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FOXP1 abnormalities in lymphoma: translocation breakpoint mapping reveals insights into deregulated transcriptional control

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Deregulation of FOXP1 expression plays an important role in lymphoma development although the underlying molecular mechanism is poorly understood. *FOXP1* is targeted by chromosome translocations in MALT lymphoma and diffuse large B-cell lymphoma, where high-level protein expression is associated with poor prognosis. Nonetheless, the incidence and nature of FOXP1 abnormalities at both the genetic and protein levels, and their correlation in these lymphomas are not well established. We investigated *FOXP1* translocation, copy number change and protein expression in MALT lymphoma ($n = 321$), MALT lymphoma with a diffuse large B-cell lymphoma component (59), nodal diffuse large B-cell lymphoma (64) and extranodal diffuse large B-cell lymphoma (151) by interphase fluorescence *in situ* hybridization and immunohistochemistry. *FOXP1* translocation was found in eight MALT lymphomas and three MALT lymphomas with diffuse large B-cell lymphoma, with all positive cases originating in the stomach. In diffuse large B-cell lymphoma, the translocation was seen in 5 cases originating in the stomach (2), tonsil (1), large intestine (1) and lymph node (1). Immunoglobulin heavy chain gene was the translocation partner in 11 of the 16 positive cases. Fluorescence *in situ* hybridization mapping revealed *FOXP1* breakpoints within the 5′ untranslated region of the gene (upstream of exon 6, the first coding exon of full-length FOXP1) in 14 cases, but downstream of exon 6 (most likely upstream of exon 8) in the remaining 2 cases. Three copies of the *FOXP1* gene were observed in MALT lymphoma (17%), MALT lymphoma with diffuse large B-cell lymphoma (12%) and diffuse large B-cell lymphoma (32%), including cases with *FOXP1* translocation (19%). Immunohistochemistry showed strong/moderate FOXP1 staining in all the cases with *FOXP1* translocation. However, FOXP1 expression was independent of *FOXP1* translocation or copy number changes. Our findings suggest that (1) *FOXP1* translocation may disrupt the full-length *FOXP1* transcript and lead to expression of *FOXP1* transcript variants with alternate 5′ ends and (2) mechanisms other than translocation and copy number changes are also responsible for FOXP1 overexpression in lymphoma.
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FOXP1 is a member of the forkhead box family of transcription factors that have diverse functions in different cell and tissue types. FOXP1 regulates Rag1 and Rag2 expression and is essential for B-cell development.[1] Apart from this, the function of FOXP1 is largely unknown. Nonetheless, FOXP1 function is unlikely to be confined to its role in

B-cell development. Gene expression profiling studies show high expression of *FOXP1* mRNA in activated B cells and activated B-cell-like diffuse large B-cell lymphomas.[2] FOXP1 protein expression was seen in 40–60% of diffuse large B-cell lymphomas[3,4] and strong expression was significantly associated with inferior overall survival.[4,5] Similarly, FOXP1 expression was found in 29% of extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT) and strong expression was associated with poor disease-free survival and transformation to diffuse large B-cell lymphoma.[6] These findings suggest a role for FOXP1 expression in lymphoma pathogenesis.

In support of the above notion, *FOXP1* genetic abnormalities were also described in lymphoma. Streubel *et al*[7] first showed that *FOXP1* was involved in t(3;14)(p13;q32) in MALT lymphoma. *FOXP1* translocation was present in 10% of MALT lymphomas, in those from the ocular adnexa (4/20 = 20%), thyroid (3/6 = 50%) and skin (2/20 = 10%), but not in those from the salivary gland, stomach and lung.[7] The translocation was subsequently found in one case of MALT lymphoma of the stomach,[8] seven cases of diffuse large B-cell lymphoma of the stomach, thyroid and lymph nodes and also in two cases of B-cell non-Hodgkin lymphoma unclassified.[8–10] In addition, *FOXP1* gene amplification was reported in one case of diffuse large B-cell lymphoma in one study,[8] but not in others,[7,9–11] The discrepancies in the incidence of *FOXP1* genetic abnormalities remain to be further investigated in a large series.

Of the 19 cases of *FOXP1* translocation-positive lymphoma reported,[7–10] *FOXP1* breakpoints were characterized in 1 gastric MALT lymphoma and 1 gastric diffuse large B-cell lymphoma, both with t(3;14)/*FOXP1-IGH*. In the gastric MALT lymphoma, the *FOXP1* breakpoint was 38 kb upstream of the first noncoding exon 1 and the remaining *FOXP1* sequence was joined to immunoglobulin heavy chain gene (*IGH*) joining segment 6.[7] Thus, *FOXP1* is overexpressed most likely as a result of strong transcriptional activity of the *IGH* enhancer. In contrast, in the gastric diffuse large B-cell lymphoma, the *FOXP1* breakpoint was in intron 2 (according to the Ensemble Genome Database), thus causing separation from its normal promoter. The remaining *FOXP1* sequence was joined to *IGH-Cα1* but in an opposite transcriptional orientation.[9] Despite this, FOXP1 protein was strongly expressed, suggesting expression of variant *FOXP1* transcripts under the control of an alternative promoter.[9] Although little is known about the transcriptional regulation of FOXP1, the different *FOXP1* breakpoints described are expected to have significantly different effects on *FOXP1* transcriptional control. However, the number of *FOXP1* translocation cases investigated for *FOXP1* breakpoints is small and characterization of breakpoints in further cases is needed to understand how the translocation impacts on *FOXP1* transcriptional regulation. Such knowledge is critical not only for understanding the role of *FOXP1* translocation in lymphoma pathogenesis, but also for studies of *FOXP1* transcriptional regulation in normal and malignant B cells lacking *FOXP1* genetic abnormalities. We have screened a large series of MALT lymphomas of various sites, and diffuse large B-cell lymphomas from nodal and extranodal sites for *FOXP1* translocation and mapped the *FOXP1* breakpoints in the 16 translocation-positive cases identified.

