Correlation of Matrix Metalloproteinase-2 and p21 Expressions with Capsular Invasion of Thymoma AB

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Abstract

Thymomas are the common neoplasms of mediastinum, and type AB is the most frequent case. All thymomas are potential for invasion to adjacent tissues and considered malignant. Capsular invasion is one of its prognostic indicators. Matrix metalloproteinase-2 (MMP-2) is a family of proteinases that has the ability to degrade a component of extracellular matrix and affect tumor invasion and or metastasis. Cytoplasmic expression of p21 protein can be significantly correlated with invasion and metastasis. The correlation of these two proteins in thymoma has not been widely studied, hence we aimed to analyze the expression of MMP-2 and p21 and investigate their correlation with capsular invasion of thymoma AB. This cross-sectional study was performed on the 24 paraffin-embedded samples of thymomectomy during January 2013-Desember 2019 at Anatomical Pathology Laboratory of Dr. Soetomo General Hospital Surabaya. The samples were devided based on capsular invasion into 2 groups. Immunohistochemical staining was performed to detect expression of p21 and MMP-2. The correlation was statistically analyzed using *Spearman* test. There was no significant difference of MMP-2 expression between thymoma with capsular invasion and without capsular invasion (p=0.839), and also no significant difference of p21 expression between thymoma with capsular invasion and without capsular invasion (p=0.816). No correlation of MMP-2 and p21 expressions in thymoma AB was revealed (p=0.255). In thymoma AB, the expression of MMP-2 and p21 were not correlate with capsular invasion. These results may contribute to the development of thymoma research.

Keywords: *Thymoma, MMP-2, p21, capsular invasion*

Introduction

Thymoma is the most common neoplasm in the mediastinum, origin from the epithelial cells of the thymus gland, and the cause has not been determined until now[1]. The incidence of thymoma is approximately 1.3-3.2 cases per 1 million world population[2]. Their management strategies have not been standardized yet and because of the rarity case[1]. Thymoma is generally an indolent neoplasm, however, all subtypes of thymoma can appear at an advanced stage and show malignant behavior[3].

World Health Organization (WHO) 2015 divides thymoma into five subtypes which type A is reported to be about 4-7% of all types of thymoma, type AB 28-34%, type B1 9-20%, type B2 20-36%, and type B3 10-14%[4]. Tumor invasion and metastasis are also thought to be more related to prognosis than with tumor histology[5]. All thymoma subtypes have the potential to invade adjacent tissue with the rate of invasion increases according to tumor type in the order of types A, AB, B1, B2, B3. In thymoma A there were no invasion cases, in thymoma AB there were 5.9% cases, B1 18.5%, B2 20.5%, and B3 41.7% cases[6]. Many researches investigate about invasion in thymoma B2 and B3 but

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no research abaout thymoma AB.

The most important part of the metastatic process is the degradation of the basement membrane and extracellular matrix. Basement membrane damage is the main predictor of tumor metastasis. Type IV collagen is the main component of the basement membrane[7]. MMP-2 has a role in cell invasion because of its ability to degrades type IV collagen[8]. Immunohistochemical examination of MMP-2 is also useful for showing a correlation with tumor stage, predicting aggressiveness (invasion) and the potential for malignancy of thymoma[5].

The p21 protein, also known as a cyclin-dependent kinase (CDK) 1 or protein-interacting 1 inhibitor, is an identified potent inhibitor of the cyclin/CDK complex. p21 in the cytoplasm has the function of promoting tumor proliferation and metastasis and in one study, it had a negative effect on thymoma survival rates, and was significantly correlated with WHO subtype, Masaoka stage and with thymoma invasion[1].

The correlation of these two proteins in thymoma, especially in thymoma AB has not been widely studied. This research aimed to analyze the expression of MMP-2 and p21 and investigate their correlation with capsular invasion of thymoma AB and get the prospect of providing prognosis of thymoma in general and thymoma AB specifically.

Materials and Methods

This study had been approved by the Health Research Ethic Committee of Dr. Soetomo General Hospital, Surabaya, Indonesia (2015/KEPK/VI/2020). This was analytic observational research with a cross-sectional approach that was performed on the 24 paraffin-embedded samples of thymomectomy during January 2013-Desember 2019 at Anatomical Pathology Laboratory of Dr. Soetomo General Hospital Surabaya. The samples were grouped based on capsular invasion into two groups. Thymoma AB with capsular invasion were 13 samples and thymoma AB with no capsular invasion were 11 samples.

Immunohistochemistry staining was performed to detect expression of p21 and MMP-2. The tissues were cut into four mm sections, deparaffinized three times with xylol for five minutes each, and rehydrated through graded alcohol. Antigen retrieval was achieved by microwave treatment in sodium citrate buffer (pH 6.0) for ten minutes. The tissue sections were then incubated with monoclonal antibodies for MMP-2 (8B4: sc-13595; dilution 1:200; Santa Cruz Biotechnology) and p21 (0.N.488: sc-71811; dilution 1:200; Santa Cruz Biotechnology) overnight, followed by secondary antibody for 10 minutes at room temperature. Sections were then counterstained with hematoxylin and dehydrated with alcohol.

