Chapter 3
CagA

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Abstract Chronic infection with *Helicobacter pylori cagA*-positive strains is the strongest risk factor for gastric cancer. *H. pylori* injects the *cagA*-encoded CagA protein into the host gastric epithelial cells. Recent studies revealed that CagA acts as a pathogenic/oncogenic scaffold, which promotes oncogenic signaling in the delivered host cells. Indeed, CagA interacts with a variety of cellular proteins and deregulates their functions. More specifically, the N-terminal structured region of CagA associates with ASPP2 and RUNX3. The C-terminal disordered region of CagA possesses multiple segments containing the Glu-Pro-Ile-Tyr-Ala (EPIYA) motif, which undergoes tyrosine phosphorylation by Src family kinases (SFKs), and the CagA-multimerization (CM) sequence. The EPIYA segments interact with SH2 domain-containing proteins such as SHP2 and Csk in a tyrosine phosphorylation-dependent manner, whereas the CM sequence binds to the polarity-regulating kinase PAR1 in a tyrosine phosphorylation-independent manner. We propose a hypothesis that mammalian proteome contains a distinct class of proteins carrying an EPIYA or EPIYA-like sequence, which are imitated by bacterial EPIYA effectors such as CagA to perturb intracellular signaling in the host cells.

Keywords CagA • Pathogenic/oncogenic scaffold • EPIYA motif/segment

3.1 Introduction

Gastric cancer is one of the most common neoplasms and the third leading cause of cancer mortality in the world. East Asian countries (Japan, Korea, and China) have the highest incidence rates of gastric cancer [1]. Chronic infection with *Helicobacter pylori* is associated with gastric diseases including cancer [2]. *H. pylori* strains that possess cytotoxin-associated gene pathogenicity island (*cag* PAI) in their genome are more virulent strains than those without *cag* PAI and are associated with more severe gastric mucosal damages. The *cag* PAI region, a ~37 kb genomic region acquired by horizontal transmission from unknown origin,

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contains ~30 genes encoding a type IV secretion system (T4SS) as well as cytotoxin-associated gene A (CagA). T4SS is a needle-shaped apparatus formed by supramolecular assembly of multiple Cag proteins [3, 4], and CagA, a 120–145 kDa protein encoded by the *cagA* gene, is delivered into the host gastric epithelial cells via T4SS [5]. Epidemiological studies revealed the increased risk for gastric carcinoma in individuals infected with *cagA*-positive *H. pylori* strains compared with those infected with *cagA*-negative strains [6–8].

3.2 Invasion of CagA into the Gastric Epithelial Cells

In normal eukaryotic cells, the outer leaflet of plasma membrane is enriched with choline-containing phospholipids and glycosphingolipids, whereas the inner leaflet is enriched with amine-containing phospholipids such as phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidic acid (PA), and phosphatidylinositol (PI) [9]. The direct attachment of *H. pylori* to gastric epithelial cells induces the transition of the plasma membrane PS to the outer leaflet. The externalization of PS upon *H. pylori* infection is rapid, transient, and independent of apoptotic process. At the interface between T4SS and host cell surface, CagA directly binds to the externalized membrane PS via the electrostatic interaction, which is critical for the delivery of CagA into host cells. Delivered CagA again binds to the membrane PS and thereby localizes to the inner face of the plasma membrane [10]. Inside the cells, CagA undergoes tyrosine phosphorylation at the Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs, which are present in a variable number in its C-terminal region, by Src family kinases (SFKs) and c-Abl kinase [7, 11, 12]. CagA promiscuously interacts with multiple cellular proteins via its C-terminal region in both tyrosine-phosphorylation-dependent and tyrosine-phosphorylation-independent manners and thereby perturbs the host cell signaling. As such, CagA is considered to act as a bacterium-derived scaffold/hub protein that potentiates oncogenic signaling in delivered gastric epithelial cells [13].

