Non-Hodgkin lymphoma – Biology & Translational Research

PB2322

NEW POTENTIAL CANDIDATE GENES IN MANTLE CELL LYMPHOMA

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Background: Mantle cell lymphoma (MCL) is a B cell non-Hodgkin lymphoma characterized by the translocation of the cell cycle regulator cyclin D1 (CCND1) under control of the immunoglobulin heavy chain (IGH) locus leading to the constitutive overexpression of CCND1 and cell cycle deregulation. The survival of MCL patients is still poor, especially for patients resistant to frontline therapy. Despite the remissions observed in patients, relapses often occur with disseminated lymphoma and are often more difficult to treat. There is a need for a better understanding of the clonal heterogeneity in MCL and to identify new genes, which could be targeted by novel drugs or be used as biomarkers to predict response to treatment.
Aims: We previously showed the genetic complexity and clonal evolution in MCL by exome analysis in paired samples at diagnosis and relapse and identified new mutations in genes involved in B-cell signaling pathways. In the current study, we have investigated the heterogeneity in gene expression in the same cohort of patients.
Methods: Malignant B cells at diagnosis and relapse from 4 MCL patients were sorted as presented at the EHA meeting in 2017 and subjected to total RNA sequencing together with CD19+ enriched B cells from 3 healthy donors. Six genes aberrantly expressed in MCL samples compared to healthy CD19+ B cells were selected and validated by qPCR in independent cohorts of diagnostic samples from MCL and chronic lymphocytic leukemia (CLL) patients. Peripheral blood mononuclear cells from 20 MCL and 20 CLL patients as well as CD19+ enriched B cells from 10 healthy donors were included. Exemption from informed consent was approved by the National Ethical Committee.
Results: The transcriptome analysis pointed to 19 upregulated genes. The expression of these genes varied between patients but also between paired diagnosis and relapse samples. This suggested clonal evolution or malignant progression and inter-patient heterogeneity, supporting our previous study. We selected 6 upregulated genes that were mainly associated to B cell signaling (CD1c, BLNK, MAP4K1, CCDC50, LILRA4 and PTPRJ) and explored the expression levels in MCL, CLL and healthy CD19+ B cells. While BLNK and CCDC50 were previously reported as highly expressed in MCL, we showed that BLNK expression was similar in MCL and CLL but slightly decreased compared to healthy CD19+ B cells. No difference was observed between MCL, CLL and healthy CD19+ B cells for the CCDC50 or MAP4K1 genes. CD1c was detected in MCL and CLL, although downregulated compared to healthy CD19+ B cells, with a lower expression in CLL than in MCL. This supports its expression in malignant B cells. Few studies detected PTPRJ and LILRA4 in MCL. We showed that PTPRJ was upregulated in MCL and CLL compared to healthy CD19+ B cells with a higher expression in MCL than in CLL. Interestingly, both MCL and CLL displayed 2 clear and different LILRA4 expression levels where the expression in healthy CD19+ B cells displayed an intermediate level.
Summary/Conclusion: Our transcriptome analysis supports the genetic complexity and the clonal evolution in MCL. It also identifies the genes CD1c, PTPRJ and LILRA4 to be aberrantly expressed in MCL with differential regulation of CD1c and PTPRJ in MCL and CLL. This study proposes new candidate genes to target with drugs or to use as biomarkers in MCL.

