells and tumor cells[27,28]. Additionally, NY-ESO1 can elicit in both cellular and humoral immune responses[29]. Serum NY-ESO1 antibodies have been identified in many tumors; including multiple myeloma[30-32]. The cytotoxic T lymphocyte that is induced by NY-ESO1 demonstrates a killer effect on tumor cells that was stronger than that of the control group in vitro studies. Therefore, NY-ESO1 may be a good candidate target for immunotherapy, because of widespread expression, limited off-target toxicities and strong immunogenic to boost immune properties[33,34]. There have been many studies regarding the correlation between NY-ESO1 expression and histopathological factors in many cancers[35,36]. For multiple myeloma, the expression of NY-ESO1 is related to relapsed disease; however, its role as a prognostic marker is still unclear[37].

Hence, the objective of this study was to identify the expression of NY-ESO1 protein, via immunostaining in the bone marrow of MM patients; in addition to correlate the expression with clinical and survival outcomes.

MATERIAL AND METHODS

Population and sample

Patients were retrospectively selected from the database of the Department of Pathology, Songklanagarind Hospital; from January 2012 untill December 2015, with clinicopathology data being collected. The inclusion criteria were: patients that were diagnosed as multiple myeloma by bone marrow biopsy in Sonklanagarind Hospital, and had adequate paraffin-embedded tissue for immunostaining. The exclusion criteria were: patients having had prior chemotherapy, second primary cancer and inadequate tissue for immunostaining. This study was approved by the Ethical committee of the Faculty of Medicine, Prince of Songkla University (Rec. 62-170-5-1)

Immunohistochemistry

The paraffin block was sectioned into a 2 μm unstained slide and baked in a microwave oven for approximately 10 minutes. Then, the slide was deparaffinized with xylene and rehydrated with ethyl alcohol. All sections were stained with monoclonal mouse primary antibodies to NY-ESO1 (clone E978). Staining was performed with the Leica BOND-MAX automated immunostainner. All sections were examined by one general pathologist and one pathological resident. The percentage

of immunoreactive cells and staining intensity were evaluated in the most representative area. The proportion between immunoreactive cells (cytoplasmic staining and/or nucleus) and all plasma cells was scored as 5 groups (score 0: <5%, score 1: 5% to <25%, score 2: 25% to <50%, score 3: 50 to <75%, and score 4: 75–100%). The intensity was scored from 0–3 (0=negative, 1=weak, 2=moderate, and 3=strong staining). The total scores were calculated by summation of the proportion score and intensity score. Cases with a total score ≥3 were interpreted as positive staining (Figure 1)[38].

Statistical analysis

Clinicopathological characteristics of the patients are presented in percent, mean and median, and compared using Ordinary least squares (OLS) regression. The Kaplan–Meier method was used to estimate the overall survival (OS) distributions, and the log-rank test was performed to compare the survival difference in each group. Differences were considered statistically significant when the p-value was less than 0.05. All statistical analyses were calculated by R studio program 3.3.1.

RESULTS

Clinicopathological data

The clinicopathological data of patients having results of NY-ESO1 staining are summarized in Table 1. There were 4 cases (6.6%) that demonstrated NY-ESO1 positive staining. No association between clinical variables and NY-ESO1 expression were identified: except serum albumin levels. Serum albumin levels in the negative staining group were higher than those of the positive staining group (p-value<0.01). Most of the patients were in tumor stage 3.

Survival analysis

Log-rank test revealed no statistical significance between survival time of NY-ESO1 positive and negative patients (p-value=0.69) (Figure 2). Additionally, univariate analysis showed no statistical significance between survival time and NY-ESO1 immunostaining results (p-value=0.99) (Table 2). The clinical factors, laboratory results and type of treatment were not associated with clinical outcome by statistical analysis. Multivariate analysis could not be evaluated due to insufficient positive cases for calculation.

