Innovations of In-vitro Diagnostics

Product Manager, Roche Diagnostics (HK) Ltd

Roche Diagnostics has in recent years launched a large number of reagent kits for disease detection based on real-time PCR. Most recently, a SARS detection kit has been developed for quantification of the SARS-CoV. The presentation will give an introduction to the new kits and instrumentation offered by Roche for real-time quantitative PCR, with emphasis on the special features of the new LightCycler SARS-CoV QV Quantification Kit.

The LightCycler SARS-CoV Quantification Kit is specially adapted for real-time, online RT-PCR in glass capillaries using the LightCycler Instrument. The kit allows the detection of RNA encoding SARS-Coronavirus and simultaneous detection of a kit-specific internal control by dual color detection.

The target region identified by the LightCycler SARS-CoV Quantification Kit was selected from a conserved region of the SARS-Coronavirus genome in different SARS-Coronavirus isolates. The selected region shows no significant sequence homology to other coronaviruses (human group 1, 2, 3 coronaviruses) or non-SARS-CoV-related viruses.

The Internal Control is amplified by same primer pair as the target sequence, but detected by a different pair of Hybridization Probes, emitting fluorescent light of a different wavelength. The approach of using identical primer pair for the Internal Control and the target sequence should allow better control against false negative results.

The analytical sensitivity of the kit was tested with the help of a serially diluted in vitro transcript derived from a SARS-Coronavirus fragment (ay283794) and was determined at approximately 20 copies per reaction.

Currently, the kit is being evaluated in major SARS testing centers in Hong Kong, China, Taiwan, Singapore, Japan, Canada and Europe. Official launch of the kit is planned to be made by end of July 2003.

Identification and characterization of a coronavirus causing severe acute respiratory syndrome.

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Severe Acute Respiratory Syndrome (SARS) is a recently emerged disease associated with pneumonia in a proportion of those human persons infected. The severe acute respiratory syndrome (SARS) was unusual for its severity and patients suffered from this disease did not response to empirical antimicrobial treatment for acute community acquired typical or atypical pneumonia. By 7th July of 2003, a cumulative total of 8439 cases and 812 deaths have been reported from 32 countries.

A novel coronavirus (SARS-CoV), genetically related distantly to group 2 members of the family Coronaviridae, was isolated from SARS patients by The University of Hong Kong. Nasophargeal aspirate samples from patients suffered from SARS were used to infected cell lines. Electron microscopy of negative stained ultracentrifuged cell culture extracts showed the presence of pleomorphic enveloped virus particles of around 80-90 nm (range 70-130 nm) in diameter with surface morphology compatible with a coronavirus. Thin section electron microscopy of infected cells and lung biopsy revealed virus particles of 55-90 nm diameter within smooth walled vesicles in the cytoplasm. Virus particles were also seen at the cell surface. The overall findings were compatible with coronavirus infection in the cells.

Total RNA from infected cells was extracted and subjected to random and gene-specific RT-PCR assays. Genetic fingerprints, which are unique in infected cells, were isolated and cloned. Sequence analyses of DNA fragments indicate this virus is close to viruses under the family of Coronaviridae. Deducted amino acid sequence of polymerase protein has the highest homology to the RNA polymerase of type-2 coronaviruses (bovine coronaviruses and murine hepatitis viruses). However, phylogenetic analysis of the protein sequences in this family (types 1-3 coronaviruses) separated the SARS virus into a distinct group.

Based on the determined viral sequences, a real-time PCR assay was developed to detect this pathogen in clinical samples. In addition, a serology test was established to detect IgG antibodies against this virus in patients' serum samples. The majority of patients with clinically defined SARS, but none of the control samples, had either serological or RT-PCR evidence of infection by this virus.

DNA Vaccination

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DNA vaccination represents a rapidly developing technology with new perspectives for the prevention and therapy of infectious diseases. The historic foundation for DNA vaccines emerged from the observations on gene transfer of "plasmid or naked" DNA and the resulting in vivo protein expression. Subsequently, DNA vaccinations were found to be able to elicit protective immunity in chickens and in mice from challenge with live influenza virus. These findings have opened up new avenues for vaccine development. Since then, a huge number of reports on use of DNA vaccinations in viral, bacterial, parasitic and less frequently fungal infections have been published and reviewed.

