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Distinct comparative genomic hybridisation profiles in gastric mucosa-associated lymphoid tissue lymphomas with and without t(11;18)(q21;q21)

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Summary

t(11;18)(q21;q21) occurs specifically in mucosa-associated lymphoid tissue (MALT) lymphoma and the translocation generates a functional API2-MALT1 fusion product that activates nuclear factor (NF)κB. t(11;18) positive lymphomas usually lack the chromosomal aberrations and microsatellite alterations frequently seen in the translocation-negative MALT lymphomas. To further understand their genetic differences, we investigated gastric MALT lymphomas with and without t(11;18) by comparative genomic hybridisation. In general, both chromosomal gains and losses were far more frequent in t(11;18)-negative (median = 3·4 imbalances) than t(11;18)-positive cases (median = 1·6 imbalances), with gains being more frequent than losses. Recurrent chromosomal gains involving whole or major parts of a chromosome were seen for chromosomes 3, 12, 18 and 22 (23%, 19%, 19% and 27% respectively). Discrete recurrent chromosomal gains were found at 9q34 (11/26 = 42%). Bioinformatic analysis of genes mapping to 9q34 revealed potential targets. Among them, TRAF2 and CARD9 are known interaction partners of BCL10, playing a role in NFκB activation. Interphase fluorescent *in situ* hybridisation confirmed genomic gain of the TRAF2, CARD9 and MALT1 loci in 5/6 and 2/2 cases showing chromosomal gains at 9q34 and 18q21 respectively. The results further highlight the genetic difference between MALT lymphomas with and without t(11;18). Moreover, our findings suggest that genomic gain of genes that modulate NFκB activation, such as *MALT1*, *TRAF2* and *CARD9*, may play a role in the pathogenesis of the translocation-negative MALT lymphoma.

Keywords: mucosa-associated lymphoid tissue lymphoma, t(11;18), comparative genomic hybridisation, *TRAF2*, *CARD9*, *MALT1*.

Three recurrent chromosomal translocations have been identified in mucosa-associated lymphoid tissue (MALT) lymphoma, namely t(11;18)(q21;q21), t(1;14)(p22;q32) and t(14;18)(q32;q21). t(11;18) involves the *API2* and *MALT1* genes and generates a functional API2-MALT1 fusion product (Akagi *et al*, 1999; Dierlamm *et al*, 1999; Morgan *et al*, 1999). t(1;14) and t(14;18) juxtapose the *BCL10* and *MALT1* gene, respectively, to the immunoglobulin gene locus in 14q32 leading to deregulated expression of the oncogene (Willis *et al*, 1999; Zhang *et al*, 1999; Sanchez-Izquierdo *et al*, 2003; Streubel *et al*, 2003). The oncogenic activities of the three chromosome translocations are linked by the physiological role of BCL10 and MALT1 in antigen receptor-mediated nuclear factor (NF)κB activation (Isaacson & Du, 2004). The three chromosome translocations occur at markedly variable incidences in MALT lymphoma of different sites but are always mutually exclusive (Isaacson & Du, 2004). Among the three chromosome translocations, t(11;18) is the most frequent, occurring most often in those from the lung (40%) and stomach (30%), moderate in those from the ocular adnexae (15%), but rarely in those from the salivary gland, thyroid and skin (Ye *et al*, 2003; Streubel *et al*, 2004a; Ye *et al*, 2005).

There is growing evidence suggesting that t(11;18) positive cases are distinct from other MALT lymphomas, including those with t(1;14) or t(14;18). t(11;18) positive MALT lymphoma rarely undergoes high grade transformation (Remstein *et al*, 2002; Chuang *et al*, 2003) despite that the translocation is significantly associated with cases at advanced

