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Helicobacter pylori Neutrophil-activating Protein: from Virulence Factors to Potential Therapeutic Targets and Diagnostic Biomarkers

Helicobacter pylori (*H. pylori*) is well recognized as a causative agent of acute and chronic gastric inflammation. Infection with *H. pylori* is associated with chronic gastritis, peptic ulcer disease, and gastric cancer. *H. pylori* neutrophil-activating protein (HP-NAP), a virulence factor of *H. pylori*, may play a crucial role in *H. pylori*-induced gastric inflammation due to its ability to attract and activate neutrophils. Due to the immunogenic and immunomodulatory properties of HP-NAP, this protein has been used for developing vaccines against *H. pylori* infection and new drugs for cancer therapy (Fig. 1). HP-NAP is also a potential diagnostic marker and drug target for *H. pylori*-associated diseases (Fig. 1). We have developed a method for one-step negative purification of recombinant HP-NAP by using diethylaminoethyl (DEAE) resin with high purity and high yield (Fig. 2) (Yang *et al*., 2015, *BMC Biotechnol*. 15: 23; Kuo *et al*., 2016, *JoVE (112)*, Hong *et al.*, 2017, *PLoS One*. 12(3), e0173632). The US and Taiwan patents have been issued for this invention in 2014. In addition, we have identified a commercial antibody being able to detect HP-NAP. This antibody also acts as a blocking antibody to inhibit HP-NAP-stimulated production of reactive oxygen species (ROS) by human neutrophils. Both Taiwan and U.S. patents for the new usage of this antibody have been issued in 2017. HP-NAP purified by this negative chromatographic method and the newly identified antibody against HP-NAP should be beneficial for the development of new drugs, vaccines and diagnostics for *H. pylori* infection or for other new therapeutic applications.

HP-NAP is a ligand of a pertussis toxin (PTX)-sensitive G protein-coupled receptor (GPCR) and Toll-like receptor 2 (TLR2). However, the identity of this PTX-sensitive GPCR is unknown. Also, how HP-NAP activates these two receptors are not clear. We have reported that HP-NAP triggers the human mast cell line-1 (HMC-1) cells to secrete histamine and interleukin-6 (IL-6) in a PTX-sensitive manner (Tsai *et al*., 2015, *Virulence* 6(8): 755). Our finding that HP-NAP stimulates histamine release from HMC-1 cells further supports the idea that HP-NAP plays a role in gastric mucosal injury by activating mast cells. Recently, we found that the engagement of this PTX-sensitive GPCR and TLR2 is respectively related to HP-NAP-induced production of ROS and IL-8 by neutrophil-like differential HL-60 promyelocytic leukemia cells (Fig. 3). In addition, we have developed a rapid mutagenesis approach to produce HP-NAP mutants for studying the interaction of HP-NAP with its receptors (Kuo *et al*., 2017, *Biol Proced Online*. 19:12). By employing these cell line models, we could like to identify and characterize the PTX-sensitive GPCR of HP-NAP. This receptor might serve as a new drug target to alleviate the disease symptoms caused by *H. pylori* infection.

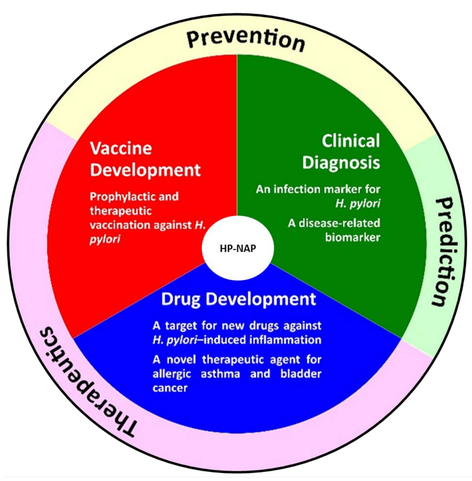

Fig. 1. Clinical applications of HP-NAP. HP-NAP is a potential candidate for development of new drugs, vaccines and diagnostics for *H. pylori* infection or for other new therapeutic applications. Such clinical applications could improve the prediction, prevention, and therapeutics of *H. pylori*-associated disease.

