Contribution of *MYC* FISH in Pathological Diagnosis of Burkitt Lymphoma

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ABSTRACT

Burkitt lymphoma (BL) is an aggressive B-cell non-Hodgkin lymphoma typically with *MYC* rearrangement. Distinction between BL and diffuse large B cell lymphoma (DLBCL) is not infrequently encountered in routine practice. In this communication, we report our experience with the *MYC* break-apart probe FISH in the diagnosis of BL in 19 cases of aggressive B-cell lymphoma. BL was the final diagnosis in 10/19 cases (52.63%), and 9 of them were shown to carry *MYC* gene rearrangement. DLBCL was the final diagnosis in 7 cases and all had negative FISH test. B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL were diagnosed in the remaining 2 cases, and one of them showed *MYC* rearrangement. To summarize, identification of *MYC* gene rearrangement by FISH assay helps supporting the diagnosis of BL in most of the cases.

INTRODUCTION

Burkitt lymphoma (BL) was first described by Denis Burkitt in 1958[1]. It is an aggressive non-Hodgkin lymphoma of B-cell lineage. Since the original description, tremendous progress has been made in understanding molecular mechanisms of the cancer. Pathological diagnostic criteria have been changed and it is now clear that traditional morphological approach alone is not sufficient to make a firm diagnosis of BL.

One of the fairly common differential diagnoses in routine pathological practice is to distinguish between BL and diffuse large B cell lymphoma (DLBCL). Since dysregulation of *MYC* gene, mostly commonly t(8;14) (q24;q32), features all cases of BL, assessment of the gene is diagnostically helpful[2]. In this communication, we report our experience with the *MYC* break-apart probe FISH in the diagnosis of BL.

MATERIALS AND METHODS

19 consecutive cases of malignant lymphoma underwent *MYC* break-apart probe FISH assay were retrieved from the pathological file at Department of Pathology, Faculty of Medicine, King Chulalongkorn Memorial Hospital, Bangkok, during 2009 to 2012.

For all cases, the histologic differential diagnosis was between DLBCL and BL. The demographic data (age, gender, and site of lesion), immunophenotype, and *MYC* FISH result were recorded. Materials used in all cases were formalin-fixed paraffin-embedded tissue. Details of the antibodies used were shown in Table 1. *MYC* FISH was performed by break-apart (split signal) DNA probe (DAKO, Code Y5410). Translocation involving the *MYC* gene was scored when a specific signal pattern (co-localization of one pair of signals and segregation of the other, see Figure 1) was identified by a non-numerical approach[3]. Final diagnosis was rendered by combining the FISH result and other parameters.

Table 1 Details of primary antibodies and probe used.

| Primary antibody/Probe	Catalog No.	Dilution	Detection system	Procedure
CD3	DAKO IR 503	RTU	EnVisionTM FLEX Visualization system	DAKO AUTOSTAINER LINK 48
CD10	DAKO IR 648	RTU	EnVisionTM FLEX Visuaizgation system	DAKO AUTOSTAINER LINK 48
CD20	DADO IR 604	RTU	EnVisionTM FLEX Visuaizgation system	DAKO AUTOSTAINER LINK 48
BCL-2	DAKO 1R614	RTU	EnVisionTM FLEX Visuaizgation system	DAKO AUTOSTAINER LINK 48
BCL-6	Leica PA0204	RTU	EnVisionTM FLEX Visuaizgation system	DAKO AUTOSTAINER LINK 48
Ki-67	DAKO 1R626	RTU	EnVisionTM FLEX Visuaizgation system	DAKO AUTOSTAINER LINK 48
MUM-1	DAKO M7259	1:200	Ultra View Universal DAB Detection Kit	Ventana Benchmark XT Autostainer
c-Myc	Ventana 790-4628	RTU	OptiView Universal DAB Detection Kit	Ventana Benchmark XT Autostainer