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stages and those not responding to *Helicobacter pylori* eradication (Liu *et al*, 2000; Liu *et al*, 2002). Cytogenetically, t(11;18) positive tumours usually do not show other chromosomal aberrations, such as trisomies 3 and 18, frequently seen in t(11;18) negative tumours including those positive for t(1;14) and t(14;18) (Auer *et al*, 1997; Ott *et al*, 1997; Barth *et al*, 2001; Barth *et al*, 2002; Remstein *et al*, 2002). Furthermore, t(11;18) MALT lymphomas do not show microsatellite alterations, which frequently occur in the translocation-negative tumours (Starostik *et al*, 2002). To further characterise the genetic differences between MALT lymphoma with and without t(11;18), we compared the chromosomal gains and losses between 9 t(11;18) positive, 2 t(1;14) positive and 15 translocation-negative gastric MALT lymphomas using comparative genomic hybridisation (CGH). Interphase fluorescent *in situ* hybridisation (FISH) was applied to confirm the recurrent chromosomal changes.

Materials and methods

Materials

A total of 26 cases of well-characterised gastric MALT lymphoma were included in this study. All these cases were previously studied for the presence of t(11;18), t(1;14) and t(14;18): t(11;18) was investigated by reverse transcription polymerase chain reaction (RT-PCR) of RNA samples extracted from frozen tissues (Ye *et al*, 2003), while t(1;14) and t(14;18) were examined by BCL10 and MALT1 immunohistochemistry followed by interphase FISH (Ye *et al*, 2003; Ye *et al*, 2005). Nine cases were positive for t(11;18), two cases positive for t(1;14) and the remaining 15 cases were negative for all the three translocations. Frozen tumour tissues were available in each case. The use of redundant archival tissues for research was approved by the local ethics committee of each authors' institution.

DNA extraction and labelling

The proportion of tumour cells was estimated on haematoxylin and eosin slides and only tissue specimens containing more than 70% tumour cells were used for DNA extraction. Where necessary, crude dissection was carried out in order to enrich the tumour cells (Pan *et al*, 1994). Tumour DNA was extracted from frozen tissue sections using the Wizard® genomic DNA purification kit (Promega, Southampton, UK). Reference DNA was prepared from normal peripheral blood lymphocytes using a QIAGEN blood and cell culture DNA kit (QIAGEN, West Sussex UK). DNA was quantified using GeneQuant pro (Amersham pharmacia biotech, Cambridge, UK). DNA from the breast cancer cell line MPE 600 containing gain of 1q, small deletion at 1pter and distal 11q, and loss of 9p and 16q (Vysis, Surrey, UK) was used as a positive control.

The tumour and reference DNAs were labelled with SpectrumRed dUTP and SpectrumGreen dUTP (Vysis, Surrey, UK), respectively, by nick translation as described previously (Lu *et al*, 1997). Briefly, the reaction was carried out in a 50 μl reaction mixture containing 1 μg tumour or reference DNA, 20 μmol/l of each dATP, dCTP and dGTP, 10 μmol/l of dTTP, 10 μmol/l of SpectrumRed dUTP (for tumour DNA) or SpectrumGreen dUTP (for reference DNA), 5 mmol/l of $MgCl_2$, 10 mmol/l of β-mercaptoethanol, 10 μg/ml of bovine serum albumin and 50 mmol/l of Tris–HCl (pH 7·2), 0·05 U DNase I (Promega, Madison, WI, USA) and 5 U DNA polymerase I (Promega, Madison, WI, USA) at 15°C for 3 h. The reaction was stopped by addition of 3 μl 0·5 mol/l of EDTA. The probe was purified with MicroSpin G-50 column (Amersham Biosciences, Piscataway, NJ, USA) and checked on 1% agarose gels. Under the above conditions, the probes were typically in the range of 0·3–3·0 kb.

