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Expression of Twist 1 in bone marrow and extramedullary lesions of patients with myeloma and its prognostic significance

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In patients with multiple myeloma (MM) extramedullary (EM) lesions could be a possible indicator of poor prognosis. Twist 1, an EMT related transcription factor, is known to play an important role in embryonic development and also in the process of tumor cell proliferation, apoptosis, metastasis etc. The Twist 1 protein expression hence has prognostic significance in MM. This study investigates expression of Twist-1 in MM patients with extramedullary lesions and its relationship with clinicopathological data and prognosis, so as to elucidate the clinical significance of Twist-1 expression. Patients with MM complicated with EM lesions were selected retrospectively, including 35 cases in the bone marrow group (MM complicated with extramedullary lesions) and 80 cases in the extramedullary group (MM combined with extramedullary lesions). The expression of Twist 1 protein was detected by immunohistochemistry. The relationship between Twist 1 protein expression and general clinicopathological data was analyzed by statistics. The relationship between Twist 1 protein expression and prognosis was analyzed by COX single factor and multivariate regression analysis. Immunohistochemical staining showed that the expression level of Twist-1 protein in the nucleus of myeloma cells in the extramedullary group was significantly higher than that of the bone marrow group. However, in the cytoplasm of myeloma cells, there was no significant difference in the expression level of Twist-1 protein between the extramedullary group and the bone marrow group. The expression level of Twist-1 protein in extramedullary tissue was not related to sex, age, D-S stage, ISS stage, immunoglobulin type, creatinine and hemoglobin. The expression level of β2-microglobulin in Twist-1 high expression group was significantly higher than that of Twist-1 low expression group. Follow-up findings discovered that overall survival (OS) of Twist 1 high expression group was significantly lower than that of Twist 1 low expression group. COX multivariate analysis showed that Twist 1 protein and International Staging System (ISS) stage were independent risk factors affecting OS for 3 years. The high expression rate of Twist 1 protein in extramedullary lesions of MM patients with extramedullary lesions is significantly higher than that in bone marrow tissues of patients with extramedullary lesions. The results suggest that high expression of Twist 1 protein is an independent risk factor affecting the prognosis of MM patients with extramedullary lesions.

Keywords: Multiple Myeloma, Plasma cells

Myeloma is a malignant proliferative disease originating from plasma cells. With the main clinical performances of clonal proliferation of plasma cells and massive secretion of monoclonal immunoglobulin, it is a common malignant disease in the hematology department. Multiple myeloma (MM) is the most common type of myeloma, ranking the second among malignant diseases of the blood system with an incidence rate of 13%[2]. Extramedullary lesions are the most common way for myeloma to metastasize when the lesions protrude from damaged bone sites. Extramedullary lesions cannot affect a radical cure with the characteristics of strong atypia and tumor cells and the low differentiated degree[3], which pose a heavy impact on patients and families. Hence, it becomes necessary to explore MM with extramedullary lesions for effective treatment. Studies have confirmed that Epithelial mesenchymal transformation (EMT), as the basis of embryonic development, is involved in a variety of biological processes, such as loss of cell-cell adhesion, tumor infiltration, tumor migration, etc.[4].

Twist 1 is an EMT related transcription factor that not only plays an important role in embryonic development, but also participates in the process of tumor cell proliferation, apoptosis, epithelial interstitial transformation, metastasis and neovascularization[5]. High expression of Twist 1 protein was found in some malignant tumors, such as lung cancer, liver cancer and prostate cancer, and its expression is reported to be closely related to poor prognosis[6]. Until now, the expression of Twist 1 protein in MM with

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extramedullary diseases and its prognostic significance have been rarely reported. In this context, here, we examined the expression of Twist 1 in bone marrow and extramedullary lesions of patients with MM and extramedullary lesions and analyzed their relationship with prognosis in order to provide more basis for the treatment of clinical myeloma.

