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Helicobacter pylori in peptic ulcer

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ABSTRACT

Helicobacter pylori is a gram-negative, curved, rod-shaped bacterium known to cause gastritis and to be an important factor in the pathogenesis of peptic ulcers. Serological testing has recently been proposed as an aid in diagnosis of H.pylori infections. Helicobacter pylori, like several other mucosal pathogens, probably uses recombination and slipped-strand mispairing within repeats as mechanisms for antigenic variation and adaptive evolution consistent with its restricted niche,

H. pylori has a few regulatory networks, and a limited metabolic repertoire and biosynthetic capacity. Its survival in acid conditions depends, in part, on its ability to establish a positive inside-membrane potential in low pH. We propose that these proteins may be retained on the outside surface of the cell membrane and contribute to the interaction between H. pylori and host cells. Studying the altered gene expression in the infected host cells can understand the regulatory mechanism that control pathological changes. H. pylori resides at the surface of gastric cells would lyse sometime after death, and the bacterial intracellular materials might also influence gastric cells. We speculate that this event is involved in the inflammantory response, which is an important factor of gastritis, ulcers and presumably, gastric cancer. At this moment, we can not make a conclusion whether or not those novels genes were specifically induced for H.pylori lysate, and we would not reject the assumption that yhose genes may be also highly induced by some other pathogenic bacteria.

Keywords: Helicobacter pylori, peptic ulcer, VacA gene

Introduction

Helicobacter pylori is a gram-negative, curved, rod-shaped bacterium known to cause gastritis and to be an important factor in the pathogenesis of peptic ulcers. Serological testing has recently been proposed as an aid in diagnosis of H.pylori infections.

Helicobacter pylori, strain 26695, has a circular genome of 1667867 base pairs and 1690 predicted coding sequences. Sequence analysis indicates that H.pylori has well-developed systems for mobilty, for scavenning iron, and for DNA restriction and modification. Many putative adhesins, lipoproteins and other outer membrane proteins were identified, underscoring the potential complexity of host-pathogen interaction. Based on the large number of sequence-related genes encoding outer membrane proteins and the presence of homopolymeric tracts and dinucleotide repeats in coding sequences, Helicobacter pylori,like several other mucosal pathogens,probably uses recombination and slipped-strand mispairing within repeats as mechanisms for antigenic variation and adaptive evolution consistent with its restricted niche, H.pylori has a few regulatory networks, and a limited metabolic repertoire and biosynthetic capacity. Its survival in acid conditions depends, in part, on its ability to establish a positive inside-membrane potential in low pH.

The protein toxin VacA, produced by citotoxic strains of H.pylori, causes a vacuolar degeneration of cells, which eventually die. VacA is activated by a short exposure to acidic solutions in the pH 1.5-5.5 range, followed by neutralization. Activated VacA has different CD and fluorescence spectra and a limited proteolysis fragmentation pattern from VacA kept at neutral pH. Moreover, activated VacA is resistant to pH 1,5 and to pepsin. Intoxication of mammalian cells with the vacuolating toxin (VacA) released by Helicobacter pylori causes the formation of large acidic vacuoles containing the vacuolar ATPase proton pump and Rab7, a late endosome marker. Scientists describe a novel subcellular fractionation procedure, and they show that nanomolar concentrations of VacA induce a clear redistribution of lysosomal membrane glycoproteins among endocytic compartments. This redistribution is an early event in the process of cellular intoxication by VacA and precedes the formation of macroscopic vacuoles. The absence of the cation independent mannose 6-P receptor and the presence of Rab7 and of lysosomal membrane proteins in the newly

formed compartment suggest that the vacuolating toxin induces the accumulation of a post-endosomal hybrid compartment presenting both late endosomal and lysosomal features.

To probe the role of Rab7 in vacuolation, HeLa cells were transfected with a series of Rab mutants and exposed to VacA. Dominant-negative mutants of Rab7 effectively prevented vacuolation, whereas homologus Rab5 and Rab9 mutants were only inhibitory or inffective. Espression of wild-type or GTPase-deficient Rab mutants synergized with VacA in inducing vacuolization.