Comparative genomic hybridisation and digital image analysis

The labelled tumour and reference DNA (500 ng each) were mixed with 25 μg Cot-1 human DNA (Invitrogen, Paisley, UK), precipitated with sodium acetate and ethanol and dissolved in 10 μl of hybridisation buffer containing 70% formamide, 10% dextran sulphate and 2 × saline sodium citrate (SSC, pH7·0). The DNA samples were denatured at 77°C for 5 min and immediately applied onto normal metaphase spreads that had been just undergone denaturation in 70% formamide/2 × SSC (pH 7·0) and dehydration in series of ethanol. The hybridisation was carried out under a sealed coverslip for 2–3 days at 37°C in a moist chamber. The slides were then sequentially washed in 0·4 × SSC/0·3% (octylphenoxy)polyethoxyethanol (IGEPAL, pH 7·0) (Sigma, St Louis, MO, USA) at 72°C for 2 min, 2 × SSC/0·1% IGEPAL at 42°C for 5 min, and in distilled water at room temperature. Finally, slides were mounted using antifade medium with 4′,6-diamidino-2-phenylindole (DAPI, Vector Laboratories Inc., Burlingame, CA, USA).

Digital images were captured using a cooled CCD-camera (Photometrics; Tuscon, AZ, USA) with QUIPS software (Version 3·1.2., Vysis, Richmond Surrey, UK) linked to a Zeiss Axioplan epifluoresence microscope (ZEISS, Jena, Germany). Between 6 and 10 images of high quality metaphases in each case were analysed using the same software package. The relative genomic gains and losses were determined by comparing the ratio of red (tumour DNA) and green (reference DNA) fluorescence intensity along all chromosomes and the thresholds used for record of chromosomal gains and losses were 1·20 and 0·80, respectively, according to previous studies (Lu *et al*, 1997; Lu *et al*, 1999). The heterochromatic regions, p-arms of acrocentric chromosomes, and the entire X and Y chromosomes were excluded from analysis.

Interphase fluorescent in situ hybridisation

For further analysis of the most frequent chromosomal gains (9q34 and 18q21) detected by CGH analysis, we performed

interphase FISH as detailed below. For chromosomal gains at 18q21, *MALT1* is probably the gene or one of the genes targeted (Sanchez-Izquierdo *et al*, 2003) and interphase FISH was carried out using the commercial LSI *MALT1* dual colour break apart probe (Vysis/Abbott Laboratories Ltd, UK).

Bioinformatic analysis identified several potential target genes in the commonly gained band 9q34. These included the genes encoding tumour necrosis factor (TNF) receptor-associated factor 2 (TRAF2), caspase recruitment domain family member 9 (CARD9), cyclin-dependent kinase 9, G protein-coupled receptor, v-abl Abelson murine leukemia viral oncogene homolog 1 (ABL), Rap guanine nucleotide exchange factor (GEF) 1, growth factor independent 1B (potential regulator of CDKN1A) and Notch homolog 1 (NOTCH1). Among them, TRAF2 and CARD9 seem to be the most relevant in view of their interaction with BCL10 and their role in NFκB activation (Bertin *et al*, 2000; Yoneda *et al*, 2000). BAC clones RP11-83N9 and RP11-100C15 as well as RP11-251M1 flanking the CARD9 and NOTCH1 loci were selected as interphase FISH probes. The clones were differentially labelled with Spectrum Green (RP11-83N9/RP11-100C15) and Spectrum Red (RP11-251M1) and pooled to obtained a break-apart assay. Similarly, BAC clones RP11-83N9/RP11-100C15 were combined with BAC clone RP11-417A4, which is located telomeric of the TRAF2 locus. In combination, both assays enable the detection of breakpoints affecting the CARD9/NOTCH1 locus or the TRAF2 locus as well as the assessment of copy number changes. Bacterial culture, BAC DNA isolation and labelling, probe preparation were preformed as previously described (Martin-Subero *et al*, 2002). These probes were first tested on metaphase spreads to confirm the location and specificity of the probe. Then they were applied to formalin-fixed and paraffin-embedded tissue sections from 8 to 10 reactive tonsils to determine the thresholds (mean of false positives + 3 SD) to diagnose chromosomal alterations in lymphoma cells (Martin-Subero *et al*, 2003).

