[課程－２]

審査の結果の要旨

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Infection with *Helicobacter pylori* (*H. pylori) cagA*-positive strain is associated with human malignancies such as gastric carcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma of B-lymphocyte origin. *H. pylori* effector protein CagA is injected by the bacterium into gastric epithelial cells via type IV secretion, where it undergoes tyrosine phosphorylation at the EPIYA motif by the host cell kinases. Tyrosine-phosphorylated CagA specifically interacts with SHP2 tyrosine phosphatase, which is known to act as a human oncoprotein. Indeed, SHP2 is a key target molecule that determines the virulence of *cagA*-positive *H. pylori.* Since SHP1 tyrosine phosphatase is a close relative of SHP2, I explored in this study the role of SHP1 in *H. pylori* CagA-mediated gastric carcinogenesis. The experiments I performed and the results I obtained in this work are as follows,

1. To examine whether CagA can form physical complex with SHP1, I transiently co-transfected CagA and SHP1 vectors in AGS human gastric epithelial cells and with the use of sequential immunoprecipitation-western blotting technique, I found that CagA physically interacts with SHP1. I performed infection experiment (MOI: 100) of BJAB human B cells with *H. pylori cagA*-positive strain or isogenic *cagA*-negative strain and found that CagA is directly delivered into B cells, where it efficiently interacted with endogenous SHP1.

2. To investigate whether the observed CagA-SHP1 interaction depends on CagA tyrosine phosphorylation, I transiently transfected a wild-type CagA or phosphorylation resistant CagA vector together with an SHP1 vector in AGS cells. Upon sequential immunoprecipitation-western blotting experiment, I found that CagA-SHP1 complex formation is independent of tyrosine phosphorylation of CagA. *In vitro* binding assay using full-length SHP1 (1-595) or SHP1ΔN [lacking the N-terminal region (247-595)], revealed that full-length SHP1 but not the SHP1ΔN bound to GST-CagA-His. This result indicated that the N-terminal region of SHP1, which contains the two SH2 domains, is required for direct binding with CagA. I also found that the CagA-SHP1 interaction gives rise to the activation of the catalytic activity of SHP1 by *in vitro* phosphatase assay.

3. Since SHP1 is a protein tyrosine phosphatase, I next examined the effect of SHP1 on the tyrosine phosphorylation status of CagA in AGS gastric epithelial cells by ectopically expressing wild-type

SHP1 or its catalytically inactive-mutants (D419A, R459M, C453S) together with CagA in AGS cells. Wild-type SHP1 but not the catalytically inactive-mutants reduced the levels of CagA tyrosine phosphorylation. Further more, *in vitro* phosphatase assay of wild-type SHP1 or catalytically inactive SHP1 (SHP1 C453S) using tyrosine-phosphorylated CagA (pY CagA-His) as a substrate also showed that wild-type SHP1 but not SHP1 C453S tyrosine-dephosphorylated CagA, indicating that SHP1 is a phosphatase that is responsible for CagA dephosphorylation at the EPIYA-motifs. To exclude the possibility that CagA was non-specifically dephosphorylated by phosphatase in *in vitro* phosphatase assay, SHP1 homologue SHP2 was subjected to *in vitro* phosphatase assay with the use of tyrosine-phosphorylated CagA as a substrate. The results of the experiment revealed that SHP2 did not dephosphorylate CagA, highlighting the specificity of SHP1 towards CagA. Additionally, I found that specific knockdown of SHP1 in AGS cells using siRNA resulted in the elevated level of tyrosine-phosphorylated CagA.

4. In AGS gastric epithelial cells, translocation and subsequent phosphorylation of CagA causes a unique cell-morphological change known as 'hummingbird phenotype', a phenotype that is also associated with cell scattering and increased cell motility. To examine whether SHP1 is capable of counteracting the ability of CagA to induce the hummingbird phenotype, I ectopically expressed CagA alone and together with SHP1 or SHP2 in AGS cells and quantified the degree of hummingbird phenotype induction using confocal microscope. As a result, I found that hummingbird phenotype induced by CagA was attenuated in AGS cells expressing SHP1 but not SHP2. Thus SHP1 acted as a negative-regulator of the *H. pylori* CagA oncoprotein in gastric epithelial cells.

In conclusion, my present work revealed that SHP1 is a phosphatase that is responsible for the dephosphorylation and thus inactivation of the *H. pylori* CagA oncoprotein, which plays a key role in the development of human gastric carcinoma. This is the first demonstration for the presence of a mammalian phosphatase that dephosphorylates the bacterial EPIYA effector protein. SHP1 may be an attractive molecular target in preventing gastric cancer as it abolishes tyrosine phosphorylation-dependent oncogenic action of CagA. This study contributes to our understanding for the mechanism underlying the functional regulation of the *H. pylori* oncoprotein CagA, which plays a key role in the development of gastric cancer, the second leading cause of cancer-related death worldwide. This study therefore deserves the award of Ph.D. degree.