THE STUDY OF ANIMAL VIRUSES, THE DEVELOPMENT OF DIAGNOSTIC REAGENTS, AND THE CONSTRUCTION OF RECOMBINANT BACULOVIRUSES FOR DEVELOPMENT OF SUBUNIT VACCINES

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The Molecular Virology and Biotechnology laboratory at NPUST is an interdisciplinary research unit combining interests in viral pathogenesis, cell biology, diagnostics and immunology. For the past twelve years, the broad research theme of this laboratory has been the study of the pathogenesis of animal viral diseases, development of diagnostic kits/reagents and construction of baculovirus display system for development of subunit vaccines, as well as screening of anti-cancer compounds from plants and studying their mechanisms of action. We have published more than 100 scientific papers and 98 conference papers. Our major achievements are shown below.

1. Viral pathogenesis and viral protein biological functional analysis

In this section the pathogenesis and protein functional analysis of several animal viruses are investigated. In avian reovirus (ARV), the major focus has been on development of diagnostic reagents and ELISA kits, construction of recombinant baculoviruses for development of subunit vaccines, the study of biological function of σ C, σ A, P10, and P17 proteins, the mechanisms of viral evolution and replication, the regulation of cell cycle and apoptosis by ARV p17 and σ C proteins respectively. Many achievements have been accomplished with regards to ARV study as evidenced by more than 40 SCI scientific papers published to-date on ARV alone.

Recently, we have been studying the genetic mutations of Newcastle disease virus (NDV), the antigenicity and pathogenicity of NDV, mutations in the heptad repeat region of the HN protein affecting the HA function of the protein, the V protein of NDV that enhances virus replication. We have discovered for the first time that genotype VI of NDV occurred in 1999 outbreaks and that the HN glycoprotein of recent Taiwanese NDV isolates carried a substitution at the amino acid residue 81 (I to M) in the heptad repeat region in the stalk domain resulting in a dramatic decrease in the activity of HA. These results argue that a specific amino acid sequence within the heptad repeat region of the stalk is important for the HA of the HN glycoprotein. It was also found that the V protein of NDV can enhance multiplication of diverse viruses up to 10-104. In the study of infectious bursal disease virus (IBDV), we have reported for the first time the phylogenetic relationship of very virulent Taiwanese IBDV and established a yeast expression system expressing the VP2 protein of IBDV. The recombinant IBDV VP2 protein is used as an antigen for development of ELISA kits.

Bovine ephemeral fever has caused the economic loss in daily industry. Only few reports regarding Taiwanese bovine ephemeral fever viruses (BEFV) have been published. To solve the clinical problems, we have established an ELISA kit and magnetic bead-based DNA probe for antibody monitoring of BEFV and virus detection. Traditionally the detection of antibodies by serum neutralization has been the gold standard for BEFV detection but it is time-consuming. We have developed a rapid, sensitive, and specific G protein-based ELISA kit to replace serum neutralization test. Since bovine ephemeral fever diagnosis based on the titers of serum neutralization tests is time-consuming, we developed the magnetic bead-based DNA probe (liquid array) to detect the BEFVs in blood samples or tissue sections. The magnetic bead-based DNA probing system is an excellent diagnostic tool with high sensitivity, specificity, and fast turnround time. In addition to development of diagnostic reagents for detection of BEFV, we have also investigated the mechanism of virus replication, BEFV-induced apoptosis, and inhibition of BEFV replication by aspirin and salicylic acid. We have discovered for the first time that gene expression and activation of Fas-mitochondria pathway are required in BEFV-induced apoptosis and that activation of mTOR/complex1 and inhibition of mTOR/complex1 are beneficial for BEFV multiplication. We also discovered that aspirin could inhibit BEFV replication by COX inactivation, but not SA. Src and JNK phosphorylation and AP1 activation were affected in dose-dependent manner by SA. SA suppressed BEFV replication through down- regulation of Src-JNK-AP1 signaling pathway.

2. Development of the magnetic bead-based probing system

We developed the magnetic bead-based probing system (liquid array) that uses a nested PCR and magnetic bead-based DNA probing assay. This system has been established to detect BEFV, NDV, ARV, and avian influenza virus (AIV). This system is an excellent diagnostic tool with high sensitivity, specificity, and fast turnround time. A minimum of 1-10 copies/ul of viruses could be detected by this system. It has higher sensitivity than that of PCR or real-time PCR up to 10-100 folds. We have published many SCI papers and patents regarding the application of this system. The principal of this system is shown in Fig. 1. The Asian News channel has introduced our new system for detection of pathogens.

3. Construction of baculovirus display system and development of recombinant baculoviruses as a subunit vaccine

We have succeeded in development of a vaccine platform, universal baculovirus surface display system (UBSDS) that can display different foreign proteins on the envelope of baculovirus. The signal sequence

(SS), transmembrane domain (TM), and cytoplasmic domain (CTD) derived from the gp64 protein of baculovirus, histidine-tagged green florescence protein (GFP) gene, and four multiple cloning sites were inserted into the pBacSC vector. GFP can be used as a reporter gene. Gp64 TM and gp64 CTD are able to express heterologous genes on the baculovirus envelope. The transmembrane domain and cytoplasmic domains of gp64 in the platform were designed in order to improve stability and quantity of foreign proteins on the envelope of baculovirus. This new system was used to construct the recombinant baculoviruses for development of subunit vaccines, including AIV HA and NA (Fig. 2A-2B), ARV sigma C and B , canine parvovirus VP2, classical swine fever virus E2 and Erns. Many patents and SCI papers regarding UBSDS and baculovirus display system have been published.

