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Fluorescence In Situ Hybridization for *TP63* Rearrangements in T-cell Lymphomas: Single Site Experience of 470 Patients and Implications for Clinical Testing

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Abstract

Aims: The aims of this study were to review our 5-year experience with clinical FISH testing for *TP63* rearrangements using both *TP63* breakapart (BAP) and *TBL1XR1/TP63* dual-fusion (D-FISH) probes, to evaluate the frequency of *TP63* rearrangements and the distribution of *TBL1XR1* versus alternate partner loci, and to assess whether both probe sets are necessary in up-front FISH testing.

Methods and results: A retrospective review of the Mayo Clinic cytogenetic database identified 470 patients evaluated by FISH testing for *TP63* rearrangements in FFPE tissue using both BAP and D-FISH probes. Of these, 25 (5.3%) had *TP63* rearrangements. All samples were being investigated for anaplastic large cell lymphoma or other T-cell lymphoma subtypes. A *TBL1XR1* partner was identified by D-FISH in 12 (48%) of 25 cases. All cases positive by *TBL1XR1/TP63* D-FISH were also positive by *TP63* BAP FISH.

Conclusion: This is the largest series of *TP63* rearrangements to date. The frequency of positive results among cases referred to a large reference laboratory for *TP63* FISH testing was 5.3%. Approximately half of *TP63* rearrangements have a *TBL1XR1* partner. *TP63* BAP FISH testing is sufficient for up-front testing of FFPE tissue samples. However, because of the genomic proximity of the *TP63* and *TBL1XR1* loci, we recommend reflex *TBL1XR1/TP63* D-FISH testing in positive and equivocal cases.

Keywords

Anaplastic large cell lymphoma; *TP63*; p63; T-cell lymphoma; chromosomal rearrangement; fluorescence in situ hybridization

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Introduction

Genetic characterization of T-cell neoplasms has diagnostic, prognostic, and therapy-related value.¹ Anaplastic large cell lymphoma (ALCL) is one of the most common forms of T-cell non-Hodgkin lymphoma and is classified based on the presence or absence of *ALK* rearrangements (ALK-positive and ALK-negative ALCL, respectively).² ALK-positive ALCLs have a favorable prognosis and can be targeted specifically using ALK inhibitors.³ Approximately 30% and 8% of ALK-negative ALCLs harbor *DUSP22* and *TP63* rearrangements, respectively.^{1,2,4,5} The accurate detection of *TP63* (3q28) rearrangements, most commonly with the *TBL1XR1* gene region associated with inv(3)(q26q28), is critical as ALK-negative ALCLs with *TP63* rearrangements have a dismal prognosis.^{4,6} *TP63* rearrangements also have been reported in rare cases of peripheral T-cell lymphoma, not otherwise specified; mycosis fungoides; diffuse large B-cell lymphoma; and lung adenocarcinoma.^{6–10} Since immunohistochemistry for p63 is not specific for *TP63* rearrangements in ALCL,¹¹ our group developed and validated a *TP63* break-apart (BAP) fluorescence *in situ* hybridization (FISH) probe and a *TBL1XR1/TP63* dual-color, dual-fusion (D-FISH) probe set for clinical use on formalin-fixed, paraffin-embedded (FFPE) tumor specimens. Herein, we describe our 5-year experience of concurrently performing *TP63* BAP and *TBL1XR1/TP63* D-FISH probe sets on FFPE specimens.

Methods

A 5-year retrospective review of the Mayo Clinic cytogenetics database (1/29/2014–2/28/2019) was performed to identify all patient cases with concurrent *TP63* BAP and *TBL1XR1/TP63* D-FISH studies performed on FFPE specimens. The start date of 1/29/2014 coincided with the initiation of clinical FISH testing for *TP63* rearrangements at Mayo Clinic. Both FISH probe sets were “laboratory developed” and validated for clinical application as previously described.^{7,12} Probe design is shown in Supplemental Figure 1 and normal cutoff values are shown in Supplemental Table 1. Additional details on the clinical validation are included in Supplemental Methods. All specimens were subjected to standard FISH pretreatment, hybridization, and fluorescence microscopy according to FFPE-specific protocols. A total of 510 patient samples were submitted for *TP63* FISH testing during this 5 year period, with 470 cases demonstrating successful results for both FISH probe sets. Five cases were successful for only the *TP63* BAP probe set and two cases were successful for only the *TBL1XR1/TP63* D-FISH probe set, while 33 cases submitted for *TP63* FISH studies could not be evaluated due to insufficient tissue or poor sample quality. The study was conducted under a protocol approved by the Mayo Clinic Institutional Review Board (#17–005929, 3/6/19).

