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Impact of Microarray Technology in Influenza Virus Research and Diagnostics

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Abstract

Influenza A viruses cause 300,000-500,000 deaths every year across the globe and are continues threat to public health. There have been five influenza pandemics till date. In spite of the recent advances in the field of virus research there is no steady vaccine against influenza A viruses. Flu shots are given every year in many countries but remain effective only for one season. One of the main reasons of vaccine ineffectiveness is the high mutation rate in the influenza A genome caused by a poor error prone replication mechanism and virus recombination in the host cells resulting in variation in antigenic proteins. In order to overcome this problem scientists have enabled different strategies to control virus infectivity. Now in place of viruses, host responses are studied and modified in order to obtain an effective immune response against the viruses. Also, new strategies like rapid large scale virus diagnosis and development of effective vaccines using reverse genetics technique are being used for improvement of antiviral counter measures. Microarray technique, which is a high throughput method for diagnosis as well as assessment of large number of biomolecules in one attempt, has played an important role in understanding the etiology and pathogenesis of influenza A viruses. In this review we have discussed significance of microarray technology in influenza virus research during last one decade and highlighted its application in extensive diagnostics and genome wide transcriptional profiling of host responses to virus infection.

Introduction

Microarrays are arrangement of biomolecules on a solid surface like glass slide. Based on the principle of homology, any target material (e.g. RNA, DNA, proteins etc.) can be detected on the basis of complementarity/homology with the spotted probes [1]. As thousands of probe molecules can be spotted on a single slide it is a high-throughput technique for detection and analysis of nucleic acids or proteins under various physiological, developmental and disease conditions. The type and sequence of the probes spotted on the array determines its application. For example, DNA microarrays are used for gene expression analysis whereas antibody based arrays are used for detection of specific proteins. With the development in this technology, study and analysis of living cells and tissues are also possible on microarrays [1,2].

Microarray technology is one of the key developments in the field of genome biology in recent years. It has shown great applications from genome wide gene expression analysis to mutation detection, genome mapping and drug discovery. The advancements in microarray technology have revolutionized research in biomedical sciences especially in the area of infectious diseases. It has not only provided a platform for large scale simultaneous detection of multiple pathogens in a single experiment but also enabled understanding of the dynamic host-pathogen interaction. Studies on genetic evolution of pathogens, host cellular responses and investigation of the determinants of pathogenicity on a whole-genome scale have been made possible by microarray technology. In recent years, infectious diseases caused by viruses have become a matter of great concern due to their high transmission, rapid mutability and lack of suitable therapeutics [1,3,4].

Influenza A viruses are members of the family *Orthomyxoviridae*, which are small enveloped viruses with a genome consisting of 8 segments of negative-sense, single-stranded RNA that encodes for 10 to 11 proteins depending on the strain [5]. Highly contagious influenza A viruses are capable of infecting a wide variety of hosts which include birds, pigs, cattles, horses and humans. They cause mortality as well as morbidity of hundreds and thousands of people every year across the world. The segmented genome and highly error-prone viral replication lead to enormous genetic flexibility to influenza viruses which results in moderate to severe mutation in the circulating viral strains. Mutations at the level of nucleotide change termed as antigenic drift or major antigenic changes due to reassortment of genome segments known as antigenic shift are the major factors in evolution and emergence of novel viral strains leading to pandemic outbreaks.

Genomic changes control the differences in virulence and host range seen among influenza A isolates. Influenza A viruses are serologically categorized based on the presence of surface glycoprotein hemagglutinin (HA) and neuraminidase (NA). So far, 16 HA and 9 NA subtypes of type A influenza virus have been identified. Although more than one hundred of the possible 144 HA-NA combinations have been found in birds (water fowl) [5], the generally accepted reservoir of influenza viruses, a few HA-NA types like H1N1, H2N2 and H3N2 have circulated in humans. To combat influenza viruses, several approaches have been made in last decades which include development of efficient vaccine, small molecule inhibitor and drug development. However genetic plasticity of influenza viruses is main burden to have a permanent success to control influenza infection. This has created a need for high throughput diagnostic tool for rapid identification and detection of known viruses for proper prophylaxis. Genomics tools

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such as high-throughput sequencing, mRNA expression profiling have contributed largely towards the understanding of the pathogens. Microarray technology has played a significant role in this regard. Because of miniaturization it can detect thousands of targets in one experiment, thus improving our power and capacity of detecting and understanding the viruses and their disease biology to a great extent.

