Aberrant promoter methylation in gastric lymphomas

Aberrant gene promoter methylation of *p15*, *p16*, *p73*, *VHL*, *caspase 8*, and *hMLH1* was investigated in gastric mucosa-associated lymphoid tissue (MALT) and diffuse large B cell (DLBC) lymphomas, with nodal marginal zone B-cell (MZBC, counterpart of MALT) and DLBC lymphomas studied in comparison. MALT/MZBC lymphomas shared similar methylation patterns, with more frequent *p15* and *p16* methylation than gastric/nodal DLBC lymphomas, which themselves had comparable methylation patterns. Therefore, gastric MALT appeared biologically similar to nodal MZCB lymphoma, but distinct from gastric DLBC lymphoma. Gastric DLBC is more prevalent than MALT lymphoma in our population. Our results suggested that this might not represent a higher transformation rate of the latter to the former.

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The stomach is the commonest site of extranodal B-cell non-Hodgkin's lymphoma (NHL). The commonest histologic subtypes are diffuse large B-cell (DLBC) lymphoma and mucosa-associated lymphoid tissue (MALT) lymphoma. In the West, MALT lymphoma associated with *Helicobacter pylori* (*H. pylori*) infection is more prevalent, occurring in 75% of cases.[1] However, DLBC lymphoma is more frequent in Chinese patients, accounting for about 60% of gastric NHL.[2] The lower frequency of gastric MALT lymphoma is not related to *H. pylori* prevalence, as the infection is as common in China as it is the West.[2] The reason for this difference remains undefined. It is known that MALT lymphomas may become *H. pylori*-independent and transform into DLBC lymphomas.[1] Therefore, the frequencies of DLBC and MALT lymphomas may be affected by this transformation. Recently, epigenetic gene silencing by CpG island hypermethylation has been shown to be important in cancer formation.[3] Using an approach involving candidate genes that have been shown to be hypermethylated in various tumors, we investigated the patterns of aberrant promoter methylation in gastric DLBC and MALT lymphoma. As a comparison, we also studied nodal DLBC and marginal zone B-cell (MZBC, the nodal counterpart of MALT) lymphomas. Furthermore, using aberrant methylation as markers, we tested the hypothesis that the lower frequency of gastric MALT as compared with DLBC lymphomas in our population might be due to a higher rate of transformation of MALT to DLBC lymphoma. Seventy-nine patients were studied (Table 1). Histologic diagnoses were based on the World Health Organization (WHO) classification scheme.[4] High molecular weight DNA was extracted from gastrectomy or lymph node specimens. Methylation-specific-polymerase-chain-reaction (MSP) for the *p15*, *p16*, *p73*, *VHL*, *caspase 8* and *hMLH1* genes was performed as previously described[5] (Figure 1). Briefly, bisulphite conversion of DNA (1 mg, CpGenome DNA Modification Kit, Intergen, Purchase, NY, USA) was followed by MSP using primers for the methylated and unmethylated gene promoters (Figure 1). DNA from normal donors was used as a negative control for the experiments. The results showed that *p15*, *p16* and *p73* were the most frequently methylated genes, while *VHL*, *caspase 8* and *hMLH1* were infrequently or not at all methylated.

Aberrant promoter methylation appeared on the whole to be more frequent in MALT/MZBC lymphomas than in gastric or nodal DLBC lymphomas (Table 1A). The *p15* gene was methylated significantly more in gastric MALT than in DLBC lymphomas, whereas the *p16* and *p73* genes were methylated significantly more in nodal MZBC than in DLBC lymphomas. However, the patterns of aberrant methylation were similar for gastric MALT and nodal MZBC lymphomas. This was also the case for gastric and nodal DLBC lymphomas, except for *p73* which was more often methylated in the stomach (Table 1B).

Table 1. The methylation status of p15, p16, p73, VHL, caspase 8 and hMLH1 as detected by methylation-specific polymerase chain reaction in gastric DLBC and MALT lymphomas, and nodal DLBC and marginal zone B-cell lymphomas.