Locus-specific interphase FISH was performed on paraffin-embedded tissue sections. This was carried out essentially as described previously (Ye *et al*, 2003). Briefly, deparaffinised sections were pre-treated by pressure-cooking for 2 min and 40 s in 1 mmol/l EDTA buffer pH 8·0 and subsequently incubated in pepsin solution (80 μg/ml) at 37°C for 20 min to increase the probes accessibility. Sections were then fixed in 1% paraformaldehyde for 1 min at room temperature, washed twice in double distilled water, dehydrated through increasing ethanol series, and air-dried. The appropriate probe mixture was applied to tissue sections and sealed with a coverslip. Both probe and target DNA were denatured at 80°C for 25 min and hybridisation was carried out at 37°C for 3 days. After hybridisation, the slides were washed with 0·4 × SSC/0·3% IGEPAL (pH 7·0) (Sigma) at 72°C for 2 min, in 2 × SSC/0·1% IGEPAL at room temperature for 1 min and finally in 2 × SSC. Slides were mounted using Vectashield anti-fade medium with DAPI (Vector Laboratories Inc.). The image acquisition and processing was performed using a fluorescent microscope with cooled CCD-camera (Olympus, BX61, Tokyo, Japan) and CYTOVISION software (Version: 2·75, Applied Imaging International Ltd, Newcastle, UK). The FISH slides were viewed and the hybridisation signals for each probe were counted from 100 cells in each case by two investigators independently.

Statistical analysis

The statistical difference in the percentage of cells showing three or more copies of a gene locus by interphase FISH among different groups was investigated by non-parametric Mann–Whitney *U*-test. *P*-values <0·05 were considered significant.

Results

Distinctive CGH profiles in gastric MALT lymphomas with and without t(11;18)

Among the 26 cases studied, one t(1;14) positive case (case 18) was shown to have trisomies 3, 12 and 18 by previous karotyping analysis (Willis *et al*, 1999). CGH analysis of the same case confirmed gains of chromosome 3, 12 and 18, and also revealed losses at 7p12-q21 (Table I).

In general, both chromosomal gains and losses were far more frequent in t(11;18) negative than positive cases, with chromosomal gains being more frequent than chromosomal losses (Fig 1, Table I). Of the nine t(11;18) positive cases, only five cases showed imbalances. Remarkably, all these imbalances were chromosomal gains. In contrast, all 17 t(11;18) negative cases exhibited chromosomal imbalances (Fig 1, Table I). The median number of imbalances was much higher in t(11;18) negative ($n = 3{\cdot}4$ imbalances) than t(11;18) positive cases ($n = 1{\cdot}6$ imbalances). The median number of chromosomal gains and losses in t(11;18) negative cases was 2·64 and 0·76 respectively.

Recurrent gains involving whole or major parts of a chromosome were seen for chromosomes 3, 12, 18 and 22, and these gains were nearly exclusively seen in t(11;18) negative cases. For example, gain of whole or major parts of chromosome 3 and 18 was found in 6/17 (35%) and 5/17 (29%) t(11;18) negative cases, respectively, including those with t(1;14), but not in any of the t(11;18) positive cases. Discrete recurrent chromosomal gains affecting the tip of 9q were detected in 11/26 cases (42%) including 4 t(11;18) positive cases (Fig 1, Table I). The minimum overlapping region was at band 9q34.

Confirmation of chromosomal gains at 9q34 and 18q21 by interphase FISH

To confirm the CGH results, interphase FISH was performed with probes for the MALT1 locus as well as two assays for 9q34 on 4 μm paraffin-embedded tissue sections. The cut-off value (mean + 3 SD) for *MALT1*, *TRAF2* and *CARD9* gene probes were 3·4%, 8·4% and 5·1% respectively.

Table I. Chromosomal gains and losses in gastric MALT lymphomas with and without t(11;18) as shown by CGH analysis.