Materials and methods

Materials

A total of 595 cases of lymphoma were studied and their histological diagnoses were reviewed. These included 321 cases of MALT lymphoma, 59 cases of MALT lymphoma with a diffuse large B-cell lymphoma and 215 cases of diffuse large B-cell lymphoma; and their tissue origins are summarized in Table 1. Formalin-fixed paraffin-embedded tissues were retrieved from each case. The use of

Table 1 Incidences of *FOXP1* translocation and numerical changes in lymphoma

Lymphoma type	*Primary site*	*No. of cases*	*No. of cases with* FOXP1 *translocation*		*No. of cases with 3 copies of* FOXP1	
MALT lymphoma	Gastric	188	8	4%	34	18%
	Non-gastric[a]	133	0	0%	22	17%
MALT lymphoma+diffuse large B-cell lymphoma	Gastric	53	3	6%	4	8%
	Non-gastric[b]	6	0	0%	3	50%
Diffuse large B-cell lymphoma	Nodal	64	1	2%	28	44%
	Extra nodal gastric	69	2	3%	16	23%
	Extra nodal non-gastric[c]	82	2	2%	27	33%

[a]MALT lymphomas from the salivary gland (40), ocular adnexa (46), lung (13), intestines (10), thyroid (7), abdominal wall, breast, bladder, tonsil, testes, liver, thymus, larynx and pharynx.

[b]MALT lymphoma with a diffuse large B-cell lymphoma component from the thyroid (4) and intestines (2).

[c]Diffuse large B-cell lymphoma from the thyroid (19), tonsils (13), intestines (21), salivary gland (3), skin, testes and nasopharynx. The two cases with *FOXP1* translocation are from the tonsil (1/13), and intestines (1/21).

Table 2 BAC clones used to generate interphase FISH assays for detection of *FOXP1* translocation

BAC Clone	*Abbreviation*	*Gene*	*Position (Mb)*[a]	*Label color*	*Probe set*
RP11-0118 O11	O11	*FOXP1*	70.63–70.79	Orange	BAP 1, fusion
RP11-01031 N18	N18	*FOXP1*	70.72–70.91	Orange	BAP 1, fusion
RP11-0430 J3	J3	*FOXP1*	70.86–71.02	Orange	BAP 1, fusion
RP11-0079 P21	P21	*FOXP1*	71.02–71.19	Orange	BAP 3
RP11-0090 H15	H15	*FOXP1*	71.17–71.30	Orange	BAP 3
RP11-0298 C2	C2	*FOXP1*	71.26–71.42	Orange	BAP 2,3
CTD-2011 M13	M13	*FOXP1*	71.51–71.66	Orange	BAP 2
RP11-0154 H23	H23	*FOXP1*	71.62–71.82	Green	BAP 1,3
RP11-0266 O22	O22	*FOXP1*	71.73–71.89	Green	BAP 1,2,3
RP11-0321 A23	A23	*FOXP1*	71.87–72.05	Green	BAP 1,2,3
RP11-150 I16	I16	*IGH*	104.38–104.53	Green	Fusion
RP11-817 G24	G24	*IGH*	104.53–104.76	Green	Fusion
RP11-937 M13	937 M13	*IGH*	104.76–104.95	Green	Fusion

BAP: break-apart probe.
[a]Positions of BACs are according to Ensembl and the UCSC Genome Browser.

