ORIGINAL ARTICLE

Unfavourable prognosis of patients with trisomy 18q21 detected by fluorescence in situ hybridisation in t(11;18) negative, surgically resected, gastrointestinal B cell lymphomas

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Background: The most frequent cytogenetic alteration in gastrointestinal (GI) B cell lymphoma (BCL) is t(11;18)(q21;q21). GI B cell non-Hodgkin lymphomas lacking this translocation vary in their biology and clinical outcome. The t(11;18) negative subgroup shows increased numerical changes of chromosome 18, although its clinical relevance remains unknown.

Methods: Thirty surgically resected primary GI BCLs were examined—11 low grade marginal zone mucosa associated lymphoid tissue (MALT) lymphomas, four marginal zone lymphomas with diffuse large BCL (DLBCL), and 15 de novo DLBCLs. Chromosome 18 aberrations were examined using interphase fluorescence in situ hybridisation. Trisomy 18 was studied applying a centromere 18 probe and a dual colour probe for the MALT1 gene at 18q21.

Results: Using the MALT1 probe, only one marginal zone MALT lymphoma had a break apart pattern, indicating t(11;18) or variants. In the GI BCLs lacking MALT1 breaks, trisomy 18q21 was seen in seven patients (four with complete trisomy 18 and three with partial trisomy of 18q21). Trisomy 18q21 was found in two of 10 low grade MALT lymphomas and five of 19 GI BCLs with large cell component. Six of 17 patients with trisomy 18q21 presented with $\geq$ stage II and one of 12 with stage I disease. Trisomy 18q21 was associated with significantly shorter disease specific survival in the whole group and GI BCLs with large cell component, but not in the low grade group.

Conclusions: Trisomy 18q21, including MALT1, may be associated with advanced tumour stage and may be a predictor of poor outcome in surgically resected primary GI BCLs.

The most frequent disease specific chromosomal aberration in mucosa associated lymphatic tissue (MALT) lymphomas is the t(11;18)(q21;q21) translocation, in which the MALT1 gene from 18q21 is fused to the API2 gene at 11q21.^{1–9} Its presence or absence discriminates two distinct biological subgroups.¹⁰ The t(11;18) translocation is restricted to cytogenetically stable, classic, low grade marginal zone lymphomas of the MALT type, which lack secondary chromosomal aberrations and are not prone to transform into diffuse large B cell lymphomas (DLBCLs).^{1–10} In contrast, t(11;18) negative MALT lymphomas constitute a biologically heterogeneous group, including a subset of t(11;18) negative extranodal, low grade, marginal zone lymphomas of the MALT type with the potential for high grade transformation, extranodal marginal zone lymphoma with coexistent transformation into DLBCL, and de novo extranodal DLBCL.¹⁰

“The most common region of gains at 18q in t(11;18) negative mucosa associated lymphoid tissue lymphomas is chromosome band 18q21, which contains the MALT1 gene”

Various chromosomal aberrations have been described in t(11;18) negative MALT lymphomas, with deletions in chromosome regions 2q, 6q, 7q, and 13q and gains in 2p, 3q, 8q, 9, 11q, 12, and 18q being the most frequent.^{10–12} Trisomy 18 was found in 48% of t(11;18) negative MALT lymphomas, particularly in those with a pulmonary localisation.¹³ The most common region of gains at 18q in t(11;18) negative MALT lymphomas is chromosome band 18q21,

which contains the MALT1 gene. To date, the prognostic value of chromosome 18 aberrations in gastrointestinal (GI) B cell lymphoma is unknown. To investigate this question we studied the prognostic impact of trisomy 18 determined by interphase cytogenetics in a well documented retrospective clinicopathological series of surgically resected GI B cell lymphomas from a single institution.

MATERIALS AND METHODS

Patients

In total, 37 patients with surgically resected GI B cell lymphomas from a single institution were analysed.¹⁴ Seven cases were excluded because they repeatedly failed to hybridise sufficiently. One additional case of low grade marginal zone lymphoma was excluded because it showed signal patterns indicating a breakpoint in the MALT1 gene, suggesting the occurrence of t(11;18)(q21;q21) or a variant. The final study group comprised 17 men and 12 women with a mean age of 69 years (range, 49–82) and 66 years (range, 41–79) for male and female patients, respectively. Ten cases were classified as low grade marginal zone B cell lymphoma of MALT type and 19 cases as GI DLBCL according to the updated World Health Organisation classification.¹⁵ In four

Abbreviations: CI, confidence interval; DLBCL, diffuse large B cell lymphoma; DSS, disease specific survival; FFS, failure free survival; FISH, fluorescence in situ hybridisation; GI, gastrointestinal; MALT, mucosa associated lymphoid tissue; OS, overall survival; PAC, phage 1 artificial chromosome

patients with GI DLBCL, a simultaneous occurrence of low grade marginal zone lymphoma indicated a transformation.