		Chromosome changes		Percentage cells showing 3 or more signals by interphase FISH		
Case no.	Translocation	Gains	Losses	LSI MALT1 (%)	RP11-83N9/ RP11-100C15 and RP11-417A4 (CARD9/NOTCH1/ TRAF2 BAP) (%)	RP11-83N9/RP11-100C15 and RP11-251M1 (CARD9/NOTCH1 BAP) (%)
Cut off				3·4	8·4	5·1
1	t(11;18)+ve			0	1	3
2	t(11;18)+ve			0	2	1
3	t(11;18)+ve			0	1	3
4	t(11;18)+ve			0	0	2
5	t(11;18)+ve	8q22, 16p		1	–	–
6	t(11;18)+ve	9q33-34, 22q		1	18	22
7	t(11;18)+ve	9q33-ter		0	20	4
8	t(11;18)+ve	1p33-ter, 9q34, 11p15, 20q, 22q		0	1	2
9	t(11;18)+ve	9q34, 17p12-ter, 17q23-ter, 22q		–	–	–
10	trans −ve	6p		–	–	–
11	trans −ve	3		3	0	2
12	trans −ve	6q24-ter, 12q24, 16, 17, 20q, 22q		2	25	11
13	trans −ve	3		0	–	–
14	t(1;14) +ve	12		68	1	2
15	trans −ve	2p13-16, 3	2p11-12, 4q, 5p15, 13q, 14q31-32	1	1	2
16	trans −ve	2p13-22, 18q, 21q	2p11-12, 8p	–	–	–
17	trans −ve	18q		–	–	–
18	t(1;14)+ve	3, 12, 18	7p12-q21	60	1	2
19	trans −ve	3, 18		53	–	–
20	trans −ve	9q	6q25-27, 8p21	–	–	–
21	trans −ve	9q34, 12q23-ter		–	–	–
22	trans −ve	3p11-14, 7q11, 9q34, 12q24, 15q25-26, 18q11-21, 22q		–	–	–
23	trans −ve	9q34, 22q		2	–	–
24	trans −ve	9q34, 14q32, 22q11·2		0	23	9
25	trans −ve	1q21-24, 9q, 10q22-24, 15q22-24, 20q	3p23-ter, 4q	1	32	6
26	trans −ve	9q33-34, 11q11-13, 16p11-ter, 17p	4q11-26	0	20	15

–, not done; CGH, comparative genomic hybridisation; MALT, mucosa-associated lymphoid tissue; FISH, fluorescent *in situ* hybridisation.

It has to be stated, that in sections of reactive tonsils used as controls, up to 38% (standard deviation = 6·56) of cells showed only 1 signal with the different FISH probes. This indicates that truncated nuclei, as a result of tissue sectioning, may lead to an significant underestimation of the true percentages of cells harbouring three or four copies of the loci investigated.

Interphase FISH with two dual colour breakapart probes for 9q34, allowing detection of breakpoints affecting the TRAF2 and CARD9 loci as well as chromosomal imbalances was performed separately in 15 cases where adequate tissues were available. These included six cases with chromosomal gain at 9q34 and 9 without evidence of chromosomal gain at this locus by CGH. Of the nine cases without CGH evidence of chromosomal gain at 9q34, one case (no. 12) showed FISH signals that indicated three or four copies of both the *TRAF2* and *CARD9* genes in a proportion of cells well above the threshold (mean + 3 SD) respectively (Table I). In contrast, 5/6 cases with chromosomal gain at 9q34 displayed FISH signals indicating three or more copies of the *TRAF2* and *CARD9* gene (Fig 2, Table I). In support of this, the percentage of cells with three or four copies of the *TRAF2* and *CARD9* gene was significantly ($P = 0{\cdot}028$ and $P = 0{\cdot}028$, respectively) higher in cases with CGH gain at 9q34 than those without CGH gain at this locus. There was no evidence for a chromosomal break in this region in all cases examined.