In 1983 an Avustralian physician, Robin Warren, and his colleague Barry Marshall, biochemist, wrote separate letters to the lancet under a single title. Warren described how, over the previous five years or so, he had observed spiral-shaped bacteria on the gastric epithelium of patients with peptic ulcers. Marshall then discussed the history of pyloric bacteria in mammals, and offered some thoughts on why these Campylobacter like organisms hadn't been reported previously. He noted that they had been found in non-human mammals, and that they were even observed long ago in human cadavers, but their existence had been rationalized as a post-mortem consequence.

The authors, scribed the varying incidence of peptic ulcers in different Australian states to environmental factors, such as contaminated drinking water. Interestingly, the epidemiological study was based entirely on records of state-mandated prescriptions for the antihistamine drug cimetidine which, according to newspaper accounts, was the most prescribed drug in the world during the 1980s. Imagine the impact of a finding that penicillin or other antibacterial agent might be a more appropriate treatment. H.pylori, as well as for cimetidine and, for the enzyme urease. The answer is that this enzyme is crucial to the survival of H.pylori in the very acid pH of the stomach. It has long been known that resident microorganisms in the stomach use the enzyme urease to convert urea to ammonia and carbon dioxide. Few other organisms can survive in this acidic environment, but H.pylori has an electropositive internal millieu which helps it to tend off the onslaught of protons in the surrounding medium. Tomb et al. have shown that the proteins in H.pylori contain twice as many of the basic amino acids arginine and lysine as proteins in other organisms.

Urease is the main antigenic activity associated with H.pylori and it is a convenient diagnostic tool, which is why there has been such an enormous increase in citations involving urease. Tomb et al. Reveal the entire restriction-modification system for recognizing and destroying foreign DNA, and outline a complete scheme of metabolism on the basis of the resident genes. They have also worked out how H.pylori mimics blood group antigens, as well as a likely molecular mechanism for encouraging immunogenic variation.

Helicobacter pylori, a Gram-negative, spiral-shaped bacterium colonizing the stomach, is involved in the pathogenesis of gastritis and gastroduodenal ulcers. Recently, such an infection has been associated to MALT lymphomas and to an increase disk of developing gastric adenocarcinoma.

Even though H.pylori shows a great genetic variability, strains isolated from human biopsies can be classified into two group. Type I strains are associated with the more serious pathologies and are characterized by the common production of a toxin, termed VacA, and of a toxin-associated antigen (CagA). Type II strains produce neither of these two antigens and lack a 40 kb. Pathogenicity island found in the genome of type I strains. VacA is synthesized as a 140 kDa precursor, which is processed to the mature 95 kDa protein during export from the bacteria. Structural features of the toxin hint at the possibility that VacA belongs to the group of bacterial protein toxins with an A-B type structural organization: protomer B binds to a membrane receptor present on the surface of target cells and mediates the translocation of the catalytic A subunit into the cell cytosol.

Helicobacter pylori bacterial extracts cause a vacuolar degenaration of epithelial cells, followed by cell death in animals and in cells in culture. Vacuolar pH is acidic, as deduced from the accumulation of neutral red, a membrane-permeant amine that protonates in the vacuolar lumen. Determination of neutral red uptake has become the standart in vitro assy of H.pylori cytotoxicity.

Baby hamster kidney (BHK) is a cell line often used in studies of the endocytic path of higher eucaryotes. The route leading endocytosed material from the plasma membrane of BHK cells, through the early endosomal compartment (Rab5+/ transferrin receptor+) to the late

endosomal compartment (Rab7+/cation independent mannose 6-P receptor CI-M6PR+), has been characterized in detain using electron microscope markers and a range of Rab mutants and/or endocytosis inhibitors.

BHK cells exposed to VacA were fractionated with a novel, isopryonic density ultracentrifugation method, optimized for the purification of late endosomes and lysosomes from BHK cells.Together with parallel immunnofluorescence staining, this procedure allowed us to obtain evidence that vacuoles are enriched in lysosomal membrane markers such as Lgp110, contain low levels of lysosomal hydrolytic activities, are characterized by the presence of the late endocytic marker Rab7, but are devoid of another late endosomal marker, the CI-M6PR.

