1362
doi:10.1017/S1431927617007474
Microsc. Microanal. 23 (Suppl 1), 2017

Characterizing the Intracellular Trafficking of *Helicobacter pylori* VacA

Nora J. Foegeding[1], Timothy L. Cover [2,3,4], Melanie D. Ohi[1,5,6]

[1]Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN, USA
[2]Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN, USA
[3]Department of Pathology, Microbiology and Immunology, Vanderbilt University School of Medicine, Nashville, TN, USA
[4]Veterans Affairs Medical Center, Nashville, TN, USA
[5]Department of Biochemistry, Vanderbilt University, Nashville, TN, USA
[6]Center for Structural Biology, Vanderbilt University, Nashville, TN, USA

Helicobacter pylori persistently colonizes the gastric mucosa of more than half of the world population, with prevalence as high as 90% in developing nations. Infection with *H. pylori* causes chronic gastric inflammation and is the leading cause of stomach ulcers and gastric cancer. As a result of increased treatment failure due to antibiotic resistance, clinicians are seeking alternative strategies that specifically target *H. pylori* virulence factors to disrupt *H. pylori* colonization and prevent disease.

H. pylori secretes a number of virulence factors to establish and maintain colonization of the host [1]. Both the ability of *H. pylori* to colonize the stomach and the risk of developing *H. pylori*-mediated disease is directly associated with the secretion of a pore-forming virulence factor called vacuolating cytotoxin A (VacA). VacA is secreted as an 88 kDa monomer that oligomerizes to form an anion-selective channel [2-4]. It is comprised of two domains: a 33 kDa (p33) domain located at the N-terminus is required for channel formation [5-6] and a 55 kDa (p55) domain located at the C-terminus is involved in receptor binding [7-8]. VacA is a multifunctional toxin that triggers diverse responses from both epithelial and immune cells. These include vacuolation, pore formation in the plasma membrane, epithelial monolayer permeabilization, alteration of endo-lysosomal function, apoptosis, disruption of mitochondrial function, and inhibition of T lymphocyte activation. In addition, VacA has been shown to target endosomes, mitochondria, and epithelial cell-cell junctions. [9]. Despite decades of research, VacA's role in the development of *H. pylori*-associated disease is not understood.

Knowing the cellular location and itinerary of a protein is critical for understanding its biological role. In the current model of VacA trafficking, VacA is internalized into early endosomes (EE) that either mature into late endosomes (LE), where VacA induces vacuolation, or are postulated to be trafficked to mitochondria to induce apoptosis [9]. The ability of VacA to directly traffic to and form a pore in mitochondrial membrane is controversial. In support of this idea, *in vitro* experiments using isolated mitochondria have reported that VacA is imported into the inner mitochondrial membrane (IMM) [10,11] and that VacA directly causes $\Delta\Psi_m$ reduction [12]. In addition, fluorescence microscopy studies using fixed cells have reported that VacA localizes to mitochondria [10,13-15]. Others propose that VacA-induced mitochondrial dysfunction is due to VacA acting indirectly by simply activating pro-apoptotic factors Bax and Bak to trigger mitochondrial-dependent apoptosis [12]. They do not observe VacA localization to mitochondria nor do they detect VacA-induced release of cytochrome *c* from isolated mitochondria [12]. Furthermore, it has been suggested that VacA may travel retrograde through the Golgi and ER [16-17]; however, this has not been investigated. Lastly, although VacA has been shown to have cytosolic activity in cell culture *if* expressed or microinjected inside the host cell [10,18], it is not known whether extracellular VacA can be internalized and released into the cytosol. Therefore, despite having a

general framework for VacA trafficking, there are still many questions that remain regarding what happens to VacA after internalization.

To clarify the biological role of VacA, we are characterizing the intracellular trafficking of VacA. We are determining how VacA is internalized by inhibiting specific endocytic pathways (Fig. 1). Additionally, we are using fixed and live cell imaging to characterize which compartments VacA travels in and which organelles VacA targets. Together, these data contribute insight into the molecular mechanism behind VacA toxicity.

References:

[1] JC Atherton and MJ Blaser, J. Clin. Invest. **119** (2009), p. 2475-2487.
[2] H Iwamoto *et al,* FEBS Lett. **450** (1999), p. 101-104.
[3] DM Czajkowsky *et al,* Proc. Natl. Acad. Sci. **96** (1999), p. 2001-2006.
[4] F Tombola *et al,* FEBS Lett. **460** (1999), p. 221-225.
[5] D Ye, DC Willhite, and SR Blanke, J. Biol. Chem. **274** (1999), p.9277-9282.
[6] MS McClain *et al,* J. Biol. Chem. **278** (2003), p. 12101-12108.
[7] X Sewald, W Fischer, and R Haas, Trends Microbiol. **16** (2008), p. 89-92.
[8] IJ Kim and SR Blanke, Front. Cell. Infect. Microbiol. **2** (2012), p. 37.
[9] NJ Foegeding *et al,* Toxins **8** (2016), p. 173.
[10] A Galmiche *et al,* EMBO J. **19** (2000), p. 6361-6370.
[11] JH Foo *et al,* J. Mol. Biol. **401** (2010), p. 792-798.
[12] E Yamasaki *et al,* J. Biol. Chem. **281** (2006), p. 11250-11259.
[13] P Jain, ZQ Luo, and SR Blanke, Proc. Natl. Acad. Sci. **108** (2011), p. 16032-16037
[14] A Oldani *et al,* PLoS Pathog. **5** (2009), e1000603.
[15] DC Willhite and SR Blanke, Cell. Microbiol. **6** (2004), p. 143-154.
[16] B Kern *et al,* Cell. Microbiol. **17** (2015), p. 1811-1832.
[17] RH Argent, C McGarr, and JC Atherton, FEMS Microbiol. Lett. **237** (2004), p. 163-170.
[18] M de Bernard *et al,* Mol. Microbiol. **26** (1997), p. 665-674.

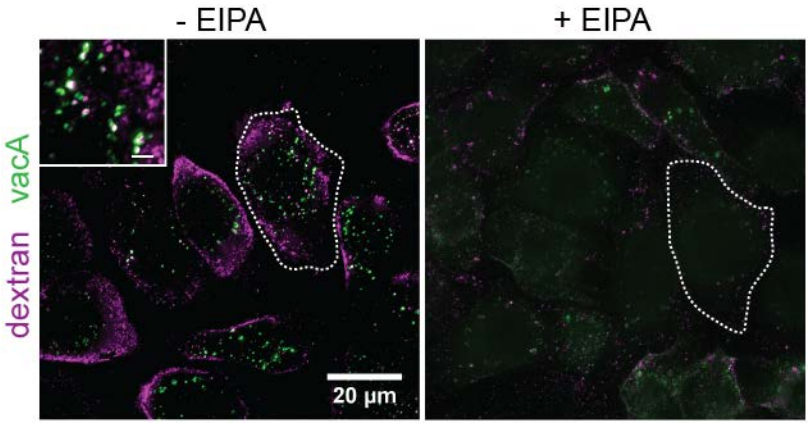

Figure 1. VacA internalization in the presence of a macropinocytosis inhibitor. AGS cells were preincubated with EIPA or a mock control. Following the pretreatment with EIPA, cells were treated with Alexa 488-VacA and 70 kDa TRITC dextran to permit cargo endocytosis and labeling of macropinosomes. Following the incubation, cells were acid washed to remove surface bound cargo, fixed, and imaged. Scale bar in zoom, 2 µm.

https://doi.org/10.1017/S1431927617007474 Published online by Cambridge University Press