3.3 Structure of CagA

CagA comprises more than 1200 amino acid residues with sequence diversity in its C-terminal region among distinct *H. pylori* isolates. Crystal structure analysis revealed that the CagA N-terminal region (~70 % of the entire protein) has a solid structure, whereas the C-terminal region (~30 % of the entire protein) containing the EPIYA motifs is intrinsically disordered (Fig. 3.1) [14, 15]. The N-terminal region of CagA is composed of three distinct domains, domains I, II, and III. Domain I, a mobile module with α-helical structure, mediates the interaction of CagA with ASPP2, a tumor suppressor apoptosis-stimulating protein of p53,

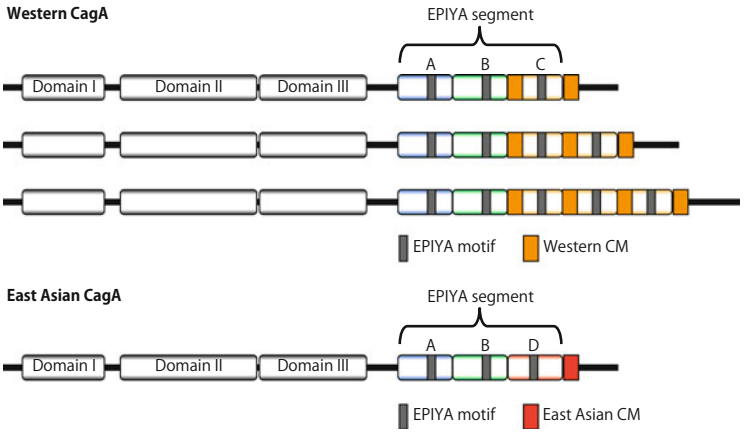

Fig. 3.1 Structure of *H. pylori* CagA. The N-terminal region of CagA contains domains I, II, and III. Based on the structural diversity of the C-terminal region, CagA is classified into Western CagA and East Asian CagA. The C-terminal region of Western CagA contains the EPIYA-A, EPIYA-B, and EPIYA-C segments. Western CagA contains at least two CM motifs. The C-terminal region of East Asian CagA contains the EPIYA-A, EPIYA-B, and EPIYA-D segments. East Asian CagA has a single CM motif

that induce the degradation of p53 (Fig. 3.2) [16, 17]. Domain I also binds to a tumor suppressor RUNX3 and thereby inactivates the tumor suppressor [18].

Domain II forms a structural core of CagA and contains the basic patch, a cluster of basic residues, with which CagA binds to the membrane PS. Domain II also contains a large antiparallel β-sheet, through which CagA associates with β1-integrin. Domain III intramolecularly interacts with the disordered C-terminal region, stabilizing the complex of CagA with multiple cellular molecules [14].

In the disordered C-terminal region, four EPIYA segments, EPIYA-A, EPIYA-B, EPIYA-C, and EPIYA-D, are classified based on the amino acid sequence surrounding each of the EPIYA motifs. The diversity of the C-terminal region is due to combination and/or order of the four distinct EPIYA segments, which serve as tyrosine phosphorylation sites [19, 20]. The EPIYA-A and EPIYA-B segments are common in almost all CagA species. The EPIYA-C segment is specific to "Western CagA," which is distributed in Europe, North America, and Australia, whereas the EPIYA-D segment is specific to "East Asian CagA," which is distributed in East Asian countries. Furthermore, the EPIYA-C segment duplicates in variable numbers (from 0 to 5) among Western CagA species [19].

The CagA-multimerization (CM) sequence, which was originally identified as a CagA sequence that is responsible for CagA multimerization (dimerization) in host cells, is a 16 amino acid stretch present within the EPIYA-C segment and in also the end of EPIYA-repeat region. Accordingly, Western CagA proteins with ABC, ABCC, and ABCCC types contain two, three, and four CM motifs, respectively. East Asian CagA has a single CM motif that is located immediately following EPIYA-D segment (Fig. 3.1) [19, 21, 22].

