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BCL6, MUM1 AND CD10 EXPRESSION IN MANTLE CELL LYMPHOMA

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Abstract

Mantle cell lymphoma (MCL) characteristically express CD20, CD5 and cyclin-D1, carries the translocation t(11;14) (q13;q32) and typically has no expression of germinal center (GC) cell markers. So-called aberrant phenotypes such as CD5 negative and cyclin-D1-negative-MCL have been described. Also few cases with CD10 and/or BCL-6 protein expression have been reported. We analyzed 127 MCL looking for the frequency of aberrant immunophenotype, CD10, BCL-6 and MUM1 expression. All cases were CD20 and cyclin-D1 positive, 96% expressed CD5 and 98% showed the t(11;14). BCL-6 expression was observed in 12 % of the cases and MUM1 in 35%. No one case showed CD10 positivity in 30% or more neoplastic cells. Only 3 cases showed 10 to 20% of tumoral cells positive for CD10. MUM1 expression was observed in 67% of the BCL-6 positive cases. 32% of the cases showed a MUM1+/BCL-6-/CD10- phenotype and 56% had a triple-negative-pattern. Aberrant phenotype is infrequent but not rare, and does not rule out a diagnosis of MCL in an otherwise typical case.

Keywords

malignant lymphoma; mantle cell lymphoma; BCL-6; CD10; MUM1; immunohistochemistry; t(11;14); FISH

INTRODUCTION

Mantle cell lymphoma (MCL) is a mature B-cell non-Hodgkin lymphoma (NHL), consistently expressing the cell cycle regulatory protein cyclin-D1 as a result of the presence of a characteristic translocation t(11;14) (q13;q32) (1-3). Most MCL are composed of small centrocyte-like B-cells that have a CD20, CD5 and cyclin D1-positive immunophenotype and lack expression of germinal center (GC) cell markers. Most cases lack significant somatic hypermutation of the immunoglobulin genes, so MCL cells are thought to derive from naive, pregerminal center and mantle zone B-lymphocytes (4,5). On the other hand, it has been demonstrated that 15 to 30% of cases carry somatic mutations of the immunoglobulin heavy chain variable genes (*IGHV*), implying that the neoplastic cells in these cases have been

exposed to the GC microenvironment, thus the neoplastic cells may be derived from GC or post-GC B-cells rather than naïve B cells (6,7,8).

Despite the usual uniform morphology and immunophenotype it is well recognized that cases of MCL may display clinical and probably biological heterogeneity. So-called aberrant phenotypes have been described, such as CD5 negative-MCL, in up to 10% of cases (9,10). Rare cases of cyclin D1 negative with absence of the typical t(11;14) but having a gene expression signature typical of MCL have been reported (11,12). There are also reports of few cases with germinal center CD10 and/or BCL6 protein expression (8,13,14,15,16). In the same way, morphological variants are recognized such as blastoid and pleomorphic, often associated with additional genetic changes (17), and with marginal-zone-like features (2).

CD10 is a zinc metallopeptidase expressed in early lymphoid progenitors and normal GC cells. The aberrant expression of CD10 by immunohistochemistry and flow cytometry has been reported in a few cases of well-studied MCL; almost one-third of them were pleomorphic or blastoid variants (7,14,15,16,18). However, both the mechanism of CD10 expression and its putative role in the pathogenesis on this subset of MCL remain unclear.

The *BCL-6* gene is located in chromosome 3 (3q27), and encodes a POZ/Zinc finger transcriptional repressor protein that is largely restricted to GC-B-cells. It is required for GC formation and the T-cell mediated immune response. In lymphoid malignancies, in addition to the constitutive *BCL-6* expression of GC B-cells, the gene can be deregulated by different mechanisms, including *BCL6* rearrangement, somatic mutations in the 5'noncoding region and accumulated mutations in the regulatory region (19,20). A few cases of MCL with BCL-6 protein expression have been reported. Some of these cases contained genetic alterations of the *BCL-6* gene and, in the few cases with *IGVH* mutational study performed, a minority showed the presence of *IGVH* somatic hypermutations (7,21).

