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GPCR signaling and endocytosis; Protein chemistry

Research Interests

One of the research interests of this laboratory is in the areas of molecular cell signaling and receptor trafficking. The focus of our current study is to characterize the molecular mechanisms of cellular signaling of protease-activated receptor 1 (PAR1). PAR1, a G protein-coupled receptor (GPCR) for the coagulant protease thrombin, mediates a variety of cellular responses important in cardiovascular biology and disease. It is activated by thrombin via an unusual proteolytic mechanism. Due to the irresveriable proteolytic activation of PAR1, lysosomal sorting of the receptor is critical for termination of PAR1 signaling. Recently, we found thatβ-arrestin was not required for the endocytosis of PAR1 but participated in the regulation of PAR1-mediated Src activation. β-arrestin acts as a scaffording protein to form the signaling complex with PAR1, Src, and activated extracellular regulated kinase (ERK) at the cell membrane and in endosomes to provide an additional PAR1-induced signaling pathway. Of interest, β-arrestin1 and β-arrestin2 play opposing roles in the regulation of PAR1-mediated Src activation. β-arrestin2 promotes the degradation of Src after PAR1 activation. Src is a critical signal transducer of PAR1-mediated cellular responses such as proliferation and angiogenesis. However, how cells terminate the PAR1 signaling at its effector level, Src, is not completed understood. We have been focusing on the investigation of the mechanisms by which PAR1 induces degradation of Src and interested in the following questions. What is the fate of Src after PAR activation? Is ubiquitin involved in PAR1-induced degradation of Src? How dose β-arrestin2 promote this event? In addition, we found that Src was re-expressed after its degradation in response to PAR1 activation. The re-expression of Src might regulate PAR1-indcued gene expression, which may be related to PAR1-promoted metastatsis of some cancer cells. We will also try to explore such possibility.

Our other research interest is focusing on the study of *Helicobactor pylori* neutrophil-activating protein (HP-NAP), a virulence factor of *H. pylori*. *H. pylori* is a microaerophilic gram-negative bacterium that colonizes the stomachs of an estimated half of all humans. Four diseases are now widely acknowledged to be caused by *H. pylori*: duodenal ulcer, gastric ulcer, adenocarcinoma of the distal stomach, and gastric mucosa-associated lymphoid tissue lymphoma. HP-NAP, a 150 kDa protein isolated from water extracts of *H. pylori*, was first found to be able to stimulate the production of reactive oxygen species (ROS) in neutrophils and promote neutrophil adhesion to endothelial cells. Now, HP-NAP is known to play a role not only in innate immunity but also in adaptive

immunity. Various reported inflammatory responses induced by HP-NAP support the idea that HP-NAP is important both for immunity and for pathogenesis. HP-NAP has been shown as a ligand binding to an unidentified GPCR and toll-like receptor 2 (TLR2). The engagement of GPCR and TLR2 seems to be related to HP-NAP-induced production of ROS and cytokines by monocytes, respectively. However, the details signaling events acting downstream of these receptors mediated by HP-NAP are not clear. We have successfully cloned, purified and characterized this protein. Pharmacological, molecular biological and biochemical approaches will be applied to explore the inflammatory mediators induced by HP-NAP and to identify the G protein-coupled receptor of HP-NAP. The findings should be able to provide the basic understanding of the cellular signaling events caused by *H. pylori* infection and new insights into the treatment and diagnosis of diseases associated with *H. pylori*.

Recent Publications

1. Wang, C-A., Liu, Y-C., Du, S-Y., Lin, C-W., and **Fu, H-W**. (2008). *Helicobacter pylori* Neutrophil-activating Protein Promotes Myeloperoxidase Release from Human Neutrophils. Biochem Biophys Res Commun. 377, 52-56.
2. Lai, C-H., Chang, Y-C., Du, S-Y., Wang, H-J., Kuo, C-H., Fang, S-H., **Fu, H-W.**, Lin, H-H., Chiang, A-S., and Wang, W-C. (2008). Cholesterol depletion reduces Helicobacter pylori CagA translocation and CagA-induced responses in AGS cells. Infect Immun. 76, 3293-3303.
3. Shu C-W., Sun, F-C., Cho J-H., Lin C-C., Liu, P-F., Chen, P-Y., Chang M D-T., **Fu, H-W.**, and Lai, Y-K. (2008). GRP78 and Raf-1 cooperatively confer resistance to endoplasmic reticulum stress-induced apoptosis. J Cell Physiol. 215, 627-635.
4. Lu, T-L., Kuo, F-T., Lu, T-J., Hsu, C-Y., and **Fu, H-W.** (2006). Negative Regulation of Protease-activated Receptor 1-induced Src Kinase Activity by the Association of Phophocaveolin-1 with Csk. Cell Signal. 18, 1977-1987.
5. Kuo, F-T., Lu, T-L., and **Fu, H-W**. (2006). Opposing Effects of β-arrestin1 and β-arrestin2 on Src Activation and Degradation Induced by Protease-activated Receptor 1. Cell Signal. 18, 1914-1923.
6. Chang, Y-S., Lee, L-C., Sun, F-C., Chao, C-C., **Fu, H-W**., and Lai, Y-K. (2006). Involvement of calcium in the differential induction of heat shock protein 70 by heat shock protein 90 inhibitors, geldanamycin and radicicol, in human non-small cell lung cancer H460 cells. J Cell Biochem. 97, 156–165.
7. Pickett, J. S., Bowers, K. E., Hartman, H. L., **Fu, H-W**., Embry, A., Casey, P. J., and Fierke, C. A. (2003). Kinetic Studies of Protein Farnesyltransferase Mutants Establish Active Substrate Conformation. Biochemistry 42, 9741-9748.

Selected Conference Presentations

1. **Fu, H-W.**, Wang, C-A., Liu, Y-C., and Du, S-Y. (2008). The Neutrophil-activating Protein of *Helicobacter pylori* Promotes Myeloperoxidase Release from Human Neutrophils. FASEB J. 275, A269. Suppl. S, *Poster Presentation (PP5B-10/132)*, The 33rd FEBS Congress & 11th IUBMB Conference, Athens, Greece.
2. **Fu, H-W.**, Jeng, K-C. G., Liu, Y-C., and Liao, P-C. (2006) Histamine and Leukotriene C_4 Release Induced by *Helicobacter pylori* Neutrophil-activating Protein in Rat Basophilic Leukemia RBL-2H3 cells. *Poster Presentation (P3-02)*, The 11th SCBA international symposium, San Francisco, CA, USA.
3. Lu, T-L., and **Fu, H-W.** (2005). Tyrosine Phosphorylation of Caveolin-1 Induced by Protease-activated Receptor 1 via Src Kinase and Gi Signaling Pathway. *Poster Presentation (29)*, The 4th World Congress of Cellular and Molecular Biology, Piotier, France.
4. **Fu, H-W.**, Liu, Y-C., Lin, C-W., and Lu, Y-L. (2005). Overexpression, Purification and Characterization of *Helicobacter pylori* Neutrophil-activating Protein. *Poster Presentation (05-071)*, VIth European Symposium of The Protein Society, Barcelona, Spain.
5. Kuo, F-T., Lee, S-B., and **Fu, H-W.** (2004) The Involvement of β-arrestin in Signaling Transduction of Protease-activated Receptor 1. *Poster Presentation (P15/16-154)*, The 1st Pacific-Rim International Conference on Protein Science, Yokohama, Japan.