Cytoplasmic staining for MMP-2 and p21 were evaluated on tumour cells. MMP-2 is considered positive if expressed in the cytoplasm >1% of tumor cells[9]. p21 is considered positive if expressed in the cytoplasm >1% of tumour cells[10]. All samples were evaluated by two pathologists in the blinded fashion. Any discordant was solved by interobserver agreement. The comparison of MMP-2 and p21 expression in thymoma AB with and without capsular invasion was tested using Mann-Whitney U test. The correlation was analyzed using Spearman test, a p-value of less than 0.05 was considered statistically significant.

Results and Discussion

The majority of patients in this reseach were aged 40-49 years (29.2%) and average age of all patients was 52.21 years. Clinicopathological characteristics of the patients are shown in Table 1. MMP-2 was expressed at cytoplasm (Figure 1). Mann-Whitney U test showed no difference in thymoma AB with and without capsular invasion (p=0.839) (Table 2). p21was expressed at cytoplasm (Figure 2). Mann Whitney U test showed no difference in thymoma AB with and without capsular invasion (p=0.816) (Table 3). Spearman correlation test showed no correlation between MMP-2 and p21 in thymoma AB (r_s = 0.242, p = 0.255) (Table 4).

Table 1. Clinicopathological characteristics of the patients.

Characteristics	n (%)
Age (years) #	
30-39	3 (12.5)
40-49	7 (29.2)
50-59	6 (25)
60-69	5 (20.8)
70-79	3 (12.5)
Gender	
Female	17 (70.8)
Female male	7 (29.2)

Note: Mean age 52.21 years, range 34-79 years.

Table 2. Comparasion between MMP-2 expression with and without capsular invasion.

	MMP-2 EXPRESSION				*P*-VALUE[#]
	Average	SD	Minimum (%)	Maximum (%)	
NO CAPSULAR INVASION	38.4	27.2	5.0	65.0	0.839
CAPSULAR INVASION	37.3	30.7	5.0	93.0	

Mann-Whitney U test applied.

p-value <0.05, considered as significant.

Table 3. Comparasion between p21 expression with and without capsular invasion.

	MMP-2 Expression				p-Value #
	Average	SD	Minimum (%)	Maximum (%)	
No Capsular Invasion	30.6	24.34	5,0	75,0	0,816
Capsular Invasion	30.4	29.59	0	90	

Mann-Whitney U test applied.

p-value <0.05, considered as significant.

Table 4. Correlation between MMP-2 and p21 expression in thymoma AB.

	p21 Expression	
MMP-2 Expression	rs	0.242
	p-value	0.25524
	n	24

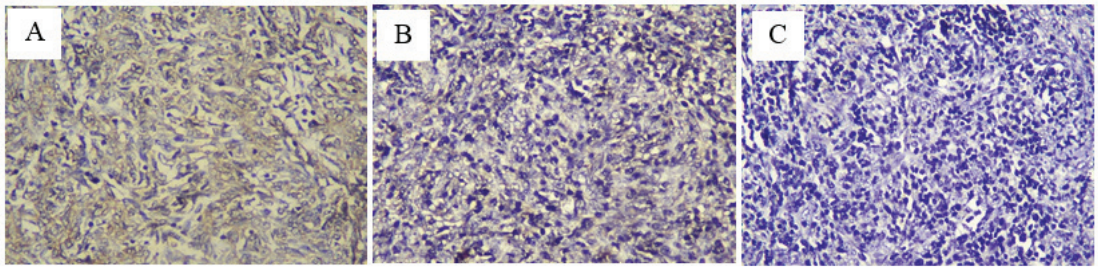

Figure 1. Immunohistochemical expression of MMP-2 in thymoma AB, 400× magnification. A: Expressed in 93% tumour cells; B: Expressed in 65% tumour cells; and C: Expressed in 0% tumour cells.

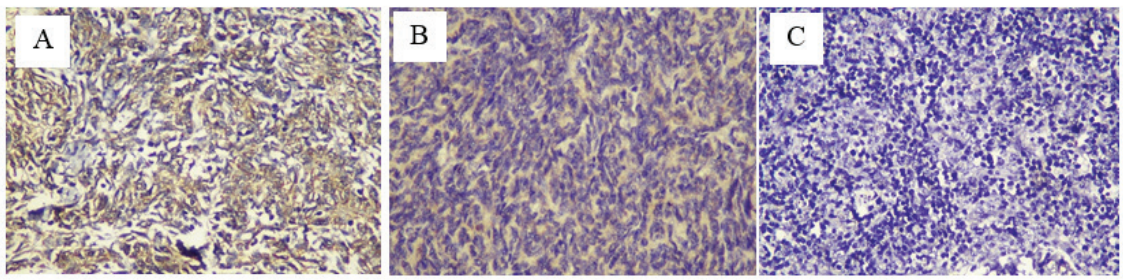

Figure 2. Immunohistochemical expression of p21 in thymoma AB, 400× magnification. A: Expressed in 90% tumour cells; B: Expressed in 60% tumour cells; and C: Expressed in 0% tumour cells.