Similarly, interphase FISH with the MALT1 probe showed extra-copy of the gene in 2/2 cases that had CGH evidence of chromosomal gain at 18q21. In contrast, only 1 of the 17 cases without CGH evidence of chromosomal gain at 18q21 showed

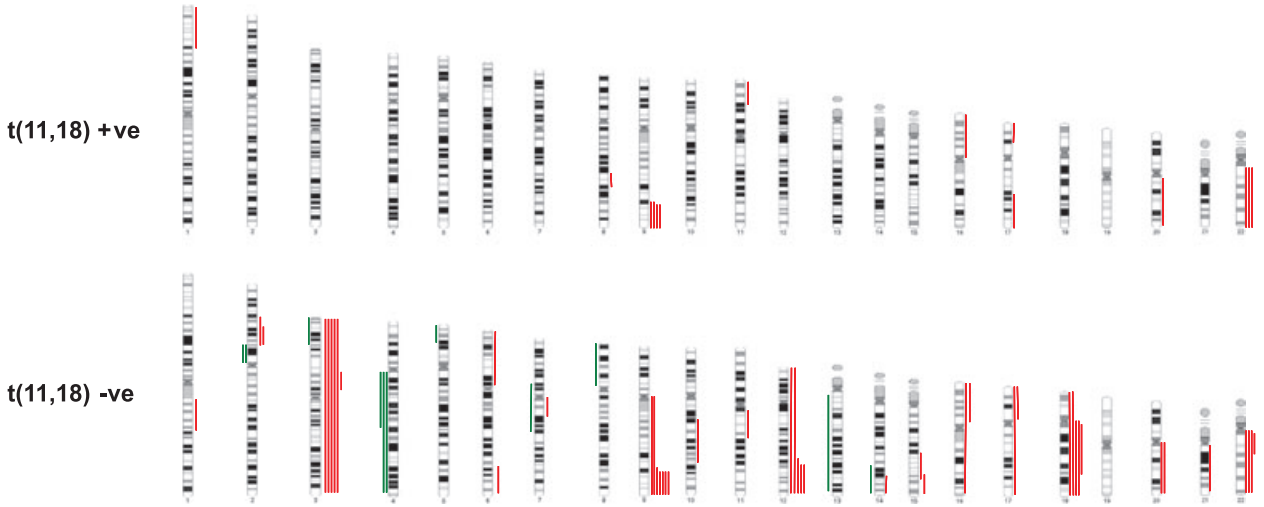

Fig 1. Comparison of chromosomal gains and losses between gastric mucosa-associated lymphoid tissue lymphomas with and without t(11;18) as revealed by comparative genomic hybridisation analysis. Both chromosomal gains (red lines) and losses (green lines) are far more frequent in t(11;18) negative than positive cases.

