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Proteomic *Helicobacter pylori* biomarkers discriminating between duodenal ulcer and gastric cancer

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ABSTRACT

Protein patterns of 129 *Helicobacter pylori* strains isolated from Korean and Colombian patients suffering from duodenal ulcer or gastric cancer were analyzed by the high-throughput methodology SELDI-TOF-MS. Eighteen statistically significant candidate biomarkers discriminating between the two clinical outcomes were selected by using the Mann–Whitney test. Three biomarker proteins were purified and identified as a neutrophil-activating protein NapA (HU HPAG1_0821), a RNA-binding protein (HPAG1_0813), and a DNA-binding histone-like protein HU, respectively (jhp0228). These novel biomarkers can be used for development of diagnostic assays predicting the evolution to gastric cancer in *H. pylori*-infected patients.

1. Introduction

The bacterium *Helicobacter pylori* is a human pathogen which colonizes the gastric mucosa and causes inflammation resulting in different clinical outcomes such as chronic and atrophic gastritis, gastro-duodenal ulcers, gastric MALT lymphoma and gastric cancer. To date, there exist no reliable diagnostics to predict, among the very large and clinically heterogeneous group of *H. pylori*-infected patients, the small group at risk for developing gastric cancer. Efforts for developing better predictive diagnostics have focused mostly on disease-related biomarkers of *H. pylori* that are explored at gene level [1–3]. However, specific gene patterns associated with a particular clinical outcome have proven with limited clinical relevance. For example, the combination of the extensively studied factors of pathogenicity *cagA*, *vacA* and *babA* cannot help to segregate a particular group of *H. pylori* virulent strains because of the wide distribution of these factors over strains [4]. With the complete sequencing of four *H. pylori* strains available on the net, (for strains 26695, J99 and HPAG1: http://cmr.jcvi.org/tigr-scripts/CMR/shared/Genomes.cgi; for strain Shi470: NCBI, accession number NC_010698), proteomic technologies hold promise for better disease-specific classification of the *H. pylori* strains. The underlying approaches are more or less straightforward. By the first approach, direct proteomics comparison is made between 2D protein maps of *H. pylori* strains [5,6]. Through using the second, indirect immunoproteomics approach, the *H. pylori* proteins are separated by 2D-E, transferred to membranes, and screened by a panel of sera collected from *H. pylori*-infected patients with different gastric pathologies. The most frequently immunorecognized spots are used for precise localisation of the protein antigens on the preparative gel [7–12]. For example, Krah et al. compared by immunoproteomics two groups of 30 sera each from patients with duodenal ulcer or gastric cancer. They used 2D-E gels of the completely sequenced *H. pylori* strain 26695 for immunostaining with patient's sera, and successfully identified 14 antigenic protein species that are discriminating between the two clinical outcomes [10]. Because of methodological limitations, most studies are based on very small number *H. pylori* strains, which cannot allow to estimate statistical significance. Although some protein biomarkers have been associated with severe gastric pathologies, a more in-depth investigation is required.

This issue can be addressed by studying subproteomes of *H. pylori* by means of the high-throughput technology, SELDI-TOF-MS,

Abbreviations: 2D-E, two-dimensional electrophoresis; ACN, acetonitrile; DU, duodenal ulcer; EDM, expression difference mapping; GC, gastric cancer; *H. pylori*, *Helicobacter pylori*; HPLC, high-performance liquid chromatography; kDa, kilodaltons; ELISA, enzyme-linked immunosorbent assay; LC–MS/MS, liquid chromatography/tandem mass spectrometry; Mr, molecular weight; S/N, signal-to-noise ratio; PMSF, phenylmethylsulphonyl fluoride; pI, isoelectric point; RP-HPLC, reversed-phase high-performance liquid chromatography; SEC, size-exclusion chromatography; SELDI-TOF-MS, surface-enhanced laser desorption/ionization time-of-flight mass spectrometry; TFA, trifluoroacetic acid.