Materials and Methods

General clinical data

From May 2011 to May 2018, 80 patients with MM complicated with extramedullary lesions were selected retrospectively, including 45 male cases and 35 female cases at an average age of 11-16 years (4~18). There were 16 cases with IgA, 47 cases with IgG, 7 cases with IgD, 4 cases with no secretion and 6 cases with light chain. All patients received bortezomib or thalidomide chemotherapy. The patients were all newly diagnosed without regular radiotherapy or any previous chemotherapy. Patients with other malignant diseases, osteoporosis, and serious infectious diseases were excluded. A total of 20 healthy bone marrow donors with matching sex and age were selected as control group. The subjects had complete clinical data. This study was approved by the hospital ethics committee and informed consent was signed by the subjects and their families. The specimens were fixed and embedded with 4% paraformaldehyde. The follow-up period starts from surgical removal of diseased tissue to December 1, with 20 deaths during the period. This study was approved by the Institutional Ethical Committee for Medical Research with necessary informed consents from the participating subjects.

Main instruments and reagents

Paraffin slicing machine (purchased from Germany Leica Company), optical microscope (purchased from Japan Olympus Corporation), Citrate antigen repair solution, DAB Chromogenic kit, SP Immunohistochemical kit, hematoxylin-eosin stain (purchased from Fuzhou MAXIM Biotechnologies Co., Ltd.).

Methods

The bone marrow tissues and extramedullary tissues treated with 4% neutral formaldehyde solution fixation, paraffin embedding, and 4 μm thickness sections, and were placed on pre-treated slides with 10% poly-lysine solution and dried before baking. The tissues were placed at 4 for standby use after reducing to the room temperature. The immunohistochemical staining was carried out according to the instructions of DAB chromogenic kit and SP immunohistochemical kit. PBS was used as the negative control instead of the primary antibody. The staining results were determined by two experienced pathologists. A comprehensive assessment of the percentage of positive cells and the extent of staining was carried out, and the colour depth score criteria were obtained: 0 (no colour), 1 (light yellow), 2 (brownish yellow), 3 (dark brown); Criteria for percentage of positive cells: 0 (no expression), 1 (1~25%), 2 (26~50%), 3 (51~75%), 4 (>75%) The scores were divided into high expression group (4~12) and low expression group (0~3).

Statistical methods

SPSS21.0 statistical software package was used for data analysis. The measurement data consistent with normal distribution were all expressed in the mean ± standard deviation. Twist 1 high expression rate and other enumeration data were expressed in [n(%)]. χ^2 test or t test was taken for inter-group differences. Non-normal distribution data are expressed as median values. U test was carried out for the difference between two. A Pearson correlation analysis method was used to analyze protein expression correlation. The survival curve was drawn by Kaplan-Meier method and Logrank test was used to compare the single-factor survival rate. COX proportional hazards regression model was used for the multivariate analysis, such as age, sex, etc. A value of P <0.05 was considered statistically significant between the groups.

Results

Expression of Twist 1 in tissues of extramedullary group and bone marrow group

Immunohistochemical staining showed that the expression level of Twist-1 protein in the nucleus of myeloma cells in the extramedullary group was significantly higher than that in the bone marrow group and the difference was statistically significant (P <0.05); in the cytoplasm of myeloma cells, there was no significant difference in the expression level of Twist-1 protein between the extramedullary group and the bone marrow group (P >0.05) (Fig. 1 and Table 1).

Relationship between Twist 1 protein expression level and clinicopathological types in extramedullary group tissues

The expression level of Twist-1 protein in extramedullary tissue was not related to sex, age, D-S stage, ISS stage, immunoglobulin type, creatinine and hemoglobin. The expression level of β2-