Staging was performed according to the Ann-Arbor system modified by Mushoff.¹⁶ Twelve patients presented with stage I disease, 15 with stage II, and two with stage IV disease. Apart from surgical resection, one patient was treated with radiotherapy, 16 with chemotherapy (plus *Helicobacter pylori* eradication in two cases), and two with both chemotherapy and radiotherapy (table 1). Of the six patients with low grade marginal zone lymphoma, three received chemotherapy according to COP and two patients according to COP/BLAM protocols. One patient was treated with cyclophosphamide, vincristin, and prednisolone. The CHOP regimen was administered to all 12 patients with GI DLBCL. Two of these patients were also treated with mitoxantron/prednimustin during the course of their disease.

Immunohistochemistry

Immunohistochemical characterisation was performed as reported previously.¹⁴

Fluorescence in situ hybridisation analysis

For fluorescence in situ hybridisation (FISH) analysis, we prepared single nuclear suspensions from 20 µm sections of the tumour blocks. After dewaxing with xylene and rehydration in a graded series of ethanol, four sections were digested in 0.005% proteinase K, pH 7.5, for 30–60 minutes at 37°C and mechanically disaggregated by pressing through a nylon mesh. The isolated nuclei were sedimented by centrifugation, washed, and suspended in phosphate buffered saline, and finally spotted on to slides and air dried. Sections were washed for 30 minutes in 2× saline sodium citrate at 37°C and dehydrated in ethanol before hybridisation. For interphase cytogenetic analysis, a centromere

specific probe for chromosome 18 (QBI0gene, Illkirch, France) and an 18q21 specific probe set with four phase 1 artificial chromosome (PAC) clones flanking MALT1 were used in each case. The dual colour probe set consisted of the red labelled PACs 83A16 and 119K19, which map proximally to MALT1, and the green labelled PACs 628B12 and 124N11, which map distally. This probe set allowed the highly sensitive detection of trisomies (three yellow or mixed signals), in addition to rearrangements (separated red and green signals) involving MALT1, indicating the occurrence of t(11;18), t(14;18), or variants.^{17 18} Hybridisation was performed according to standard methods. Experiments were evaluated using an epifluorescence microscope connected to a CCD camera. In each case, at least 100 cell nuclei were counted. As controls, five samples from normal gastric mucosa and 10 cases of *H pylori* associated gastritis were studied.

Statistical analysis

Statistical analysis was performed using SPSS software for Windows, version 9.0. Correlations between trisomy 18 and stage were analysed by means of the Pearson χ^2 test. Overall survival (OS), disease specific survival (DSS), and failure free survival (FFS), defined as the time from the primary diagnosis to the last follow up or death, to death because of lymphoma, and to disease relapse or death because of lymphoma, respectively, were analysed by means of the Kaplan–Meier method and compared by the log rank test.¹⁹ Cox regression analysis was performed including the variables age, stage, grade, treatment, and trisomy 18q21 (including all cases with trisomic status at the MALT1 locus independent of whether derived from complete or partial trisomy 18). p Values < 0.05 were considered significant.²⁰

Table 1 Clinical and molecular cytogenetic data of patients with t(11;18) negative gastrointestinal B cell lymphoma

