Chapter 5
Autophagy

Hitoshi Tsugawa and Hidekazu Suzuki

Abstract Autophagy is a eukaryotic, nonspecific degradation mechanism and serves as part of the innate immune system of host cells. In *Helicobacter pylori*-infected host cells, autophagy is activated by the bacterial vacuolating cytotoxin A (VacA) via the following pathway: VacA binds to low-density lipoprotein receptor-related protein-1, induces intracellular glutathione deficiency, and enhances activation of protein kinase B (Akt), which in turn induces Mdm2-mediated p53 degradation and then activates autophagy. Translocated bacterial proteins, VacA and CagA, are degraded by autophagy. However, autophagy is not activated in the CD44v9-expressing gastric cancer stem-like cells, leading to the specific accumulation of intracellular CagA. Therefore, the presence of CD44v9-expressing cells in *H. pylori*-infected patients is associated with the risk of developing gastric cancer. Autophagy in the host gastric epithelial cells plays an important role in *H. pylori* infectious disease via degradation of VacA and CagA.

Keywords VacA • CagA • Cancer stem cells • CD44

5.1 Induction of Autophagy by VacA in Host Epithelial Cells Infected with *Helicobacter pylori*

Autophagy is a nonspecific degradation mechanism seen in eukaryotic cells. Targets for degradation by autophagy include intracellular components such as mitochondria, where targets are surrounded by the cellular double membrane to form transient autophagosomes and are degraded by fusion with lysosomes. Autophagy is an important cellular recycling mechanism to overcome temporary starvation, in addition to removing intracellular foreign materials. In particular, intracellular parasitic bacteria such as group A streptococci, *Shigella* spp., *Salmonella* spp.,

H. Tsugawa (✉)
Department of Biochemistry and Integrative Medical Biology, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan
e-mail: h.tsugawa@a6.keio.jp

H. Suzuki
Medical Education Center, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo, 160-8582 Tokyo, Japan

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and *Listeria monocytogenes* are degraded by autophagy. In fact, autophagy serves as part of the innate immune system of host cells. In recent years, it has been revealed that autophagy is activated in *Helicobacter pylori*-infected cells, interestingly, by an exotoxin known as vacuolating cytotoxin A (VacA), which is produced by the bacterium and is involved in ulceration. VacA is biologically versatile and shows apoptosis-inducing activity via mitochondrial pathway [1]. In a recent study, Terebiznik et al. indicated that VacA induces autophagy [2, 3]. VacA is composed of p55 fragments involved in receptor recognition and an N-terminal (p33) fragment involved in vacuolization. Further, the p55 fragment can be divided into m1 type (m1VacA) and m2 type (m2VacA) due to the difference in primary structure. Receptor protein-tyrosine phosphatase (RPTP) α and β have been identified as receptors for VacA [4, 5]. Additionally, Yahiro et al. recently indicated that low-density lipoprotein receptor-related protein-1(LRP1) is a receptor for VacA and this binding is important for induction of autophagy [6]. Our study demonstrated that although m1VacA bound to LRP1, m2VacA did not [7]. Thus, m2VacA probably does not induce autophagy.

Interestingly, m1VacA causes a reduction in intracellular glutathione (GSH) levels in the host epithelial cells, resulting in the accumulation of intracellular reactive oxygen species (ROS) [7]. It is well known that accumulation of intracellular ROS activates autophagy. In fact, autophagy induced by m1VacA is repressed by treatment with antioxidants such as N-acetylcysteine [7]. In addition, m1VacA also induces p53 downregulation during autophagy. Tasdemir et al. reported that p53 inactivation by chemical inhibition or knockdown induces autophagy via inhibition of mTOR [8]. Our study showed that p53 downregulation by m1VacA is necessary for the induction of autophagy [7]. In fact, the autophagic pathway via m1VacA is as follows: m1VacA induces GSH deficiency by binding to LRP1 and enhances activation of protein kinase B (Akt), which in turn induces Mdm2-mediated p53 degradation, activating autophagy [7] (Fig. 5.1).

Recent studies have revealed that p62 binds to LC3 on the autophagosomal membrane to target ubiquitinated aggregates for selective degradation [9]. Yahiro

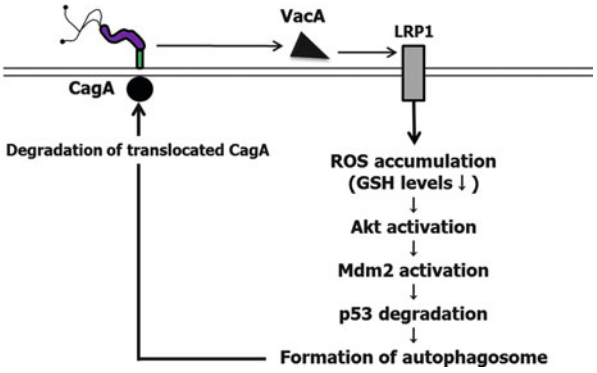

Fig. 5.1 Autophagy leading signals by VacA. VacA induced GSH deficiency via binding to LRP1 and then enhances activation of Akt. Activation of Akt induces Mdm2-mediated p53 degradation and then activates autophagy

et al. demonstrated that LC3-II is colocalized with p62 on the autophagosomal puncta induced by VacA [6]. Therefore, autophagy activated by VacA is considered selective and involves targeting by ubiquitination. Further studies are needed to clarify the selective mechanisms of VacA-activated autophagy.