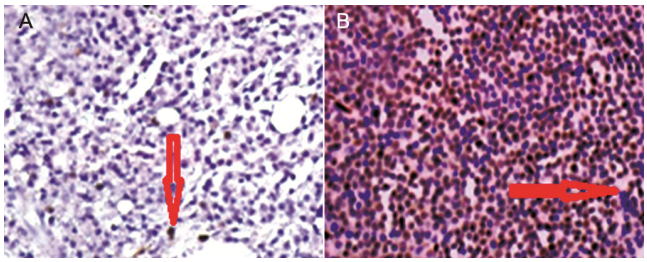

Fig. 1 — Immunohistochemical staining of Twist 1 in (A) Bone marrow group; and (B) Extramedullary group

Table 1 — Expression of Twist 1 in tissues of extramedullary group and bone marrow group

Group	No.	Twist 1	
		High nuclear expression	High intracellular expression
Bone marrow group	35	3 (8.6)	14 (40.0)
Extramedullary group	80	20 (25.0)	43 (53.8)
χ^2		4.107	1.841
P		0.043	0.175

Table 2 — Relationship between Twist 1 protein expression level and clinicopathological types in extramedullary group tissues

Parameter	Twist 1 low expression N=60	Twist 1 high expression N=20	P
Age	12 (4~18)	15 (7~16)	0.420
Male	34	11	0.799
Duie-Salmon (DS) staging			0.201
I	15	2	
II	21	7	
III	24	11	
International Staging System (ISS) staging			0.061
I	12	2	
II	25	5	
III	23	13	
Immunoglobulin class			0.129
IgA	12	3	
IgG	33	8	
IgD	3	4	
No secretion type	4	0	
Light chain type	8	5	
Creatinine (μmol/L)			0.430
>176.8	4	3	
≤176.8	56	17	
Hemoglobin (g/L)	120.72 (76.19-152.73)	117.34 (75.34-160.27)	0.221
β2-microglobulin (μg/mL)	4.32 (1.12-12.21)	7.0 (2.38-9.1)	0.011

microglobulin in Twist-1 high expression group was significantly higher than that in Twist-1 low expression group and the difference was statistically significant (P <0.05) (Table 2).

Effect of high expression of twist 1 protein on prognosis

Follow-up findings discovered that the three-year overall survival rate (OS) (55.00%) of patients in high protein expression group Twist 1 was obviously lower than that in Twist 1 low expression group (85.00%) and the difference was statistically significant (P <0.05) (Fig. 2).

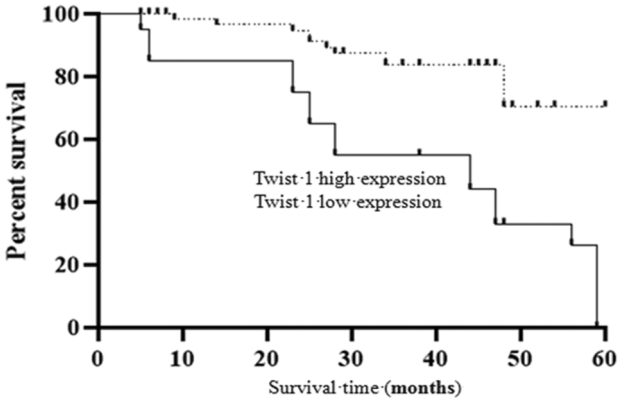

Fig. 2 — Curve diagram of Twist 1 high protein expression to 3-year survival rate

Relationship between high expression of Twist 1 protein and prognosis analyzed with COX single factor and multiple factors

Univariate analysis found that DS staging, ISS staging, β2- microglobulin, Twist 1 protein were all related to the patient's 3-year OS. Multivariate analysis showed that Twist 1 protein and ISS staging were independent risk factors affecting 3-year OS (P <0.05) (Table 3).

Discussion

Multiple myeloma (MM) is one of the most common malignant tumors in the blood system, characterized by clonal proliferation of plasma cells and the secretion of many monoclonal immunoglobulin. Patients are prone to extramedullary infiltration, extensive late metastasis, and poor prognosis. With no radical curve method, MM becomes a major disease threatening the health and safety of patients[7]. Radiotherapy and chemotherapy are commonly used in clinical treatment, but the individual sensitivity of drugs varies greatly, and the 5-year survival rate has not been greatly improved[8]. Therefore, it is of great significance for MM patients to study the molecular mechanism of MM etiology and to seek a new direction of new targeted drugs for MM therapy.