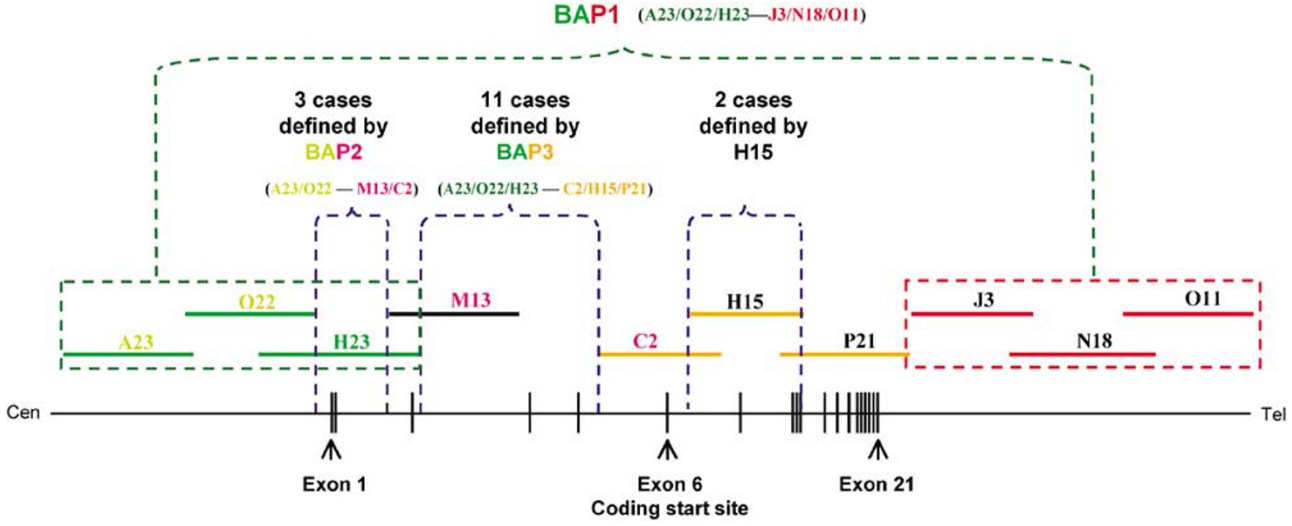

Figure 1 Schematic representation of *FOXP1* genomic structure and the BAC clones used for detection of *FOXP1* translocation and mapping of *FOXP1* breakpoints. Please refer to Table 2 for the full name and position of the BAC clones.

such archival tissues for research was approved by the local ethics committees of the authors' institutions, where required.

Fluorescence *In Situ* Hybridization

A series of 10 BAC clones mapping to various positions of the *FOXP1* gene were used to form three break-apart (BAP) fluorescence *in situ* hybridization (FISH) assays to detect *FOXP1* translocation and to map *FOXP1* breakpoints (Table 2; Figure 1). The 3 BAC clones telomeric of the *FOXP1* gene were combined with 3 BAC clones centromeric of the *IGH* locus (kindly provided by Professor Reiner Siebert) to form a FISH assay to detect *FOXP1–IGH* fusion. BAC DNA extraction and labeling were performed essentially as previously described.[12] The specificity and efficacy of the probes were tested on metaphase slides and on formalin-fixed paraffin-embedded tissue sections of a MALT lymphoma with t(3;14)(p13;q32)/*FOXP1-IGH*.

Interphase FISH was performed as described previously.[12] For each probe set, the mean plus three standard deviations of false positive signals in 100 nuclei from seven reactive tonsils was used as the cut-off value for the diagnosis of chromosomal aberrations.[12] *FOXP1* translocation-positive cases were further screened with *IGH* (Vysis/Abbott Laboratories Ltd, UK), *IG-κ* and *IG-λ* BAP probes (kindly provided by Professor Reiner Siebert) to identify potential translocation partners. All MALT lymphomas with *FOXP1* translocation were investigated with a *MALT1* BAP probe, whereas diffuse large B-cell lymphomas with *FOXP1* translocation were investigated with *BCL6*, *BCL2* and *c-MYC* BAP probes (Vysis/Abbott Laboratories Ltd).

Immunohistochemistry

FOXP1 immunohistochemistry was performed on formalin-fixed paraffin-embedded tissue sections using the JC12 mouse monoclonal antibody, which recognizes specifically an epitope in the C-terminus (within exons 18–21) of FOXP1. Various conditions including different antibody dilutions and antigen retrieval methods were tested and the optimized conditions are described below. Briefly, sections were pretreated in a microwave for 15 min in DAKO Low Target Retrieval Solution and then incubated with the JC12 antibody (1/150 dilution) for 45 min, followed by incubation with secondary antibody and visualization by the streptavidin-immunoperoxidase method. FOXP1 immunostaining was evaluated independently by three assessors (AG, CMB, MQD) and scored according to the percentage of positivity ($<30\%$, 30–70%, $>70\%$ cells) and the intensity of immunostaining (weak, moderate, strong) as described previously.[4,6]

CD10, Bcl-6 and MUM1 were similarly immunostained in diffuse large B-cell lymphomas with *FOXP1* translocation and the staining was assessed semiquantitatively by visual estimation. Cases were considered positive if 30% or more of the tumor cells were stained. Diffuse large B-cell lymphomas were subclassified into the germinal center B-cell-like or non-germinal center B-cell-like subtypes as described previously.[3]

Statistical Analysis

The χ^2-test was used to analyze the difference in FOXP1 staining between MALT lymphoma and diffuse large B-cell lymphoma.

Results

FOXP1 Genetic Aberrations in Lymphoma

In total, 16 of the 595 cases of lymphoma studied with *FOXP1* BAP probe 1 showed evidence of a *FOXP1* translocation. These included eight MALT lymphomas, three MALT lymphomas with a diffuse large B-cell lymphoma component and five diffuse large B-cell lymphomas (Table 1). In the three cases of MALT lymphoma with a diffuse large B-cell lymphoma component, *FOXP1* translocation was detected in the MALT lymphoma but presence of the translocation in the diffuse large B-cell lymphoma component could not be investigated due to the absence of diffuse large B-cell lymphoma in the tissues available for research. All cases of MALT lymphoma and MALT lymphoma with a diffuse large B-cell lymphoma component harboring *FOXP1* translocation were from the stomach, accounting for 4 and 6% of the corresponding lymphoma subtype of the stomach, respectively. The five translocation-positive diffuse large B-cell lymphomas were from the stomach ($n=2$, 3%), intestine ($n=1$, 5%), tonsil ($n=1$, 8%) and lymph node ($n=1$, 2%).

