Synopsis

Characterization of a novel genotype Rotavirus and Investigations on signalling pathways in Rotavirus infected MA104 cells

Rotavirus is the most important non-bacterial diarrhea causing agent. It is responsible for about 600,000 deaths annually with most deaths occurring in the developing countries. Detailed molecular epidemiological studies have been carried out by our laboratory during the last 15 years. These studies have revealed many interesting aspects about rotavirus diversity in this country. During a limited epidemiological survey, several strains that did not react with a panel of available rotavirus serotyping antibodies were identified from a cattle farm in Hesaraghatta, Bangalore.

A prototype of these non-typeable strains, Hg18, was characterized in the present work. In order to understand the genotype of this strain, the genes encoding the outer capsid proteins VP4 and VP7 were cloned and sequenced. Analysis of the nucleotide and deduced amino acid sequences of the outer capsid proteins revealed high divergence from those strains representing the well established 14 serotypes, exhibiting less than 78% amino acid identity. Since >89% amino acid identity is required to classify a strain into a known serotype, Hg18 was proposed to represent a new genotype/serotype, G15, P[21]. Identification of a novel serotype bovine rotavirus has epidemiological significance in this country where a close association of humans with cattle exists in rural areas.

The mechanism of entry of rotavirus into the cell is highly complex and the events occurring at the cell surface and post entry are poorly characterized. Many viruses have been shown to modify cellular components to suit their needs and enhance their replication. Signalling pathways exercise gross control over the cell machinery and phosphorylation of the components of a pathway suggests the activation status. Several reports have revealed viruses modulating signalling pathways. We have carried out preliminary studies to identify the signalling pathways and components critical for rotavirus replication. In order to determine the signaling pathways important for replication in rotavirus infected MA104 cells, we employed a number of kinase inhibitors that include Genistein, PD98059, U0126, LY294002, Rapamycin and Staurosporine that target different kinases that are components of diverse signalling pathways. MA104 cells were preincubated with inhibitors at different concentrations at which cytopathic effects were not observed, for different time periods, either in the presence or absence of serum. The pretreated cells were then infected with activated virus for 1hr after which the cells were incubated in presence of the inhibitor. The number of viral foci formed, were counted using an immunoperoxidase staining assay. We found U0126 as the most effective inhibitor of rotaviral replication in MA104 cells. The inhibition of rotaviral replication by U0126 indicated involvement of MEK1/2. Although other inhibitors were less effective, some components of other pathways might also influence viral replication.

To identify cellular proteins that are differentially phosphorylated in infected cells, we analysed total phosphorylated proteins 6hr and 12hrs post-infection (PI) using...
phosphotyrosine, phosphoserine and phosphothreonine antibodies. A few proteins were found to be differentially phosphorylated in 6hr and 12hr PI lysates when compared to control lysate. One of the differentially phosphorylated proteins showing a MW of 128kDa in the phosphotyrosine blots was speculated to correspond to Focal Adhesion Kinase (FAK). FAK is a downstream effector of integrins which were identified as cellular receptors for rotavirus. We analysed the phosphorylation status of FAK and the downstream signaling components. Our results indicate that FAK-Raf-ERK pathway is activated in infected cells. We also analysed the status of a number of other signaling components, translation factors and apoptotic markers to identify other kinases that might affect viral replication. Interestingly, the translation initiation factor eIF4GI was observed to undergo cleavage in virus infected cells, the significance of which in viral mRNA translation needs to be examined. Although caspase-8 was cleaved in infected cells but no cleavage of caspase-3 was detected suggesting that rotavirus infected cells probably undergo cell death by necrosis and not apoptosis.

Viral replication causes huge stress to the cell. Further, Hsc70 was identified as a coreceptor for rotavirus entry. Hence we examined some of the heat shock proteins in MA104 cells during rotoviral replication. While we observed upregulation of Hsc70, Hsp70, Hsp27, Bip, Calnexin, other Hsps like Hsp60 and Hsp75 were downregulated during the course of infection. Hsc70 migrated into the nucleus during the later stages of infection. Induction of these Hsps could play a chaperone role in viral morphogenesis and in suppression of apoptosis.

Since the FAK-Raf-MEK-ERK pathway appears to be critical for viral replication we wanted to determine the role of FAK in viral replication using a dominant negative construct of FAK. MA104 cells were transiently transfected with dominant negative FAK DNA for 40hrs and then infected with rotavirus. The number of foci formed were counted using immunoperoxidase assay. Cells transfected with FAK-ΔN showed 70-80% reduction in viral foci formation. We determined the levels of viral structural and nonstructural proteins in FAK-ΔN and control infected cells and found a general reduction in levels of viral proteins. We also analysed if the inhibitory action of FAK-ΔN was at binding of virus to the receptor or a postbinding event. Our results indicate that rotavirus binding to MA104 cells is not affected when transfected with FAK-ΔN constructs.

This study reveals that the FAK-Raf-MEK-ERK pathway is activated during rotovirus entry. Rotavirus appears to utilize this pathway for its replication and inhibitors of the components of the pathway severely affected virus replication. These studies provide insights towards development of alternative therapeutic strategies against rotavirus disease.