RTU=Ready to use
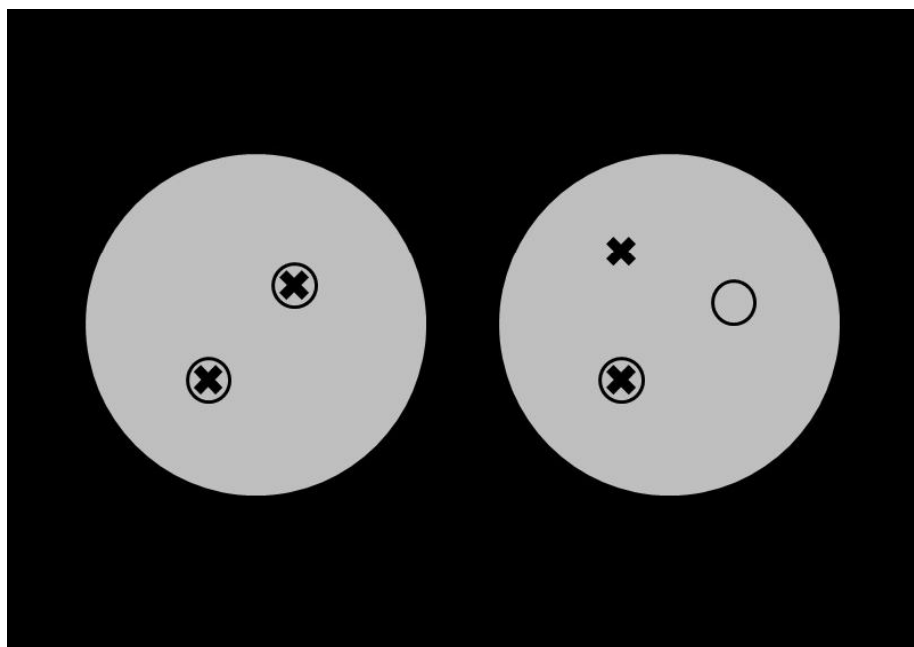
Figure 1   Diagram showing *MYC* gene evaluation by break-apart (split signal) DNA probes. The human *MYC* gene consists of 3 exons spanning a region of ~5 kb on chromosome 8 band q24. The FISH DNA probes are a mixture of a Texas Red-labeled DNA probe (*MYC*-Downstream) covering 418 kb telomeric to the *MYC* breakpoint cluster region (O), and a fluorescein-labeled DNA probe (*MYC*-Upstream) covering 652 kb centromeric to the *MYC* breakpoint cluster region (X). In normal cells, two fused signals are noted (left cell in figure). In cells with *MYC* gene rearrangement, 1 fused and 2 split signals are seen (right cell).

RESULTS

The patients' age ranged from 9-89 years (mean = 47.47). There were 5 females and 14 males. Anatomical site, histopathology (nuclear size and starry-sky pattern), immunophenotype, *MYC* FISH result, and the final diagnosis of all cases were shown in Table 2. One BL (case # 19) tested for EBV showed positive result of EBER in situ hybridization.

Table 2 Details of cases and results

Case No.	Age (y)	Site	Histopathology Cell size	SSP	Immunophenotype	FISH MYC	Final Diagnosis
1	9	Lymph node, right submandibular	M and L	+	CD3-, CD10+, CD20+, BCL2-, BCL6+, Ki-67+ (100%), Tdt-, MUM1-, c-Myc+/-	+	BL
2	15	Lymph node, left groin	M and L	+	CD3-, CD10+, CD20+, CD43-, BCL2-, BCL6+, Ki-67+ (100%), MUM1-, c-Myc+	+	BL
3	17	Brain, left cerebrum	M	+	CD3-, CD10+, CD20+, BCL2-, BCL6+, Ki-67+ (100%), MUM1-, c-Myc-	+	BL
4	32	Pelvis	M and L	+	CD3-, CD10-/+, CD20+, BCL2-, BCL6+, Ki-67+ (100%), MUM1-, c-Myc+/-	+	BL
5	33	Soft tissue, left side of neck	M and L	+	CD3-, CD10+, CD20+, BCL2-, BCL6+, Ki-67+ (100%), MUM1+, c-Myc+	+	BL
6	36	Liver	M and L	+	CD3-, CD10+, CD20+, BCL2-, BCL6+, Ki-67+ (95%), MUM1-, c-Myc+	+	B-cell L, inter
7	37	Lymph node, inguinal	M and L	−	CD3-, CD10+, CD20+, BCL2-, BCL6+, Ki-67+ (>90%), MUM1-, c-Myc-	−	DLBCL
8	44	Lymph node	M and L	−	CD3-, CD10-, CD20+, BCL2-, BCL6+, Ki-67+ (100%), MUM1-, c-Myc-	−	DLBCL
9	44	Right breast	M	+	CD3-, CD10+, CD20+, BCL2-, BCL6+, Ki-67+ (80-90%), MUM1+, c-Myc-	−	B-cell L, inter
10	46	Left retroperitoneum	M and L	−	CD3-, CD10+, CD20+, BCL2-, BCL6+, Ki-67+ (100%), MUM1-, c-Myc+	−	DLBCL
11	48	Pancreas	M and L	−	CD3-, CD10+, CD20+, BCL2-, BCL6+, Ki-67+ (100%), MUM1-, c-Myc+	+	BL
12	50	Nasopharynx, right	M and L	−	CD3-, CD10+, CD20+, BCL2-, BCL6+, Ki-67+ (>90%), MUM1-, c-Myc+	+	BL
13	54	Stomach	M and L	−	CD3-, CD10+, CD20+, BCL2-, BCL6+, Ki-67+ (100%), MUM1-, c-Myc+/-	−	BL
14	56	Lymph node, axilla	M and L	+	CD3-, CD10-, CD20+, BCL2-, BCL6-/+, Ki-67+ (90%), MUM1+/-, c-Myc-	−	DLBCL
15	61	Lymph node, right cervical	M	+	CD3-, CD10-, CD20+, BCL2+, BCL6-, Ki-67+ (60%), MUM1+, c-Myc-	−	DLBCL
16	71	Lymph node, right inguinal	M and L	+	CD3-, CD10-, CD20+, BCL2+/-, BCL6+, Ki-67+ (80%), MUM1+, c-Myc+	−	DLBCL
17	75	Bone marrow	M and L	−	CD3-, CD10+, CD20+, BCL2-, BCL6+, Ki-67+ (100%), MUM1-, c-Myc-	+	BL
18	85	Lymph node	M and L	+	CD3-, CD10-, CD20+, BCL2+, BCL6-, Ki-67+ (>90%), MUM1+, c-Myc-	−	DLBCL
19	89	Base of tongue	M	+	CD3-, CD10+, CD20+, BCL2-, BCL6+, Ki-67+ (100%), MUM1-, c-Myc-	+	BL

