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title: FOXP1 Expression in Monoclonal Gammopathy of Undetermined Significance and Multiple Myeloma

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running title: FOXP1 in neoplastic plasma cells

ABSTRACT

Multiple myeloma (MM) is clonal disorder of terminally differentiated B cells. In some cases premalignant patient's state is monoclonal gammopathy of undetermined significance (MGUS). Neoplastic plasma cells in both entities carry multiple and complex chromosomal abnormalities that make understanding of the disease development difficult. New insight in malignant mechanisms that underlay multiple myeloma may come from FOXP1 analysis in neoplastic plasma cells. FOXP1 is known to be important for B-cell maturation and differentiation and could have significant role in plasma cell tumors.

This is an analysis of FOXP1 protein presence and *FOXP1* gene abnormalities in 13 cases of MGUS and 60 case of MM.

Our results have shown that FOXP1 protein is expressed in neoplastic plasma cells, unlike in their normal counterparts, and that additional *FOXP1* gene copies could be found in both MGUS and MM.

Based on FOXP1 presence in MM and its role in diffuse large B-cell lymphoma and MALT type lymphoma, we speculate that FOXP1 might have important role for in plasma cell neoplasm.

key words: FOXP1, IGH translocations, MGUS, multiple myeloma, plasma cells

INTRODUCTION

Multiple myeloma (MM) is clonal disorder of antibody-secreting terminally differentiated B cells, plasma cells that home to and expand multifocally in the bone marrow. [1] In some patients the associated precursor condition for MM is monoclonal gammopathy of undetermined significance (MGUS). [2]

Considering MM tumor cells have complex genetic abnormalities which make understanding of a disease difficult, new insights on oncogenetic changes are needed. One of possible players in disease development is FOXP1. FOXP1 protein is known to be upregulated after the B-cell activation; it is expressed in mature B-lymphocytes, but not in normal plasma cells, and is connected with B-cell proliferation and differentiation. [3-6] It is explored in DLBCL and MALT lymphomas where it has a connection to prognosis. [7-10] In this case-series study we show *FOXP1* gene abnormalities as well as FOXP1 protein presence in neoplastic plasma cells.

PATIENTS AND METHODS

Patients

60 formalin fixed, paraffin embedded bone marrow trephines and/or bone marrow aspirate smears taken from patients with MM and 13 formalin fixed, paraffin embedded bone marrow trephines and/or bone marrow aspirate smears taken from patients with MGUS were analyzed. Immunohistochemical data of characteristic protein expression analysis as well as data about most common gene aberrations obtained by FISH method in MM were available for all patients, except data about del(13), which were available only for 26 MM and four MGUS patients (Table 1). MM group consisted of 30 females and 30 males, median age 68 years. 39 MM cases had non-diffuse and 21 had diffuse type of bone marrow infiltration (Figure 1a and b). 44 MM cases had low grade

plasma cells and 16 had high grade plasma cells (Figure 1c and d). MGUS group consisted of six females and seven males, median age 67 years. All samples were taken at the time of the diagnosis. Histological samples were routinely processed with HE, Giemsa and assessed by a qualified pathologists (AŠ and MD). Final diagnoses were made by hematologists (DRK and RA) in University Hospital 'Merkur' and University Hospital 'Dubrava' according to World Health Organization criteria. [1] All MM patients had at least one major and one minor or three minor criteria including marrow plasamcytosis between 10 and 30% and M-component present less than 3.5 g/dl IgG and 2g/dl IgA in serum or 1g/24h of BJ protein in urine. MGUS patients had less M-component than MM patients, presented less than 10% of bone marrow plasmacytosis, showed no bone lesions and had no myeloma related symptoms.

This study is part of a project approved by the Medical School Ethics Committee, University of Zagreb, Croatia.

Methods

Immunostaining

Immunostaining was performed on 4 μm thick; formalin fixed paraffin embedded bone marrow trephine sections using a LSAB/HRP kit (Dako, Denmark), according to the manufacturer's instructions. The JC12 anti-FOXP1 antibody was acquired from Dr. Alison Banham, Oxford, UK.

Fluorescent *in situ* hybridization (FISH)

FISH analysis for defining aberrations involving the *FOXP1* locus was performed on 4 μm thick, formalin fixed paraffin embedded bone marrow trephine sections according to

the protocol described by Ventura et al. [11] (http://jmd.amjpathol.org). *FOXP1* aberrations were detected with the previously described break apart probe. [12]

Statistics

χ^2 test was calculated in STATISTICA7 (StatSoft Pacific Pty Ltd., Melbourne, Australia). The level of statistical significance was set at 0.05.