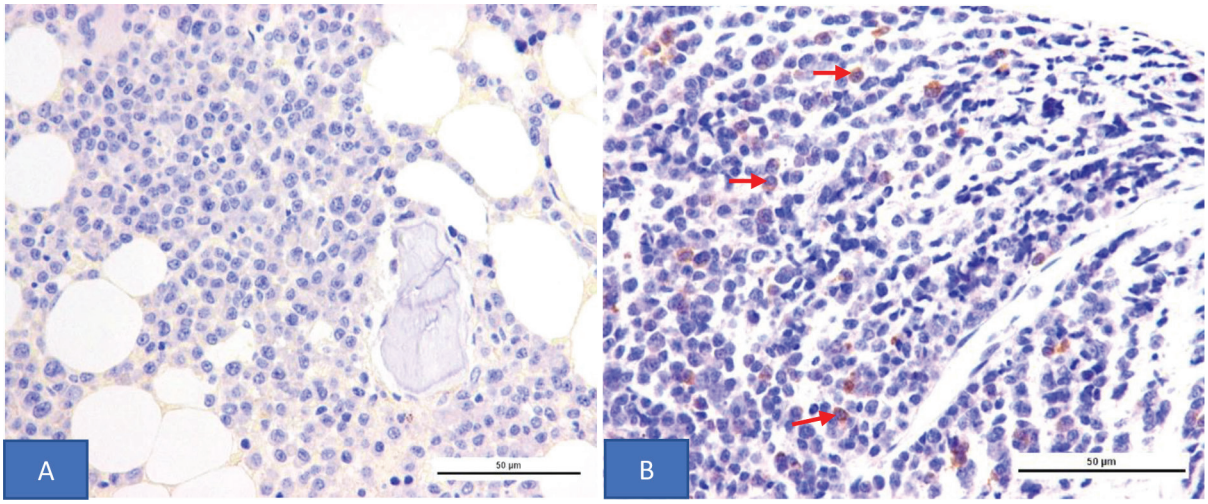

Figure 1 (A) NY-ESO1 immunohistochemistry staining showed negative results in the bone marrow of multiple myeloma patients (B) NY-ESO1 immunohistochemistry staining showed positive staining in cytoplasm of plasma cells (arrow)

Table 1 Demographic data of patients and New York esophageal squamous cell carcinoma 1 (NY-ESO1) immunostaining results

Variable	NY-ESO1 negative (n=57)	NY-ESO1 positive (n=4)	p-value*
Age	39-87 year (Mean 61.80)	43-76 (Mean 59.50)	0.68
Gender			
Male	26 (45.6%)	3 (75.0%)	0.34
Female	31 (54.4%)	1 (25.0%)	
Stage			
I	7 (12.3%)	1 (25.0%)	0.60
II	18 (31.6%)	1 (25.0%)	
III	32 (56.1%)	2 (50.0%)	
Serum Beta2 microglobulin (median)	5.6 mg/L (IQR=4.1, 9.6)	4.2 mg/L (IQR=4.3, 15.7)	0.69
Serum albumin (mean)	3.4 g/dL (S.D.=0.7))	2.4 g/dL (S.D.=0.9)	0.004
Serum creatinine (median)	1.1 mg/dL (IQR=0.8, 1.8)	1.1 gm/dL (IQR=0.9, 1.5)	1
Treatment			
Chemotherapy	26 (45.6%)	2 (50.0%)	1
Immunotherapy	23 (40.5%)	2 (50.0%	
Bone marrow transplant	8 (13.9%)	0 (0.0%)	
Light chain restriction			
Kappa	34 (59.6%)	2 (50.0%)	1
Lambda	23 (40.4%)	2 (50.0%)	
Status after treatment			0.74
Alive	15 (26%)	0 (0.0%)	
Death	7 (12%)	0 (0.0%)	
Unknown	35 (62%)	4 (100.0%)	