Gain of one or more extra copies of the *FOXP1* gene was seen in 17% of MALT lymphomas, 12% of MALT lymphomas with a diffuse large B-cell lymphoma component and 33% of diffuse large B-cell lymphomas (Table 1). These included three cases (one thyroid MALT lymphoma with diffuse large B-cell lymphoma, one intestinal diffuse large B-cell lymphoma and one gastric diffuse large B-cell lymphoma) showing four or more extra copies of the *FOXP1* gene. Gain of an extra copy of the *FOXP1* gene, but not the translocated allele, was seen in 3 of the 16 *FOXP1* translocation-positive cases (19%).

Further interphase FISH with *MALT1* BAP probe showed that none of the *FOXP1* translocation positive MALT lymphomas or MALT lymphomas with diffuse large B-cell lymphoma had a translocation involving the *MALT1* locus. For diffuse large B-cell lymphoma with *FOXP1* translocation, interphase FISH was performed with *BCL2, c-MYC* and *BCL6* BAP probes, respectively, and only one case (no. 14) was found to harbor a *BCL6* translocation.

Clinicopathological Features of Lymphomas with *FOXP1* Translocation

There were neither apparently uniform clinical nor histological features associated with these *FOXP1* translocation-positive cases (Table 3). All MALT lymphoma cases showed characteristic histology and immunophenotype. Follicular colonization and prominent plasma-cell differentiation were each seen in three cases, with one case displaying both histological features. One case of stage-I gastric MALT lymphoma (no. 2) was treated by *Helicobacter pylori* eradication but showed no response to this first-line treatment.[14,15] Interestingly, one case of stage-I MALT lymphoma with a diffuse large B-cell lymphoma component (no. 9) showed complete remission following *H. pylori* eradication. With the exception of one patient with gastric MALT lymphoma who died of postsurgical infection, all other cases of MALT lymphoma with or without diffuse large B-cell lymphoma showed favorable responses to treatment.

Of the five cases of diffuse large B-cell lymphoma, two cases were of germinal center B-cell-like subtype and the remaining three were of non-germinal center B-cell-like subtype.[3] The histological and clinical features of these cases are summarized in Table 3.

Identification of the Translocation Partner of *FOXP1*

Interphase FISH with an *IGH* BAP probe showed a break in the *IGH* locus in 11 of the 16 *FOXP1* translocation-positive cases (Table 3). Further FISH with the *FOXP1–IGH* fusion probe confirmed *IGH* as the translocation partner in each of these 11 cases. Of the remaining five cases in which *FOXP1*

Table 3 Summary of cases with *FOXP1* translocation

Case no.	*Site*	*Age/sex*	*Histological diagnosis*	*Histological features*	*CD10/BCL6/ MUM1*	*Clinical stage*[1]	*Treatment and follow-up*	FOXP1 *and other genetic abnormality*	FOXP1 *breakpoint*	*FOXP1 immunohisto-chemistry*
1	Stomach	31/F	MALT lymphoma	Centrocyte-like cells, lymphoepithelial lesions		n/a	n/a	*FOXP1-IGH*, an extra copy of *FOXP1*	18 kb upstream of exon 1 to 54 kb downstream of exon 2	75%, strong staining in most positive cells, including those in lymphoepithelial lesions
2	Stomach	47/F	MALT lymphoma	Small and medium-sized centrocyte-like cells, lymphoepithelial lesions		I	Failed to respond *H. pylori* eradication, total gastrectomy, CR for 48 months, alive	*FOXP1-IGH*	18 kb upstream of exon 1 to 54 kb downstream of exon 2	n/a
3	Stomach	62/M	MALT lymphoma	Atypical medium-sized lymphoid cells, lymphoepithelial lesions, follicular colonisation		I	Subtotal gastrectomy, 130 months, alive	*FOXP1-IGH*	Intron 3 to 13 kb downstream of exon 5	80%, strong staining in majority of positive cells
4	Stomach	16/F	MALT lymphoma	Centrocyte-like cells, monocytoid cells, lymphoepithelial lesions, follicular colonisation		I	Subtotal gastrectomy, 117 months, alive	*FOXP1-IGH*	Intron 3 to 13 kb downstream of exon 5	80%, strong staining in most positive cells
5	Stomach	16/M	MALT lymphoma	Centrocyte-like cells, lymphoepithelial lesions, prominent plasma cell differentiation		II-1	Subtotal gastrectomy, 240 months, died of accident without evidence of lymphoma	*FOXP1* translocation with unknown partner	Intron 3–13 kb downstream of exon 5	n/a
6	Stomach	64/M	MALT lymphoma	Centrocyte-like cells, lymphoepithelial lesions, prominent plasma cell differentiation, follicular colonisation		II-1	Subtotal gastrectomy, lost in follow-up	*FOXP1-IGH*	Intron 3 to 13 kb downstream of exon 5	90%, strong staining in most positive cells, including those in IELs
7	Stomach	64/M	MALT lymphoma	Centrocyte-like cells, lymphoepithelial lesions, prominent plasma cell differentiation, pleomorphic monocytoid cells		II-2	Total gastrectomy, 2 months, died of postoperative infection	*FOXP1* translocation with unknown partner, but not with *IGH* and *IGκ* loci, an extra copy of *FOXP1* and *MALT1*	Intron 3 to 13 kb downstream of exon 5	50%, moderate staining in most positive cells
8	Stomach	48/F	MALT lymphoma	Centrocyte-like cells		I	6 cycles chemotherapy (CEOP-B), CR, but lost in follow-up	FOXP1-IGH	Intron 3 to 13 kb downstream of exon 5	n/a
9	Stomach	50/M	MALT lymphoma+diffuse large B-cell lymphoma	Small lymphocytes, aggregates of large lymphoid cells		I	*H. pylori* eradication, CR, 44 months, alive	FOXP1-IGH an extra copy of *MALT1*	Intron 3 to 13 kb downstream of exon 5	Assessed in MALT lymphoma, 60%, strong staining in majority of positive cells
10	Stomach	50/F	MALT lymphoma+diffuse large B-cell lymphoma	Centrocyte-like cells, foci of diffuse large B-cell lymphoma		n/a	n/a	*FOXP1-IGH*	60 kb upstream of exon 7 to exon 10	Assessed in MALT lymphoma, 85%, strong staining in most positive cells
11	Stomach	70/M	MALT lymphoma+diffuse large B-cell lymphoma	Centrocyte-like cells, areas with numerous large cells, foci of diffuse large B-cell lymphoma		I	Subtotal gastrectomy+multiagent chemotherapy, CR, 48 months, alive	*FOXP1* translocation with unknown partner, but not with *IGH*, *IGκ* and *IGλ*	60 kb upstream of exon 7 to exon 10	Assessed in MALT lymphoma, 85%, moderate to strong staining in most positive cells