MUM1/IRF4 has received attention in the last years through its inclusion in immunohistochemical profiles for the identification of the diffuse large B-cell lymphoma (DLBCL) subtype with a non-germinal center-like phenotype (22). Its expression has been demonstrated in plasma cell proliferations, chronic lymphocyte leukemia (CLL), DLBCL and Burkitt lymphoma (BL) (23). MUM1 is expressed in late GC or post-GC B-cells and plasma cells but in contrast to what might be expected, MUM1 expression is not always associated with mutated *IGHV*, being observed in both mutated and non-mutated *IGVH* B-cell neoplasms (24). On the other hand, gene expression data has demonstrated overexpression of MUM1/IRF4 in naïve B-cells exposed to foreign antigens, supporting the hypothesis that MUM1 expression represents a molecular mechanism for integrating information received from multiple stimuli. Downstream, MUM1 regulates transcription in several different pathways including those that lead to immunoglobulin (IG) somatic hypermutation, class switch recombination, plasma cell differentiation, cell proliferation, apoptosis and chemotaxis (24, 25). MUM1 has been proposed to play an important role in mediating B-cell activation and differentiation (25). There are few survey studies evaluating MUM1 in B-cell lymphomas, so the incidence of MUM1 expression in MCL has not been well studied. Martinez et al found 3 in 28 cases (11%) of MUM1 expression in MCL (26). On the other hand, Tumwine et al. reported an absence of MUM1 expression in 4 cases of mantle cell lymphoma studied (21). In this study, we describe the clinico-pathological and immunophenotypic characteristics of 127 cases of MCL, in order to determine the frequency of aberrant immunophenotype and the expression of GC and post GC markers, including CD10, BCL-6 and MUM1.

MATERIAL AND METHODS

A total of 127 cases of MCL were obtained retrospectively from the files of *Consultoria em Patologia*, a large reference consultation service in anatomic pathology located in Brazil. The study group included consecutively studied cases of MCL with available representative material formalin-fixed and paraffin-embedded received in consultation between January, 2005 and December, 2007. Both nodal and extra-nodal MCL cases were included. Clinical data including gender, age at diagnosis and anatomic tumor location were obtained from the referring pathologists/oncologists and/or pathology reports. Available hematoxylin and eosin stained slides of each case were reviewed by two of the authors (GG, CEB) and representative areas were selected for TMA. A morphologic sub-classification of the cases was performed, considering variants included in WHO (2008) classification as follows: classic, blastoid, pleomorphic and marginal zone-like (2).

Tissue microarray construction

Two tissue microarray (TMA) blocks were constructed, using a tissue arrayer (Beecher Instruments, Sun Prairie, WI, USA). Each individual case was represented by three tumor cores of 0.6 mm that were taken from the original paraffin blocks. Serial sections of 3µm were cut from the tissue array blocks and used for immunohistochemical and fluorescence in situ hybridization (FISH) analysis. Proper positive and negative controls cores for each marker were also included in the array block to provide adequacy of the antibodies used in the immunohistochemical study.

Immunohistochemistry

An immunohistochemistry study was performed for each TMA using Novolink polymer® (Novocastra, Newcastle Upon Tyne, UK) as the detection system and an epitope-retrieval method was applied as needed for each specific antibody; diaminobenzidine (DAB) was the chromogen. Table 1 shows the primary antibodies used. MUM1, CD10 and bcl-6 were considered to be positive when, 30% or more of the neoplastic cell nuclei stained for each marker. The intensity of the staining was recorded for MUM1 as weak, moderate and strong. The Ki-67 proliferative index (PI), using the monoclonal antibody MIB1, was assigned a percentage value that was calculated by scoring 500 tumor cell nuclei for the presence of nuclear expression.

Fluorescence in situ hybridization analysis (FISH)

Interphase FISH analysis was performed on formalin –fixed, paraffin-embedded tissue included in TMA. For detection of the t(11;14)(q13;q32), a commercially available LSI IGH/CCND1 double-color-double-fusion probe was used (Abbott, USA). Whenever possible, at least 100 cells were analyzed. The threshold for positivity was established from a group of immunophenotypically characterized samples (tonsils) that did not contain the translocation of interest. A positive case was defined when the mean number of positive tiles detected was 3 standard deviations above the mean of this negative control group; this threshold established was 21.15%.