The identification of the molecular targets of bacterial toxins has contributed greatly to the present understanding of cell phsiology. Clostridial neurotoxins were shown to block neuroexocytosis, a highly regulated vesicular trafficking process, by cleaving specifically three proteins involved in synaptic vesicle blocking and fusion. It is expected that the elucidation of the mechanism of action of VacA will also contribute to the understanding of membrane traffic in eukaryotic cells.

Vacuoles appear as round structures of up to several micrometres in diameter, a size which can be reached only upon fusion of several smaller compartments. The toxin could promote endosome-endosome fusion either directly or through stimulation of a fusogenic protein. VacA could inhibit directly or indirectly the transport from late endosomes to lysosomes.

The large amount of enherichment of Rab 7 on vacuolar membranes prompted us to investigate to role played by this small GTPase in the genesis and growth of vacuoles induced in cells by VacA.

The Rab family of Ras-related GTP binding proteins are known to regulate the extent and the specificity of intracellular membrane traffic in eukaryotic cells (Nowick and Brenwald, 1993; Simons and Zerial, 1993; Zerial and Stenmark, 1993; Pfeffer, 1994). These proteins are anchored to the membrane by a geranyl-geranyl group that is added to the C-terminal cysteines and that is important for their function.

cDNAs for Rab7 have been previously isolated from rat, dog, mouse, yeast and plants. Functional data on yeast and plant Rab7 suggested an important role of this protein in the late endocytic pathway. The fundamental role of Rab7 in the late endocytic pathway has been demonstrated also in mammalian cells, using mutants that interfered with the ability of the protein to bind or hydrolyze GTP.

The late endosomal compartment, where Rab7 resides, appears to be involved in the site of some human and mammal disease such as the Chediak-Higashi syndrome or the vacuolar degeneration of epithelial cells induced by the VacA toxin of Helicobacter pylori. In both cases the formation of giant late endocytic organelles that bear Rab7 on them was observed. The involvement of Rab7 in the cellular vacuolation induced by the H.pylori VacA cytotoxin has recently been demonstrated. Dominant negative mutants of Rab7 prevented vacuolisation.

The genom of H.pylori strain 26695 consists of a circular chromosome with a size of 1667867 base pairs and average G+C content of 39%. Five regions within the genome have a significiantly different G+C composition. Two of them contain one or more copies of the insertion sequence (IS605) and are flanked by a 5S rRNA sequence at one end and a 521 bp repeat near the other. Thirty-six tRNA species were identified using tRNAscan-SE. These organized into 7 clusters plus 12 single genes.Two separate sets of 23S-5S and 16S rRNA genes were identified, along with one orphan 5S gene and one structural RNA gene. Associated with each of the two 23S-5S gene clusters is a 6 kb repeat containing a possible operon of ORFs that have no database matches. Two distinct insertion sequence (IS) elements are present.

There are five copies of the previously described IS605 and two of a newly discovered element designated IS606. In addition, there are eight partial copies of IS605 and two partial copies of IS606. Both elements encode two divergently transcribed transposases (TnpA and TnpB). IS606 has less than 50% nucleotide identity with IS605 and the IS606 transposases have 29% amino-acid identity with their IS605 caunterpart. Both copies of the IS606 TnpB may be non-functional owing to frame shifts.

Most pathogens show tropisim to specific tissues or cell types and often use several adherence mechanisms for successful attachment. H.pylori may use at least five different adhesins to attach to gastric epithelial cells. One of them, HpaA (HP0797), was previously identified as a lipoprotein in the flagellar sheath and outer membrane. In addition to the HpaA orthologue, we have identified 19 other lipoproteins. Two adhesins, one of which mediates attachment to the Lewisb histo-blood group antigens, belong to the large family of outer membrane proteins (OMP). It is conceivable that other members of these closely related proteins also act as adhesins. Given the large number of sequence-related genes encoding putative surface exposed proteins, the potential exists for recombinational events leading to mosaic organization. This could be the basis for antigenic variation in H.pylori and an effective mechanism for host defence evasion.

At least one other mechanism for antigenic variation could operate in H.pylori. The DNA sequence at the beginning of eight genes, including five members of the OMP family, contain stretches of CT or AG dinucleotide repeats. In addition, poly (C) or poly (G) tracts occur within the coding sequence of nine other genes.