Table 3 Continued

Case no.	*Site*	*Age/sex*	*Histological diagnosis*	*Histological features*	*CD10/BCL6/ MUM1*	*Clinical stage*[1]	*Treatment and follow-up*	FOXP1 *and other genetic abnormality*	FOXP1 *breakpoint*	*FOXP1 immunohisto-chemistry*
12	Stomach	48/F	Diffuse large B-cell lymphoma	Medium-sized and large pleomorphic cells	Germinal center B-cell-like (CD10+, BCL6+, MUM1-)	II-1	Subtotal gastrectomy+multiagent chemotherapy, CR, 84 months, alive	FOXP1-IGH an extra copy of *MALT1*	Intron 3 to 13 kb downstream of exon 5	75%, moderate staining in most of positive cells
13	Stomach	28/F	Diffuse large B-cell lymphoma	Large atypical cells	Germinal center B-cell-like (CD10-, Bcl6+, MUM1-)	II-2	Subtotal gastrectomy, 6 cycles of CEOP-B, then 4 cycles of ESHAP, 1 cycle IVAM, died 11 months after diagnosis	*FOXP1* translocation with unknown partner, but not with *IGH*, *IGκ* and *IGλ*, an extra copy of *FOXP1* and *BCL6*	Intron 3 to 13 kb downstream of exon 5	70%, moderate to strong staining in most positive cells
14	Large intestine	58/M	Diffuse large B-cell lymphoma	Large centroblastic and pleomorphic cells	Non-germinal center B-cell-like (CD10-, Bcl6+, MUM1+)	I	Right-colectomy, then 6 cycle CEOP-B chemotherapy, 28 months later, lymphoma relapse in nasopharynx, cervical and inguinal lymph nodes, treated with ESHAP but lymphoma relasped in nasal cavity, further treated with IVAM and R-COP, CR for 38 months in the last follow-up	FOXP1-IGH *BCL6* translocation	Intron 3 to 13 kb downstream of exon 5	75%, strong staining in majority of positive cells
15	Tonsil	43/M	Diffuse large B-cell lymphoma	Medium-sized atypical cells	Non-germinal center B-cell-like (CD10-, Bcl6-, MUM1+)	I	n/a	*FOXP1* translocation with unknown partner, but not with *IGH*, *IGκ* and *IGλ* an extra copy of BCL6	Intron 3 to 13 kb downstream of exon 5	95%, strong staining in most positive cells
16	Lymph node	47/M	Diffuse large B-cell lymphoma (cervical lymph nodes) relapsed as follicular lymphoma grade 3a+diffuse large B-cell lymphoma (axillary lymph nodes)	Diffuse large B-cell lymphoma: centroblastic and multilobated cells. follicular lymphoma: centrocytes and centroblasts	Non-germinal center B-cell-like (CD10-, Bcl6+, MUM1+)	II	6 cycle of CEOP-B, relapse, then treated with multiagent chemotherapy, CR, but lymphoma relapsed again and died of disease at 52 months follow-up	*FOXP1-IGH*	18 kb upstream of exon 1 to 54 kb downstream of exon-2	50%, moderate staining in majority of positive cells

CEOP-B: cyclophosphamide, epirubicin, vincristine, prednisolone and bleomycin; CR: complete remission; ESHAP: etoposide, methylprednisolone, high-dose cytarabine and cisplatin; IVAM: ifosfamide, etoposide, cytarabine and methotrexate; n/a: not available; R-COP: rituximab, cyclophosphamide, vincristine and prednisolone.