This study found that the high expression rate of Twist 1 protein in the nucleus of tumor cells in MM patients with extramedullary infiltration was significantly higher than that in their bone marrow tissue. Multivariate risk regression analysis showed that high expression of Twist 1 protein in extramedullary tissue was an independent risk factor for short PFS and

Table 3 — Relationship between high expression of Twist 1 protein and prognosis analyzed with COX single factor and multiple factors

Parameter	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* value	HR (95%CI)	*P* value
Age	1.649 (0.600~4.492)	0.299		
Sex	1.540 (0.847~2.670)	0.219		
DS staging	1.980 (1.299~3.000)	0.002	1.63 (0.841~3.410)	0.140
ISS staging	2.730 (1.676~4.450)	<0.001	2.62 (0.99~6.68)	0.043
Immunoglobulin typing	0.871 (0.690~1.085)	0.810		
β2-microglobulin	3.431 (1.830~6.413)	<0.001	1.901 (0.876~3.501)	0.182
Hemoglobin	0.598 (0.327~1.079)	0.586		
Creatinine	1.671 (0.230~12.299)	1.678		
Twist 1 protein	3.350 (1.741~6.069)	<0.001	3.63 (1.701~8.093)	0.013

OS in patients. The results of the present study provide a useful reference for evaluating the prognosis of patients with MM and extramedullary infiltration.

Twist 1 is a conservative transcription factor in evolution, belonging to the basic helix-loop-helix protein family[9]. Existing studies suggest that Twist 1 not only plays an important role in embryonic development, but also participates in the proliferation, apoptosis, epithelial interstitial transformation, metastasis, neovascularization and other processes of tumor cells. High expression of Twist 1 protein was found in some malignant tumors, such as lung cancer, liver cancer and prostate cancer, and its expression was closely related to poor prognosis[10]. During the progression of cancer cells, through strengthening nucleosome remodeling and recruitment of deacetylase complex, Twist 1 can directly inhibit E-cadherin expression, cause epithelial mesenchymal transformation, and then promote tumor metastasis[11]. In patients with some mesenchymal tumors such as osteosarcoma, both progression-free survival and overall survival rates are low and closely related to metastasis in patients with high Twist 1 expression. There is a high expression of Twist 1 in melanoma cells. Compared with the primary site, the expression level Twist 1 of the metastatic site is higher[12]. Some scholars have established Twist 1 protein stable expressions with virus vector and found that the up-regulation of Twist 1 protein can induce stem cell-like changes in cells, and tumor blood vessels and tumor globular changes can be found in cell mass. In the process of participating in EMT as embryonic development genes, anomalous expression of Twist 1 protein in breast cancer and prostate cancer was confirmed[13]. With regard to diseases of the blood system, high expression of Twist 1 protein was found in leukemic cells such as acute myeloid leukemia and its proliferation ability was enhanced and apoptosis was inhibited[14]. With regard to MM, under hypoxic conditions, EMT protein may be activated, which can reduce the expression of E-cadherin protein and promote the infiltration and metastasis of myeloma cells[15]. Twist 1 is an important transcription factor to maintain the basic characteristics of the mesophyll of tumor and synovial sarcoma. The current study has shown that 3-year overall survival (OS) of Twist 1 high expression group was significantly lower than that of the low expression group (P <0.05). This suggests that Twist 1 is not only an important participant in the development of epithelial tumors, but also plays an important role in the progression of mesenchymal tumors such as MM with extramedullary degenerative diseases.