RESULTS

Of the 127 MCL cases, 92 (71%) were male and 35 (28%) female, with a 2.6:1 M:F ratio. The median age was 66 years and mean age was 58.7 years, with an age range of 36 to 90 years old. An extranodal presentation was present in 18% of the cases (25 cases), and lymph node primary involvement was observed in 82% (102 cases). The most common extranodal anatomic presentation was the digestive tract: 19 cases (Table 2).

Morphologic features

In the original material reviewed to select the areas for TMA construction, 123 cases showed a diffuse architecture and four cases exhibited a nodular pattern (more than 75% of nodular architecture). Most of the cases showed a classic morphologic pattern, (113 cases, 89%). Morphologic variants were distributed as follows: 11 cases of the blastoid type (9%), two cases of the pleomorphic variant (1.5%) and only one case with a marginal-zone-like pattern (0.75%).

Immunohistochemistry

Intense and diffuse expression of CD20 and cyclin D1 was present in all 127 cases of MCL. CD5 was observed in 122 cases (96%). All five CD5-negative cases were cyclin-D1 positive and 3 of them also expressed CD43. Considering all cases, CD43 was co-expressed in 84 cases (66%). All cases were negative for CD23.

GC and post-GC markers were observed in a variable number of cases: BCL-6 in 15 cases (12%) and MUM1 in 45 cases (35%). Although CD10 was negative in all cases for a 30% cut off, there were three cases with 10 to 20 % of the cells expressing moderate to weak CD10 (2%), these cases expressed CD5 and CD43. One of them showed blastoid morphology. CD138 was negative in all cases. In the group of BCL-6 positive cases (15 cases), 14 of them expressed CD5, 10 cases had CD43 co-expression, and 9 cases also showed MUM1 expression. The blastoid and pleomorphic cases were negative for BCL-6, except in two cases.

MUM1 expression was characterized by moderate or strong nuclear positivity in lymphoma cells in the majority of all MUM1-positive cases; all these cases were cyclin-D1 and CD5 positive and in the MUM1 positive group (45 cases) 2 expressed CD10 in 20% of the cells, 9 were BCL-6-positive and 34 were CD43-positive. MUM1-positive cases were extranodal in nine cases, and nodal in 36 cases; corresponding to 36% and 35% of all extranodal and nodal cases, respectively. Expression of MUM1 was observed in 9 (60%) of the BCL-6 positive cases. It is worth noting that 34 cases (28%) showed a MUM1+/BCL-6-/CD10- phenotype (post-GC phenotype) and only 78 cases (56%) were “triple-negative”, consistent with a “true mantle zone cell phenotype”. The remaining 15 cases had a GC or late GC-phenotype, including 6 cases that were BCL-6+, with absence of MUM1 (GC pattern) and 9 cases (24%) with presence of MUM1 (a late GC pattern). Six of the nine BCL-6+, MUM1+ cases expressed CD43. The case that was negative for CD5 and bcl-6+ showed CD43 expression. The Ki-67 proliferation index varied from 15 to 90%, with a mean of 38%, Blastoid and pleomorphic cases had a mean index of 70%, while MCL excluding the blastoid and pleomorphic cases had a mean index of 35%.

FISH analysis for the t(11;14)(q13;q32)

The FISH analysis was performed in all cases and 118 cases displayed the *IGH/CCND1* fusion. In three cases the result was negative, and six other cases had inconclusive results, due to detachment of the tissue cores during the FISH analysis (no more tissue was available in these cases). The BCL-6 positive cases showed positive FISH results in 14 cases, while one had an inconclusive result. All three negative cases for t(11,14) had typical morphology and immunophenotype for MCL, including expression of cyclin-D1 protein (Table 2). All those cases with CD10 partially expression presented the translocation.