RESULTS

Immunostaining

JC12 staining was present in one MGUS (Figure 2a) and 12 MM (Figure 2b) cases (Table 2). Cases were regarded as positive if more than 30% of neoplastic plasma cells were immunohistochemicaly positive for the JC12 antibody.

Fluorescent *in situ* hybridization

Aberrations involving the *FOXP1* locus were observed in five MGUS (all cases with one additional fused signal) and 24 MM (20 cases with one additional fused signal, one case with four fused signals, three cases with ≥5 fused signals) cases (Table 2).

Statistics

MGUS

FOXP1 protein expression and/or *FOXP1* gene changes showed no statistical significance when compared with available data listed in Table 1.

MM

FOXP1 gene aberrations showed statistical significance when compared with bone marrow infiltration. *FOXP1* locus showed additional copies in cases with diffuse bone marrow infiltration (*P*=0.000) (Table 3). Significant results were also obtained between *FOXP1* gene changes and plasma cell morphology and BCL1 expression. High grade

plasma cells (*P*=0.020) (Table 3) and BCL1 expression (*P*=0.012) go with additional copies of *FOXP1* locus.

DISCUSSION

Results obtained on 13 MGUS and 60 MM samples showed FOXP1 protein expression in both plasma cell neoplasm. *FOXP1* gene aberrations are similar in MGUS and MM – in both groups there are no translocations; only additional copies of the gene could be found. Some difference between MGUS and MM could be suspected because of amplification (5 and more *FOXP1* copies) in MM and only one additional *FOXP1* copy in MGUS. As differentiation between amplification and aneuploidy was not done, we can not assume if there is only gene amplification and not chromosome 3 gain. Whatever the cause is, it seems that more *FOXP1* gene copies represent some trigger for its protein expression, although significant χ^2 value between additional *FOXP1* gene copies and FOXP1 protein expression was not shown.

In MGUS, statistically, no significant χ^2 result was shown.

In MM, statistics linked *FOXP1* gene aberrations to diffuse type of bone marrow infiltration, higher plasma cell grade and expression of BCL1 protein. Additional *FOXP1* gene copies or chromosome 3 gain, thus come with advanced stage of disease or maybe just more complex intracellular events in a tumor plasma cell at the beginning of the disease.

Our data gained on relatively small groups of patients indicate FOXP1 presence and involvement in plasma tumor cells development. As normal plasma cells do not express FOXP1 protein, it could be assumed that FOXP1 is important for B-cell differentiation and that final B-cell, plasma cell, does not require FOXP1 for its function. Therefore, our findings about FOXP1 presence in MM and MGUS may point out direction of plasma cell

changes in MM: tumor cells might regress or they might originate from B-cell stage that has a stop in development just before final plasma cell is produced. Further studies for analyzing possible value of these findings in treatment of MM patients are required as well as in other malignancies as suggested by Koon et al in 2007. [13]

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Figure 1. Bone marrow sections of multiple myeloma patients with a) non diffuse infiltration pattern, b) diffuse infiltration pattern and bone marrow aspirate smears with c) low grade and d) high grade morphology of tumor plasma cells.

Figure 2. JC12 immunohistochemical positivity based on peroxidase reaction in a) monoclonal gammopathy of undetermined significance and b) multiple myeloma cases.

Table 1. Immunohistochemical findings and genetic aberrations data (detected by FISH method) of multiple myeloma and monoclonal gammopathy of undetermined significance patients.

	multiple myeloma			monoclonal gammopathy of undetermined significance		
Σ	60			13		
	+	-	NI†	+	-	NI
BCL1 expression	23	37	0	5	8	0
PAX5 (BSAP) expression	3	57	0	0	13	0
CD20 expression	11	49	0	5	8	0
del(13)	4	22	34	0	4	9
IGH/*BCL1*	9	51	0	2	11	0
IGH/*FGFR3*	19	40	1	1	12	0
IGH/*MAF*	4	55	1	0	13	0
IGH/*c-MYC*	13	44	3	1	11	1
PAX5 translocation	12	43	5	2	11	0

†NI (not informative) – cases which were not analysed because of a lack of material or cases that were uninterpretable due to low sample quality

Table 2. Summarized JC12 immunostaining and *FOXP1* gene aberrations data for 13 monoclonal gammopathy of undetermined significance and 60 multiple myeloma patients.

