Request for Report for Projects Awarded in 2013 and 2014 by

Mississippi Center for Food Safety and Post-Harvest Technology

Title: Defining physiological functions of deubiquitinating enzymes as potential drug targets in foodborne disease salmonellosis

Award year: 2013

PI: Mariola J. Edelmann

Co-PI: Attila Karsi

Collaborator:

1. Objectives.
   a) Defining biological function of chicken deubiquitinases, especially in the context of enteric infections
   b) Optimization of new proteomic methods for biomarker research in chicken and catfish infectious diseases

2. New Accomplishments toward objectives. Please indicate if all objectives listed were completed.

a) We measured inhibition of UCH-L5 in chicken HD11 and human THP1 macrophages. We measured the effect of UCH-L5 on inflammation by cytokine measurement and cell viability and established a strong connection.

b) We used proteomic techniques to discover new biomarkers in catfish relevant in foodborne disease. We analyzed proteome from catfish infected or not infected with *Edwardsiella ictaluri*. While the genome still has not been available and we are waiting on final version, the initial protein search revealed several important differences between infected and non-infected animals.

3. Objectives not accomplished and impediments to meeting objectives.
4. Lack of availability of catfish genome

5. If continuing project, when will new and/or long term objectives be completed?
   Not continuing

6. Students supported

   a. n/a

7. Leveraged Funds: External Competitive Funding Applied and Awarded based on findings from this project.
   a. Applied for:
      i. Funding agency NIH
      ii. Program R03
      iii. Funding request ($$) $50,000

b. Awarded:
    i. Funding agency USDA
    ii. Program AFRI
    iii. Funding awarded ($$) $150,000

8. Outputs – In addition to the above, please populate the following sections to be included in a report to be compiled in a FSI Research Accomplishment Booklet. The project report will also be posted in a FSI website to be developed.

Please submit reports in Microsoft Word Document (except the published journal articles in pdf format) to Ms. Kaila Peggs by May 15.

Project Summary (Issue/Response)

Approximately 42 million cases of bacterial foodborne diseases are reported annually, and pathogenic Salmonellae are one of the leading causes (Lynch et al., 2006; Mead et al., 1999). While salmonellosis can be treated with antibiotics, the high incidence of the multidrug-resistant strains means that development of new treatments is essential (Karon et al., 2007). Bacteria corrupt numerous biochemical pathways of the host, and understanding of these interactions contributes to drug discovery and facilitates research on established drug targets (Alksne, 2002). However, host-pathogen protein interactions in Salmonella infection remain mostly unknown (McGhie et al., 2009). Deubiquitinating enzymes (DUBs) regulate ubiquitination, a modification that controls protein function, stability and localization (Acconcia et al., 2009; Kirkin and Dikic, 2007), and DUBs are universally involved in health and disease (Hussain et al., 2009; Singhal et al., 2008). To date, substrates and functions of the majority of human DUBs are also poorly understood (Reyes-Turcu et al., 2009), and only recently have some of them been found critical in bacterial infection (Edelmann and Kessler, 2008). Based on our preliminary data, our published work (Edelmann et al., 2010) and precedent (Basseres et al.), our working hypothesis was that the activity and expression level of specific deubiquitinating enzymes is altered during *Salmonella* infections of chicken macrophages, and that these deubiquitinating enzymes regulate the function of proteins critical in innate immunity. Ubiquitin has been shown to be widely involved in regulation of critical host responses during *Salmonella* infection, but the function of host deubiquitinating enzymes are largely unknown. Our goal is to test how by interference with the activity of the deubiquitinating enzymes one can affect the host responses to bacterial infection.

Project Results/Outcomes

Our study pertaining to *Salmonella* infection was to identify the functions of host deubiquitinases with altered activity and stability during *Salmonella* infection in HD11 macrophages. By using label-free chemical proteomics with ubiquitin-specific active-site probe, we detected down-regulation of UCH-L3 and up-regulation of UCH-L5 in HD11 macrophage cells infected with Salmonella Typhimurium. We also showed that inhibition of UCH-L5 enzyme by siRNA and b-AP15, a cell-permeable UCH-L5-selective inhibitor with prospects of use in cancer (D'Arcy et al., 2011), leads to reduced uptake of bacteria into the HD11 chicken macrophages. Higher micromolar concentrations of b-AP15 lead to increased cell death during infection, but not in uninfected cells, while lower concentration of b-AP15 did not have any measurable effect on cell death (data not shown), but it did cause

decreased bacterial uptake. Furthermore, this effect was not attributable to effects of UCH-L5 on proteasomal activation, as we did not detect any differences in proteasomal activity in siRNA-deficient cells. Moreover, higher levels of UCH-L5 led to significant loss in viability after two days of overexpression, which was related to the catalytic activity of UCH-L5, since by inhibiting activity of UCH-L5 with b-AP15 in UCH-L5-overexpressing macrophages, this effect on viability was lost.We demonstrated that overexpression of UCH-L5 leads to increase of caspase-1 activity (data not shown), while caspase-3 activity is not only not elevated in these cells but it is lower than in control cells, which indicates that the cell death that is caused by UCH-L5 is not apoptosis. Combined, these results might mean that UCH-L5 overexpression might trigger pyroptosis but not apoptosis. Recent study indeed suggested that down-regulation of UCH-L5 by DUB inhibitors does negatively impacts inflammasome assembly and Interleukin-1 beta release (Lopez-Castejon et al., 2013). In summary, we showed that host DUBs, such as UCH-L5 and UCH-L3 regulate Salmonella uptake into macrophages and that UCH-L5 is a novel player in innate immunity.

