論文の内容の要旨

論文題目

Regulation of the *Helicobacter pylori* CagA oncoprotein by the SH2 domain containing protein tyrosine phosphatase SHP1

(SH2 ドメイン含有チロシンホスファターゼSHP1によるピロリ菌がんタンパク質CagAの制御)

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Helicobacter pylori (*H. pylori*) is a gastric pathogen that colonizes approximately 50% of the world's population. Chronic Infection with *H. pylori cagA*-positive strain is associated with human malignancies such as gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma of B-lymphocyte origin. The *cagA* gene, which is located at *cag* PAI in the *H. pylori* chromosome, encodes a 120-to 145-kDa immunodominant protein, CagA. CagA is injected directly from the bacterium into gastric epithelial cells via type IV secretion system and localized to the inner side of the plasma membrane, where it undergoes tyrosine phosphorylation by Src family kinases (SFKs) at the Glu-Pro-Ile-Tyr-Ala (EPIYA) sequence that is present in variable numbers in its C-terminal region. Depending on the geographic region four distinct EPIYA-sites have been described, EPIYA-A, -B, -C and –D each of which is conserved. Remarkably, the EPIYA-A and EPIYA-B motif are found in strains throughout the world, but EPIYA-C is mainly present in strains from western countries (Australia, Europe and North America) and some Asian countries (India and Malaysia), while the EPIYA-D motif predominates in the far East (China, Japan and Korea). Tyrosine-phosphorylated CagA specifically binds to SHP2, a cytoplasmic Src-homology 2 (SH2) domain-containing protein tyrosine phosphatase and stimulates the phosphatase activity of SHP2. Deregulation of SHP2 by CagA induces abnormal proliferation and movement of gastric epithelial cells promoting the acquisition of a cellular transformed phenotype known as hummingbird phenotype. Perturbation of SHP2 by CagA is thought to be substantially involved in

pathological processes that are associated with *cagA*-positive *H. pylori* infection. CagA transgenic mice studies showed induction of abnormal proliferation of gastric epithelial cells as well as hematopoietic cells, followed by the development of gastrointestinal carcinomas and leukemias /lymphomas in a tyrosine phosphorylation-dependent manner.

SHP2 and its structural close relative SHP1 comprise a unique subfamily of vertebrate protein tyrosine phosphatases (PTPs). The PTPs with SH2 domains, SHP1 and SHP2, are prone for interaction with tyrosine-phosphorylated proteins and have been shown to bind to multiple receptor species including transmembrane tyrosine kinases, cytokine receptors, antigen receptors, and adhesion molecules. SHP1 (also termed as PTPN6) is expressed predominantly in hematopoietic cells of all lineages and all stages of maturation, however it is also expressed in epithelial cells. In contrast, the structurally close relative SHP2 (also termed as PTPN11) is widely expressed. Both SHP1 and SHP2 are composed of a central catalytic domain, two SH2 domains at their N-termini and a C-terminus with potential tyrosine phosphorylation sites and are involved in the regulation of cellular proliferation and survival. Generally SHP1 has been shown to acts as a negative regulator of signal transduction in hematopoietic cells, terminating signals from a diverse range of signaling molecules including interleukin-3 receptor, c-Kit, colony-stimulating factor-1 receptor, B and T cell antigen receptors and receptor-associated JAK kinases. The essential role of SHP1 as a negative regulator of signal transduction is consistent with the multiple defects in hematopoietic cells observed in motheaten mice, which lack functional SHP1. Motheaten mice die soon after birth due to over-proliferation and accumulation of macrophages in the lungs. However functional role of SHP1 expressed in epithelial cells remains largely unknown.

In this study I explored the role of SHP2-related SHP1 tyrosine phosphatase in *H. pylori* CagA-mediated gastric carcinogenesis. The key findings in this work are the identification of SHP1 as the major and specific PTP that dephosphorylates *Helicobacter pylori* CagA oncoprotein and attenuates CagA-mediated pathogenesis in gastric epithelial cells. Here I found a novel interaction between SHP1 and *Helicobacter pylori* CagA, in gastric epithelial cells and B cells. Unlike CagA-SHP2 interaction the association between CagA-SHP1 is independent of the tyrosine phosphorylation of CagA at the EPIYA-motifs. I also demonstrated that SHP1 directly binds with CagA and this binding is mediated by N-terminus, containing in-tandem SH2 domains of SHP1. And such CagA-SHP1 interaction resulted in the activation of

enzymatic activity of the phosphatase in *in vitro* phosphatase assay.