Microarray as Diagnostic Tool for Influenza A Viruses

Global surveillance, early detection and rapid intervention are critical for pathogen mediated disease management especially in case of influenza infection because of its rapid transmissibility, rapid mutation and recombination between serotypes. DNA microarrays are proving themselves as powerful and attractive tools for diagnostic applications like virus identification, sequencing and subtyping. These initiatives have become more important in recent times because of growing threat of interspecies transmission of pathogens resulting in the emergence of new infectious diseases in humans as well as in domestic and wild animals. Microarray technique can provide a rapid high throughput diagnostic tool for enhanced influenza surveillance [2].

Microarray Chips for Identification and Sub Typing of Influenza Viruses

Influenza viruses are traditionally detected using specific antibody based immunoassays or immunofluorescence assays. The antibodies against influenza surface antigens like HA and NA are used for the assays. PCR and RT-PCR based methods using specific primers against viral nucleic acids were more advanced, specific and comparatively faster methods of influenza virus detection but suffer from low throughput and limited multiplexing [6-8]. As influenza viruses have multiple types and subtypes which show high amount of genetic variation and mutability, sequencing or protein blotting based analysis is further needed to verify the PCR results. Microarrays are the new diagnostics tools for identification and quick subtyping of influenza viruses. For this low-density oligonucleotide microarrays containing limited number of highly multiplexed unique conserved genome "signatures" sequences as probes are used [9-13]. These microarrays are used to detect both influenza A and B viruses as well as various subtypes of influenza A viruses on the basis of three gene targets the HA, NA and M (Matrix) gene segments [6,10,14,15]. Numerous short DNA capture sequences were designed and used to both identify and subtype influenza A viruses on the basis of sequence similarity and differences. The identification process mainly involved RT-PCR amplification of influenza RNA to be identified using universal primers, fluorescent labeling of PCR product using fluorescently labeled dNTP based PCR, fragmentation of PCR products, microarray hybridization. For detection, fluorescent intensity patterns are used to determine the type and subtype of influenza virus. A summary of some of the studies carried out on microarray platforms used for influenza detection and subtyping is given in Table 1.

Microarray Designing and Application

Probe selection and design are the most challenging part in designing microarray chips for identification of influenza viruses. Li et al. in 2001 developed cDNA arrays by immobilizing sequences approx. 500 nucleotides in length derived from influenza viral cDNA onto glass slides. For identification of viruses the samples were labeled with fluorescent dyes and allowed to hybridize onto the array. The study could identify 3 unknown influenza viruses; however the technique suffered from high degree of mismatches (approx. 14%) [13]. In order to resolve this problem smaller oligonucleotide based arrays were designed containing conserved viral genome for greater specificity. Kessler et al. used capture sequences of approx. 45-65 nucleotides selected from conserved regions in the genomes of seven specific pre-1998 influenza A viruses. The method could identify 4 different subtypes viz. H1N1, H3N2, H1N2 and H5N1 of influenza A viruses as well as influenza B viruses. However, low-level sequence specific false-positive signals were present on almost 50% of their arrays [16]. Sengupta et al. used even smaller probes (approx. 21 nucleotides) as capture sequences obtained from VirOligo primer database. They developed microarrays containing 476 probes based on HA (Hemagglutinin) and NA (Neuraminidase) surface proteins and could identify viruses of H1N1 subtypes and recommended use of smaller oligonucleotides for influenza virus detection [12]. At present, various software and algorithms are used for designing specific probes against influenza viruses and different combinations of oligonucleotides are used for virus detection [14,17,18]. Recently probes based on amino acid sequences of influenza surface proteins were used to design universal microchip that could determine all sub-types of HA and NA of influenza A virus. As, influenza genome based microarray chips showed lot of false-positive results probes based on most specific representative of amino acid sequences specific to each subtype were used to design oligonucleotide microarrays [19].

Two types of microarrays, sequencing and hybridization arrays are used for viral sub typing of influenza viruses. "Sequencing" microarrays use tiled sets of 10^5 to 10^6 probes of either 25-mers or 29-mers, containing one perfectly matched and three mismatched probes per base for both strands of target genes. This format in combination with specific PCR is ideal for single nucleotide polymorphism (SNP) genotyping and phylogenetic analysis [20,21]. Since variations in pathogen sequence affects hybridization patterns, these approaches used differential measures of specific pathogen hybridization patterns to identify individual sequence variants. This method requires prior knowledge of a differential hybridization pattern that is empirically determined in control experiments and thus not very useful in identification of new or unknown viruses.