Patient	Age	Sex	Location	Subtype	Stage	Treatment	Recurrence (month)	FU	Result	Chrom 18 aberration
1	80	m	Stomach	Low	EI 1	GE		219	DWOD	
2	41	f	Stomach	Low	EI 1	GE		115	AWOD	
3	81	m	Stomach	Low	EI 1	GE		39	AWOD	
4	61	m	Stomach	Low	EI 2	GE+CT		8	DWOD	
5	49	m	Large bowel	Low	EII 1	HCE+CT+RT	Locally (22)	22	DWD	+18q
6	66	f	Stomach	Low	EII 1	GE		52	DWOD	
7	66	f	Stomach	Low	EI 1	GE+CT		55	AWOD	+18
8	66	m	Stomach	Low	EII 1	GE+CT+ER		60	AWOD	
9	79	m	Stomach	Low	EIV	GE+CT+ER	Locally (10)	12	DWD	
10	70	m	Small bowel	Low	EIV	IR+CT		5	DWD	
11	51	m	Stomach	DLBCL+L	EI	GE		96	AWOD	
12	70	f	Stomach	DLBCL+L	EII 1	GE+CT		177	AWOD	+18
13	62	m	Stomach	DLBCL+L	EI 1	GE		12	DWOD	
14	71	f	Stomach	DLBCL+L	EII 1	GE+CT		57	AWOD	
15	70	f	Large bowel	DLBCL	EI 2	HCE		160	DWOD	
16	50	f	Stomach	DLBCL	EI 2	GE+RT		290	DWOD	
17	70	m	Stomach	DLBCL	EI 2	GE		1	DWD	
18	79	f	Stomach	DLBCL	EI 2	GE+CT	Brain (48)	78	AWD	
19	62	f	Stomach	DLBCL	EI 2	GE		76	AWOD	
20	74	m	Stomach	DLBCL	EI 2	GE+CT		75	AWOD	+18
21	76	f	Stomach	DLBCL	EI 2	GE+CT+RT	Locally (72)	109	AWOD	
22	68	m	Stomach	DLBCL	EII 1	GE+CT		80	AWOD	
23	82	m	Stomach	DLBCL	EII 1	GE		1	DWD	+18/+18q*
24	71	f	Stomach	DLBCL	EII 1	GE+CT	Locally (6)	6	DWD	+18q
25	68	m	Stomach	DLBCL	EII 1	GE+CT	Locally (10)	58	DWD	
26	68	m	Stomach	DLBCL	EII 1	GE+CT		80	AWOD	
27	73	f	Stomach	DLBCL	EII 1	GE+CT		24	DWOD	
28	76	m	Stomach+bowel	DLBCL	EII 1	GE+IR+CT	Locally (11)	24	DWD	+18q
29	64	m	Stomach	DLBCL	EII 1	GE+CT		24	DWOD	

FU, follow up (in months); chrom, chromosome; m, male; f, female; low, low grade marginal zone lymphoma of MALT type; DLBCL+L, diffuse large B cell lymphoma with coexistent low grade marginal zone lymphoma of mucosa associated lymphoid tissue type; DLBCL, diffuse large B cell lymphoma; GE, gastrectomy; CT, chemotherapy; HCE, hemicolectomy; RT, radiotherapy; ER, eradication of *Helicobacter pylori*; IR, intestinal resection; DWD, dead with disease; DWOD, dead without disease; AWD, alive with disease; AWOD, alive without disease; +18, complete trisomy of chromosome 18; +18q, partial trisomy of chromosome 18; +18/+18q*, mosaic pattern with 33% +18 and 40% +18q.

RESULTS

The presence of complete and partial trisomy 18 was studied by interphase FISH applying a centromere 18 probe and a dual colour probe mix for the MALT1 gene at chromosome band 18q21. In five samples of normal gastric mucosa and 10 samples of *H. pylori* associated gastritis, more than two fluorescence signals for the MALT1 probe mix or the centromere 18 probe were detected in a mean of 3.4% (SD, 3.7%) and 0.9% (SD, 1.6%) of the interphase nuclei, respectively. The cut off values for both probes, defined as mean of false positive + 3 SD, was 14.5% and 5.7% for the centromere 18 and MALT1 probes, respectively. Based on these results, and assuming a tumour cell content of at least 50% in all samples based on morphological assessment for both probes, a cut off value of 15% of nuclei with more than two signals was set for the detection of complete or partial trisomy 18 to prevent false positive results.

Only one case of low grade marginal zone lymphoma of MALT showed a break apart pattern with the MALT1 probe, probably because of a t(11;18) translocation. Of the remaining 29 GI B cell lymphomas studied, seven showed at least 23% of cells with a signal pattern indicating trisomy 18(q21) and, thus, clearly above the detection limit. A mean of 40% (range, 17–55%) and 46% (range, 23–73%) of the nuclei displayed more than two signals for the centromere 18 and the MALT1 probes, respectively. The seven cases with trisomy 18(q21) comprised two of the 10 low grade marginal zone lymphomas and five of the 19 GI MALT lymphomas with a diffuse large B cell component, indicating a similar frequency of 18q21 gains in both morphological subtypes.