[a]According to Lugano International Conference classification for clinical staging of gastrointestinal lymphoma.[13]

Table 4 Correlation of FOXP1 expression and its genetic abnormalities in MALT lymphoma and diffuse large B-cell lymphoma

FOXP1 *genetic changes*	*No. of cases*	*Negative or FOXP1 expression in <30% cells*		*FOXP1 expression in 30–70% cells*				*FOXP1 expression in >70% cells*			
				Weak staining		*Moderate or strong staining*		*Weak staining*		*Moderate or strong staining*	
MALT lymphoma											
Translocation	5	0	0%	0	0%	1	20%	0	0%	4	80%
3 copies	19	8	42%	4	21%	3	16%	0	0%	4	21%
Normal	95	45	47%	8	8%	15	16%	3	3%	24	25%
Diffuse large B-cell lymphoma											
Translocation	5	0	0%	0	0%	1	20%	0	0%	4	80%
3 copies	32	9	28%	3	9%	5	16%	0	0%	15	47%
Normal	102	31	30%	10	30%	12	11%	2	2%	47	46%

translocation was not associated with *IGH*, further interphase FISH with *IG-κkappa* and *IG-λ* BAP probes were performed in four cases where materials were available (Table 3), and the results were negative.

Mapping *FOXP1* Breakpoints

To map the location of *FOXP1* breakpoints, further interphase FISH was performed using *FOXP1* BAP probes 2 and 3, and additionally with BAC clones C2/H15/P21. Three cases (nos. 1, 2 and 16) showed a split signal with BAP probe 2, indicating breakpoints between BAC clones O22 and M13, corresponding to the region spanning 18 kb upstream of exon 1 to 54 kb downstream of exon 2 (Figure 1). Although the precise positions of the breakpoints in these cases were unknown, the relative sizes of the exons and introns in this region suggest that these breakpoints most likely reside downstream of exon 1. Eleven cases (nos. 3–9 and 12–15) displayed a split signal with BAP probe 3, indicating breakpoints between H23 and C2, corresponding to the region spanning from intron 3 to 13 kb downstream of exon 5 (Figure 1). The remaining two cases (nos. 10 and 11) showed no evidence of split signals with BAP probes 2 and 3, but displayed split signals with BAC clones C2/H15/P21. Further interphase FISH localized the breakpoint to the region covered by H15, which spans 60 kb upstream of exon 7 to exon 10 (Figure 1). In view of the long introns 6–7 but short exons 8–10 and introns 8–9, it is likely that the breakpoints in these two cases are located upstream of exon 8.

There was no difference in the location of *FOXP1* breakpoints between MALT lymphoma and diffuse large B-cell lymphoma, or between cases with and without *IGH* as the translocation partner.

Correlation of FOXP1 Protein Expression with Its Gene Abnormalities

FOXP1 protein expression was investigated by immunohistochemistry in 119 cases of MALT lymphoma, 3 cases of *FOXP1* translocation positive MALT lymphomas with diffuse large B-cell lymphoma and 139 cases of diffuse large B-cell lymphoma, where tissues were available. Strong uniform FOXP1 staining was found in most *FOXP1* translocation-positive cases of MALT lymphoma and diffuse large B-cell lymphoma (Tables 3 and 4; Figure 2). However, such strong homogenous FOXP1 staining was also frequently seen in *FOXP1* translocation-negative lymphomas, irrespective of the presence of normal or increased copy number of the *FOXP1* gene, being significantly more frequent in diffuse large B-cell lymphoma (46%) than in MALT lymphomas (25%; $P<0.0005$; Table 4).

Discussion

Unlike t(11;18)(q21;q21)/*API2-MALT1*, t(1;14)(p22;q32)/*BCL10-IGH* and t(14;18)(q32;q21)/*IGH-MALT1* which are specifically associated with MALT lymphoma,[12,16,17] *FOXP1* translocation has been found in MALT lymphoma and diffuse large B-cell lymphoma of nodal and extranodal sites.[7–10] However, similar to these three translocations, *FOXP1* translocation also occurs at variable frequencies in MALT lymphoma of different sites. Our results, based on screening a large series of MALT lymphomas of various sites, showed that *FOXP1* translocation was associated with those of the stomach, but not other mucosal sites. In line with this, two of the five extranodal diffuse large B-cell lymphomas with *FOXP1* translocation were also from the stomach. These findings are in keeping with the results of the recent studies by Wlodarska *et al*[8] and Haralambieva *et al*.[10] In addition, we also found rare cases of MALT lymphoma and diffuse large B-cell lymphoma showing 4 or more extra copies of the *FOXP1* gene, suggesting the presence of chromosome polysomy or *FOXP1* gene amplification.[8] Nonetheless, the incidences of *FOXP1* translocation or gene amplification in MALT lymphoma and diffuse large B-cell lymphoma are low.