DNA vaccines consist of the gene of interest cloned into a bacterial plasmid. It comprises a bacterial origin of replication allowing for growth in bacteria. A multiple cloning site is added for insertion of the foreign gene. A bacterial antibiotic resistance gene is included to ensure only plasmid containing bacteria will propagate during culture for subsequent purification of the plasmid. The plasmid should possess a transcriptional promoter such as the cytomegalovirus (CMV) intron. A promoter for optimal gene expression in mammalian cells. The promoter is linked to a region encoding an immunogenic protein of the pathogen of interest preceded by a gap site for correct mRNA initiation. The expression gene is completed by incorporation of polyadenylation sequences either from bovine growth hormone or from simian virus 40 to allow correct termination of the mRNA.

Immunization with plasmid DNA has been shown to activate both humoral and cellular immune responses, including the generation of antigen specific CD8+ cytotoxic T cells as well as CD4+ T helper cells. In these regards, DNA vaccination can compensate for the weakness of most of the currently available vaccines, which are efficient in inducing antibody response, but cannot induce cellular immunity efficiently, something that is particularly crucial for combating against intracellular pathogens. Furthermore, DNA vaccinations have the advantage of being non-infectious and act to focus the immune response on only those antigens desired for immunization. Clinical DNA vaccine trials are currently performed for HIV, HBV and Plasmodium falciparum.

The precise mechanisms involved in the activation of immune responses in vivo following injection of plasmid DNA have not yet been determined. A number of studies have been carried out in order to elucidate the immune mechanisms. The use of DNA as a mean of vaccination offers potential benefits in protective efficacy, cross-strain applicability, development speed, and manufacturing cost as compared with conventional vaccines. With more understanding of the immunology of DNA vaccinations and the advances in molecular biology, DNA vaccines could be an effective and promising ammunition against infectious diseases that keep people in the burden of death.

Epigenetics of Natural Killer Cell Malignancies

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Natural Killer (NK) cell lymphomas are a group of rare but highly aggressive malignancies. Both cytogenetics and comparative genomic hybridization show that chromosomal regions involving 6q, 11q, 13q and 17q are commonly deleted. The frequent DNA losses suggest that the presence of putative tumour suppressor genes at these regions is important in NK cell transformation. Other than DNA loss, aberrant methylation of promoter CpG regions is an alternative mechanism whereby tumour suppressor genes are inactivated. We used a candidate gene approach to investigate the patterns and significance of this epigenetic change in NK cell malignancies. Thirty-three patients were studied for promoter methylation in five putative tumour suppressor genes by methylation-specific polymerase chain reaction (MSP). The p73 gene was methylated in 94% of cases, a frequency that is the highest known for any human malignancy. Other methylated genes included hMLH1 (63%), p16 (63%), p15 (48%), and RAR beta (47%). Methylation of two or more genes occurred in 88% of cases. We conclude that NK cell malignancies show a specific pattern of promoter methylation, with p73 being consistently involved. The demonstration of p73 methylation may serve as a potential molecular tool for NK cell lymphoma detection.

Chromosomal Aberrations in Childhood De novo Acute Myeloid Leukemia

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Acute myeloid leukemia (AML) is an uncommon but heterogeneous hematologic malignancy in children. The majority of reports were studies on Caucasians. Using G-band karyotyping supplemented with multi-color fluorescent in-situ hybridization (MFISH) and reverse-transcriptase polymerase chain reaction (RT-PCR), we investigated the cytogenetics of 38 consecutive Chinese children with de novo AML (17 males and 21 females) of median age of 10 years (range: 0.75-15) at diagnosis. Clonal aberrations were noted on 68.4% (26/38) patients. MFISH refined the compositions of abnormal G-band karyotypes of seven AML and revealed cryptic rearrangements in three karyotypically normal AML. RT-PCR illustrated t(15;17) in an AML with normal karyotype. Among patients with clonal aberrations, the incidences of t(8;21), 11q rearrangement, and inv(16) were 15.4%, respectively, whereas t (15;17) was 19.2%. Hypodiploidy with the loss of sex chromosome was prominent on M2/M4 harboring t(8;21). Three of seven M7 were not associated with Down's syndrome. Four patients harboring inv(16) did not present as M4 eosinophilia. The total or partial losses of chromosome 5 and chromosome 7 were infrequent, at 3.8%, respectively. However, a clustering of the preponderance of aberrations resulting from unbalanced rearrangement of group C chromosomes was noted and chromosome 7 was most frequently involved. Complex karyotypes prevailed at a rate of 30.8%. Results of this study may provide more data to the literature on the leukemogenesis of children de novo AML.