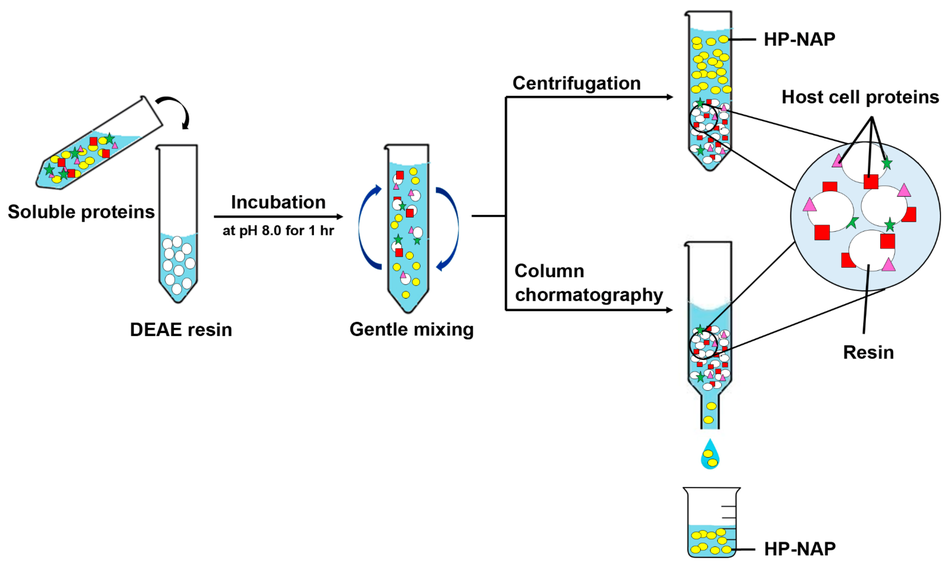

Fig. 2. Schematic outline of the negative purification of HP-NAP by DEAE resin in batch mode. The soluble protein fraction containing HP-NAP is added to the DEAE resin pre-equilibrated with Tris-buffer at pH 8.0. The proteins/resin slurries were then incubated at 4 ˚C for 1 hour with gentle mixing. The host cell proteins bind to the resins during the incubation. HP-NAP present in the unbound fraction is obtained by either collecting the supernatant after centrifugation or flow-through fraction from a column run by gravity flow.

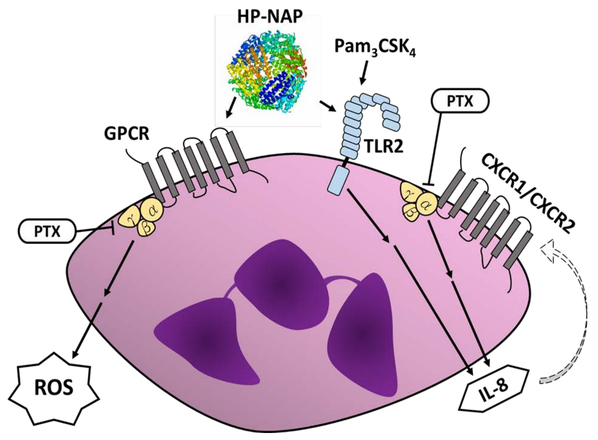

Fig. 3. Schematic representation of molecular mechanism involved in ROS production and IL-8 secretion induced by HP-NAP in ATRA-induced differentiated HL-60 cells. HP-NAP induces production of ROS in ATRA-induced differentiated HL-60 cells through an PTX-sensitive GPCR. HP-NAP-induced TLR2 activation triggers IL-8 secretion. Similarly, Pam_3CSK_4-induced TLR2 activation also triggers IL-8 secretion. The secretion of IL-8 induced by HP-NAP and Pam_3CSK_4 are almost completely inhibited by PTX. It is possible that HP-NAP-dependent secretion of IL-8 could induce autocrine signaling through CXCR1/CXCR2 in ATRA-induced differentiated HL-60 cells. The activation of CXCR1/CXCR2 further augments IL-8 secretion.

Molecular Regulation of Cellular Trafficking of Src Mediated by Thrombin Receptor

Thrombin, a multifunctional serine protease generated at sites of vascular injury, plays a critical role in hemostasis and thrombosis and perhaps in inflammatory and proliferative responses. Cellular responses to thrombin are mediated at least in part by a family of G protein-coupled protease-activated receptors (PARs). PAR1, the prototypical member of this family, is irreversibly activated upon thrombin cleavage. Src is an important downstream effector of PAR1-mediated cellular signaling. However, the regulation of Src kinase activity after PAR1 activation is very complex. Upon PAR1 activation, Src is recruited to the receptor by β-arrestins to form signaling complexes (Kao *et al.*, 2006, *Cell Signal.* 18:1914-1923). Src is then trafficked together with activated PAR1 to the endocytic vesicles and then sorted together with PAR1 to lysosomes for degradation (Fig. 4). PAR1-induced lysosomal degradation of Src depends on the endocytosis of activated PAR1 (Tasi *et al.*, 2019, *FEBS Lett.* 586:2351-2359) and appears to be promoted by β-arrestin2 (Kao *et al.*, 2006, *Cell Signal.* 18:1914-1923). This endocytosis-dependent lysosomal degradation of Src triggered by PAR1 occurs in both human embryonic kidney 293 and human umbilical vein endothelial cells (Tasi *et al.*, 2019, *FEBS Lett.* 586:2351-2359). Our findings provide a new paradigm for how an irreversibly activated receptor regulates its downstream signaling. We hypothesize that β-arrestin2 may serve as an adaptor for an E3 ubiquitin ligase to induce ubiquitination of Src for directing its lysosomal sorting induced by PAR1 (Fig. 5). We will further investigate this possibility by addressing the following questions. Is Src ubiquitinated after activation? Do ubiquitin E3 ligases and/or lysosomal sorting machinery involved in this event? How does β-arrestin2 promote this event?