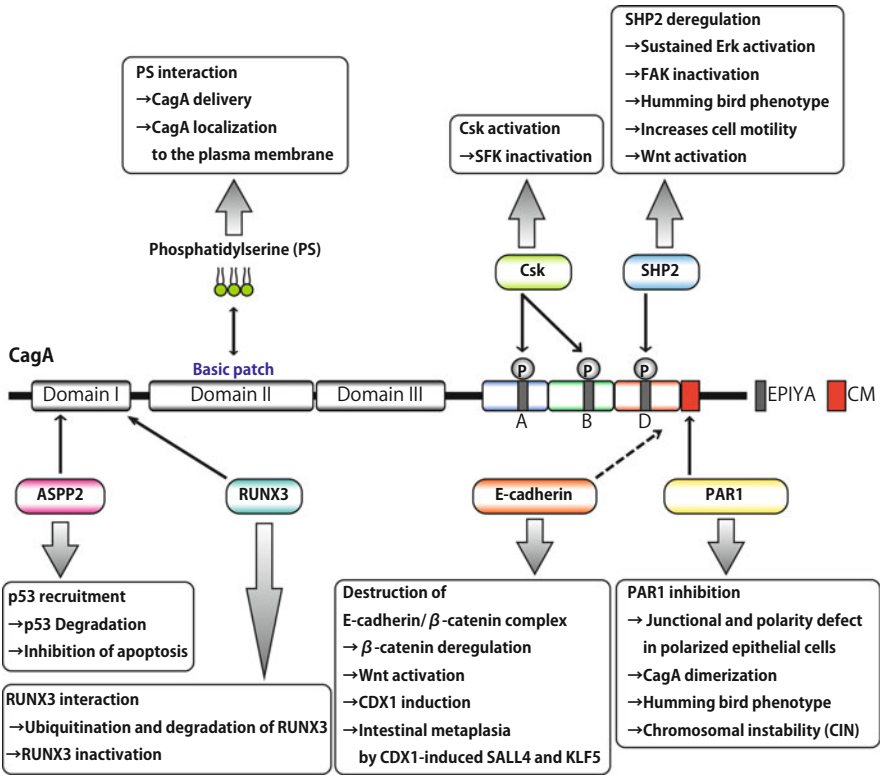

Fig. 3.2 *H. pylori* CagA as a pathogenic/oncogenic scaffold. Domain I of CagA binds to ASPP2. CagA-ASPP2 complex recruits p53 and induces degradation of p53 to inhibit apoptosis. Domain I also binds to RUNX3 and thus inactivates its tumor-suppressive function via degradation. Domain II of CagA contains basic patch, which is required for CagA delivery via T4SS and localization to the plasma membrane. Tyrosine-phosphorylated EPIYA motifs can bind to Csk and SHP2. The CagA-Csk complex activates Csk, which in turn inhibits SFK activity. The CagA-SHP2 complex deregulates the pro-oncogenic SHP2 phosphatase activity. CagA associates with E-cadherin in a phosphorylation-independent manner, thereby inducing aberrant Wnt activation that leads to the development of intestinal metaplasia. The CM motif binds to and inhibits PAR1. This interaction causes junctional and polarity defects while inducing chromosomal instability

3.4 EPIYA-Dependent CagA Function

CagA is phosphorylated at the EPIYA segments in the C-terminal region by SFKs and c-Abl [7, 23]. Upon tyrosine phosphorylation, CagA interacts with the SHP2 protein tyrosine phosphatase via the EPIYA-C or EPIYA-D segment (Fig. 3.2) [20, 24, 25]. SHP2 possesses two SH2 domains, which can interact with tyrosine-phosphorylated peptides, in the N-terminal region, followed by a protein tyrosine phosphatase (PTP) domain. SHP2 is required for the full activation of the Ras-Erk pathway and is known as a *bona fide* oncoprotein because gain-of-function

mutations in SHP2 are associated with various human malignancies [26, 27]. CagA deregulates SHP2 phosphatase activity through complex formation and aberrantly activates Erk [24, 25]. Also, CagA-deregulated SHP2 dephosphorylates to inactivate focal adhesion kinase (FAK) [28]. FAK is a tyrosine kinase that regulates the turnover of focal adhesion spots [29]. Inactivation of FAK by CagA induces elongated cell shape known as the hummingbird phenotype and increases cell motility [28, 30].

Approximately 70 % of Western CagA species have a single EPIYA-C segment, and some have two or three [19, 31]. CagA with an increased number of EPIYA-C segments shows an increased ability to bind to and thereby deregulate SHP2 in a tyrosine phosphorylation-dependent manner. In contrast to the molecular polymorphism in Western CagA, almost all East Asian CagA have a single EPIYA-D segment [19, 31]. Notably, the EPIYA-D segment binds to SHP2 more robustly than the EPIYA-C segment does [20].