Results

A total of 470 unique individuals with successful *TP63* BAP and *TBL1XR1/TP63* D-FISH studies performed on FFPE tumor specimens were identified, including 291 males and 179 females (mean age, 59 years; range, 5–100 years). Of the 470 cases, 25 (5.3%) demonstrated a *TP63* rearrangement with the BAP probe, including 12 men and 13 women (mean age, 65

years; range, 38–87 years). The indication for testing, based on review of the accompanying pathology report and the reason-for-referral field of the FISH requisition, was ALCL or other T-cell lymphoma in 24 cases and was not indicated in 1 case (Table 1). Of the 25 cases, 12 (48%) demonstrated *TBL1XR1/TP63* fusion by D-FISH and 13 (52%) lacked evidence of *TBL1XR1/TP63* fusion (Figure 1). No cases were identified with *TBL1XR1/TP63* fusion by D-FISH with a negative BAP *TP63* result. All 5 specimens in which only BAP FISH was successful were negative for *TP63* rearrangement, while 1 of 2 specimens in which only D-FISH was successful demonstrated *TBL1XR1/TP63* fusion.

Of 445 cases with concurrent *TP63* BAP and *TBL1XR1/TP63* D-FISH studies that lacked *TP63* rearrangement, copy number data were available in 434 cases. Of these, 157 cases (36.2%) demonstrated polysomy 3 (or gains of 3q) indicated by extra *TP63* and *TBL1XR1* signals, and 4 cases (0.9%) demonstrated monosomy 3 (or loss of 3q) indicated by loss of *TP63* and *TBL1XR1* signals (data not shown). Two cases (0.5%) demonstrated loss of the *TP63* gene region and gain of the *TBL1XR1* gene region.

Discussion

Our 5-year review of concurrent *TP63* BAP and *TBL1XR1/TP63* D-FISH studies performed on FFPE specimens identified 25 of 470 cases (5.3%) with *TP63* rearrangements, which is similar to the overall frequency of *TP63* rearrangements (5–8%) previously reported in ALK-negative ALCL.^{1,2,4,5} These cases represent the largest series of *TP63* rearrangements to date.

Of the 25 cases with *TP63* rearrangements, 12 (48%) demonstrated *TBL1XR1/TP63* fusion by D-FISH, slightly lower than our previous report indicating the frequency of the *TBL1XR1* partner (64%).⁷ Alternate *TP63* fusion partners have not been comprehensively studied, but include *ATXN1* and *FOXK2*.^{7,13} It remains unclear whether the *TP63* partner locus has prognostic or other clinical significance because of an insufficient number of published cases with outcome data.

TP63 encodes p63, a member of the p53 family that also includes p73. Like p53, full-length p63 isoforms that contain the N-terminal transactivation (TA) domain have tumor suppressor properties, and also play a role in cell development and differentiation.¹⁴ In contrast, isoforms that lack the TA domain and have an altered N-terminus (Np63 isoforms) have oncogenic properties. *TP63* rearrangements that have been sequenced to date share similar breakpoints and encode putatively oncogenic fusion proteins lacking the p63 transactivation (TA) domain.^{7,9,13} However, p63 fusion proteins also lack the N domain and are not recognized by antibodies to Np63 isoforms such as p40.¹¹ Immunostaining using pan-p63 antibodies is consistently positive in cases with *TP63* rearrangement but lacks specificity because some ALCLs without *TP63* rearrangements express full-length p63 (TAp63 isoforms), a finding associated with extra intact copies of the *TP63* locus.¹¹ Therefore, p63 (but not p40) immunohistochemistry can be used as a screening test to select cases for subsequent FISH testing but cannot be used as a surrogate for FISH.¹¹ We were not able to assess the correlation between *TP63* FISH results and p63 immunohistochemistry findings in the present series since most of the cases were submitted for FISH testing only through

our reference laboratory. Similarly, follow-up data were not available in most cases to evaluate associations with outcome.