'Hybridization' microchips are the more commonly used microarrays for viral subtyping. These microarrays use sets of immobilized oligonucleotide probes that form complexes with virus specific cDNA tagged with, a fluorescent label [8,11,12]. These microchips are used for the determination of different influenza virus types [13] and also for sub-typing majority of HA and NA influenza virus variants [21]. Hybridization microchip contains sets of HA (H1-H13, H15 and H16) and NA (N1-N9) oligonucleotide probes to be classified into different HA-NA subtypes of influenza viruses. For the detection of the newly emerged latest pandemic H1N1 swine influenza viruses additional sets of probes are needed [22]. Overall, 20 to 50–mer probes are used to identify each subtype of HA and NA. With more development more advanced electronic microchips with more specific shorter probes and automated detection system were used for influenza virus sub typing [22, 23].

Mchip

The Matrix (M) protein/gene of influenza viruses can be used to identify viruses belonging to influenza A, B or C classes/types. The M gene segment alone contained enough genetic diversity between subtypes to provide subtype information. The conserved regions of M

Study	Capture sequences as Probes	Solid surface	Virus subtypes detected/targets used	Detection methods
Li et al., 2001	Multiple fragments of the hemagglutinin, neuraminidase, and matrix protein genes converted into cDNA of average size of 500 bases were used as probes.	Glass slides	Influenza A H1N1, H3N2 and H2N2 viruses. Viral RNAs were reverse transcribed and amplified by PCR, and the products were labeled with cyanine dyes.	GenePix (Axon Instruments) or ChipReader (Virtek) confocal scanners, and the fluorescence were quantitated using ImaGene software (Biodiscovery).
Sengupta et al., 2003	A collection of 476 influenza virus-specific oligonucleotides was spotted onto glass slides as probes (21 nucleotide in length designed using VirOligo database).	Glass slides	H3N8, H3N2 and H1N1 influenza A viruses. Viral RNAs were reverse transcribed and amplified by PCR, and the products were labeled with cyanine dyes.	Packard BioScience Scanarray 3000; Perkin-Elmer, Boston, Mass).
Kesseler et al., 2006 (Flow-Thru-Chip)	Twenty-nine oligonucleotide probes (45 to 65 bp) were designed for recognition of seven different genes: the matrix protein (MP) gene of influenza A virus (MP/A); the nonstructural (NS) gene of influenza B virus (NS/B); hemagglutinin genes H1, H3, H5; and neuraminidase genes N1 and N2. Highly conserved viral sequences were obtained from the National Center for Biotechnology Information (NCBI) database and segmented into two to seven overlapping pieces. The length of the overlapping domain between two consecutive gene segments was generally 10 to 15 bp, but exceptionally shorter (5 bp) or longer (25 bp) lengths were designed as a function of the sizes of the specific gene segments.	silicon wafer	Influenza A H1N1, H5N1, H3N2 and influenza B, B/Yamanashi/166/98 viruses. Viral RNAs were reverse transcribed and amplified by PCR using biotinylated primers, and the products were labeled with Horse radish peroxidase-streptavidin.	MGX 2000 instrument and placed in the MGX 1200CL detection unit for chemiluminescence (CL) detection
Townsend et al., 2006 (FluChip-55)	103 capture-label pairs based on HA, NA, M and NP gene sequences were used as probes.	Glass slides	Influenza A H1N1, H3N2, H5N1 and influenza B viruses. Influenza virus RNA was extracted, reverse transcribed with universal influenza A virus gene-specific primers (12) that contained a T7 promoter, amplified by PCR, transcribed back to RNA, and then fragmented. Labeled with a fluorophore.	Bio-Rad Laboratories (Hercules, CA) VersArray scanner with detection at 532 nm, a laser power of 60%, a photo multiplier tube sensitivity of 700 V, and a 5-µm resolution. Image contrast was optimized by using Photoshop (Adobe, San Jose, CA).
Han et al., 2008	Fifty-two oligonucleotide probes (detective probe, DP) specific for all 25 subtype viruses were designed according to the gene sequences of the amplified target cDNAs and avian influenza A subtypes in the GenBank database using DNAstar, Bioedit, Primer 5.0, and OMIGA software.	Glass slides	Influenza A H1N1, H3N8, H4N6, H2N3, H5N2, H6N8, H7N1, H8N4, H9N2, H10N4, H11N9, H12N5, H13N6, H14N5 and H15N8 viruses. Viral RNAs were reverse transcribed and amplified by multiplex PCR using TAMRA-tagged primers.	PerkinElmer Scan Array Gx plus scanner with detection at 532nm and laser power of 90%.
Gall et al., 2009	The array consists of one probe for the conserved matrix gene and 97 probes targeting the HA0 cleavage-site region.	NanoChip 400 electronic microarray system	Influenza A H1N1, H2N2, H2N3, H3N8, H4N6, H5N1, H5N2,, H5N9, H6N2, H6N5, H7N1, H7N3, H7N7, H9N2, H10N4, H10N8, H11N6, H12N5, H13N2, H13N8, H14N3, H15N6 and H16N3 viruses. Target RNA was reverse transcribed, amplified and biotin-labeled by a one-step pan haemagglutinin RT-PCR modified by use of biotinylated primers.	NanoChip 400 system (Nanogen Inc.).
Gall et al., 2009	99 Influenza A-specific oligonucleotide probes	ArrayTube platform	Influenza A H1N1, H2N2, H2N3, H3N2, H3N8. H4N6, H5N1, H5N2, H6N2, H6N5, H7N1 and H7N7 viruses. Target RNA was reverse transcribed, amplified and biotin-labelled by a one-step pan haemagglutinin RT-PCR modified by use of biotinylated primers. *In vitro*-transcribed RNA from a synthetic gene was applied as a labeled positive control.	ATR03 transmission reader (Clondiag)
Huang et al.,2009	Eight targets: the matrix gene segment (M1) of influenza A virus; the nonstructural gene segment (NS) of influenza B virus; the HA genes H1, H3, and H5; and the NA genes N1 (H1), N2, and N1 (H5). Sequences were obtained from GenBank and the Influenza Sequence Database and were then aligned. Highly conserved regions were selected for primers and for capture and discriminator oligonucleotides.	Electronic microarray	Influenza A H1N1, H3N2, H5N1 and influenza B, B/Shanghai/361/ 2002 viruses. Five capture mixes, each containing 100 nM concentrations of two or three biotinylated capture oligonucleotides complementary to two different amplicons, were sequentially addressed for 15 s at 350 nA to a number of electrode sites equal to the number of samples being analyzed.	Nanochip 400 (NC400) instrument (Nanogen, San Diego, CA).