Conclusion

Twist 1 protein is mainly expressed in the tumor nucleus of Multiple myeloma (MM) patients. It is highly expressed in bone marrow lesion tissue and extramedullary lesion tissue, and the high expression rate of Twist 1 protein in extramedullary lesion tissue is significantly higher than that in bone marrow tissue. High expression of Twist 1 protein is an independent risk factor for prognosis of MM patients with extramedullary lesions. A subgroup analysis is not possible to further describe the process in terms of EMT and tumor angiogenesis due to limited sample size and lack of molecular biology and genetics data. Much information and samples are still needed to clarify the specific mechanism of Twist 1 and MM.

Conflict of Interest

All authors declare no competing interests.

References

1 Lifan X, Jianping L & Hujun L, Lentivirus-mediated shRNA interference with ADAM10 gene on the proliferation of multiple myeloma MM.1S cells. *Exp Hematol*, 4 (2017) 1074.

2 Zhiyao Z, Lili C & Guoqin F, Preliminary study on the effect and mechanism of GRK6 on the proliferation of MM1R cells in multiple myeloma. *Exp Hematol*, 5 (2016) 1421.

3 Rajkumar SV & Kumar S, Multiple myeloma current treatment algorithms. *Blood Cancer J*, 9 (2020) 1.
4 Li G, Song YP, Lv Y, Li ZZ & Zheng YH, Clinical Characteristics and Prognostic Analysis of Multiple Myeloma with Extramedullary Disease: A SEER-Based Study. *J Oncol*, 30 (2021) 6681521.
5 Bethany S & Neil B, Role of EMT in Metastasis and Therapy Resistance. *J Clin Med*, 5 (2016) 17.
6 Shibue T & Weinberg RA, EMT, CSCs, and drug resistance: the mechanistic link and clinical implications. *Nat Rev Clin Oncol*, 14 (2017) 611.
7 Ye X, Tam WL, Shibue T, Kaygusuz, Y, Reinhardt F, Eaton, EN & Weinberg RA, Distinct EMT programs control normal mammary stem cells and tumour-initiating cells. *Nature*, 7568 (2015) 256.
8 Bill R & Christofori G, The relevance of EMT in breast cancer metastasis: Correlation or causality? *FEBS Lett*, 589 (2015) 1577.
9 Dong CY, Liu XY, Wang N, Wang LN, Yang BX, Ren Q, Liang HY & Ma XT, Twist-1, A Novel Regulator of Hematopoietic Stem Cell Self-Renewal and Myeloid Lineage Development. *Stem Cells*, 32 (2014) 3173.
10 Abdelrahman AE, Arafa SA & Ahmed RA, Prognostic Value of Twist-1, E-cadherin and EZH2 in Prostate Cancer: An Immunohistochemical Study. *Turk Patoloji Derg*, 1 (2017) 198.
11 Wang N, Guo D, Zhao YY, Dong C, Liu X, Yang B, Wang S, Wang L, Liu Q, Ren Q & Lin Y, TWIST-1 promotes cell growth, drug resistance and progenitor clonogenic capacities in myeloid leukemia and is a novel poor prognostic factor in acute myeloid leukemia. *Oncotarget*, 6 (2015) 20977.
12 Krossa S, Schmitt AD, Hattermann K, Fritsch J, Scheidig A J, Mehdorn HM & Held-Feindt J, Down regulation of Akirin-2 increases chemosensitivity in human glioblastomas more efficiently than Twist-1. *Oncotarget*, 6 (2015) 21029.
13 Heidari N, Vosoughi T, Asl JM, Malehi SA & Saki N, How is the relationship between TWIST-1 and BCR-ABL1 gene expressions in chronic myeloid leukaemia patients? *Biomarkers*, 23 (2018) 1.
14 Camp E, Anderson PJ , Zannettino ACW & Gronthos S, Tyrosine kinase receptor c-ros-oncogene 1 mediates TWIST-1 regulation of human mesenchymal stem cell lineage commitment. *Bone*, 94 (2016) 98.
15 Jacobsen M, Sandusky G & Shah K, Abstract 4940: Use of whole slide digital image analysis for determination of expression of Twist-1 in normal panceata and stage IV pancreatic ductal adenocarcinomas. *Cancer Res*, 76 (2016) 4940.