Four and three cases displayed signal patterns indicating complete trisomy 18 or partial trisomy 18q21 including the MALT1 locus (fig 1), respectively. In one case with supernumerary signals for the centromere 18 probe, greatly increased numbers of nuclei with three signals for the MALT1 probe suggested coexistence of cells with complete and partial trisomy 18. FISH with centromere and locus specific probes for other chromosomes (CEP3, CEP7, MYC (8q24), CEP12, IGH (14q32), and TP53 (17p13)) ruled out hyperploidy as the cause of the trisomy 18(q21) in the seven

Figure 1 Detection of partial trisomy 18q21 in cell nuclei isolated from one case with low grade marginal zone lymphoma of the mucosa associated lymphoid tissue (MALT) type by dual colour fluorescence in situ hybridisation (FISH) using MALT1 flanking probes. In red, phage 1 artificial chromosomes (PACs) 83A16 and 119K19 proximal to MALT1; in green, PACs 628B12 and 124N11 distal to MALT1. Several DAPI stained nuclei (blue) with three copies of MALT1 (mixed red/green or yellow signal) are shown, whereas FISH with the centromere 18 probe on nuclei from this same case showed normal disomy (not shown).

cases with increased copy numbers of the MALT1 locus (data not shown).

Trisomy of the MALT1 locus, derived from either complete or partial trisomy 18, was associated with disease stage, although the results did not reach significance ($p = 0.051$); six of 17 patients who presented with at least stage II disease were trisomic, but only one of 12 patients with stage I disease was trisomic.

The median follow up time of the complete study population was 56 months (mean, 69; range, 1–290). During the period evaluated, 16 patients died, eight from the underlying GI lymphoma and eight from other causes without clinical evidence of residual lymphoma. One patient with active disease was alive after 78 months and the remaining patients were alive without evidence of disease (table 1).

Figure 2 (A) Kaplan-Meier curve of disease specific survival of all patients with gastrointestinal (GI) B cell lymphoma with regard to MALT1 status ($p = 0.0458$). (B) Kaplan-Meier curve of disease specific survival of patients with GI B cell lymphoma and a large cell lymphoma component with regard to MALT1 status ($p = 0.0447$).

Increased copy number of the MALT1 locus did not influence FFS ($p = 0.153$) or OS ($p = 0.469$) when compared by means of the log rank test, but was significantly associated with a decreased DSS (DSS for patients with a normal copy number of the MALT1 locus, 82%; mean survival time, 234 months; 95% confidential interval (CI), 184 to 284 months; DSS for patients with an increased copy number of the MALT1 locus, 43%; mean survival, 83 months; 95% CI, 23 to 144 months; $p = 0.0458$; fig 2A). There was no association between increased MALT1 gene dosage and decreased DSS time in the subgroup of low grade marginal zone lymphoma of MALT type ($p = 0.722$), although such an association was seen in the subgroup of GI lymphomas with large cell component—that is, de novo extranodal DLBCL and low grade marginal zone lymphoma with transformation into DLBCL (DSS for patients with a normal copy number of the MALT1 locus, 86%; mean survival time, 245 months; 95% CI, 189 to 302; DSS for patients with an increased copy number of the MALT1 locus, 40%; mean survival time, 77 months; 95% CI, 5–149 months; $p = 0.0447$; fig 2B). Applying Cox regression analysis to detect the independent prognostic value of age, stage (I/II v III/IV), grade (low v high), treatment (surgical resection v surgical resection + chemotherapy), and trisomy at the MALT1 locus with regard to OS, FFS, or DSS, only stage with regard to OS was significant (relative risk, 4.793; $p = 0.029$). Nevertheless, because of the small population size, the power of these Cox regression analyses was restricted and results have to be interpreted with caution.

DISCUSSION

Our study detected an unfavourable prognosis for patients with trisomy 18(q21) including the MALT1 locus in surgically resected t(11;18) negative GI B cell lymphomas using FISH analysis. In particular, GI B cell lymphomas with a large cell component showed a decreased DSS compared with patients with classic low grade marginal zone lymphoma of MALT type. Most frequently, trisomy 18(q21) was found in stage II of the disease.