	Gastric lymphoma			*Nodal lymphoma*		
A	*DLBC (n=22)*	*MALT (n=15)*	*p#*	*DLBC (n=30)*	*Marginal zone BC (n=12)*	*p#*
p15	6 (27%)	9 (60%)	0.047*	10 (33%)	5 (42%)	NS (0.611)
p16	12 (55%)	11 (73%)	NS (0.247)	15 (50%)	10 (83%)	0.047*
p73	9 (41%)	9 (60%)	NS (0.254)	3 (10%)	7 (58%)	0.001*
VHL	0	1 (7%)	NS	0	3 (25%)	NS
Caspase 8	0	0	–	0	1 (8%)	NS
hMLH1	0	0	–	0	0	–

	DLBC lymphoma			*MALT /marginal zone B-cell lymphoma*		
B	*Gastric*	*Nodal*	*p#*	*Gastric*	*Nodal*	*p#*
p15	6 (27%)	10 (33%)	NS	9 (60%)	5 (42%)	NS
p16	12 (55%)	15 (50%)	NS	11 (73%)	10 (83%)	NS
p73	9 (41%)	3 (10%)	0.009*	9 (60%)	7 (58%)	NS (0.930)
VHL	0	0	–	1 (7%)	3 (25%)	NS
Caspase 8	0	0	–	0	1 (8%)	NS
hMLH1	0	0	–	0	0	–

*#:Significance evaluated by the $\chi 2$ test. *denotes statistically significance difference (p<0.05). Primer sequences for the methylated and unmethylated alleles of p15, p16, p73 and hMLH1 were as previously reported.[5] Primers for Caspase 8: unmethylated (forward: 5'-TAG GGG ATT TGG AGA TTG TGA-3', reverse: 5'- CCA TAT ATA TCT ACA TTC AAA ACA A-3') and methylated (forward: 5'-TAG GGG ATT CGG ACA TTG CGA-3', reverse: 5'-CGT ATA TCT ACA TTC GAA ACG A-3'). Primers for VHL : unmethylated (forward: 5'-GTT GGA GGA TTT TTT TGT GTA TGT-3', reverse: 5'-CCC AAA CCA AAC ACC ACA AA-3') and methylated (forward: 5'-TGG AGG ATT TTT TTG CGT ACG C-3', reverse: 5'-GAA CCG AAC GCC GCG AA-3').*

This study highlights a number of interesting features. Firstly, we showed that methylation patterns were different between MALT/MZBC lymphomas and gastric/nodal DLBC lymphomas. Secondly, MALT and MZBC lymphomas shared similar methylation patterns, and gastric and nodal DLBC lymphomas also shared other similar patterns. These results suggested that gastric MALT might be biologically distinct from DLBC lymphomas, but similar to nodal MZBC lymphomas. Similarly, gastric DLBC might be biologically similar to nodal DLBC lymphomas, and different from gastric MALT lymphomas. Furthermore, aberrant gene methylation appeared to be less frequent in gastric DLBC than in MALT lymphomas. As selective demethylation of the genes analyzed in this study is not known to occur during tumor progression, our observations suggest that most of the gastric DLBC lymphomas were not transformed from MALT lymphomas. However, the results would not preclude the possibility that some DLBC lymphomas with aberrant gene methylation were actually derived from MALT lymphomas.

