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CE - LETTER TO THE EDITOR

cagA structural types of *Helicobacter pylori* strains isolated from patients with gastric carcinoma and chronic gastritis only

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Gastric carcinoma (GC) is an important cause of death and is associated with infection by *Helicobacter pylori* (*H. pylori*) strains expressing CagA, an oncoprotein encoded by the *cagA* gene (for a comprehensive discussion of *H. pylori* infection, see Ref. [1]).

Recent studies have shown that CagA can be injected into the colonized mucocytes through a conjugative apparatus encoded by *cag* genes upstream *cagA*. Once inside the cell, CagA undergoes phosphorylation by host cell kinases. Phosphorylated CagA is capable of altering the cell morphology and disturbing host-signaling pathways, which ultimately may lead to the development of GC.

The fragment of the *cagA* gene encoding the carboxyl-terminal end of CagA is polymorphic, as it presents numerous genomic repetitions that contain the tyrosine EPIYA motif, a 5-amino acid sequence (Glu–Pro–Ile–Tyr–Ala), which is the site where CagA undergoes phosphorylation. In 1998, Yamaoka et al. [2] observed that strains with a high number of genomic repetitions in the *cagA* variable region were isolated more frequently from patients with GC. They classified the *cagA* structural types in A, B, C and D types, on the basis of the amplicon sizes obtained with primers spanning the entire *cagA* variable region. Types B and D have similar sizes and can be differentiated by sequencing the amplicons. Later on, the dissection of the *cagA* 3′ repeat region of numerous *H. pylori* strains indicated that the occurrence of gastric mucosa atrophy, a lesion considered a pre-cancerous condition, was more likely when patients were infected by strains possessing a high number of repeats, i.e. strains with type C *cagA* gene [3].

In the scientific literature, there are only few reports about the possible association between clinical features and the CagA structural variability. In the present study, we investigated on the possible relationship existing between the polymorphism of the *cagA* 3′ variable region and the occurrence of GC. We also determined the CagA mass expressed by different strains to ascertain whether the CagA size corresponded to the length of the amplicons.

Forty *H. pylori* strains were isolated from resected gastric mucosa of patients with GC and 46 strains from gastric biopsies of patients with chronic gastritis only (CGO). Strains were identified by the Gram stain and oxidase, catalase and urease tests and stored at −80°C until the characterisation of the *cagA* polymorphism was performed.

The *cagA* structural types were determined by polymerase chain reaction (PCR) using the following primers spanning the *cagA* variable region [2]; the different sizes of the amplicons obtained range from 642 to 834 bp:

CAG A sense: AACCCTAGTCGGTAATGGGTTAT
CAG A antisense: GTAATTGTCTAGTTTCGC

To increase the PCR specificity, all amplified *cagA* structural types have been obtained in the tests of internal

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amplification (nested PCR) following PCR assays performed with external cagA1 and cagA2 primers:

cagA1: AGCGGTATCAATGGCTAAAGC
cagA2: CCTGCTCCATTCTGGATATTG

Such method enables one to consider the internal fragment as totally specific for *cagA* of *H. pylori*. PCR amplification of the *cagA* variable regions was followed by agarose gel electrophoresis.

The concordance between the size of *cagA* and the mass of the CagA protein expressed was verified by Western blotting (WB): the proteins of the different strains were resolved electrophoretically in acrylamide–bisacrylamide gel, transferred electrophoretically onto a nitrocellulose sheet and let react with a rabbit polyclonal antibody (diluted 1:2,000), raised against purified CagA (gift of R. Rappuoli, Novartis, Siena). The immunoreaction was detected using a goat anti-rabbit IgG serum conjugated with peroxidase and revealed by the addition of substrate.

cagA structural type A was characterized by an amplified PCR product of 650 bp ca. (Fig. 1) and was found in 18 GC and 16 CGO cases. Type B/D *cagA* gene consisted of a 755 bp ca. PCR product (Fig. 1) and was seen in 12 GC and 12 CGO cases. Type C *cagA* gene consisted in a PCR product of 810 bp ca. (Fig. 1) and was observed in three GC and two CGO cases. Four strains from patients with GC (10.0%) and 14 CGO strains (30.4%) had a *cagA* variable region shorter than the *cagA* structural type A (Fig. 1); such kind of *cagA* was named as type A(I).

