Molecular aspects of *Helicobacter pylori* and its relation to gastric cancer

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Molecular aspects of *H. pylori* (general)

H. pylori (*HP*) has been associated with a number of gastric diseases, such as Chronic Gastritis, Peptic Ulcer, Gastric Adenocarcinoma (GA) and Gastric MALT Lymphoma. Furthermore, other links to extra-gastric cancer (such as pancreas), or even cardiovascular diseases, have been postulated.

This plethora of pathological associations is mirrored by the intrinsic complexity of the numerous molecular aspects of the disease. Indeed, we can understand the molecular pathogenesis of *HP* from the point of view on microbiology, molecular diagnostics, oncology, pathology and disease prevention, as it is highlighted in figure 1.

An important aspect of this complex picture is the interconnection between these many different aspects, highlighted by the external set of arrows in figure 1. The focus of this lecture is in the oncopathologic aspects of the disease.

Molecular aspects of *HP* and gastric adecocarcinoma

The earliest evidence of the association between *HP* and GA was epidemiological (EUROGAST Study Group, *Lancet* 1993;341:1359-1362), highlighting a six-fold increased

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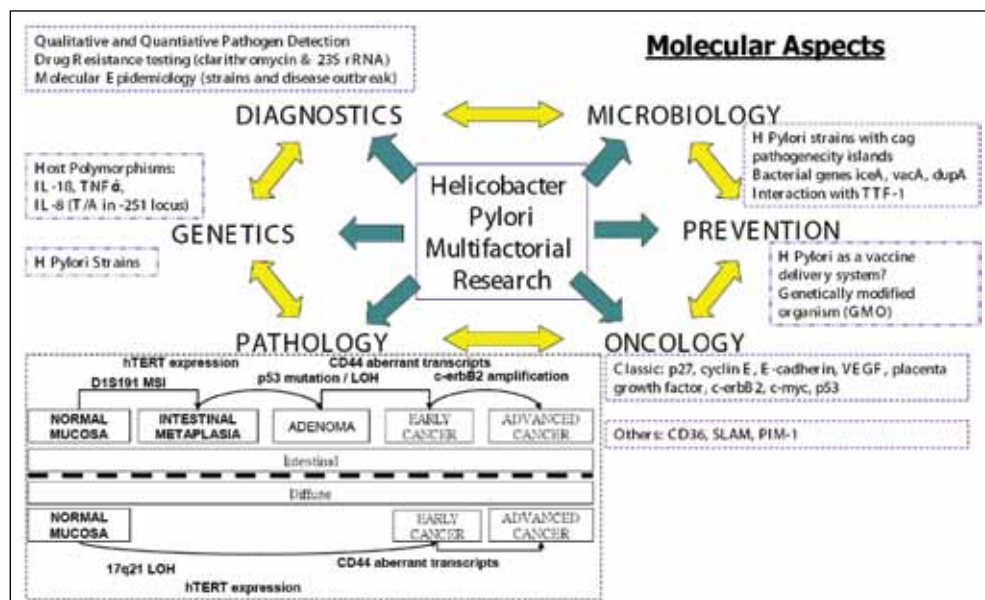
Figure 1.

risk of gastric cancer in populations with 100% *HP* infection compared with populations that have no infection. The molecular basis of this relation *per se* has been comprehensively reviewed elsewhere (*Gastric Cancer* 2004;7:61-77), showing a very complex set of molecular associations which include general molecular processes such as cell proliferation and apoptosis, as well as specific biomarkers such as E-cadherin protein, α-catenin, p53 [mutated alone or in relation to CagA(+) *HP*], ras, p21, HGF or TGF-α.

The existence of these many candidates, and the unequivocal specific weight of many of them, has suggested the importance of environmental factors in the *HP*-GA relation which, in turn, may explain a difference between the two main histological subtypes of GA, namely intestinal and diffuse types. The molecular basis of this hypothesis has been reviewed (*Aliment Pharmacol Ther* 2003;17(Suppl 2):65-71), and the results suggest that *HP* plays a key role in both types of gastric cancer by giving rein to the genetic predisposition in the case of the diffuse type, and inducing molecular alterations in the case of the intestinal type. Thus, histomorphological analysis may not be sufficient to address the specific role of *HP* in GA development.