Further progress towards completion of goals entailed establishing novel methods to prepare samples, for chemical proteomics. In designing this new method, two main criteria were used: (1) reproducibility, (2) ability to retain the catalytic activity of enzymes studied. After testing several other methods, including classical homogenization, mortar and pestle-based tissue lysis and Covaris-based sample preparation, we concluded that Covaris-based sample preparation method is compatible with chemical proteomics and it also is reproducible in our assays. Covaris is a relatively new sample preparation device, which utilizes Adaptive Focused Acoustics (AFA), and delivers precise, specifically targeted high frequency energy into the sample (Pei et al., 2010). Use of Mortar and Pestle as well as Covaris gave the best results in terms of reproducibility. To apply one of these techniques in proteomics, we used bovine tissue sections. After applying Covaris to obtain tissue lysate, we performed a reaction with an activity-based probe that specifically reacts with deubiquitinases. This reaction was subjected to quantitative LC-MS/MS analysis. We identified five bovine deubiquitinases (UCH15, UCH19, USP5 isoform 1, UCH-L1, and UCH-L3). Importantly, UCH19 was previously only reported to be predicted on a protein level, similarly for USP5 isoform 1, which was only predicted as a Third Party Annotation (TPA). We utilized this method to find novel catfish deubiquitinases, which was successful.

Project Impacts/Benefits

Pathogenic Salmonellae are one of the leading causes of enteric infections in USA (Lynch et al., 2006) and likely bio-weapon agents (Burrows and Renner, 1999; Glasner et al., 2008). The high incidence of the multidrug-resistant strains highlights the need for the development of alternative treatments (Karon et al., 2007). Salmonel-lae utilize virulence factors to subvert the critical pathways of the host to invade the cells and interfere with the innate immune responses (Valdez et al., 2009), which include Salmonella's deubiquitinating enzyme (DUB), SseL. Removal of ubiquitin by DUBs is an important biochemical step with which pathogenic bacteria can interfere and as DUBs provide a localized and quick modulation of protein stabilization, localization and signaling (Reyes-Turcu et al., 2009). Our long-term goal is to identify the host-pathogen interactions in the context of the ubiquitin proteasome system in Salmonella infections. Working towards this goal, in this project we identified DUBs modulated in Salmonella infection, and we performed several targeted assays to uncover their function in anti-bacterial responses. This contribution is significant because we will improve the current understanding of the involvement of ubiquitin in the pathogenesis associated with *Salmonella* infection, and identify potentially novel drug targets. The benefits of our study include characterization of novel host responses to enterobacterial infection and a better characterization

of chicken DUBs, which will provide new insights into the host-pathogen interplay and significantly contribute to finding novel chemotherapeutics to target DUBs.

Project Deliverables

Narayanan, Lakshmi A., and Mariola J. Edelmann. "Ubiquitination as an efficient molecular strategy employed in Salmonella infection." Frontiers in Immunology 5 (2014): 558.

E. Kummari, N. Alugubelly, C Hsu, B Dong, B Nanduri and MJ Edelmann. Activity-based proteomic profiling of deubiquitinating enzymes in Salmonella-infected macrophages reveals involvement of UCH-L5 in inflammasome activation. PLOS ONE (minor revision).

N. Alugubelly, B. Nanduri and M.J. Edelmann. "Analysis of differentially expressed proteins in Yersinia enterocolitica-infected HeLa cells". BMC Genomics (revision).

