RESEARCH COMMUNICATION

Diagnostic and Clinical Significance of KIT(CD117) Expression in Thymic Epithelial Tumors in China

Nan Song[1], Gang Chen[2], Peng Zhang[1], Ming Liu[1], Wen-Xin He[1], Ge-Ning Jiang[1]*

Abstract

Aims: To study KIT (CD117) expression in thymic epithelial tumors in China, and investigate diagnostic and clinical significance. Material and Methods: Thymic epithelial tumors (TETs) from 102 patients (3 type A, 29 type AB, 5 type B1, 22 type B2, 29 typeB3 and 16 thymic carcinomas) were examined. Immunohistochemical staining with an antic-kit monoclonal antibody was performed on a tissue microarray. Relationships between KIT positive expression and the TET clinical characteristics (WHO histologic classification and Masaoka stage system) were analysed. Results: The KIT positive expression rate was significantly higher in thymic carcinoma (60%, 9/16) than in thymoma (8%, 7/86), a strong correlation being found with the WHO classification, but not the Masaoka tumor stage. The overall survival for patients with KIT positive lesions was significantly worse. Conclusions: KIT is a good molecule marker to differentially diagnose thymic carcinoma from thymoma, while also serving as a predictor of prognosis for TETs. Further research into KIT mutations in Chinese TETs should be conducted to assess the efficacy of targeted therapy.

Keywords: Thymoma - thymic carcinoma - thymic epithelial tumors - KIT

Asian Pacific J Cancer Prev, **13**, 2745-2748

Introduction

Thymic epithelial tumors (TETs) are rare anterior mediastinal tumors which represent about 0.2% to 1.5% of all malignancies (Jong et al., 2008). TETs are mainly divide into thymoma and thymic carcinoma, both are derived from thymic epithelium. TETs encompass a wide variety of histologic patterns that are associated with diverse outcomes. Now days, although the 2004 World Health Organization (WHO) histologic classification is widely used to classify TETs by pathologist (Muller-Hermelink et al., 2004), the combination of number and letter is still obscure for clinical doctors and sometimes different pathologist will make totally different pathological diagnosis for the same patient according to the TETs WHO classification. So some molecule markers should be found to distinguish particular TETs type to each other and predict the prognosis.

KIT (CD117) is a transmembrane tyrosine kinase receptor protein encoded by the proto-oncogene c-kit that maps to chromosome 4 (4q11–12) (Vandenbark et al., 1992; Heinrich et al., 2002). Several study had reported that KIT is a useful immunohistochemical marker for the diagnosis of thymic carcinoma and an independent prognostic factors for TETs (Nakagawa et al., 2005; Aisner et al., 2010; Petrini et al., 2010), also can be considered as a potential target for therapy in selected cases (Giaccone et al., 2009; Ströbel et al., 2010). In our study, we evaluated the KIT (CD117) expression in a series of 104 TETs in China, in order to investigate it's expression states in Chinese TETs and corroborated the work of others before.

Materials and Methods

Patient samples

Thymic epithelial tumors from 104 patients treated at Shanghai Pulmonary Hospital, Shanghai, China during the period 1998-2007 and 10 normal thymus tissue from comprised the study material. In 104 TETs, there are 56 males and 51 females which age range from 21 to 81.

We reviewed hematoxylin-eosin-stained sections of each specimen to determine its histologic subtype on the basis of the WHO histologic classification (Muller-Hermelink et al., 2004), and found TETs type A in 3 cases, type AB in 29 cases, type B1 in 5 cases, type B2 in 22 cases, type B3 in 29 cases, and thymic carcinoma in 16 cases. Tumor staging was according to the revised Masaoka system (Masaoka et al., 1994), and found Masaoka stage I in 15 cases, stage II in 44 cases, Stage III in 33 cases and stage IV in 12 cases.

Construction of Tissue Microarray

TETs specimens were assessed for quality and adequacy of fixation and storage. A tissue microarray block containing tissue from 104 TETs cases was generated. In brief, two punches of 0.79 mm^2 (1 mm in diameter) were taken from different intratumoral areas in each tumor sample and arranged in the recipient tissue

[1]Department of Thoracic Surgery, [2]Department of Pathology, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai, China *For correspondence: jgnwp@ymail.com

Table 1. Expression of KIT TETs of Different WHO Histologic Classification

WHO		Cases No.	KIT Immunoreactivity				Positive cases
			-	+	++	+++	
Thymoma	Type A	3	3(100%)	0	0	0	0
	Type AB	29	28(96.5%)	1(3.5%)	0	0	1(3.5%)
	Type B1	5	4(80%)	1(20%)	0	0	1(20%)
	Type B2	22	21(95.4%)	1(4.5%)	0	0	1(4.5%)
	Type B3	28	24(85.7%)	4(14.3%)	0	0	4(14.3%)
	Totle thymoma	86	80(92.0%)	7(8.0%)	0	0	7(8.0%)
Thymic Carcinoma		16	6(40.0%)	5(33.3%)	1(6.7%)	3(20%)	9(60%)
Totle		102	86(84.3%)				16(15.7%)