On the other hand, tyrosine-phosphorylated EPIYA-A or EPIYA-B segment serves a binding site for C-terminal Src kinase (Csk) (Fig. 3.2) [7, 32]. Csk is a non-receptor protein tyrosine kinase that phosphorylates the negative regulatory site of SFKs to inhibit their kinase activities [33]. CagA recruits Csk to the plasma membrane, where it activates Csk to inhibit membrane-anchored SFKs. As CagA is phosphorylated by SFKs, Csk activation at the membrane causes reduction of CagA tyrosine phosphorylation. CagA-mediated Csk activation therefore attenuates CagA-SHP2 signaling. This negative feedback loop may enable long-term colonization of *H. pylori* in the stomach without eliciting fatal damages to the host [32].

In addition, tyrosine-phosphorylated CagA promiscuously binds to a number of host proteins possessing SH2 domain(s) such as CrkI, CrkII, CrkL, Grb7, PI3-kinase, and Ras-GAP. The CagA-Crk complex also enhances cell scattering, motility, and proliferation. Whereas the functions and biological significances of these complexes have yet to be elucidated, it appears that CagA mimics tyrosine-phosphorylated host proteins to perturb intracellular signaling in the host gastric epithelial cells [13, 34].

The adaptor protein Grb2 binds to CagA via EPIYA region in a tyrosine phosphorylation-independent manner. Grb2 recruits Grb2-associated Sos, a guanine-exchange factor of the small GTPase Ras. Accordingly, the CagA-Grb2-Sos complex stimulates Ras-Erk pathway, which also promotes cell scattering and cell proliferation [35].

3.5 CM Motif-Dependent CagA Function

CagA directly interacts with a polarity-regulating serine/threonine kinase, partitioning-defective 1 (PAR1)/microtubule affinity-regulating kinase (MARK) via the CM motif in a tyrosine phosphorylation-independent manner (Fig. 3.2) [36]. CagA inhibits PAR1 kinase activity by binding to the catalytic domain of PAR1. Since PAR1 is a master regulator of cell polarity [37], the inhibition of

PAR1 activity by CagA causes junctional and polarity defects in polarized epithelial cells [36].

In mammals, PAR1 comprises four homologues (PAR1a/MARK3, PAR1b/MARK2, PAR1c/MARK1, and PAR1d/MARK4). CagA interacts with all the members of the PAR1 family kinases, among which PAR1b is the strongest binding partner [38]. Because PAR1 exists as a homodimer in cells, two CagA molecules passively dimerize through binding to PAR1. CagA dimerization stabilizes CagA-SHP2 complex and thereby potentiates SHP2 deregulation, causing enhancement of the hummingbird phenotype by CagA [39].

PAR1 phosphorylates microtubule-associated proteins (MAPs) on their tubulin-binding repeats to destabilize microtubules [40]. Microtubules are required for not only polarity regulation but also the formation of mitotic spindles. Inhibition of PAR1 kinase activity by CagA perturbs microtubule stability and causes microtubule-based spindle dysfunction. Accordingly, CagA-expressing cells display a delay in the transition from prophase to metaphase during mitosis, showing spindle misorientation. Thus, CagA gives rise to chromosomal instability (CIN) [41].

In non-polarized epithelial cells, CagA deregulates Erk signaling and induces senescence-like proliferation arrest via accumulation of p21 cyclin-dependent kinase (CDK) inhibitor [42]. In polarized epithelial cells, CagA causes disruption of tight junctions and loss of epithelial polarity [36, 43]. CagA-expressing cells are extruded from the polarized monolayer and undergo multiple rounds of cell divisions while overcoming senescence. In polarized epithelial cells, CagA-induced Erk signals prevent accumulation of p21 by activating a guanine nucleotide exchange factor-H1 (GEF-H1)-RhoA-RhoA-associated kinase (ROCK)-c-Myc pathway [42, 44]. CagA-expressing cells also show morphological changes resembling the epithelial-mesenchymal transition (EMT) [42, 45]. Recent study reported that CagA-induced EMT is caused by stabilization of Snail, a transcriptional repressor of E-cadherin [46]. Mechanistically, CagA binds to GSK-3, which phosphorylates and destabilizes Snail, at the CagA N-terminal region, causing GSK-3 translocation and inactivation.

c-Met receptor tyrosine kinase also interacts with CagA via the CM motif. This interaction causes deregulation of c-Met that aberrantly stimulates PI3-kinase/Akt kinase signaling. Hence, CagA-c-Met interaction activates both Wnt signaling and NF-κB [13, 34].