DISCUSSION

MCL represents about 3 to 10% of all NHL (2), frequently presenting as a disseminated disease, with an aggressive course and a short response to treatment. On the other hand, cases of MCL with a more indolent course have been described, suggesting that the biology of these lymphomas may be more heterogeneous than initially thought (2,3). The mean age of diagnosis

reported is near 60 years of age, with a male predominance. In our patients, the demographic data were coincident with the literature (1,2). The growth pattern is usually diffuse, but may be vaguely nodular or mantle zone-type, and only rarely presents a truly follicular pattern. We found only four cases with more than 75% of well-defined nodular pattern. The neoplastic cells in MCL are mature B-cells with an immunophenotype similar to normal CD5-positive B-lymphocytes, a subpopulation of naïve cells, which is considered to be the normal counterpart of MCL cells (3). It has been postulated that the t(11;14) translocation occurs initially in an immature B-cell in the bone marrow, and that the selective oncogenic advantage of this chromosomal aberration fully develops when these cells attain the differentiation stage of mature naïve pre-germinal center B-cells (5,7). The origin in pre-germinal center cells is also supported by the fact that most MCL have no or very few somatic mutations in V-gene sequences of the immunoglobulin heavy chain genes. However, it has been demonstrated that 15-40% of cases of MCL may carry somatic hypermutation, indicating that some tumors may originate in cells that have undergone the influence of the mutational machinery of the follicular GC (5,7). The typical immunophenotype include positivity for CD20, cyclin-D1, CD5 and usually CD43 (2). We found 100% of expression for both CD20 and cyclin-D1, 96% for CD5 and 65% for CD43.

The GC-associated antigens CD10 and BCL-6 are usually not expressed in MCL. However, occasional cases of MCL expressing CD10 or BCL-6 have been recently described. The largest series of CD10-positive MCL included 13 cases (16). CD10+/CD5+ cases represents 0.4% of cases in a large series of B-cell lymphomas; 25% of them were MCL (14). At least one-third of the reported cases of CD10+ MCL have been blastoid or pleomorphic variants (7,13,15,16,18,26).

Fifteen of our cases showed expression of BCL-6, including none with CD10 focal co-expression, and all with cyclin-D1 overexpression and demonstration of the t(11;14), with the exception of one case with an inconclusive FISH result. This finding was previously reported by Camacho et al (8), in which the authors reported five cases of BCL-6 positive MCL. In these cases, they found either a *BCL-6* gene translocation or an extra copy of the gene, and in one case there was a high mutational index in the *IGVH* gene.

Zanetto et al (16), in their 13 cases of CD10-positive MCL, found no evidence of *IGVH* mutation in 4 of 5 tested cases and only in one case found a low level of *IGVH* mutation. Five of these cases also showed BCL-6 expression; some of these cases carried *BCL-6* gene translocations or amplification, including the case with *IGVH* mutation. The BCL-6 expression found in these cases could be the result of chromosomal alterations involving the *BCL-6* gene, rather than programmed expression of BCL-6 resulting from a GC origin of the lymphoma. MUM1/IRF4 is a transcription factor that may contribute to tumorigenesis when overexpressed. It has been demonstrated that MUM1 oncogenic activity *in vitro* is induced by antigen receptor-mediated stimuli and plays a crucial role in cell proliferation, differentiation and survival (27,28,29). MUM1 is considered a histogenetic marker of the transition from BCL-6 positivity (GC B-cells) to subsequent steps of B-cell maturation toward plasma cells (30,31) so that MUM1 is expressed not only in post-GC cells but also in final stages of the intra-GC phase (28). By PCR analysis of single MUM1+ cells from GC, Falini et al (32), demonstrated that they contained rearranged *IGH* genes with varying number of *VH* somatic mutations, suggesting that MUM1-positive cells may represent surviving centrocytes and their progeny may be committed to exit the GC to differentiate into plasma cells. The post-germinal phenotype, MUM1+/BCL-6-/CD10-, differs from that of most GC cells, which is CD10+ or MUM1-/BCL-6+, and from the MUM1-/BCL-6-/CD10- mantle cell phenotype. Unlike normal GC B-cells, in which the expression of MUM1 and BCL-6 are mutually exclusive, tumor cells, in approximately 50% of MUM1-positive DLBCL (32) and 40% of BL (23), express both, suggesting that the expression of these proteins may be deregulated in B-cell neoplasms.