Y = year; M = medium; L = large; SSP = starry-sky pattern; BL = Burkitt lymphoma; DLBCL = Diffuse large B cell lymphoma; B-cell L, inter = B-cell lymphoma, unclassifiable with features intermediate between DLBCL and BL. For immunophenotype; +, +/-, -/+, and − indicate that >90%, 50-90%, <50%, and <10% of tumor cells show immunoreactivity, respectively.

DISCUSSION

Distinction between diffuse large B cell lymphoma (DLBCL) and Burkitt lymphoma (BL) is a fairly common differential diagnosis in daily practice, and it is crucial since the latter requires more aggressive treatment. BL is high grade B-cell lymphoma that is characterized histologically by monomorphic proliferation of medium-sized non-cleaved malignant cells, with round nuclei, clumped chromatin, two to five nucleoli, medium-sized para-central nucleoli, cytoplasmic vacuoles, and an appreciable rim of basophilic cytoplasm. Nuclear and cell membrane of the individual tumor cells frequently show molding or "Square-off". High mitotic rate with "starry-sky" pattern imparted by tangible-body macrophages is characteristics, but not pathognomonic. DLBCL, despite the name implies ("large" cell), the size of tumor cells may be overlapped with that of BL. Furthermore, starry-sky pattern could also be encountered in DLBCL[2].

Diagnosis of BL is currently based on several parameters, including histomorphology, immunophenotype, cytogenetic, molecular study, and gene expression profiles; since there is no single test that can be used as gold standard for the diagnosis[4]. Immunohistochemical study is helpful, to some extent, in the differential diagnosis between DLBCL and BL, and the typical profile is summarized in Table 3.

Table 3 Immunophenotype of DLBCL and BL[2]

	DLBCL	BL
CD20	+	+
BCL-2	+/-	-
BCL-6	+/-	+
MUM-1	+/-	-
CD10	+/-	+
Ki-67	40-100%	>90%
c-Myc	+/-	+

Despite the IHC panel, diagnosis can still be ambiguous, and assessment of *MYC* gene rearrangement is needed. Having said that, translocation involving *MYC* gene, though characteristic, is not specific for BL. Furthermore, diagnosis of BL can still be made when *MYC* FISH test is negative if all other features (morphology, CD20+, CD10+, BCL6+, BCL2-, and Ki67+ >95%) fit well with the diagnosis [2,5].

In 2008, a new category of B cell neoplasm, "B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL" was introduced by the World Health Organization[2]. Diagnostic criteria of the entity include (1) morphologic overlap between DLBCL and BL with typical immunophenotype of BL, (2) morphologic features of BL with atypical immunophenotype, and (3) *MYC* rearrangement with concomitant BCL2 and/or BCL6 rearrangement (so called double-hit lymphomas)[2,6].

In our cohort of 19 B-cell lymphomas with the differential diagnosis between DLBCL and BL, positive *MYC* FISH supported the diagnosis of BL in 9 out of 10 cases. For the remaining BL case (#13) where FISH was negative, the diagnosis was based on clinical data, histomorphology and immunohistochemistry (IHC). 7/19 patients were diagnosed as having DLBCL and all of these tumors showed negative result of *MYC* FISH. It is important to note that discrepancy between c-Myc IHC and *MYC* FISH was found in 6/19 cases (31.58%): 3 cases with positive IHC but negative FISH (#10, 13, 16), and the others with the opposite result (#3, 17, 19). Mechanisms other than gene rearrangement may underlie the positive IHC in the former 3 cases whereas antigen loss may have resulted in the negative IHC (positive FISH) in the latter. B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL were diagnosed in 2 cases (cases # 6 and 9). The former case had overlapping morphological features between DLBCL and BL but

typical immunophenotype of BL and positive FISH result; the latter showed typical BL morphology but atypical immunoprofile and no *MYC* rearrangement.

In summary, several parameters are required for the distinction between diffuse large B cell lymphoma (DLBCL) and Burkitt lymphoma (BL). In addition to morphology and immunoprofile, identification of *MYC* gene rearrangement by FISH assay helps supporting the diagnosis of BL in most cases.

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