Kang et al., 2010	The oligonucleotide 59-mer probes were designed for Novel H1, N1 and M gene sequences, all other human seasonal influenza H1 and N1 sequences and swine H1 and N1 sequences were selected. Likewise, all human influenza virus isolates of subtypes H3N2 and H5N1 were selected.	Glass slides	Influenza A subtypes H1N1, H5N1, H7N7 and H9N2. Purified multiplex PCR product with incorporated aminoallyl-dUTP was further labeled with Cy3 or Cy5.	ScanArray Gx PLUS (PerkinElmer). The fluorescence intensity was quantified by Genepix Pro5.0 software (PerkinElmer).
Ryabinin et al., 2011	One 16X9 spot sub-array contained NA-specific probes, while another 16X24 spot sub-array contained HA-specific probes	Glass slides	Influenza A H5N1, H5N3, H1N1, H6N1, H7N1, H3N8, H10N7, H13N8 and H12N2. Cy- dyes were used to label the targets	Slides were scanned using ScanArray Express 2.0 (Perkin Elmer) at 543 nm (Cy3) and 633 nm (Cy5). The images were analyzed with ScanArray Express software (Perkin Elmer).
Tao et al., 2011 (VereFlu)	Clinically important influenza virus subtypes, including the 2009 pandemic influenza (H1N1) and H5N1 viruses.	VereFlu chip	H1N1,H3N2, H5N1 and influenza B viruses	The entire instrument consisted of a computer, a thermal control system (TCS), which enables PCR thermal cycling, and a microarray optical reader (Lean s.r.l., Medolla, Italy).

Table 1: List of selective studies carried out on microarray platforms used for Influenza virus identification and subtyping.

gene sequence were thus utilized for synthesis of M chips which could identify viruses of different influenza types (1998 to 2005). The Mchip was utilized to examine many specimens and it could discriminate between human H1N1, H3N2 and avian H5N1 influenza subtypes with greater specificity [10,15].