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THE ROLE OF MULTIPARAMETER FLOW CYTOMETRY IN THE WORK-UP OF IGM-MONOCLONAL GAMMOPATHIES

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Background: IgM-Monoclonal Gammopathy of Undetermined Significance (MGUS) accounts for 15 to 20% of all MGUS cases and it poses a unique diagnostic challenge as it can be associated with a broad spectrum of pathological processes including both B-cell lymphoproliferative disorders (LPD), and monoclonal-IgM related-disorders (IgM-RD). Among all, IgM-MGUS mostly progresses into Waldenström's macroglobulinemia (WM). According to the II International Workshop on WM (IWWM), IgM-MGUS and WM can be differentiated by absence *versus* presence of marrow infiltration by malignant B-cells, while smoldering WM (sWM) from sympomatic WM are differentiated by presence *versus* absence of clinical findings due to bone marrow (BM) infiltration.
Aims: Based on previous literature data, we aimed to evaluate the contribute of multiparameter flow cytometry (MFC) to discriminate between IgM-MGUS and WM by searching for clonal B-lymphocytes in BM and PB samples.
Methods: A total of 102 patients (64/38, M/F) with a median age of 70 yrs (36-89) were investigated. They were selected among patients with an IgM monoclonal gammopathy not associated with B-cell LPD other than WM. Median serum monoclonal (M)-protein level was 1.0 g/dl (0.2-3.9) and light-chain was kappa in 75% of cases. According to the II IWWM criteria, 52 patients were diagnosed as having MGUS, 23 sWM, and 27 WM. Fifty-one BM aspirates and 85 PB samples were immunophenotyped for evaluation of lymphocyte subsets and detection of clonal restriction by Ig K and λ light chain analyses on B-cell surface, irrespectively from B-cell proportion; all PB samples resulted positive for clonal restriction, and all BM aspirates were further analyzed with a large panel of MoAbs; data acquisition was performed on a Beckman Coulter Navios flow cytometer and analyses were performed by using Kaluza software.
Results: The median percentage of mature B-cells was found 1.8 (0-50) in PB, and 16 (1.3-50) in BM samples. Overall, in 56 out of 104 patients, clonal B-cells were detected; in particular, clonal restriction was demonstrated in 79% of BM and 40% of PB samples. The FCM results are summarized in the following table with regard to the different diagnosis. Of note, the identification of clonal populations required accurate gating strategies in 5 PB and 1 BM samples because of the presence of low clonal B-cell number within total B-lymphocytes.

Table 1.

	MGUS		sWM		WM	
	PB	BM	PB	BM	PB	BM
Number	47	12	16	15	32	24
Median lymphocytes % (range)	25 (12-38)	24 (8-41)	28 (7-51)	21 (12-34)	29 (10-65)	39 (13-61)
Median B-lymphocytes % (range)	3 (0-14)	3 (1-16)	1.5 (0-25)	4 (1-7)	1.4 (0-50)	26 (4-50)
Cases with clonal restriction (%)	9 (19)	5 (42)	10 (62)	12 (80)	15 (68)	24 (100)
Cases with involved/uninvolved light chain >10	3 (6)	4 (33)	3 (19)	5 (33)	7 (32)	21 (87)

Summary/Conclusion: Our results suggest a pivotal role of MFC in the diagnostic work-up of patients with IgM monoclonal gammopathies. Despite in most cases malignant cells display a non-specific phenotype, accurate gating strategies can enable to identify low-sized clonal populations within normal B-cell background in BM as well as in PB samples. In addition, further studies could establish a possible role of PB FCM studies in the management of patients with IgM monoclonal gammopathies.

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SARCOPENIA IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA IS CAUSED BY THE DEFICIENCY OF ESSENTIAL AMINO ACID TRYPTOPHAN

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Background: Sarcopenia, which is defined as the depletion of skeletal muscle, is associated with unfavorable outcomes in patients with some types of cancer including hematological malignancies. We previously reported that sarcopenia was a poor prognostic factor in patients with diffuse large B-cell lymphoma (DLBCL). However, the mechanism of development of sarcopenia has not been fully understood. Essential amino acid tryptophan is metabolized by indoleamine 2,3-dioxygenase (IDO) via kynurenine pathway. IDO is one of the immunosuppressive factor in tumor microenvironment. IDO was expressed in DLBCL tumor sites, and high IDO expression was a poor prognostic factor.
Aims: We hypothesized that the sarcopenia could be caused by the deficiency of essential amino acid tryptophan.