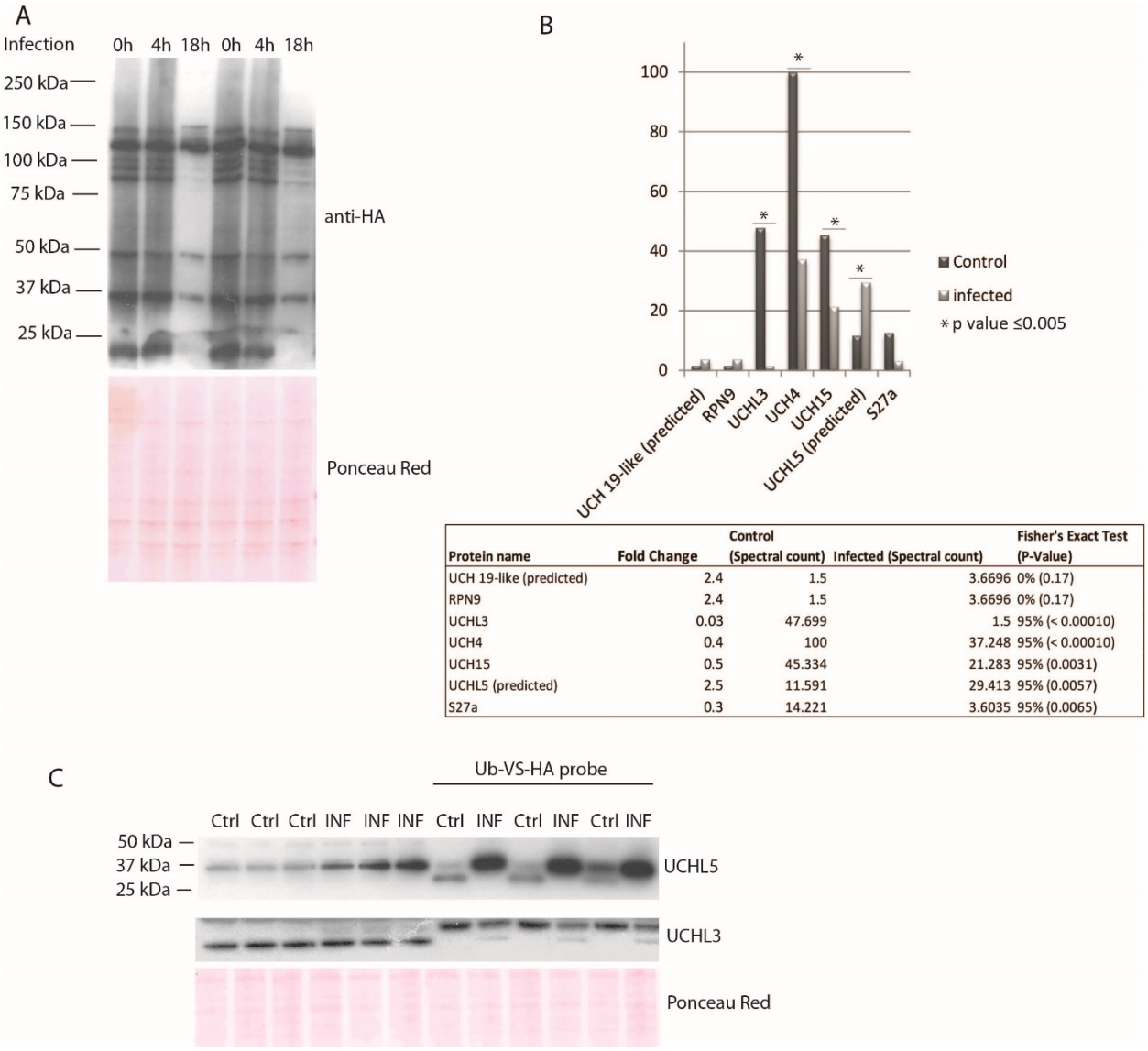
K. Hercik and MJ Edelmann. “Listeria and -Omics Approaches for Understanding its Biology”; a book chapter in “Food Safety - Emerging Issues, Technologies and Systems”. To be published by Elsevier (received proofs).

Presentations:

Edelmann MJ*, The growing role of functional proteomics in combating infectious diseases, Invited Seminar, University of Florida, May 11th 2015

Kummari E.*, Alugubelly N.*, Lee J.H. , Mangum L. , Borazjani A., Ross M., Edelmann, M.J.* Characterization of prostaglandins released from human macrophages infected with enteric bacteria. The 2nd Annual SECIM Metabolomics Workshop and Symposium, SECIM, FL, May 11-14 2015

Graphics



Protein name	Fold Change	Control (Spectral count)	Infected (Spectral count)	Fisher's Exact Test (P-Value)
UCH 19-like (predicted)	2.4	1.5	3.6696	0% (0.17)
RPN9	2.4	1.5	3.6696	0% (0.17)
UCHL3	0.03	47.699	1.5	95% (< 0.00010)
UCH4	0.4	100	37.248	95% (< 0.00010)
UCH15	0.5	45.334	21.283	95% (0.0031)
UCHL5 (predicted)	2.5	11.591	29.413	95% (0.0057)
S27a	0.3	14.221	3.6035	95% (0.0065)

Figure 1. Profiling of active deubiquitinases in HD11 macrophages infected with Salmonella Typhimurium. *(A) The western blot showing activity-based profiling of deubiquitinases in macrophages infected for 0, 4 and 18 hours with Salmonella. Ponceau Red is indicating loading control. (B). Quantitative proteomics of deubiquitinating enzymes in macrophages infected for 0 and 18 hours with Salmonella. (C). Validation of the proteomics results by using ubiquitin-specific probe (Ub-VS-HA) and anti-UCH-L3 and anti-UCH-L5 western blotting.*