Detailed breakpoint analysis of t(3;14)/*FOXP1-IGH* was performed in two cases in previous studies.[7,9] In one case, the *FOXP1* breakpoint was

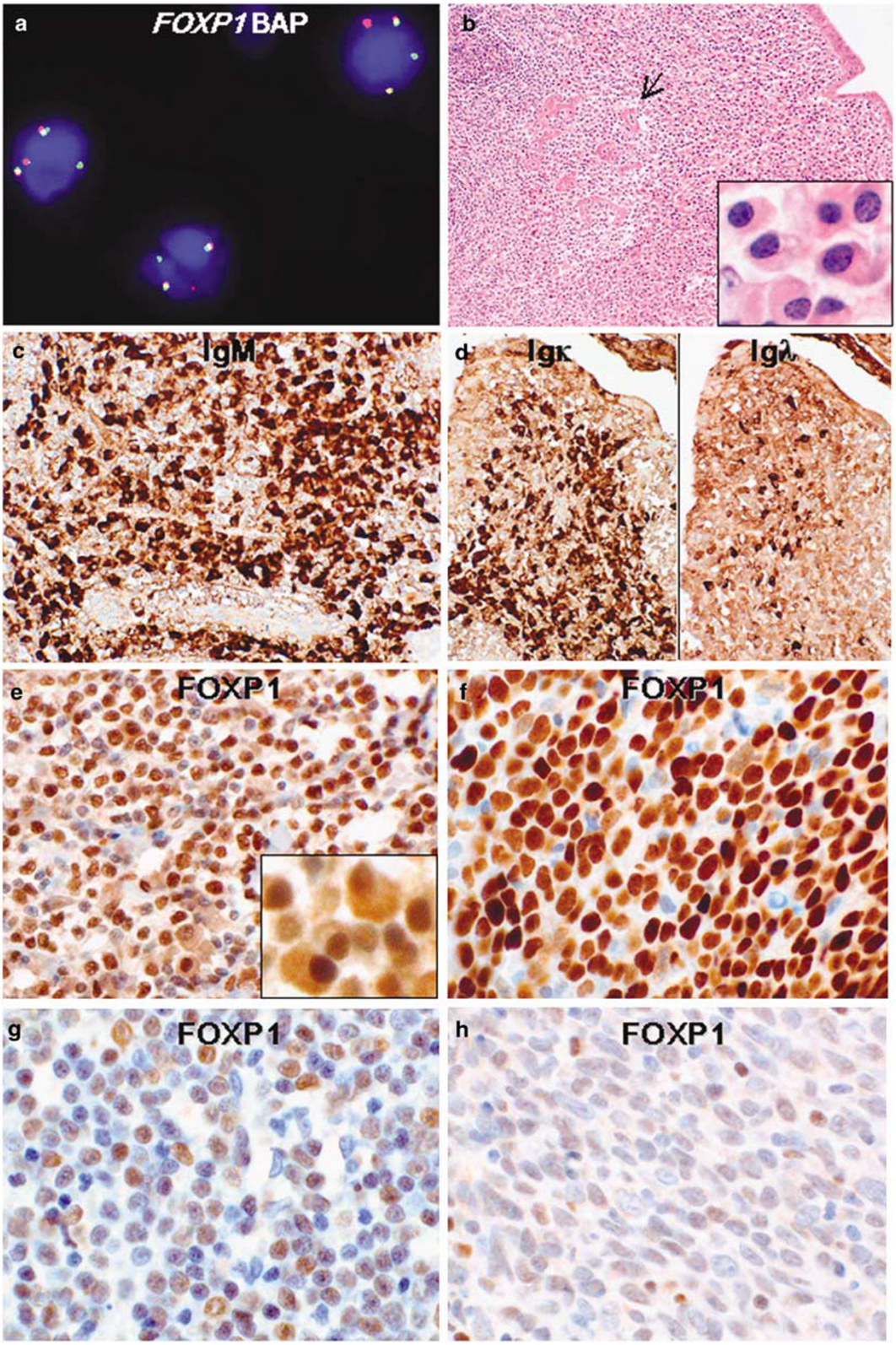

Figure 2 Examples of *FOXP1* FISH and FOXP1 immunohistochemistry. *FOXP1* interphase FISH with break-apart probe (BAP) set 1 shows a split signal and presence of an extra copy of the gene in a case of gastric MALT lymphoma (**a**). A *FOXP1* translocation positive gastric MALT lymphoma shows a diffuse infiltrate of centrocyte-like cells, prominent plasma-cell differentiation and lymphoepithelial lesions (indicated by arrow) (**b**). The neoplastic nature of plasma cells is indicated by IgM expression (**c**) and Igκ light chain restriction (**d**). FOXP1 immunohistochemistry shows strong nuclear staining in both neoplastic centrocyte-like cells and plasma cells (**e**). Examples of diffuse large B-cell lymphoma display strong (**f**), moderate (**g**) and weak (**h**) FOXP1 staining.

38 kb upstream of the first noncoding exon 1 with the remaining *FOXP1* sequence joined to *IGH* joining segment 6,[7] whereas in the other, the breakpoint was in intron 2 with the remaining *FOXP1* sequence joined to *IGH-Cα1* but in an opposite transcriptional orientation.[9] Here we show, based on analysis of 16 cases with *FOXP1* translocation, that the *FOXP1* breakpoint was within the 5′ region of the gene in most, if not all, cases. In 11 cases, the breakpoint was mapped to the region spanning intron 3–13 kb downstream of exon 5. In three cases, the breakpoint was mapped to the region spanning 18 kb upstream of exon 1–54 kb downstream of exon 2, and the relative lengths of the exons and introns in this region suggest that the breakpoints in these cases are likely to be located downstream of exon 1. In the remaining two cases, the breakpoints were mapped to a region spanning from 60 kb upstream of exon 7 to exon 10, and the relative sizes of the introns and exons in this region suggest that the breakpoints are likely to be located upstream of exon 8. Significantly, these breakpoints fall within the coding sequence of full-length *FOXP1*, which begins in exon 6. Interestingly, Wlodarska *et al*[8] reported a t(2;3)(q36;p13) involving *FOXP1* and an unknown partner gene in a case of small B-cell non-Hodgkin lymphoma, in which the *FOXP1* breakpoint was similarly mapped to the region spanning exon 5 to exon 7. Thus, most, if not all, of these *FOXP1* breakpoints separate the downstream *FOXP1* sequence from its normal promoter. Irrespective of these different breakpoints, immunohistochemistry with JC12, a monoclonal antibody to the C-terminus of FOXP1, demonstrated high-level expression of the protein in each of these translocation positive cases.