WHO, WHO histologic classification

Table 2. Expression of KIT TETs of Different Masaoka Stage

Masaoka stage	KIT Immunoreactivity				Totle
	-	+	++	+++	
I	14(93.3%)	1(6.7%)	0	0	15
II	38(88.3%)	5(11.7%)	0	0	43
III	24(72.7%)	5(15.2%)	1(3.0%)	3(9.1%)	33
IV	10(90.9%)	1(9.1%)	0	0	11

χ^2=9.895, P=0.359

array block. A pathologist verified the presence of tumor tissue on a hematoxylin-eosin-stained tissue microarray slide. Samples were considered adequate if tumor occupied one or both of two punches. The contrast group of 10 normal thymus tissue were embedded by paraffin and made ordinary pathological section.

Immunohistochemistry

Expression of KIT was analyzed by IHC. TETs tissue microarrays were cut at 4 μm for serial section, deparaffinized with xylene, and rehydrated in graded ethanol. The tissue microarrays sections were autoclaved for 10 min in 10 mmol/L citrate buffer (pH, 6.0) for antigen retrieval before incubation with a primary antibody. The monoclonal antibody, anti-KIT (Long Island; Shanghai, China) was used as primary antibody. Immunoreaction was detected by a labeled streptavidin-biotin method and was visualized with 3,3'-diaminobenzidine, followed by counterstaining with hematoxylin. Degree of immunostaining was scored as follows by one pathologist who was blinded to the patients' information: -, negative staining; 1+, staining < 10% of tumor cells; 2+, staining≥10% but >=50% of tumor cells; or 3+, staining ≥ 50% of tumor cells (Tsuchida et al., 2008). Positive were considered samples with at least 1+. KIT staining was judged to be positive when unequivocal membranous staining was observed along the cell membrane.

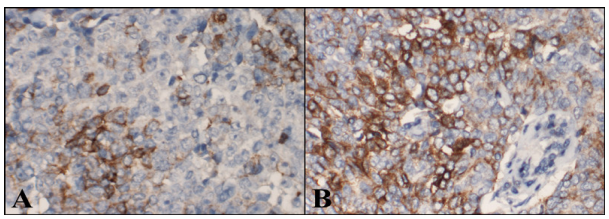

Figure 1. (A) 1+ Staining KIT Positive Expression in Type B2 Thymoma, ×400 Original Magnification. (B) 3+ Staining KIT Positive Expression in Thymic Carcinoma, ×400 Original Magnification

Statistical Analysis

The statistical analysis was performed with the SPSS version 17.0 (SPSS Inc., Chicago, IL). Clinical and biologic features were compared using the t-test and Chi-square tests, when appropriate. Survival curves were generated using the Kaplan-Meier method, and differences between curves were analyzed using the Log-Rank test. P<0.05 was considered as significant.

Results

Of the 104 TETs specimens in tissue microarray block, one was verified having no tumor cells, one was verified consisting a large number of necrotic cells, and all the other 102 TETs specimens can be used for statistical analysis.

We studied the expression of KIT in 102 TETs tissue and 10 normal thymus tissue. There was no KIT expression in 10 normal thymus tissue. Of the 102 TETs, 16 (15.7%) cases were positive for KIT expression and 86 (84.3%) were negative. The KIT expression and staining degree in different WHO histologic classification types of 102 TETs were reported in Table 1. We found that there was significant statistical differences of KIT expression among different types of WHO histologic classification (Chi-square tests, χ^2=37.041, P=0.001), and KIT expression was significantly more frequent in thymic carcinoma than in thymoma (Chi-square tests, χ^2=26.111, P<0.001). All the seven KIT positive cases of thymoma were 1+ staining, while there were one 2+ (6.7%) and three 3+ (20%) staining in the 9 KIT positive thymic carcinoma cases. One case of 1+ staining KIT positive type B2 thymoma is shown in Figure 1A, and one case of 3+ staining KIT positive thymic carcinoma is shown in Figure 1B.

The KIT expression and staining degree in different Masaoka stage TETs were reported in Table 2. We found that there won't significant statistical differences of KIT expression among different Masaoka stages (Chi-square tests, χ^2=9.895, P=0.359).

The Log-Rank test showed that overall survival for patients with KIT positive expression (median 41.6 months) was worse than that of patients without KIT expression (median 79.2 months), and the difference was statistically significant (log-rank test, χ^2=18.474, P=0.000). The survival curve is shown in Figure 2.