4. Isolation and identification of an anti-cancer compound "Damnacanthal" and the study of its antiproliferative activity machanisms

We have isolated and identified a natural compound Damnacanthal from Morinda citrifolia and investigated its antiproliferative activity in SKHep 1 and diverse cell lines. We have demonstrated that Damnacanthal-induced apoptosis through activation of p53 and p38 signaling pathways via TNF and TRAIL receptors (Fig. 3). Animal model and preclinical tests are currently being carried out.

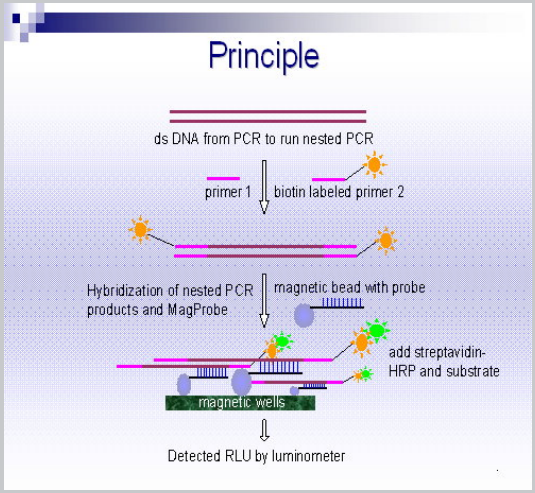

Fig. 1. A rapid, sensitive, and specific assay, RAPID-BAP was developed. The procedures of RAPID-BAP is shown. This new technique uses a nested PCR and magnetic bead-based DNA probe that enhances the sensitivity and specificity for detection of pathogens. In this technique, magnetic beads covalently coupled to amine-containing olgonucleotides through their surface carboxylate groups were used. The small size allows the magnetic beads to remain in suspension for several hours, which is more sufficient for assay setup and analysis, and also provides near-fluid-phase reaction kinetic. A luminometer is used to detect and measure luminescence that normally comes from chemical or biological reactions.

(A)The avian influenza virus NA/ recombinant baculovirus (BacSC-NA)

(B) The avian influenza HA/recombinant baculovirus (BacSC-HA)

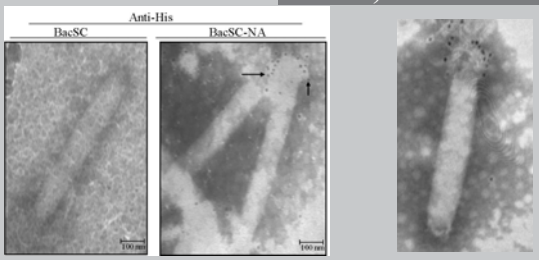

Fig.2. Immunogold electron micrographs of recombiant baculoviruses using anti-His6 monoclonal antibody as the primary antibody and anti-mouse IgG conjugated with 5-nm gold particles as the secondary antibody. Two avian influenza virus/ recombinant baculoviruses (BacSC-NA and BacSC-HA) were constructed successfully Arrows indicate the NA (A) or HA proteins (B) of avian reovirus displayed on baculoviruses envelop. BacSC is a negative control that did not display any foreign proteins on baculoviruses envelop.

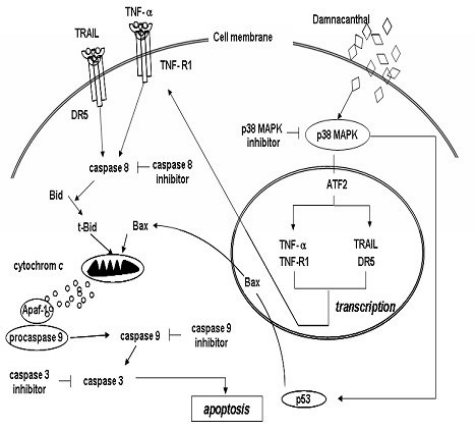

Fig.3. A proposed model for the Damnacanthal-induced apoptosis in human hepatoma SKHep 1 cells is shown. The damnacanthal (Dam), an anthraquinone, was isolated from Morinda citrifolia. Treatment of SKHep 1 with Dam resulted in the inhibition of viability and induction of apoptosis in a dose-dependent manner. Caspase-3,-8,-9 inhibitors, anti-TRAIL R2, anti-TNFR1 and anti-Fas antibodies reversed the cell apoptosis induced by Dam. Dam induces an up-regulation of pro-apoptotic p53, caspase 8, Bid, Bax, AIF, activates caspase-9 and PARP expression. In addition, Dam dissipates the mitochondrial membrane potential. Western blot analysis indicated that DAM induced an up-regulation of p53, caspase 8 and Bid. Bax translocation from cytosol to mitochondria and cytochrome c but not AIF or Smac was released from mitochondria into the cytoplasm and apoptosis protease-activating factor 1 (Apaf-1) is up-regulated following the cytochrome c release to cytoplasm were also observed. Moreover, death receptor TNFR1 and DR5 were up-regulated in Dam treated cells. Dam activated p38 MAPK, leading to transcriptional induction of TNFR1/TNF , DR5/TRAIL and the p53-regulated Bax genes. Dam-dependent expression of death receptors and their corresponding ligands induces caspase 8 activation and Bid cleavage. t-Bid, together with Dam-induced Bax, promotes cytochrome c release and caspase 9 activation, leading to apoptosis.