The virulence of individual H.pylori isolates has been measured by their ability to produce a cytotoxin-associated protein (CagA) and an active vacuolating cytotoxin (VacA). The CagA gene, though not a virulence determinant, is positioned at one end of a pathogenecity island containing genes that elicit the production of interleukin (IL)-8 by gastric epithelial cells. Consistent with its more virulent character, H.pylori strain 26695 contains a single contiguous PAI region.

VacA induces the formation of acidic vacuoles in host epithelial cells, and its presence is associated epidemiologically with tissue damage and disease. VacA may not be the only ulcer-causing factor as 40% of.H.pylori strains don’t produce detectable amounts of the cytotoxin in vitro. Sequence differences at the amino terminus and central sections are noted among VacA proteins derived from Tox+ and Tox- strains.This Tox+ H.pylori strain contains the more toxigenic Sia/ml type cytotoxin and three additional large proteins with moderate similarities to the carboxy-terminal end of the active cytotoxin.

However, they lack the paired cysteine residues and the cleavage site required for release of the VacA toxin from the bacterial membrane. We propose that these proteins may be retained onthe outside surface of the cell membrane and contribute to the interaction between H.pylori and host cells.

The surface-exposed lipopolysaccharide (LPS) molecule plays an important role in H.pylori pathogenesis. The LPS of H.pylori is several orders of magnitude less immunojenic than that of enteric bacteria and the O antigen of many H.pylori isolates is known to mimic the human Lewisx and Lewisy blood group antigen. Genes for synthesis of the lipid A molecule, the core region, and the O antigen were identified. Two genes with low similarity to fucosyltransferases (HP379, HP651) were found and may play a role in the LPS- Lewis antigen molecular mimicry. Analysis also suggests that three genes, two glycosyltransferases (HP208 and HP619) and fucosyltransferase (HP379), may be subject to phase variation.

As with other pathogens, H.pylori probably requires an iron scavenging system for survival in the host. Genome analysis suggests that H.pylori has several systems for iron uptake. One is analogous to the siderophore-mediated iron-uptake fec system of E.coli, except that it lacks the two regulatory proteins (FecR and FecI) and is not organized in a single operon. Unlike other studied systems, H.pylori has three copies of each of fecA, exbB and exbD.

A second system, consisting of a feoB-like gene without feoA,suggests that H.pylori can assimilate ferrous iron in a fashion similar to the anaerobic feo system of E.coli . H.pylori contains NapA, abacterioferritin, and pfr, anon-haem cytoplasmic iron-containing ferritin used for storage of iron. The global ferric uptake regulator (Fur) characterized in other bacteria is also present in H.pylori.

H.pylori, motility is essential for colonization. It enables the bacterium to spread into the viscous mucous layer covering the gastric epithelium. At least forty proteins in the H.pylori genome appear to be involved in the regulation, secretion and assembly of the flagellar architecture. As has been reported for the flaA and flaB genes and identified sigma 28 and sigma 54-like promoter elements upstream of many flagellar genes,

underscoring the complexity of the transcriptional regulation of the flagellar regulon.

H.pylori is unusual among pathogenic bacteria in its ability to colonize host cells in an environment of high acidity. As it enters the gastric environment by oral ingestion, the organism is transiently subjected to the extreme pH of the lumen side of gastric mucous layer (pH@2). The survival of H.pylori in acidic environments is probably due to its ability to establish a positive inside-membrane potential and subsequently to modify its micro-environment through the action of urease and the release of factors that inhibit acid production by parietal cells.

A positive cell interior can be created by the active extrusion of anions or by a proton diffusion potential. The latter model appears more likely as no clear mechanism for electrogenic anion efflux is apparent in the genome. A proton diffusion potential would require the anion permeability of the cytoplasmic membrane to be low and, thus far, only three anion transporters have been identified. Additional mechanisms of pH homeostasis may well contribute to H. pylori survival. A change in protein content observed in response to a shift of extracellular pH from 7.5 from to 3 suggests the presence of an acid-inducible response.