The t(11;18) translocation occurs in approximately 30% of cases of low grade MALT lymphoma according to recent series,^{1–9} but in our entire group of 11 patients with low grade marginal zone lymphoma only one carried a break point in the MALT1 locus, which is in agreement with the findings of Barth *et al* (12%).¹¹ Interestingly, studies with a higher frequency of pulmonary marginal zone lymphoma showed an increased rate of up to 48%.¹³ An approximately equal sensitivity between FISH analysis and reverse transcriptase polymerase chain reaction was found when both techniques were compared.^{2,9} This was confirmed in our study by external control examinations (data not shown).

“To the best of our knowledge, our data suggest for the first time a prognostic impact of trisomy 18 in t(11;18) negative gastrointestinal B cell lymphomas, especially in those with a large cell component”

Wotherspoon and co-workers were the first to report increased occurrence of trisomy 18, as detected by interphase FISH, in nine patients in a large series of 70 classic low grade marginal zone lymphoma of MALT type.²¹ In the GI tract, the authors detected trisomy 18 in 27% of patients. These findings were extended by other authors to extranodal DLBCL.^{12–21–23} In addition, comparative genomic hybridisation analysis also revealed chromosome 18 aberrations in extranodal marginal zone lymphomas and extranodal DLBCL, but to a lesser extent.¹¹ In a careful analysis with discrimination of t(11;18) positive lymphomas, an increased trisomy 18 rate

Take home messages

- Using a centromere 18 probe, together with MALT1 probes, we detected an increased rate of partial trisomies of the long arm of chromosome 18, including band q21, in which the MALT1 gene locus is situated in patients with gastrointestinal (GI) B cell lymphomas
- Trisomy 18 was associated with a significantly shorter disease specific survival time in t(11;18) negative GI B cell lymphomas, especially in those with a large cell component
- Trisomy 18q21 affecting the MALT1 locus might serve as a predictor for poor outcome and increased tumour stage in these patients

of 48% was reported for pulmonary lymphomas, similar to the t(11;18) translocation and to the initial data of Wotherspoon.^{13–21} We examined lymphomas of the GI tract exclusively and found chromosome 18 aberrations in almost one quarter of the samples, with approximately equal percentages in low grade marginal zone lymphomas (two of 10) and in GI B cell lymphomas with a large cell component (five of 19). This is a higher rate than in most other studies with GI B cell lymphomas (10%), possibly because we used both a centromere 18 probe and a dual colour probe mix for the MALT1 gene in chromosome band 18q21, which also detects partial trisomies.^{21–27} The long arm of chromosome 18 with the MALT1 locus is of special interest, because an increased copy number of chromosome 18 might represent an alternative mechanism for deregulation of MALT1 expression via a gene dosage effect in patients with t(11;18) negative GI MALT lymphomas. This hypothesis is supported by a recent study, which confirmed that amplification of the MALT1 locus leads to deregulation of MALT1 expression, and may be an important oncogenic event in the pathogenesis of GI B cell lymphoma.¹⁸ In addition, interactions with abnormalities of other chromosomes such as chromosomes 3, 7, and 12 may be possible.^{21–23}

Compared with their nodal counterparts, GI B cell lymphomas show a less aggressive clinical behaviour, with five year survival rates of almost 80%.^{28–30} There has been much controversy over the prognostic impact of different factors for primary GI B cell lymphomas during the past few years.^{28–30–37} Although most studies conclude that stage is an important predictor of survival, results concerning histological subtype are contradictory.^{28–32–37–41} To date, no tumour specific markers have been detected in GI B cell lymphomas, with the exception of p53 expression as an indicator of poor prognosis.^{14–42} Therefore, our finding of decreased DSS in patients with chromosome 18 aberrations indicate that such aberrations could provide the first molecular marker in GI B cell lymphomas with diffuse large cell component. In our study, we found similar incidences of trisomy 18(q21) in low grade marginal zone lymphoma (two of 10), in DLBCL with low grade lymphoma component (one of four) and pure DLBCL (four of 15).

In summary, to the best of our knowledge, our data suggest for the first time a prognostic impact of trisomy 18 in t(11;18) negative GI B cell lymphomas, especially in those with a large cell component. Using a centromere 18 probe, together with MALT1 probes, we detected an increased rate of partial trisomy of the long arm of chromosome 18, including band q21, in which the MALT1 gene locus is situated. Future studies using similar probe sets need to be undertaken in larger and differently treated series of patients with GI B cell lymphoma to confirm whether or not trisomy

18q21 affecting the MALT1 locus might serve as predictor for poor outcome and increased tumour stage.

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