3.6 Perturbation of β-Catenin Signal by CagA

β-catenin is well known to be involved in canonical Wnt signaling, which is critical for growth and differentiation in various types of cells [47]. β-catenin is localized to adherence junction by interacting with E-cadherin. The E-cadherin/β-catenin complex plays an important role as a major component of the adherence junctions [48, 49]. The oncogenic *H. pylori* strain activates β-catenin in gastric epithelial cells

in Mongolian gerbils [50]. CagA interacts with E-cadherin in a tyrosine phosphorylation-independent manner (Fig. 3.2) [51, 52]. The interaction of CagA with E-cadherin destabilizes the E-cadherin/β-catenin complex and thereby promotes cytoplasmic/nuclear accumulation of β-catenin [51]. The CagA CM motif is required for deregulation of β-catenin. Deregulated β-catenin by CagA aberrantly activates β-catenin-dependent Wnt target genes including *CDX1*. *CDX1* encodes caudal-related homeobox transcription factor CDX1, which is specifically expressed in the intestine and plays a crucial role in development and maintenance of intestinal epithelia.

Intestinal metaplasia of gastric epithelium is an *H. pylori*-associated precancerous lesion. Although CDX1 is not expressed in normal gastric epithelium, chronic infection with *H. pylori* induces CDX1 expression in the gastric epithelial cells via nonphysiological Wnt activation by CagA [51]. Ectopically expressed CDX1 then induces the expression of *SALL4*, a zinc finger transcription factor playing an important role in maintaining self-renewal and pluripotency. CDX1 also upregulates *KLF5*, a member of the KLF family of transcription factors that are associated with stemness [53]. CagA-expressing gastric epithelial cells therefore dedifferentiate into tissue stemlike progenitor cells by CDX1-induced SALL4 and KLF5 and then transdifferentiate into intestinal epithelial cells. This provides a mechanism that underlies the development of intestinal metaplasia in stomach [53].

β-catenin controls embryogenesis and homeostasis as an effector of the canonical Wnt pathway, where Wnt ligand binds to Frizzled receptor to stabilize β-catenin. Stabilized β-catenin then translocalizes into the nucleus, where it interacts with transcription factors such as T-cell transcription factor (TCF)/lymphocyte enhancer factor (LEF) to upregulate Wnt target genes [47, 54]. Recent studies reported that SHP2, a primary target of CagA, is present not only in the cytoplasm but also in the nucleus. In the nucleus, SHP2 dephosphorylate parafibromin/Cdc73, a core component of the RNA polymerase II-associated factor (PAF) complex. Dephosphorylated parafibromin can bind to β-catenin, and the parafibromin/β-catenin complex induces expression of Wnt target genes such as *cyclin D1* and *c-myc* [55]. SHP2 also binds to transcriptional coactivators YAP and TAZ, which are targeted by the mammalian Hippo pathway that controls cell proliferation and apoptosis. At low cell density, YAP/TAZ localizes to and recruits SHP2 to the nucleus [56]. Nuclear SHP2 stimulates transcription of Wnt target genes by dephosphorylating parafibromin.

3.7 In Vivo Oncogenic Activity of CagA

In Mongolian gerbils, gastric adenocarcinoma can occur upon long-term infection with *H. pylori* [57]. The production of CagA and T4SS potentiates colonization of *H. pylori*, epithelial cell proliferation, significant atrophy, and mucous gland metaplasia in the stomach body [58]. Moreover, a particular *H. pylori* adapted to host

Mongolian gerbils can rapidly induce the development of gastric dysplasia and adenocarcinoma [50].