MUM1 expression is observed consistently and strongly in multiple myeloma and lymphoplasmacytic lymphoma, many cases of DLBCL (75%) and BL (40%), some cases of CLL and marginal zone lymphoma, most cases of classical Hodgkin lymphoma, as well as most lymphoproliferations occurring in the settings of post-transplantation and acquired immunodeficiency syndrome (23,24,32). Falini et al (32) demonstrated nuclear positivity in 2 (33%) of 6 MCL analyzed, in more than 30% of the cells but mostly weak. On the other hand Martinez and coworkers (26) observed three of 28 MCL cases MUM1 positive and none of 4 cases in the study of Tumwine et al (21). Tsuboi et al (33) using different antibody reported that none of five cases expressed MUM1. Our results showed a higher frequency of MUM1 expression (35%) similar to that reported by Falini using the same antibody (32). Although the normal mantle cells are believed to transition into the follicle B-cell program upon stimulation, this transition not involve the induction of MUM1 (27,28). MCL not reflect the immunophenotype of normal mantle cells and if it possible that MUM1 expression may represent an earlier pregerminal/germinal center transition state, as it has seen during early B-cell development (26).

It has been demonstrated in DLBCL that the overexpression of MUM1 protein is not correlated with the presence of genetic alterations (34), and the mechanism to explain this up-regulation has yet to be elucidated. In BL, the MUM1 overexpression has been related to *C-MYC* translocation (27,28).

Our study reaffirms the concept of heterogeneity in MCL that has been previously observed in morphologic and molecular grounds (3,4). The recently proposed variant of cyclin D1-negative-MCL, which has a characteristic MCL gene expression signature but is negative for t(11;14)q12,q32 and cyclin D1 protein expression (35), also advocates in favor of MCL heterogeneity. In future studies we will be analyzing the *IGVH* mutational state in all of these particular subsets of MCL showing an aberrant phenotype. Knowledge of the spectrum of morphology and phenotype of MCL is critical in order to better understand the biology of MCL, as well as to establish a correct diagnosis, particularly when restricted immunohistochemical panels are applied.

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Figure 1. Mantle cell lymphoma (case 112); 1A: hematoxylin and eosin, $\times 200$; 1B: BCL-6 expression, $\times 200$.

Figure 2.

Mantle cell lymphoma (case 121); 2A: hematoxylin and eosin, $\times 200$; 2B: expression of MUM1, $\times 200$.

Table 1

Primary antibodies used.

Antigen	Clone	Dilution	Antigen retrieval	Source
CD45RB	PD7	1:500	MW CB	Dako
CD20	L26	1:1200	MW CB	Dako
CD3	SP7	1:200	S CB	Neomarkers
CD10	56C6	1:100	S CB	Novocastra
Cyclin D1	SP4	1:100	S CB	Neomarkers
CD5	4C7	1:1600	S CB	Novocastra
bcl-6	PG-B6P	1:100	T+ S TRIS	Dako
IRF4/MUM1	MUM1P	1:1200	S CB	Dako
Ki-67	MIB-1	1:4800	PC CB	Dako
CD23	SP23	1:400	MW CB	Neomarkers
CD43	L60	1:3200	MW CB	BD Pharmingen

DAKO, Carpinteria, CA, USA; Neomarkers Lab Vision Corporation, Fremont, CA, USA; Novocastra, United Kindom; Becton-Dickinson Biosciences, Mountain View, CA, USA; BD Pharmingen-BD Bioscience, San Jose, CA, USA.

Heat-induced epitope retrieval was employed: MW- microwave oven, PC- pressure cooker, S- steamer, T- trypsin and CB- citrate buffer.

Table 2

Clinical and immunophenotypical features of 127 cases of MCL in relation with FISH t(11;14)(q13;q32) results.

	N° of cases	FISH positive	FISH negative
Female	35 (27.6%)	35	0
Male	92 (72.4%)	83	3
Mean Age	59 yo	59 yo	69 yo
Nodal	102 (80.3%)	94	3
Extranodal	25 (19.7%)	24	0
Classic morphology	113	110	3
Blastoid variant	11(9%)	11	0
Pleomorphic variant	2(1.5%)	2	0
Marginal-zone-like	1(0.75%)	1	0
CD20+	127	118	3
BCL-1 (cyclin D1)+	127	118	3
CD5+	122 (96%)	113	3
BCL-6+	15 (11.8%)	14	0
MUM1+	45 (35.4%)	44	1
CD43+	84 (66%)	79	2
Mean PI Ki-67	38.2	38	42
Total	127	118 (97.7%)	3 (2.3%)