*statistical significance at a p-value<0.05

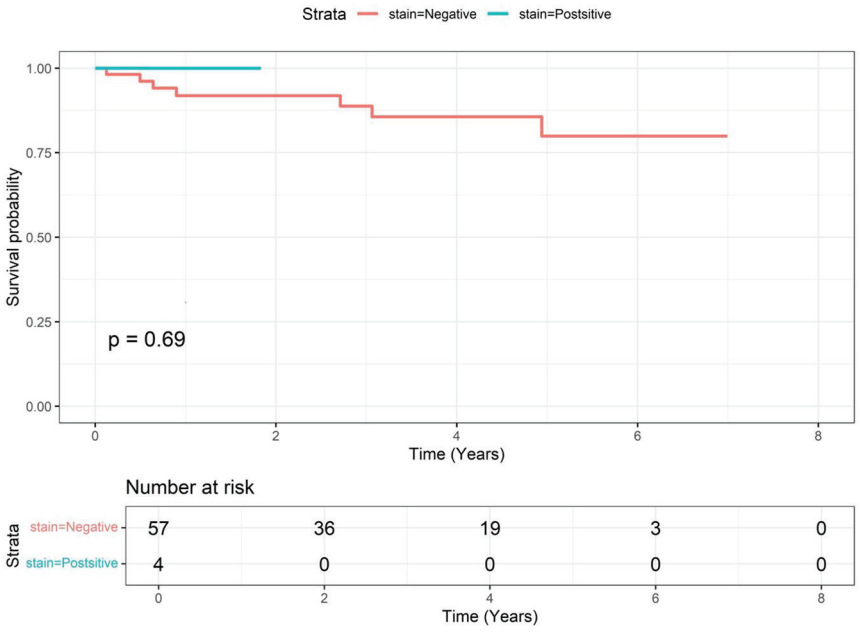

Figure 2 Kaplan-Meier curve showed no statistical difference between survival time of New York esophageal squamous cell carcinoma 1 (NY-ESO1) positive and negative patients

Table 2 Univariate analysis (Cox's proportional hazard model) of demographic data and New York esophageal squamous cell carcinoma 1 (NY-ESO1) immunostaining results

Variable	Hazard ratio (95% CI)	p-value (Wald's test)
Age	1.02 (0.94, 1.1)	0.61
Gender (male vs female)		
Male	Ref=1	
Female	5.63 (0.67, 47.04)	0.11
Stage		
Stage I and II	Ref=1	
Stage III	1.34 (0.3, 6.01)	0.71
Serum Beta2 microglobulin (median)	0.99 (0.91, 1.07)	0.73
Serum albumin (mean)	1.78 (0.55, 5.78)	0.34
Serum creatinine (median)	0.86 (0.51, 1.44)	0.56
Treatment		
Chemotherapy	Ref=1	
Immunotherapy	2.04 (0.37, 11.18)	0.41
Bone marrow transplant	1.07 (0.1, 11.96)	0.95
Light chain restriction		
Kappa	Ref=1	
Lambda	1.30 (0.29, 5.83)	0.73
NY-ESO1 results		
Negative	Ref=1	
Positive	0 (0, Inf)	0.99

95% CI=95% confidence interal

DISCUSSION

NY-ESO1 protein expression in this study (6.6%) was lower than the range of expression in previous studies (9.7-40%[22-26]). NY-ESO1 plays a role in decreasing cell cycle progression. Expression of NY-ESO1 protein was found in multiple myeloma patients, with advanced stage disease having cytogenetic abnormalities[37]. In this study, approximately 50% of positive NY-ESO1 positive cases were in the advanced stage; this correlates with other studies. Decreasing NY-ESO1 expression inhibited multiple myeloma growth and osteolytic lesions. On the other hand, high NY-ESO1 expression was associated with relapsing multiple myeloma. Thus, NY-ESO1 plays a role in cell cycle progression, which could explain the poor outcome of the patients[39]. However, the results of this study showed no correlation between clinical characteristics and expression of NY-ESO1. These results could be from the limitation of the small number of cases; hence, the need for confirmation in further studies having a larger group of this population.

NY-ESO1 expression in this study showed an inverse correlation with serum albumin levels; however, an explanation for this correlation is still unclear. There has been a study that showed correlation between low serum albumin levels and high serum levels of interleukin-6 (IL-6) that promoted proliferation of plasma cells in bone marrow. Low levels of serum albumin have also been associated with poor prognostic outcomes[40]. Expression of NY-ESO1 in neoplastic plasma cells might represent the high aggressiveness of neoplastic cells that are associated with increased levels of IL-6, and may indirectly decrease the level of serum albumin. However, due to the limited number of NY-ESO1 expression cases, the correlation between NY-ESO1 expression and serum albumin could not be completely concluded. Hence, further studies to evaluate the correlation between serum IL-6 and expression of NY-ESO1 is required to confirm this hypothesis.

The limitations of this study were its small population, and no positive NY-ESO1 cases being followed up for more than 3 years. Therefore, multivariate analysis could not be calculated. Furthermore, the number of NY-ESO1 expression cases in this study were limited to 4 cases. As this might affected the survival analysis further studies containing a large population group is suggested to improve power of the study.

CONCLUSION

The expression of the NY-ESO1 protein in MM patients was low, and was not associated with survival time.

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CONFLICT OF INTEREST

No conflicts of interest to be declared.

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