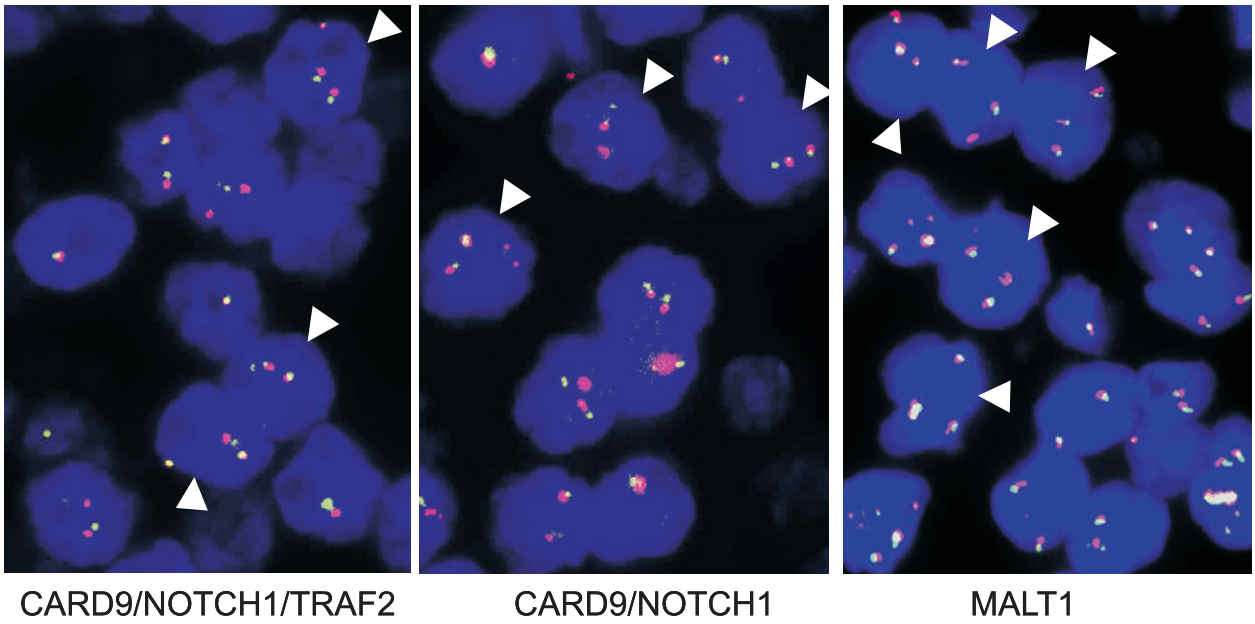

Fig 2. Confirmation of chromosomal gain at 9q34 by interphase fluorescent *in situ* hybridisation (FISH) with probes flanking the TRAF2 and CARD9 loci, and chromosomal gain at 18q21 by interphase FISH with MALT1 probe. A t(11;18) negative case (no. 26) with comparative genomic hybridisation (CGH) gain at 9q34 showing three co-localised green (BAC clones RP11-83N9/RP11-100C15) and red (RP11-417A4) signals (arrowheads), indicating three copies of the 9q34 region including CARD9, NOTCH1 and TRAF2 (left panel). The same case displays three co-localised green (BAC clones RP11-83N9/RP11-100C15) and red (RP11-251M1) signals (arrowheads) for the probe flanking the CARD9 and NOTCH1 genes (centre panel). A t(11;18) negative case (no. 18) with CGH gain at chromosome 18 shows three co-localised green and red signals with MALT1 probe in several cells (indicated by arrowheads), indicating gain of extra-copy of the *MALT* gene (right panel).

more than two copies of the *MALT1* gene. The percentage of cells with three or four copies of the *MALT1* gene was significantly higher in cases with CGH gain at 18q21 than those without ($P = 0{\cdot}04$). As expected, all cases with known t(11;18), but not those without the translocation showed signal split with the MALT1 probe.

Discussion

Our present CGH analysis showed further evidence of genetic differences between gastric MALT lymphomas with and without t(11;18). In general, chromosomal gains and losses, particularly the former, are a feature of t(11;18) negative

gastric MALT lymphoma, but not of those with the translocation. Our findings are in line with previous observations that trisomies 3, 12 and 18 are frequently seen in t(11;18) negative MALT lymphoma, but rarely in those positive for this translocation (Auer *et al*, 1997; Ott *et al*, 1997; Remstein *et al*, 2002; Streubel *et al*, 2004a).

Given that t(11;18) is most probably the primary genetic event underlying the malignant transformation, it was intriguing to see no accumulation of further chromosomal abnormalities in t(11;18) positive tumour cells. A possible explanation is that the factors promoting the acquisition of genetic abnormalities, such as oxygen reactive species generated during the inflammatory processes, are no longer evident in the microenvironment where t(11;18) positive tumour cells expand. In support of this hypothesis, t(11;18) positive gastric MALT lymphomas gain "autonomous growth" and are no longer critically dependent on antigenic stimulations as shown by their resistance to *H. pylori* eradication (Liu *et al*, 2000; Liu *et al*, 2002). Histologically, t(11;18) positive MALT lymphomas are characterised by more homogenous tumour cells, lack of transformed blasts, as compared with translocation negative cases, suggesting that chronic antigenic stimulation/inflammatory process is unlikely to be prominent in the translocation positive cases (Okabe *et al*, 2003).