5.2 Significance of Autophagy in Host Epithelial Cells Infected with *H. pylori*

H. pylori is a significant risk factor for the development of peptic ulcers and gastric cancer; therefore, in this chapter, we focus on the role of autophagy in *H. pylori* infection.

VacA binds to host cell-surface receptors and is internalized via endocytosis. An in vitro study demonstrated that VacA induces vacuolization and apoptosis in host epithelial cells [1]. These activities have been implicated to cause induced gastric mucosal injury in gastric ulcers during *H. pylori* infection [10]. Recently, it has been reported that intracellular VacA is degraded by autophagy activated by VacA itself [2, 3]. Therefore, decreased activation of autophagy by genetic polymorphisms of autophagy-related genes *ATG16L1* contributes to increase in VacA-mediated toxicity [2, 3].

The CagA effector protein is delivered into *H. pylori*-attached host epithelial cells via type IV secretion system (TFSS). Translocated CagA binds to and dysregulates SHP-2 tyrosine phosphatase and specifically interacts with the polarity-regulating kinase partitioning-defective 1 (Par1b)/microtubule affinity-regulating kinase 2 (MARK2) to disrupt tight junctions and cause a loss of epithelial apical-basolateral cell polarity. Additionally, it has been reported that *CagA*-transgenic mice developed gastrointestinal carcinomas. These observations indicate that CagA effector protein is a bacterial oncoprotein, involved in gastric carcinogenesis. However, translocated CagA is degraded by autophagy induced by VacA, and, as a result, CagA does not persist in host gastric epithelial cells [7].

Thus, it is thought that autophagy induced by VacA contributes to a reduction in gastric mucosal injury and gastric cancer risk associated with *H. pylori* infection, via degradation of VacA and CagA. On the other hand, characteristic alterations in the host cell, involved in the repression of autophagy, induce the accumulation of intracellular VacA and CagA. Thus, it is presumed that inhibition of autophagy may lead to increased gastric cancer risk via specific accumulation of CagA. Therefore, autophagy response in host gastric epithelial cells is considered to play an important role in the development of gastric carcinogenesis.

5.3 Suppression of Autophagy and Accumulation of CagA in CD44v9–Expressing Cancer Stem-Like Cells

CD44 is a cell-surface marker associated with cancer stem cells in various tumors. Gastric cancer stem-like cells express a variant isoform of CD44 (CD44v9). A recent study reported that the recurrence rate of early gastric cancer (EGC) is significantly higher in CD44v9-positive individuals than in CD44v9-negative individuals [11]. CD44v9-expressing gastric cancer cells suppress ROS accumulation via control of intracellular GSH levels by stabilizing xCT, a cystine transporter [12]. Since induction of autophagy by VacA requires the reduction of GSH, we hypothesize that autophagy is not activated by VacA in CD44v9-expressing cells. In fact, intracellular GSH levels in CD44v9-expressing cells are not decreased by VacA [7]. Further, increase of Akt, Mdm2 phosphorylation, and p53 degradation are also not observed in CD44v9-expressing cells; hence, autophagy is not induced by VacA [7]. In addition, translocated CagA accumulates in CD44v9-expressing cells (Fig. 5.2), which is reversed with sulfasalazine, a potent xCT inhibitor and a well-known anti-inflammatory drug for rheumatic arthritis and inflammatory bowel disease. Moreover, autophagy is activated in CD44v9-expressing cells by treatment with sulfasalazine [7]. These observations reveal that specific accumulation of intracellular CagA in CD44v9-expressing cancer stem-like cells is caused by the repression of autophagy. Therefore, the presence of CD44v9-expressing cells in *H. pylori*-infected patients is associated with the risk of developing gastric cancer.

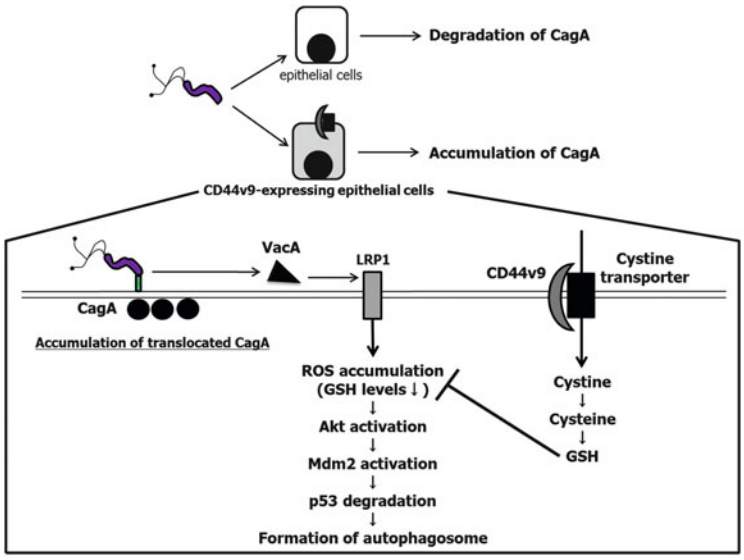

Fig. 5.2 Accumulation of intracellular CagA in the CD44v9-expressing cells through the repression of autophagy

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