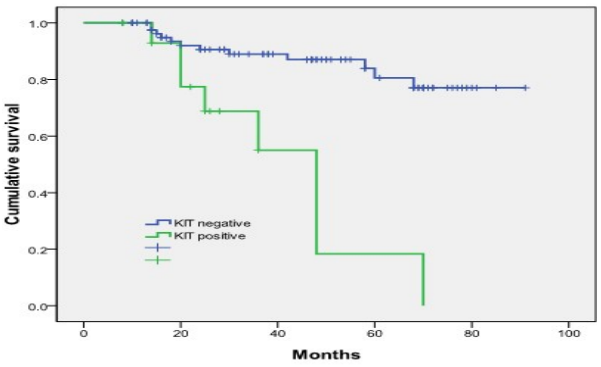

Figure 2. The Survival Curves of TETs for KIT Expression

Discussion

KIT is a type III cytokine receptor expressed on the membrane of hematopoietic stem cells, mast cells, melanocytes, and interstitial cells of Cajal (Edling et al., 2007), It was reported that overexpression of KIT was observed in many of human malignancies, such as gastrointestinal stromal tumors (GISTs), chronic myeloid leukemias, mast cell neoplasms, melanomas, and seminomas (Pan et al., 2004). And the biological characteristics of malignancies with KIT positive expression seem more invasive and have poor prognosis (Arbiser et al., 2002). KIT has been seemed as the target spot of kinase inhibitor imatinib for GISTs targeted therapy (Corless et al., 2004). Several study had reported that KIT is a useful immunohistochemical marker for the diagnosis of thymic carcinoma, and an independent prognostic factors for TETs (Nakagawa et al., 2005; Aisner et al., 2010; Petrini et al., 2010), the result of our study evaluated the KIT expression in TETs in China, and corroborated the work of others before.

KIT positive expression in thymoma was reported range between 0% and 5%, and in thymic carcinoma was reported range between 50% and 88% (Nakagawa et al., 2005; Aisner et al., 2010; Petrini et al., 2010). In our study of 102 TETs in China, the KIT positive expression rate in thymoma is 8% (7/86) and in thymic carcinoma is 60% (9/16)(reported in Table 1). And KIT expression was significantly more frequent in thymic carcinoma than in thymoma ($P<0.001$). ALL the KIT positive cases of thymoma were 1+ staining, while there were much more 2+ and 3+ staining of KIT overexpression in thymic carcinoma cases. So our result confirmed the KIT overexpression more often in thymic carcinoma than tymoma in China, it's similar to the result reported before (Nakagawa et al, 2005; Aisner et al, 2010; Petrini et al, 2010). But the KIT positive expression rate in thymoma in our study is much higher (8%) than reported before (0%-5%). Of the seven KIT positive tymoma cases, there were four type B3 cases (4/7), at the mean time, from type A to thymic carcinoma, the KIT positive rate increased from 3.5% to 60% (Table 1), so it may imply that the malignancy degree of the KIT positive TETs is higher. The difference of KIT expression among different WHO classification types also be significant statistical, we can use KIT as molecule marker to distinguish particular TETs type to each other.

Although it's reported that the GISTs with KIT positive expression are more invasive and easy to metastasize, no correlation was found between KIT expression and TETs Masaoka tumor stage in our study. It's similar to the result reported by Petrini et al. (2010). But the Log-Rank test mentioned overall survival for patients with KIT positive was worse, and the difference between the survival curves was statistically significant. It's need to do the further research to investigate whether KIT can be used as molecule marker to predict the prognosis of TETs.

So far targeted therapy has yielded modest results in the treatment of thymic malignancies in patients who have failed chemotherapy. Several case reports had described the use of imatinib for targeted therapy of selected advanced thymic tumors (Ströbel et al., 2004; Giaccone et al., 2009; Ströbel et al., 2010). Giaccone reported that in their study, two patients had stable disease and five progressed after using imatinib, no KIT mutations were detected in their 2 B3 thymomas and 5 thymic carcinomas, and KIT expression was found in only one of four samples by immunohistochemistry (Giaccone et al., 2009). Because lack of large sample, prospective studies evaluating imatinib for treatment of TETs have yielded disappointing results until now. Our result indicated the overexpression of KIT in thymic carcinoma and positive in type B3 thymoma. Further research of c-kit mutation status in large sample of advanced thymic tumors should be done to predict the efficacy of targeted therapy.

In conclusion, KIT is a good molecule marker to identify thymic carcinoma from thymoma and distinguish particular TETs type to each other. TETs with KIT positive expression may have worse prognosis. Further research of KIT mutation in Chinese TETs should be done to predict the efficacy of targeted therapy.

Acknowledgements

The author(s) declare that they have no competing interests.

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