The present study provided the evidence that SHP1 directly dephosphorylates *H. pylori* oncoprotein CagA in gastric epithelial cells and in *in vitro* dephosphorylation system. Expression of wild-type SHP1 not the phosphatase-dead form of SHP1 (C453S, D419A, R459M) resulted in the dephosphorylation of CagA, suggesting that the dephosphorylation is indeed depends on the catalytic activity of SHP1, indicating that the effect seen with SHP1 was specific. I also demonstrated that SHP1 but not its closest structurally related family member SHP2 is the specific phosphatase for CagA, further highlighting the specificity of SHP1 on CagA as a substrate. Furthermore, knockdown of SHP1 in AGS cells resulted in the up-regulation of tyrosine phosphorylation of CagA. These data collectively indicating that CagA indeed be a direct target for SHP1. To our knowledge no specific tyrosine phosphatase for *H. pylori* CagA has been reported. This is the first demonstration for the presence of a mammalian phosphatase that dephosphorylates the bacterial EPIYA effector protein CagA.

Tyrosine phosphorylation of CagA is an essential prerequisite for CagA-SHP2 complex formation and subsequent induction of unique cell-morphology termed hummingbird phenotype, a phenotype associated with cell scattering and increased cell motility. Inhibition of CagA tyrosine phosphorylation or disruption of the CagA-SHP2 complex formation might result in the attenuation of *H. pylori* CagA function. CagA-SHP2 complex formation plays the initial and major step in the pathogenesis of *H. pylori* CagA. I found that expression of SHP1 resulted in the tyrosine dephosphorylation of CagA and thereby inhibited the 'hummingbird phenotype' caused by tyrosine-phosphorylated CagA, further highlighting the importance of SHP1-mediated CagA dephosphorylation in gastric epithelial cells. Recently it was reported that SHP1 expression is suppressed during the process of gastric carcinogenesis via promoter hyper-methylation and suggesting the role of SHP1 as a tumor suppressor in the gastric epithelium. In this study I found that SHP1 tyrosine phosphatase effectively tyrosine-dephosphorylated *H. pylori* oncoprotein CagA in gastric epithelial cells, thereby inhibiting or attenuating the pathophysiological action of CagA in gastric epithelial cells. Attenuation of *H. pylori* CagA activity by C-terminal Src kinase (CSK) has been reported previously. In that case CSK inhibits the kinase activity of Src, which tyrosine-phosphorylates CagA. My findings provide an important role of SHP1 in down-regulating *H. pylori* CagA-mediated gastric carcinogenesis.

CagA-mediated cellular pathogenesis depends at least partly on relative levels of SHP2 expression in the host cells. In normal gastric epithelial cells SHP2 expression is relatively high compared to SHP1 (http://157.82.78.238/refexa/main_search.jsp, Laboratory for Systems Biology and Medicine at RCAST, The University of Tokyo). Therefore it might be expected that the relative low expression of SHP1 in gastric epithelial cells might contribute to the development of CagA-mediated pathogenesis where SHP2 is predominantly expressed. On the other hand overexpression of SHP1 almost completely abolished the phosphorylation of CagA and attenuate CagA-mediated gastric pathogenesis. Since SHP1 is abundantly expressed in hematopoietic cells including B-lymphocytes to which CagA can also be delivered by *H. pylori*. And it is possible that high expression levels of SHP1 in lymphocytes might be one reason that explains the low incidence of MALT lymphoma compared to gastric cancer in patients infected with *cagA*-positive *H. pylori*. Despite low levels of expression in gastric epithelial cells, SHP1 might be an attractive molecular target in preventing gastric cancer as it abolishes tyrosine phosphorylation-dependent oncogenic action of CagA. Development of a small compound that stimulates the phosphatase activity of SHP1 may be applicable to the prophylaxis of gastric cancer in individuals infected by *H. pylori cagA*-positive strains. Given these it will be very helpful for our understanding of the mechanism of regulation of *H. pylori*-related gastric diseases, especially the gastric cancer, the second leading cause of cancer-related death worldwide and also MALT Lymphoma.