Oncogenic potential of CagA in vivo was directly investigated by employing mice transgenically expressing CagA throughout the body [59]. The CagA transgenic mice develop gastric carcinoma, small intestinal carcinoma, or hematological malignancy. However, these neoplastic changes do not occur in transgenic mice expressing a CagA mutant that does not undergo tyrosine phosphorylation. These observations indicate that tyrosine phosphorylation of CagA is critical for the development of malignant neoplasias in CagA transgenic mice.

In zebrafish model, transgenic expression of CagA induced hyperproliferation of intestinal epithelial cells and activates Wnt target genes *cyclinD1*, *axin2*, and zebrafish *c-myc* ortholog *myca* in a tyrosine phosphorylation-independent manner. Long-term expression of CagA causes hyperplasia in a phosphorylation-dependent manner [60]. These in vivo models clearly demonstrate the oncogenic potential of the bacterial protein in metazoans.

3.8 Bacterial EPIYA-Containing Effectors and Mammalian EPIYA-Containing Proteins

It has been reported that several pathogenic bacteria produce the effector proteins that contain multiple EPIYA or EPIYA-like motifs as is the case of *H. pylori* CagA (Table 3.1). These EPIYA effectors include Tarp of *Chlamydia trachomatis*, which causes sexually transmitted diseases and blindness; BepD, BepE, and BepF of *Bartonella henselae*, which causes human diseases such as cat-scratch disease, bacillary angiomatosis, bacillary peliosis hepatis, and neuroretinitis; AnkA of *Anaplasma phagocytophilum*, which causes human granulocytic anaplasmosis; LspA1 and LspA2 of *Haemophilus ducreyi*, which causes the sexually transmitted disease chancroid; and Tir of enteropathogenic *Escherichia coli* (EPEC). These bacterial EPIYA effectors are delivered into host cells by type III or IV secretion system. Inside the cells, these effectors undergo tyrosine phosphorylation at the EPIYA motifs by host cell kinases to acquire the ability to interact with multiple SH2 domain-containing cellular proteins and thereby to perturb host cell signaling [13, 61]. These bacterial EPIYA effectors share no sequence homology with each other except for the EPIYA motifs, indicating that they didn't molecularly evolve from a common ancestor. As the bacterial EPIYA effectors do not undergo tyrosine phosphorylation inside the bacteria and the bacterial proteome does not have SH2 domain-containing proteins, it was reasonable to assume the presence of a mammalian EPIYA-containing protein(s), the function of which is mimicked by the bacterial effectors [61].

A proteome analysis revealed that some mammalian proteins such as Pragmin/Sgk223, p140Cap/SRC kinase signaling inhibitor 1, and Partitioning defective 3 homolog B/PAR3β have the EPIYA or EPIYA-like motifs (Table 3.2)

Table 3.1 Bacterial effectors that contain EPIYA (or like) motifs

Pathogen	Effector protein	EPIYA (or like) motifs
H. pylori	CagA	EPIYA
C. trachomatis	Tarp	ENIYE
B. henselae	Bep	EPLYA
		EVVYA
		TPLYA
		EPLYA
A. phagocytophilum	AnkA	ESIYE
		EDLYA
		ESIYA
		EPIYA
H. ducreyi	LspA	EPIYG
		EPVYA
EPEC	Tir	VNPYA
		EHIYD
		EPIYD

[62]. A pseudokinase Pragmin/Sgk223, which was originally identified as an effector of Rnd2 GTPase, contains a single EPIYA motif at the N-terminal region. Pragmin binds to Rnd2 to stimulate RhoA activity [63]. Pragmin also undergoes tyrosine phosphorylation on the EPIYA motif by SFKs [64]. Tyrosine-phosphorylated Pragmin binds to and sequestrates Csk in the cytoplasm, thereby maintaining SFKs active [64]. A recent study demonstrated that Pragmin acts as a Notch activation complex kinase (NACK) and a Notch transcriptional coactivator and that homozygous loss of *Pragmin* causes embryonic lethality [65]. p140Cap/SRC kinase signaling inhibitor 1 has two EPIYA-like motifs (EPLYA and EGLYA), where it is tyrosine phosphorylated [66]. p140Cap binds to and activates Csk dependently of tyrosine phosphorylation at the EPIYA-like motifs. p140Cap regulates integrin signaling via p140Cap-Csk complex formation [67]. Partitioning defective 3 homologue B/PAR3β has a single EPIYA-like motif (EGLYA). PAR3β was originally identified as a homologue of the cell polarity protein PAR3 [68] and is essential for mammary gland stem cell maintenance [69]. Although the EPIYA-like motif of PAR3β is known to undergo tyrosine phosphorylation, its functional significance has yet to be elucidated [62].