Another intrguing finding is the frequent methylation of *p73* (≈60%) in MALT/MZBC lymphomas. There are few data on *p73* methylation in lymphomas. Corn *et al*. showed that *p73* was methylated in approximately 30% of primary acute lymphoblastic leukemias and Burkitt's lymphomas.[6] We have also

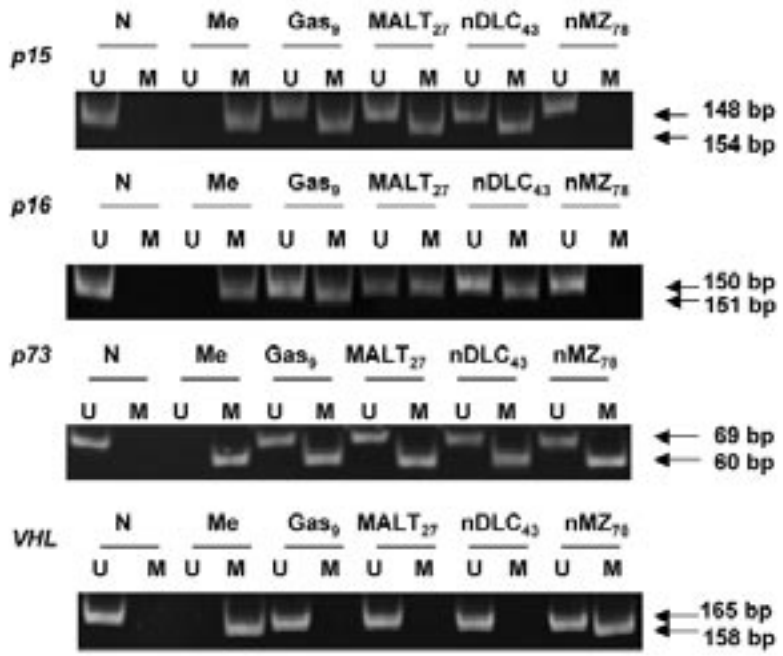

Figure 1. MSP of *p15*, *p16*, *p73* and *VHL* genes. N: normal control DNA, showing positive amplification with the unmethylated primers (U) but not with the methylated (M) primers; Me: methylated DNA, showing positive amplification with the M but not the U primers; Gas_9: gastric diffuse large B-cell lymphoma (case 9); $MALT_{27}$: gastric MALT lymphoma (case 27); $nDLC_{43}$: nodal diffuse large B-cell lymphoma (case 43); nMZ_{78}: nodal marginal zone B-cell lymphoma (case 78). For the *p15* gene, Gas_9, $MALT_{27}$ and $nDLC_{43}$ were methylated, but nMZ_{78} was not. For the *p16* gene, Gas_9, $MALT_{27}$ and $nDLC_{43}$ were methylated, but nMZ_{78} was not. For the *p73* gene, Gas_9, $MALT_{27}$ and $nDLC_{43}$ and nMZ_{78} were all methylated. For the *VHL* gene, only the nMZ_{78} was methylated.

shown that *p73* was very frequently methylated in natural killer cell lymphomas.[5] In lymphoma cell lines, methylation of *p73* correlated with down-regulation of the *p73* protein, and promoter demethylation led to re-expression of *p73*.[5,7] Interestingly, our results also showed that *p73* was frequently methylated in DLBC lymphoma in the stomach but not the lymph node. The significance of this in gastric lymphomagenesis merits further investigation.

Finally, the significance of gene methylation in MALT/MZBC and DLBC lymphomagenesis will need to studied by demonstrating that methylation-induced gene suppression contributes to cellular growth dysregulation or transformation.

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Rapid genotyping of *Xba*I and *Msp*I DNA polymorphisms of the human factor VIII gene: estimation of their combined heterozygosity in the Argentinean population

In hemophilia A, indirect analysis using factor VIII gene polymorphisms is particularly valuable to obtain rapid information for genetic counseling. Herein, we describe an alternative route to investigate two intron 22 DNA polymorphisms (*Xba*I and *Msp*I) using an intragenic 12kb-long amplimer. The estimated heterozygosities on 37 haplotypes from the Argentinean population were *Xba*I (49%), *Msp*I (50%), and combined *Xba*I+*Msp*I (63%).

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Hemophilia A (HA) is an X-linked inherited bleeding disorder due to deficiency in the coagulation factor VIII (FVIII).