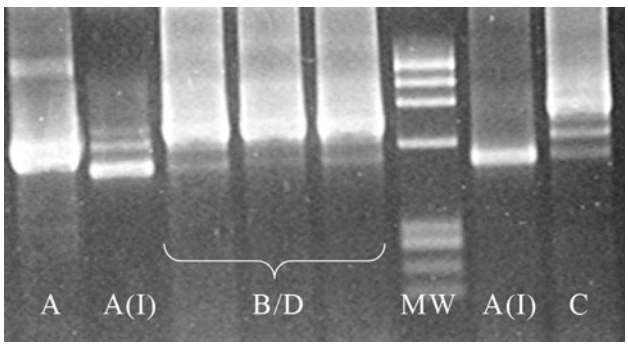

Fig. 1 Amplicons of different sizes obtained after PCR amplification and agarose gel electrophoresis. *MW* standard molecular weights

(I) stands for Italy, because, to the best of our knowledge, this *cagA* type has not been observed in other nations before. These amplicons have a deletion of approximately 100 bp; thus, their size is approximately 550 bp.

The distribution of the different *cagA* structural types in patients with GC and CGO was similar, with the exception of the *cagA* A(I) type, which was found significantly more often in CGO strains, respect to strains isolated from patients with GC ($p = 0.020$, OR = 3.94, CL 1.06–15.93; Chi-square test with the Mantel–Haenszel correction).

Nested PCR did not produce any amplicon in five strains, three from patients with GC and two from patients with CGO, although PCR carried out with primers specific for the entire *cagA* gene had shown the presence of such gene. These strains expressed CagA, as shown by WB. WB confirmed the concordance between the size of the *cagA* variable region and the mass of the homologous CagA proteins (Fig. 2).

The heterogeneity in *cagA* gene might be an important contributing factor in the outcome of *H. pylori* infection [2]; however, the possibility that different *cagA* variants may be preferentially associated with specific clinical outcomes was not confirmed in this study; in particular, the prevalence of *cagA* type C strains isolated from GC patients was similar to that of CGO cases. In other words, the number of phosphorylation sites in the *cagA* gene of our strains does not appear to be crucial for the outcome of infection in GC. Nevertheless, phosphorylation of CagA is a critical process in the development of gastric tumors: only phosphorylated CagA is able to activate the SHP2 oncoprotein, cause oxidative DNA damage in gastric mucosa and prevent apoptosis in a subpopulation of epithelial cells, which therefore can undergo malignant transformation [4]. A possible reason why our results differ from those of Yamaoka et al. [2] may consist in the fact that clones isolated in different areas may have different nucleotide sequences. The possibility that the genomic patrimony of Italian strains differs from that of Japanese helicobacters is suggested by the failure to produce amplicons in five cases using the primers designed on East Asian strains [2]. However, results similar to those obtained in Japan were attained in South Africa with strains isolated locally [5].

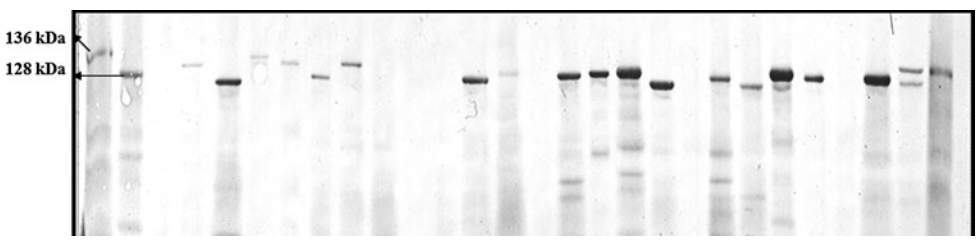

Fig. 2 Western blotting showing CagA with different masses, ranging from 110 to 140 kDa. The large CagA proteins were encoded by type C structural *cagA* gene, the smallest CagA by the type A(I) *cagA* gene

The main result of the present study consists in the increased prevalence of strains with *cagA* subtype A(I) in CGO cases. The fact that these strains were defective in 100 bp ca. suggests that deletions in the *cagA* variable region may possibly reduce the cancerogenic potential of *H. pylori*.

In conclusion, variations in genes that subsequently alter the proteins they encode have been shown to correlate with clinical outcome of disease [2, 5]. The routine characterization of *H. pylori cagA* structural types, as well as the CagA protein size, may help evaluating the risk of GC. The detection of strains with internal deletion within the *cagA* variable region may be an excellent negative marker for GC.

Conflict of interest None.

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