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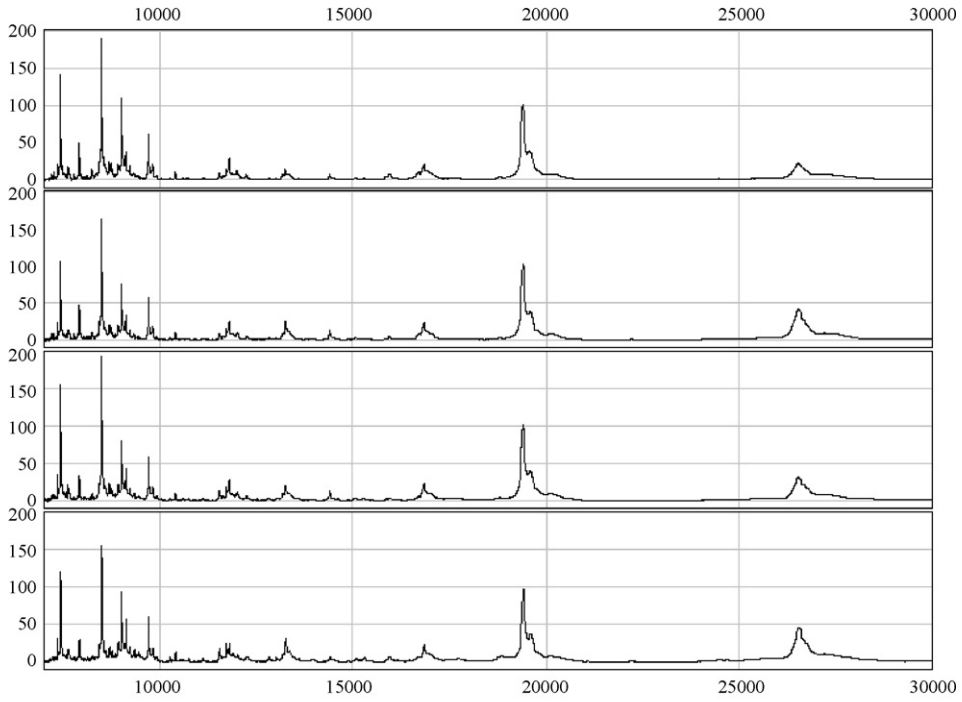
(A)



(B)

Chip array	Mr Range	Nb of Peaks	CVp
Q10	3-30 kDa	15	20%
CM10	3-30 kDa	10	22%

(C)

Chip array	Mr Range	Nb of Peaks	CVp type	CVp
CM10	3-30 kDa	21	Inter-array	16%
CM10	3-30 kDa	17	Intra-array	16%
Q10	5-25 kDa	21	Inter-array	16%
Q10	5-25 kDa	17	Intra-array	19%

Fig. 1. Reproducibility of protein profiles of bacterial cultures: (A) spectra of eight independent cultures (i.e. cultured on different times, each culture—started from a separate frozen aliquote) of one and a same *H. pylori* strain (Dual 45) tested on the Q10 surface. Four from the eight obtained spectra are represented; (B) 10–15 peaks (mass range: 3–30 kDa, $S/N \geq 5$) are used from the eight spectra of strain Dual 45 to calculate the pooled coefficients of variance (CVp) of the Q10 and CM10 surfaces. Reproducibility of the SELDI methodology: (C) intra- and inter-array reproducibility of the Q10 and CM10 surfaces as calculated by CVp of 17–21 peaks from one and a same aliquot applied 8 times on 8 spots of the same array (intra-array), as well as on one spot per array of 8 different arrays (inter-array).

which allows the generation of protein patterns from hundreds of strains within a same experiment [13]. In the present study, we used more than hundred *H. pylori* strains from two distinct geographic origins, Colombia and South Korea. The *H. pylori* strains were obtained from patients with gastric cancer (GC) or duodenal ulcer (DU). The latter serves as a control group, since it is well documented that patients with duodenal ulcer practically never develop GC [14,15]. Eighteen biomarkers were selected in this way and three of them were purified and identified.

2. Materials and methods

2.1. Bacterial strains and cell growth conditions

Characterization [1,3,16] and cell growth conditions [17] of the 129 *H. pylori* strains used in this study have been described previously. Bacterial cells, controlled for the presence of more than 95% bacillary forms by Gram staining, were harvested and washed in 10 mL of PBS, pH 7.4 containing 1 mM of PMSF. After centrifugation at 2000 × *g* for 15 min, the cell pellet was frozen and stored at −80 °C.

2.2. Protein extract

The bacterial cell pellets of three Petri plates (9 cm in diameter) were thawed and resuspended in 1 mL of lysis buffer (16 mM Na_2HPO_4, 4 mM NaH_2PO_4, 150 mM NaCl, 1% Triton X-100, 1 mM PMSF). The cell suspension was transferred into a FastProtein Blue tube and homogenized in a FastPrep apparatus (MP Biomedicals) according to the following protocol: four cycles, 40 s each at power setting 6.5; between each cycle—cooling of the tubes in ice for 5 min. After centrifugation at 15,000 × *g* for 15 min, supernatants