The lack of additional chromosomal abnormalities in t(11;18) positive MALT lymphomas may explain, at least partially, the finding that these tumours rarely undergo high grade transformation (Remstein *et al*, 2002; Chuang *et al*, 2003). The chromosomal aberrations found in t(11;18) negative MALT lymphomas also provide clues for the pathogenesis of these tumours. Among the recurrent changes, gains at chromosome 18q and 9q34 are particularly interesting. For chromosomal gain at 18q21, *MALT1* gene has been proposed as the target gene or one of the target genes (Sanchez-Izquierdo *et al*, 2003). *MALT1* gene amplification has been demonstrated in cell lines from splenic marginal zone B-cell lymphoma and Burkitt's lymphoma (Sanchez-Izquierdo *et al*, 2003). Although *MALT1* gene amplification is rarely seen in primary lymphoma (Sanchez-Izquierdo *et al*, 2003), gain of an extra copy of the gene is a frequent event in MALT lymphoma as shown in the present study and others (Murga Penas *et al*, 2003; Remstein *et al*, 2004; Streubel *et al*, 2004b).

For chromosomal gain at 9q34, our data suggest that *TRAF2* and *CARD9* may be the target genes. Similar to the *MALT1* gene, gain of additional copy of *TRAF2* and *CARD9*, but not its amplification, was seen in 7/17 (41%) of t(11;18) negative gastric MALT lymphomas. In a parallel study, we also found gain of additional copy of chromosome 9q34/TRAF2/CARD9 and 18q21/*MALT1* in the majority of salivary gland MALT lymphomas, which are negative for t(11;18) (Zhou *et al*, 2004). Chromosomal gains at 9q34 and 18q21 have been previously demonstrated in cases of MALT lymphoma (Dierlamm *et al*, 1997). Gains at 9q34 is also a feature of enteropathy type T-cell lymphoma (Zettl *et al*, 2002).

The role of the above chromosomal gains in the pathogenesis of t(11;18) negative MALT lymphoma is unclear. They may exert their oncogenic activities by enhancing NFκB activation similar to that implicated in the translocation-positive MALT lymphoma. Mounting evidence indicates that the oncogenic activity of t(11;18), t(1;14) and t(14;18) are linked by the physiological role of BCL10 and MALT1 in antigen receptor-mediated NFκB activation (Isaacson & Du, 2004). These different chromosomal translocations are believed to exert their oncogenic activities by constitutive activation of NFκB, a transcriptional factor for a number of growth factors, cytokines and apoptosis inhibitors. In this context, it is worth noting that both TRAF2 and CARD9 have been shown to interact with BCL10 and activate NFκB (Bertin *et al*, 2000; Yoneda *et al*, 2000). TRAF2 plays a central role in TNF receptor-mediated NFκB activation during the innate immune responses (Chen, 2005). Recent studies suggest that TRAF2 may also play an important role in antigen receptor-mediated NFκB activation during adaptive immune responses. It is believed that TRAF2 may, like TRAF6, catalyse the Lys 63-linked polyubiquitination of NEMO (IKKγ) in response to upstream signals from BCL 10/MALT1, which is a crucial step leading to NFκB activation (Sun *et al*, 2004; Chen, 2005). Although TRAF2 expression in MALT lymphoma is unknown, the protein is abundantly expressed in various lymphoma cell lines and in Hodgkin/Reed–Sternberg cells of Hodgkin lymphoma (Zapata *et al*, 2000; Murray *et al*, 2001). The role of CARD9 in NFκB activation pathway remains to be investigated. Nonetheless, a recent study has shown over-expression of *CARD9* mRNA in gastric MALT lymphoma (Nakamura *et al*, 2005), in keeping with the notion proposed in the current study.

It has been shown that positive regulators, such as BCL10 and MALT1, have synergistic effects in their activation of the NFκB pathway (McAllister-Lucas *et al*, 2001). Furthermore, these positive regulators are also synergistic with physiological stimulations, such as CD40 in NFκB activation (Ho *et al*, 2004). It is tentatively speculated that gain of extra copy of *TRAF2*, *CARD9*, *MALT1* and others yet to be identified may bear synergistic effects among themselves as well as with physiological stimulations in NFκB activation and lead to the same biological consequence as that of the chromosomal translocations discussed above.

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