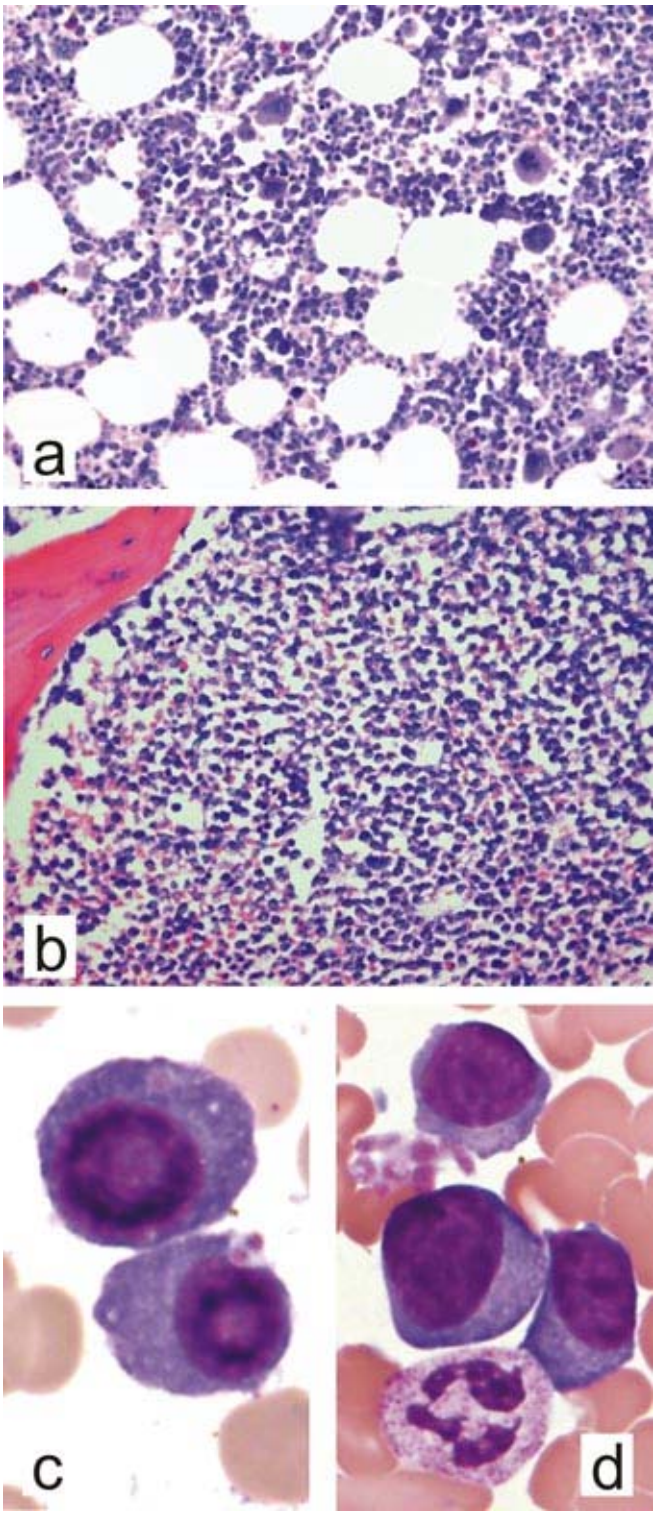
		multiple myeloma	monoclonal gammopathy of undetermined significance
Σ		60	13
FOXP1 protein expression[†]	+	12	1
	-	48	12
FOXP1 gene changes		20 with one additional copy; 1 with two additional copies; 3 with ≥5 copies; 36 without changes	5 with one additional copy; 8 without changes

[†] >30% of neoplastic plasma cells positive by immunohistochemistry with JC12 antibody

Table 3. Chi-square test between bone marrow infiltration pattern and myeloma cell grading of 60 multiple myeloma patients in relation to FOXP1 protein expression and *FOXP1* gene changes.

			FOXP1 protein expression X^2	*FOXP1* gene changes X^2
bone marrow infiltration grading of tumor cell morphology	diffuse	21	0.242 (*P*=0.622)	12.777 (*P*=0.000)
	non-diffuse	39		
	low	44	0.392 (*P*=0.822)	7.864 (*P*=0.020)
	high	16		

Figure 1. Bone marrow sections of multiple myeloma patients with a) non diffuse infiltration pattern, b) diffuse infiltration pattern and bone marrow aspirate smears with c) low grade and d) high grade morphology of tumor plasma cells.



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