Although H.pylori lacks most orthologues of the genes that are acid induced in E.coli and Salmonella typhimurium, including the amino-acid decarboxylases and formate hydrogen lyase,certain virulence factors, outer membrane proteins, sensor-regulator pairs and other proteins may be acid anduced.

Bacteria regulate the transcription of their genes in response to many environmental stimuli, such as nutrient availability, cell density, pH, contact with target tissue, DNA-damaging agents, temperature and osmolarity. In the case of pathogens, the regulated expression of certain key genes is essential for successful evasion of host responses and colonization, adaptation to different body sites, and survival as the pathogen passes to new host.

Metabolic pathway analysis of the H.pylori genome suggests the following features. H.pylori uses glucose as the only source of carbohydrate and the main source for substrate-level phosphorylation. It also derives energy from the degradation of serine, alanine aspartate and proline. The glycolysis-gluconeogenesis metabolic axis constitutes the backbone of energy production and the start point of many biosynthetic pathways.

The biosynthesis of peptidoglycan, phospholipids, aromatic amino acids, fatty acids and cofactors is derived from acetyl-CoA or from intermediates in the glycolitic pathway. The metabolism of pyruvate reflects the microaerophilic character of this organism. Neither the aerobic pyruvate dehydrogenase (aceEF) nor the strictly anaerobic pyruvate formate lyase (pfl) associated with mixed -acid fermentation are present.

The analysis of degradative pathways, uptake systems and biosynthetic pathways for pyrimidine, purine and haem suggest that H.pylori uses several substrates as nitrogen source, including urea, ammonia, alanine, serine and glutamine. The assimilation of ammonnia, an abundant product of urease activity, is achieved by the glutamine synthase enzyme and µ-ketoglutarate is transformed into glutamate by glutamate dehydrogenase rather than by the glutamate synthase enzyme.

Discussion

Little is known about the choice of which genes are expressed in gastric cells in response to H.pylori insult. Studying the altered gene expression in the infected host cells can understand the regulatory mechanism that control pathological changes. H.pylori resides at the surface of gastric cells would lyse sometime after death, and the bacterial intracellular materials might also influence gastric cells.

Therefore, scientists used the sonicated H.pylori lysate as the stimulus to represent the effect of total virulence factors on the host's gene expression. Bacterial pathogens share some comman virulence factors and also possess unique pathogenic factors, responses of host cells to bacterial insults on the

gene expression some are specific and some are non-specific for the invaded bacterium.

The induction of numerous large intracellular vacuoles is the only known effect of the cytotoxin VacA H.pylori a major determinantof gastric ulcers.Studies show that Rab7 plays an essential role in the development of these vacuoles. Cell expressing Rab7 mutants either defective in guanine nucleotide binding or stabilized in the GDP-bound form don't develop vacuoles when exposed to VacA. Rab7 mutants behave as dominant-negative with respect to the phenotype induced by the cytotoxin. Conversaly, Rab7 mutants stabilized in the GTP-bound active form have a slight but significant stimulatory effect on the same cellular response.

The effect of Rab7 on vacuolation correlates with that shown on late endosome fusion in a cell-free system. Cytosol from cells overexpressing positive Rab7 mutants enhanced fusion between late endosomes, whereas cytosol derived from cells overexpressing negative Rab7 mutants had an opposite effect. These Rab7 mutants ere inactive on the same assay performed on early endosomes.Results suggest that vacuoles arise from late endosomes in a process that is controlled by Rab7 in a positive way.

The Rab5 and Tab7 proteins appear to regulate endosome fusion sequentlly, since they found no effect of Rab7 on early endosome fusion as well as no effect of Rab5 on late endosome fusion.

Cell vacuolation is the major detectable activity of VacA, which determines its name of vacuolating cytotoxin. Previous experiments aimed at the characterization of vacuoles and at the study of the mechanism of vacuolation employed toxin enriched bacterial extract or high concentrations of purified toxin to emphasize the phenomen by inducing a rapid and massive cell vacuolation.

In vivo, type I H.pylori strains release VacA, as clearly deduced from the presence of anti-VacA antibodies in the serum of infected people or animal models. Although the quantity of the toxin released in vivo hasn't yet been detemined, vacuoles in number and dimension comparable with those induced in cultured cells are rarely detected in humangastric biopsies.

