GEORGE MASON UNIVERSITY
COLLEGE OF SCIENCE
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"Host communication mechanisms during infection with pathogenic agents"

There is a gap of knowledge in the mechanistic understanding of both the intracellular and the intercellular communication mechanisms of the host during infection with pathogenic agents. Advances in early diagnostics and effective countermeasures are urgently needed and a deep understanding of the host response mechanisms can pave the way for significant progress in these areas. Our main focus is addressing this gap of knowledge for the Tier 1 biodefense agents, including the bacterium *Yersinia pestis* (Yp), which causes the re-emerging human disease plague (also known as the Black Death). Reports of Yp infection were in the news again recently as the Chinese government sealed off a city of more than 100,000 people in 2014 from the fear of the spread of a plague outbreak, and the island nation of Madagascar also reported cases of both bubonic and pneumonic plague in 2014.

i) Intracellular Communication: In one approach, we employed the Reverse Phase Protein Microarray (RPMA) technology to quantitatively reveal the dynamic states of host signaling changes in response to Yp infection of human lung epithelial cells (16HBE14o-) and also human macrophage (U937) and mouse macrophage (RAW 264.7) cell lines. This profiling of the intracellular communication changes by RPMA was performed at different times post infection and for different treatment conditions, which included infection with the virulent Yp strain CO92, infection with the avirulent derivative CO92 (Pgm- , Pst-), and treatments with heat inactivated CO92 or LPS. Responses to 111 validated site-specific antibodies were profiled, leading to discovery of 12 novel protein hits. These include activation of AKT, p53, and inhibition of AMPKα-1, all of which suggest a model of negative regulation of the autophagy pathway. Consistent with this model, strong cytoplasmic localization of p53 protein and reduced conversion of LC3-I protein to LC3-II was observed in Yp-infected cells. In a complimentary approach, we employed quantitative phosphoproteomic analysis of response to Yp infection in highly purified CD14+ primary human monocytes; consistent with the RPMA data, this analysis also demonstrated the activation of AKT as part of the host response. The RPMA results also point to strong modulation of host survival/ apoptosis and cell growth pathways during infection.

The RPMA analysis also identified several protein hits that have been previously reported in the context of infection with other Yersinia species, and in addition confirmed several hits in the context of infection with Yp that had been previously reported through recombinant protein or transfection studies.

ii) Intercellular Communication: It was recently discovered that when cells are stressed by outside stimuli (such as infection) they communicate with other cells through release of "bioactive" vesicles called "exosomes", leading to an explosion of exosome research during the past few years that has shown their critical importance for a number of different diseases, including several infectious diseases. We have found that exosomes are important for the process of Yp infection. Our results show that exosomes released from Yp-infected cells (designated EXi) significantly slow down the growth of recipient uninfected cells, a phenotype that is identical to when the cells are infected with Yp. We have also demonstrated that specific pro-inflammatory and anti-inflammatory cytokines are released by cells that are treated with EXi. We are currently performing detailed cell cycle analyses to determine how EXi affect the growth of their recipient cells and also plan to analyze the signaling events in these recipient cells that coordinate cellular activities and functions such as the cell cycle.

The identification of critical host communication changes during Yp infection will lead to a much deeper understanding of the pathogenesis mechanisms and should allow discovery of innovative approaches for prevention, early diagnosis, and treatment of plague.

TUESDAY February 17, 2015
3:00-4:15 PM
Johnson Center Room Meeting Room F