Importantly, among the cases of *TBL1XR1/TP63* fusion we identified by D-FISH, none was negative for *TP63* rearrangement by *TP63* BAP studies. This finding indicates that *TP63* BAP FISH alone is sufficient for the detection of *TP63* rearrangements in most cases. It is important to note, however, that in some cases it may be challenging to distinguish separation of the *TP63* BAP probe associated with *TBL1XR1/TP63* fusion from a normal signal pattern due to the close spatial proximity in the interphase nucleus of *TBL1XR1* (3q26.32) and *TP63* (3q28). Based on our results, we recommend up-front *TP63* BAP FISH testing alone for the evaluation of *TP63* rearrangements in FFPE tissue samples. It is difficult to provide quantitative recommendations for the distance between signals that should be considered broken apart or fused given the multiple variables involved in scoring interphase FISH in FFPE tissue. Therefore, we recommend reflex *TBL1XR1/TP63* D-FISH testing in cases with BAP signal patterns that yield unclear results (“equivocal”), whether due to unusual signal patterns, suboptimal hybridization, or other interpretive difficulties. In addition, confirmatory FISH testing may be considered in all cases with *TP63* rearrangement by BAP FISH because of the profound prognostic significance of this finding.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Representative cases of ALK-negative anaplastic large cell lymphoma with *TP63* rearrangement. Top panels, *TBL1XR1/TP63* fusion. H&E of ALCL shows sheets of pleomorphic cells (Case 3, Table 1). *TP63* BAP FISH demonstrates separation of red (5') and green (3') signals (arrows). The degree of separation of the probes is limited by the relative proximity of *TBL1XR1* (3q26.32) and *TP63* (3q28). *TBL1XR1/TP63* D-FISH shows multiple fusion signals consistent with 2 copies of *inv(3)(3q26.32q28)*. Bottom panels, *TP63* rearrangement with alternate partner. H&E image of ALCL with sheets of pleomorphic cells (Case 20, Table 1). *TP63* BAP FISH demonstrates separation of red and green signals (arrows). *TBL1XR1/TP63* D-FISH shows multiple red and green signals without fusion signals.

Table 1.Clinicopathologic features and FISH results in 25 cases with *TP63* rearrangements

Patient	Gender	Age (years)	Specimen	RFR	TP63 rearrangement (BAP) (%)	TBL1XR1/TP63 fusion (D-FISH) (%)
1	M	45	Gingiva	ALK-negative ALCL	+ (64)	+ (62)
2	F	40	Soft tissue, abdomen	ALK-negative ALCL	+ (100)	+ (100)
3	F	66	Nasal cavity	ALK-negative ALCL	+ (88)	+ (98)
4	F	62	Soft tissue, buttock	ALK-negative ALCL	+ (86)	+ (90)
5	M	60	LN, axillary	ALK-negative ALCL	+ (92)	+ (100)
6	F	75	LN, cervical	CD30+ T-cell lymphoma	+ (100)	+ (100)
7	F	53	LN, cervical	ALK-negative ALCL	+ (62)	+ (85)
8	M	74	Tonsil	ALK-negative ALCL	+ (100)	+ (100)
9	M	59	Soft tissue, groin	CD30+ T-LPD	+ (100)	+ (100)
10	F	76	LN, groin	ALK-negative ALCL	+ (45)	+ (43)
11	F	62	LN, supraclavicular	PTCL, NOS	+ (100)	+ (100)
12	F	80	LN, axillary	CD30+ T-cell lymphoma	+ (100)	+ (100)
13	M	75	LN, axillary	ALCL	+ (66)	-
14	M	67	LN, groin	ALK-negative ALCL	+ (100)	-
15	M	70	LN, cervical	ALK-negative ALCL	+ (100)	-
16	F	61	LN, cervical	ALK-negative ALCL	+ (96)	-
17	M	76	Soft tissue, paraspinal	ALK-negative ALCL	+ (62)	-
18	F	38	LN, cervical	Rule out <i>TP63</i> rearrangement	+ (100)	-
19	F	73	Soft tissue, neck	ALK-negative ALCL	+ (94)	-
20	F	60	LN, cervical	ALK-negative ALCL	+ (100)	-
21	F	64	Skin, cheek	CD30+ T-LPD	+ (85)	-
22	M	87	Pleural effusion	ALK-negative ALCL	+ (100)	-
23	M	59	Soft tissue, thigh	ALK-negative ALCL	+ (66)	-
24	M	69	Liver	ALK-negative ALCL	+ (92)	-
25	M	71	Axillary mass	ALK-negative ALCL	+ (100)	-

RFR, reason for referral; BAP, break-apart probe; D-FISH, dual-color dual-fusion probe; LN, lymph node; ALCL, anaplastic large cell lymphoma; ALK, anaplastic lymphoma kinase; PTCL, NOS, peripheral T-cell lymphoma, not otherwise specified; T-LPD, T-cell lymphoproliferative disorder; +, positive; -, negative.