In view of the evidence stated above, a key question arises: *why, despite the fact that there are many plausible links between HP and GA, there is no clear molecular biomarker that explains that link, and offers a target for treatment?* To answer this question, we need to reflect upon the way biomarker discovery has occurred to date. Much of the evidence

stated above was generated by the test of single genes (or single pathway) in a disease setting. An alternative mode is to use techniques that will allow us to have a snapshot of the overall molecular activity of a disease. Indeed, the area of high-throughput analysis of clinical samples and cell lines has developed significantly in the last few years. A series of biochip-based techniques such as single-nucleotide polymorphism microarrays, cDNA microarrays, proteomics biochips and tissue microarrays have been applied to many cancer types. To understand their relevance to the question in hand, namely the *HP*-GA association, we would like to briefly review two papers centered on cDNA microarray work:

1. The paper by Sepulveda et al (*Aliment Pharmacol Ther* 2002;16(Suppl 2):145-157) examined broad patterns of gene expression induced by *H. pylori* (CagA+ strain - ATCC 43504) in the gastric cancer cell line 1739-CRL AGS using the U95A microarray. The result showed that nearly 6000 genes present in the array were expressed by AGS cells, 200 of which showed the most marked changes. This study reports, for the first time, the induction of the serine threonine Kinase PIM1 and ATF3 by *HP* infection of AGS cells.

2. In the work by Chen et al (*J Clin Oncol* 2005;23:7286-7295), 18 pairs of normal-tumour samples with known long-term survival were analysed by cDNA microarrays, followed by supervised analysis, RT-PCR to confirm the microarray data and further analysis to identify those genes predicting good versus poor survival. The results showed that a survival prediction model consisting of three genes (*CD36*, *SLAM*, *PIM1*) can accurately predict surgery-related outcome in gastric cancer patients.

   Taken together, this work shows the possible importance of PIM1 as a gene that can a) explain the *HP*-GA link, and b) serve as a therapeutic target.

PIM1, RUNX3, *H. pylori* and gastric cancer

The human pim-1 (recently reviewed in *Eur J Cancer,* 2008, Aug 18, Epub ahead of print) is an oncogene localized on chromosome 6p21.2. The PIM1 protein is a serine/threonine kinase, expressed ubiquitously in both normal and transformed cells. In humans, the PIM1 oncogene is expressed in lymphoid and haematopoietic malignancies, prostate cancer, squamous cell carcinomas of head & neck region, gastric carcinoma and colorectal carcinomas. PIM1 expression is regulated by cytokines, growth factors and hormones. PIM1 is also involved in conditions such as ischaemia & cellular hypoxia, and infections (Epstein-Barr virus and *HP*). More importantly, PIM1 inhibitors may be useful in the clinical practice (LY294002).

To start investigating the role of PIM1 in the *HP*-GA link, we analysed the expression of PIM1 protein in a series of clinical samples by immunohistochemistry. These samples were divided in two groups:

1. 108 cases representing Chronic Gastritis, Gastric Intestinal Metaplasia and GA, in a tissue microarray format, independent of *HP* status. This analysis showed a significant increase of PIM1 protein expression from chronic gastritis to GA (43%, 53% and 66% respectively).

2. 56 cases of samples from patients with Chronic Gastritis, Chronic Gastritis and Intestinal Metaplasia and Chronic Gastritis and Atrophy (with and without *HP*). This analysis showed a significant increase of PIM1 in those cases with *HP*, in all 3 sample types.

Hence, we have preliminary evidence to support the possible role of PIM1 in GA progression and in *HP*-related GA. Interestingly, this possible role of PIM1 may be directly related to another, established tumor suppressor gene in GA. In 2005, we reported that downregulation of the RUNX3 gene (*Cancer Res* 2005;65(17):7743-7750) is frequently inactivated in more than 80% of GA. Subsequently, a direct relation between RUNX3 and PIM1 has been postulated (*BMC Cell Biol* 2006;7:21). This interesting RUNX3-PIM1 interaction in the context of GA is now one of the areas of interest in our laboratory.

Conclusion

Both *H. pylori* infection and gastric cancer are complex diseases. There are many possible single molecular players linking *HP* and gastric cancer. Gene expression array studies may unravel genetic links unknown to date. PIM1 may be relevant in explaining the transition from *HP*-related gastritis to gastric cancer, and may represent a target for disease prevention. The effect of PIM1 may be exercised through the TGF-beta / RUNX3 pathway.