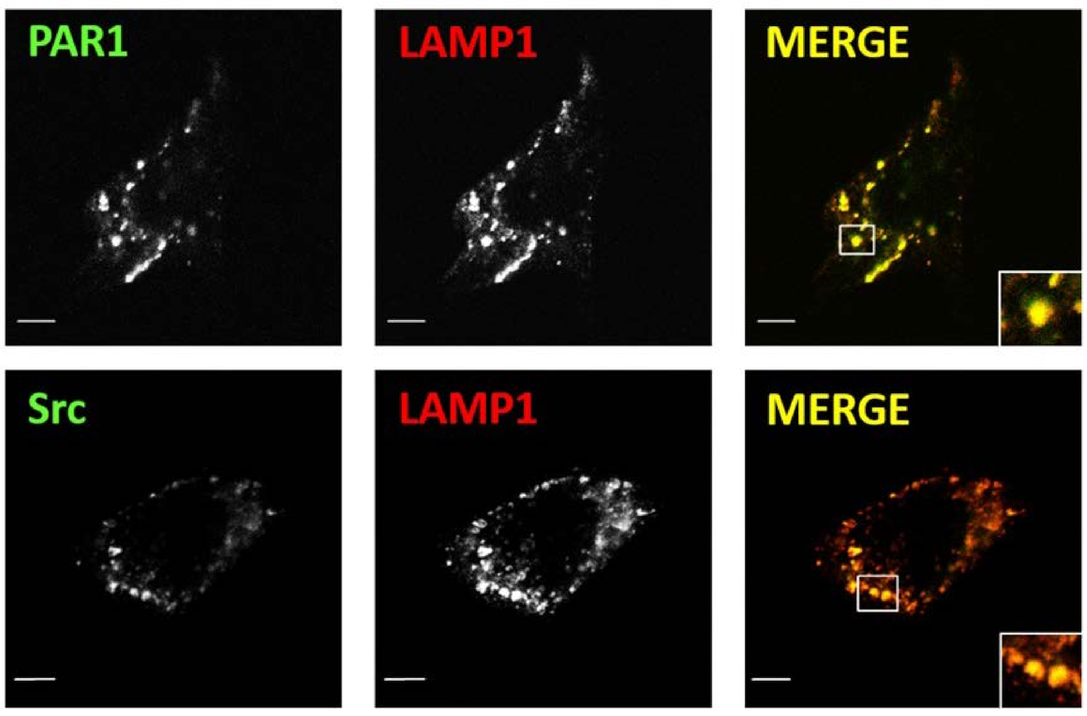

Fig. 4. PAR1-mediated lysosomal sorting of Src. HEK293 cells stably expressing FLAG-tagged PAR1 were serum-starved for 1 hour. Cells were then stimulated with 100 μM SFLLRN at 37 ˚C for 30 minutes. The cells were then incubated with anti-PAR1 1809 antiserum at 4 ˚C for 1 hour. Cells were fixed, permeabilized and immunostained with antibody against Src or lysosome-associated membrane protein 1 (LAMP1), a lysosomal marker, at 4 ˚C for 2 hours. Co-localization of LAMP1 with PAR1 or Src is represented as yellow in the merge images. Scale bar 5 μm. The images were collected by confocal microscopy.

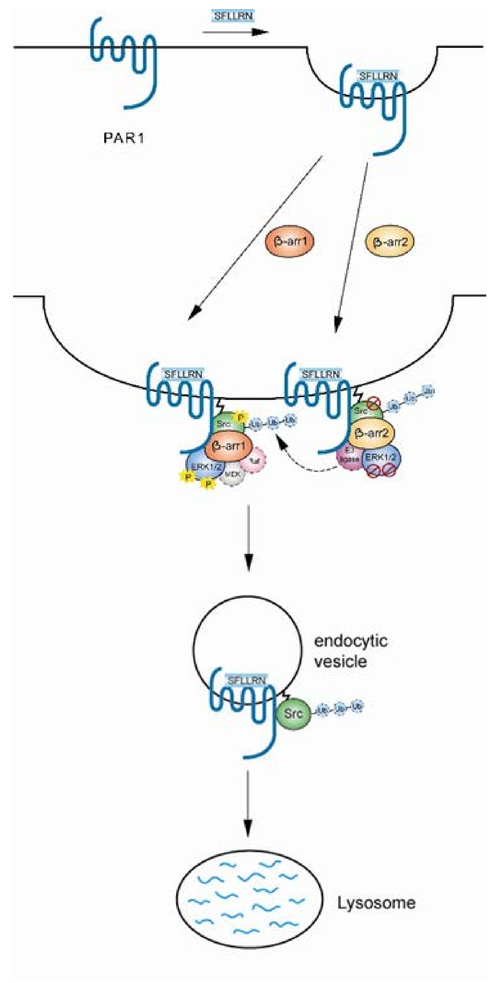

Fig. 5. Schematic representation of a possible molecular mechanism by which PAR1-mediated endocytosis-dependent lysosomal degradation of Src. Stimulation of PAR1 by its peptide agonist SFLLRN recruited β-arrestins to the receptor to form a signaling complex. β-arrestin 2 may serve as an adaptor for an E3 ubiquitin ligase to induce ubiquitination of Src for directing its lysosomal sorting induced by PAR1.