The 5′ end of the *FOXP1* gene might also be disrupted by other means during oncogenesis. In one study *FOXP1* was one of the most frequent targets of retroviral integration in myeloblastosis-associated virus type-2-induced chicken nephroblastoma.[18] Significantly, all the integration events were clustered around the second coding exon (corresponding to human *FOXP1* exon 7).[18] Typically, the retrovirus exerts its oncogenic potential by deregulating cellular gene expression or causing alterations in the cellular gene product, consequently changing its function.

The similar disruption of the 5′ sequence of the *FOXP1* gene by various chromosome translocations in human lymphoma and by retroviral integrations in the chicken nephroblastoma model suggests that these different events may activate the oncogenic potential of FOXP1 through a common mechanism of deregulating *FOXP1* transcriptional control. As mentioned above, most, if not all, of these *FOXP1* breakpoints separate the downstream *FOXP1* sequence from its normal promoter. Thus *FOXP1* translocation could result in the loss of normal full-length *FOXP1* mRNA expression, and expression of variant *FOXP1* transcript species with alternate 5′ ends, most likely under the control of an alternative internal promoter. In support of this speculation, there are 14 probable alternative promoters for *FOXP1* according to AceView (http://www.humangenes.org). In addition, at least in the case of *FOXP1* translocation with *IGH* as the translocation partner, the probability of promoter substitution is unlikely due to the opposite transcriptional orientation of the *FOXP1* and *IGH* genes as demonstrated in detail by Fenton *et al*.[9] More importantly, we have recently identified several *FOXP1* transcript variants utilizing novel 5′ noncoding exons including two variants using an alternative exon 6b and four variants using an alternative novel exon 7(a–c) in activated B-cell-like diffuse large B-cell lymphoma.[19] Furthermore, high-level expression of smaller FOXP1 proteins was found in activated B-cell-like diffuse large B-cell lymphoma by western blotting.[19] Among *FOXP1* transcript variants identified, those using an alternative novel exon 7 contain the translation initiating site (two ATG) in exon 8, and thus encode N-terminally truncated proteins (lacking approximately the first 100 amino acids of the full-length FOXP1), which was consistent with the detection of smaller FOXP1 proteins by western blotting.[19] Although little is known about the function of the human N-terminally truncated *FOXP1* isoforms, the murine FOXP1D that lacks the N-terminal polyglutamine domain exhibits altered transregulatory properties.[20] Thus, it is tempting to speculate that *FOXP1* translocations, at least those with a breakpoint mapped to intron 6–7, may lead to expression of FOXP1 isoform(s) with a truncated or alternate N-terminus.

Among the 16 cases of lymphoma with *FOXP1* translocation studied, there were no obviously uniform clinical or histological features associated with the translocation. All the MALT lymphomas with *FOXP1* translocation showed classic histological and immunophenotypic features of MALT lymphoma. Clinically, all four cases of *FOXP1* translocation-positive gastric MALT lymphoma with long-term follow up showed durable complete remissions following gastrectomy. Of the five cases of diffuse large B-cell lymphoma with *FOXP1* translocation, the histological, immunophenotypic and clinical features were also not remarkable. The markers, including CD10, Bcl6 and MUM1, commonly used for subclassification of diffuse large B-cell lymphoma into germinal center B-cell-like and non-germinal center B-cell-like subtypes were heterogeneously expressed in these cases, as described previously.[10]

Apart from deregulation of FOXP1 expression by chromosome translocation, overexpression of the protein was also frequently seen in MALT lymphoma and diffuse large B-cell lymphoma lacking the translocation and this was independent of *FOXP1* gene copy number change. Interestingly, strong uniform FOXP1 expression was seen more

frequently in diffuse large B-cell lymphoma (46%) than in MALT lymphomas (25%; Table 4), as demonstrated previously.[3–6] These data, together with detection of *FOXP1* translocation and gene amplification in these lymphomas suggest that FOXP1 deregulation may play an important role in their development. The findings that FOXP1 overexpression is independent of its gene translocation and copy number changes indicate the presence of other mechanisms that deregulate FOXP1 expression in lymphoma. As discussed above, we have recently identified several *FOXP1* transcript variants utilizing novel 5′ noncoding exons and high-level expression of smaller FOXP1 protein isoforms in activated B-cell-like diffuse large B-cell lymphoma.[19] Significantly, the expression of these transcript variants and the smaller protein isoforms can be induced in normal B cells following antigenic or mitogenic stimulation.[19] Our localization of *FOXP1* translocation breakpoints and the implications of these breakpoints for the transcriptional deregulation of *FOXP1* in lymphoma provide timely and important insights to guide future investigations of the role of FOXP1 in both B-cell biology and lymphoma development.

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