Remerkabley, there appears to be a non-parallel delivery of luminal and membrane components of the lysosomes in VacA induced vacuoles because they have a low content of hydrolytic enzymes. Such a phenomenon has already been described in the biogenesis of phagosomes in macrophages.

Conclusion

Our whole genome analysis of H.pylori gives new insight into its pathogenesis, acid tolerance, antigenic variation and microaerophilic character. The availability of the complete genome sequence will allow further assessment of H.pylori genetic diversity.

This is an important aspect of H.pylori epidemiology as allelic polymorphism within several loci has already been associated with disease outcome. The extent of molecular mimicry between H.pylori and its human host, an underappreciated topic, can now be fully explored.

The identification of many new putative virulence determinants should allow critical tests of their roles and thus new insight into mechanisms of initial colonization, persistence of this bacterium during long-term carriage, and the mechanisms by which it promotes various gastroduodenal diseases.

The analysis showed two mRNA for Rab7 of about 2.5 and 1.7 kb similarly to what was been previously found in mouse and rat. The two mRNAs were present in all the cell lines examined . However there was a strong difference in the amount of mRNAs among the different cell lines. The two mRNAs for Rab / were very abundant in A1251 cells while they were barely visible in HepG2. The fact that the expression of the Rab7 mRNAs is altered in A1251 and HepG2 cellsthat come from two different kind of tumours, could suggest an important role of this protein in these pathologies

The high degree of conservation between the humen and the yeast Rab7 protein indicates a critical structure-function relationship in the Rab7 protein conserved throughout the evolation similarly to what happens for other GTP-binding proteins of the same family.

The present data demonstrate, for the first time, a functional involvement of the endocytic pathway in vacuolar cell degeneration due to VacA. Efficient membrane flow from early to late endosomes and a functional Rab7 are necessary for vacuole formation and growth. The present data suggest that Rab7 is essential for the homotypic fusion of late endosomes, which appears to generate vacuoles and support their growth.

Future Plan

The significance of these anomalies is not clear, the possibility of rampant horizontal gene-transfer is unnerving to a community that hopes to reconstruct the history life on the basis of amino-acid sequence comparison. To test the point we will examine a number of bacterial urease sequences including H.pylori, and a number of plant urease sequences including plants.

We can study antibody response to different strains of H.pylori and other bacterial species (with different process).

We will find several H.pylori strains specific proteins of which bands B, C, D,E, and L may be of interest as antigen preparations for future gastric ulcers. Whether one protein or a larger number of the proteins should be included or whether they should be used separately or as a mixture is still to be settled. Studies suggest, however, that morethan one protein should be used for the antigen peparation. A combination of the H, I, J, K, and L bands had a high level of discrimination between H.pylori-positive and negative patients, and this combination of proteins will be studied further.

We will study about VacA and Rab7 because they are not yet known which molecules besides Rab7 can serve as a target for VacA.

The isolation of the human Rab7 cDNA will allow us to study in detail the role of this protein in the endocytic pathway both in human cell lines and in normlaor pathological states.this could be very significiant especially considering that the late endosomal compartment appears to be involved in the pathogenesis of several important human diseases.

In all studied prokaryotes the two genes are contiguous, but separate and are part of the same transcriptinal unit. Whether tis gene fusion in H.pylori results in a fused protein, or whether the transcriptional or translational product of the fusion is subject to splicing, is currently not known.

Further work to clone the full length cDNAs of these genes are needed to clarify the identify of nucleotide sequence and to study their function as the pathogenic factors in gastric diseases. Although we don't know the role of expression of host cellular genes in the pathogenesis of gastric diseases associated with H.pylori infection, we speculate that this event is involved in the inflammantory response, which is an important factor of gastritis, ulcers and presumably, gastric cancer.

At this moment, we can not make a conclusion whether or not those novels genes were specifically induced for H.pylori lysate, and we would not reject the assumption that yhose genes may be also highly induced by some other pathogenic bacteria. However, the success of H.pylori but not almost other pathogenic bacteria residing in stomach makes gene induction by H.pylori lysate have an unique and specific pathogenesis meaning.

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