Although each mammalian EPIYA-containing protein specifically interacts with particular SH2 domain-containing protein(s) to control the intracellular molecular network, the bacterial EPIYA effectors promiscuously interact with a number of host proteins. It is therefore suggested that bacterial EPIYA effectors act as "a master key" that picks the host protein-protein interactions and perturb multiple host signaling pathways involved in a broad range of physiological functions. In this regard, *H. pylori* CagA is the most investigated and archetypal bacterial EPIYA

Table 3.2 Mammalian proteins that contain EPIYA (or like) motifs

EPIYA (or like) motifs	Protein name
EPIYA	Pragmin/Sgk223
	General transcription factor TFIIE, α subunit
	Solute carrier family 2
	Transmembrane protein 218
	Coiled-coil domain-containing protein 146
EGLYA	p140Cap/SRC kinase signaling inhibitor 1
	Oxidative stress-induced growth inhibitor 1
	Short transient receptor potential channel 3, 6, 7
	Zinc finger SWIM domain-containing protein 4
	Zinc finger SWIM domain-containing protein 5
	Partitioning defective 3 homologue B/PAR3β
	Citron Rho-interacting kinase
	Filamin B
	Beta/gamma crystallin domain-containing protein 3
	Striated muscle preferentially expressed protein kinase
	WD repeat and FYVE domain-containing protein 3
	Malonyl-CoA-acyl carrier protein transacylase, mitochondrial
EPLYA	p140Cap/SRC kinase signaling inhibitor 1
	Putative fidgetin-like protein 2
ESIYE	Retinal dehydrogenase 2
	Carcinoembryonic antigen-related cell adhesion molecule 20
	Trafficking protein particle complex subunit 8
EDLYA	Myotubularin-related protein 6
	Myotubularin-related protein 8
	Early endosome antigen 1
	Pre-mRNA-processing-splicing factor 8
	Piezo-type mechanosensitive ion channel component 2
ESIYA	Solute carrier family 25 member 38
EHIYD	Protein unc-119 homologue A
	Occludin
	Clathrin interactor 1
EPIYD	Nuclear receptor ROR-beta
	CUB and sushi domain-containing protein 3
ENIYE	Torsin-1A
	Zinc finger protein 674
	Zinc finger protein 699
	Zinc finger protein 568
	Sodium/hydrogen exchanger 9
	MICAL-like protein 2
	Putative ATP-dependent RNA helicase DHX30
	Testis- and ovary-specific PAZ domain-containing protein 1

(continued)

Table 3.2 (continued)

EPIYA (or like) motifs	Protein name
EPVYA	Palmdelphin
	Tripartite motif-containing protein 1b Tripartite motif-containing protein 1b-like protein
	Rho GTPase-activating protein 27 isoform
EVVYA	Probable ubiquitin carboxyl-terminal hydrolase FAF-Y
TPLYA	39S ribosomal protein L3, mitochondrial
	Zinc finger MIZ domain-containing protein 2
	Bile salt export pump
	Xin actin-binding repeat-containing protein 2
	Zinc finger protein 469

effector [34]. Possibly, pathogenic bacteria exploit the EPIYA effectors to achieve a successful colonization in the host.

3.9 Conclusion

The chronic infection with *H. pylori cagA*-positive strains is the strongest risk factor of gastric cancer. *H. pylori* delivers CagA into host gastric epithelial cells via T4SS. Upon delivery, CagA promiscuously interacts with a number of host cell proteins in both phosphorylation-dependent and phosphorylation-independent manners to subvert physiological cell functions. Carcinogenesis requires two major events. One is the activation of oncoprotein and the other is the inactivation of tumor suppressor. *H. pylori* CagA interacts with both of them and successfully disturbs their functions. Human gastric organoid (hGO) method has recently been reported [70]. This newly developed experimental approach may provide additional insights into our understanding of gastric carcinogenesis mediated by *H. pylori* CagA.

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