The metastatic process is undoubtedly an important phase of neoplastic disease and develops when tumor cells acquire the specific ability to leave the primary tumor, invading the surrounding matrix[11]. Matrix metalloproteinases (MMPs) and tissue metalloproteinase inhibitors (TIMPs) represented a critical role in tumor invasion and metastasis. In particular, gelatinase including gelatinase A (MMP2) can degrade the extracellular matrix (ECM) and basement membrane. Very few studies have been conducted on thymoma, and fewer papers have discussed the expression of the MMP molecule and Its significance for predicting biological behavior in thymoma. It is known that the gelatinolytic activity of MMP2, known as gelatin zymography, is associated with thymoma invasion and is thought to have the potential for malignancy[12]. Capsule invasion is a factor that can affect the prognosis of thymoma[13].

The age of the patients observed in this study ranged from the age of 34-75 years (mean 52.21). Most belonged to a grup of the 40-49 years (29.2%) and the majority occur in women (70.8%). This is consistent with a study stated that type A and AB patients were significantly older than patients B1-3 by a median of 60 versus 52[14] and the majority patients are women with an overall mean age of 51.0 ± 14.3 years[15]. The results of this study indicated that there was no significant difference between the expression of MMP-2 and capsular invasion in AB thymoma. According to another research conducted by Takahashi, the expression of MMP-2 showed varying results, namely in type A thymoma, expression 20%, type AB 8.3%, type B1 18.2%, type B2 27.3%, type B3. 77.8%, type C 55.6%. Researchers also conducted research on TIMP-2 which the results were positive in 40%, 58%, 73%, 82%, 78%, and 67% respectively of types A, AB, B1, B2, B3, and C thymoma. The MMP-2 expression showed a weak correlation with TIMP-2 where the Spearman correlation coefficient is 0.362[5].

While other studies mentioned, all thymomas A expressed MMP-2, 67% expressed TIMP-2, and about 66% of thymomas expressed both. About 60% of thymoma B1 and 25% of thymoma AB expressed TIMP-2 and MMP-2, and 50% both expressed strongly. Most of thymomas B2, B3, and C (83-100%) expressed MMP-2 and TIMP-2. The result of the study, in non-invasive (stage I) thymoma the positive staining rates for MMP-2 and TIMP-2 were very low (10% and 0%, respectively), and in invasive (stage II-IV) tumors, the positive staining rates for MMP-2 and TIMP-2 were very high (91% and 97%, respectively). There was a significant difference in the expression levels of MMP-2 and TIMP-2 between

non-invasive and invasive thymoma (Fisher exact test; P = 0.0001)[16].

MMP activity is a balance between MMPs/TIMPs and is a determining factor for maintaining the stability and integrity of the extracellular matrix. The roles of MMPs in tumor metastasis were not relied on the exact concentration of MMPs in the local area, but on the ratio of MMPs/TIMPs[17]. TIMP is a specific regulator of MMP. Decreased TIMP production can also result in more effective enzyme activity and is potentially invasive. Overexpression TIMP in tumor cells can inhibited tumor invasion and metastasis[18]. MMP-2 and TIMP-2 imbalance is very important for tumor cells to have a strong invasive potential. Thus, exact concentration of MMPs in the local area has no significance and cannot reflect tumor potential. MMPs/TIMPs can act as a prognostic factor indicating invasiveness and metastasis[17]. The unexpressed MMP-2 in this study could be due to the inhibitory activity of TIMP-2, but the previous MMP-2/TIMP-2 ratio data were not known.

Regarding p21 expression, there was also no significant difference between the expression of p21 and capsular invasion in AB thymoma. This result is in accordance with study by Kuhn and Wistuba that stated the expression of p21 was minimally increased (16%) in the 31 examined thymomas[19] and according to Omatsu, there was no cytoplasmic p21 expression in normal thymic epithelial, thymoma, and thymic carcinoma[20]. Also, p21 expression was detected in small amounts in thymoma and it has been reported that p21 expression is increased in thymic carcinoma than in thymoma B3. The combination of high p53, low p21 and p27 low expression has the potential to predict the biologically aggressive behavior of thymoma[21] and it is found more frequently in invasive than non-invasive thymomas[22].

The results of this study indicate that there is no relationship between MMP-2 and p21 expression in thymoma AB. A research suggests the expression of TIMP2 at p21 reduction, while TIMP2 is described as the component responsible for removing MMP. Taken together, reduction of p21 leads to attenuated cell motility and invasiveness of the different cell lines, possibly by ERK3 scattering which in turn results in decreased levels of the MMP2 and TIMP2 genes. In summary, the data suggest that p21 reduction has a negative impact on cell motility and invasion that is mediated, at least in part, by the ERK3 / MMP2 / TIMP2 pathway[23].

Conclusion

In thymoma AB, the expression of MMP-2 and p21 were not correlate with capsular invasion. These findings can contribute to an improvement of thymoma research.

Conflict of Interest: The authors declare that they have no conflict of interest.

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