RESEARCH ABSTRACT

SWINE HEALTH

Title: Identification of host factors interacting with classical swine fever virus proteins: development of novel anti-viral therapeutics." **NPB# 09-111**

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Scientific Abstract: Classical swine fever (CSF), classified as a notifiable disease to the OIE (World Organization for Animal Health), is a highly contagious, economically significant viral disease of domestic and wild pigs. The causative agent, classical swine fever virus (CSFV), is a member of the genus *Pestivirus* of the family *Flaviviridae*. Viral mechanisms involved in CSFV induction of disease, generalization of infection, tissue tropism, host range, and induction of immune responses are not well understood. The spread of CSFV into non-enzootic regions with high-density pig farming is of major concern. Current vaccines, either lack antigenic markers, a fact that impedes their use during a disease outbreak in CSFV free areas, or are poor inducers of early immunity, a critical factor during a disease outbreak. Therefore, it is important to explore the possibility of developing novel approaches to prevent or restrain the progress of the infection in the affected animal. The main goal of this project is to identify host proteins directly involved in the process of virus infection. Currently, there is almost no information available regarding host factors directly interacting with the CSF viral proteins during its replication cycle. This study should contribute in the understanding of the pathogenesis of the disease and provide valuable information for the development of novel alternatives to control (such as novel vaccines and anti-viral therapeutics) the spread of the infection in the natural host. We specifically focus in the identification of host proteins interacting with virus structural proteins (SP) using as a tool a yeast two-hybrid system (YTH). The YTH as used for screening a swine primary macrophage cDNA library to identify CSFV SP-host protein-protein interactions. Structural components of the CSFV virion include the nucleocapsid Core protein and envelope glycoproteins E^{rns}, E1, and E2. We have screened all 4 virus SP. We have identified 11 host proteins interacting with CSFV Core protein and over 50 others interacting with E2. Technical limitations of the YTH system made difficult to obtain clear results with E^{rns} and E1. Three of the 11 Core binding host proteins were selected for further work: Two of these proteins are involved with the cellular sumoylation pathway, named SUMO-1 (small ubiquitin-like modifier) and UBC9, a SUMO-1 conjugating enzyme. The third host protein is named IQGAP. In all three cases critical amino acid residues in Core protein interacting with each of the host proteins were identified using sets of Core proteins harboring punctual amino acid substitutions in designed areas. Once the critical binding areas in Core protein were identified, to further assess the role of these three host protein interactions in the CSFV growth cycle, alanine substitutions in those critical areas were introduced in Core gene of highly virulent CSFV strain Brescia (BICv). Mutant virus harboring mutation that affect interactions between Core and the host proteins were attenuated in swine. Interestingly, data demonstrated a clear correlation between the failure of Core protein on binding SUMO-1, UBC9 or IQGAP and the attenuation of the virus in swine. These results strongly suggest that interaction of CSFV Core protein with host SUMO-1, UBC9 or IQGAP are a critical step in the pathogenesis of CSF in swine.