Bchip

Both A and B type of influenza viruses can infect human beings leading to disease and recurrent annual epidemics in humans. Influenza A viruses can infect several species but influenza B viruses are almost entirely restricted to humans and causes comparatively mild infection. Although influenza B viruses do not show the same degree of antigenically distinct subtypes, they are subject to antigenic drift through the accumulation of point mutations, with a slightly lower evolutionary rate than type A. Dankbar et al., reported development of a microarray (BChip) designed to target the influenza B gene segments HA, NA and M and provide lineage information. They analyzed two major circulating lineages, Yam88 (B/Yamagata/16/88) and Vic87 (B/Victoria/2/87). In their experiment the array included 62 different influenza B virus samples, as well as negative control samples of influenza A and parainfluenza 1 [24].

Lab-On-Chip Influenza Assay: Vereflu

VereFlu assay, a recent addition in the field of influenza virus diagnosis which is based on multiplex reverse transcription (RT)-PCR followed by microarray amplicon detection by hybridization to a DNA microarray. This is the first commercially available integrated lab-on-chip device for the specific detection of clinically important influenza virus subtypes. This assay simultaneously detects five subtypes of influenza virus which includes seasonal H1N1, H3N2, H5N1, 2009 pandemic H1N1 and influenza B virus. Teo et al. [25] tested different influenza subtypes and reported an overall clinical sensitivity of 94.3% and specificity of 96.8%. It involves a portable, fast (3 hours detection time) device which is particularly suited for diagnostic applications which includes detection, differentiation and identification of human influenza virus subtypes in fields.

Glycan Microarrays

The binding of viral coat glycoprotein HA to sialylated glycan receptors on host epithelial cells is the critical initial step in the infection and transmission of influenza viruses. Specificity of influenza A virus for the host is mediated by the viral surface glycoprotein HA, which binds to receptors containing glycans with terminal sialic acids. Avian influenza viruses prefers alpha2-3-linked sialic acids on receptors of intestinal epithelial cells, whereas human viruses are specific for the alpha2-6 linkage on epithelial cells of the lungs and upper respiratory tract [26,27]. Glycan array technologies can rapidly assess virus receptor specificity and the potential emergence of human-adapted H5N1 viruses. Receptor preferences of a number of human and avian influenza viruses including the 1918 H1N1 pandemic strains by analyzing their HA were determined using a recently developed glycan array [28]. These arrays can provide information about virus-host interaction and its evolution. Additionally these arrays can also detect carbohydrate modifications like sulphation and fucosylation on the host cells [28, 29].

Protein Arrays

Microarray platform is now being used for immobilization of proteins or antibodies against viral antigens. These arrays can greatly enhance the diagnosis process. Now viruses can be directly detected using antibodies against specific strain or subtype of influenza virus [30].

Study of Host Cellular Responses to Influenza A Infection Using DNA Microarrays

Microarray technology is being widely used in understanding the pathogenesis of influenza A viruses by studying host cellular responses during virus infection. High-density microarray-based analysis of mRNA expression and single nucleotide polymorphisms are providing unprecedented ways to analyze the diversity of the genomes of influenza viruses as well as the molecular basis of the host response to them [4,31,32].

Because of its small genome which is segmented in nature, influenza viruses have mutability which makes it resistant to the existing vaccines. Influenza vaccines are therefore needed to be given every season in order to maintain immunity. Thus, an alternate antiviral strategy could be better understanding the critical host factors that are influenced and required by the virus for its efficient propagation. Although genome of cell remains relatively constant, the transcriptome and proteome varies greatly due to its biochemical interactions with the genome, as well as the external and internal environment. The mRNA expression of a cell is dependent on the location of the cell, different stages of its life cycle, and different environmental conditions. Virus infection requires the

host cell's machinery to replicate which in turn modulate the cellular environment including transcriptome and proteome and reflects the specific alterations of the cellular pathways. Hence virus infection mediated host expression analysis is critically important to dig out information for developing antiviral strategies.

Microarray-assisted mRNA expression profiling of the infected hosts (cells, tissues or entire organism) can provide valuable information about the pathogenesis, mechanism of host response and prophylaxis of the virus infection. By comparing mRNA expression in individuals infected with the virus to the uninfected or control individuals, researchers can generate a "molecular signature" of the host response genes or pathways specifically involved in the host responses to the virus infection. The same principle can be used for identification of any new emerging virus in terms of host responses. For example, when microarray expression profile of human lung epithelial cells (A549) infected with different subtypes of influenza A viruses was compared, it showed characteristic difference in the expression of specific set of genes specially involved in immune response which can be used as specific markers for individual virus infections (Figure 1) [33]. Also, detailed analysis showed contrasting differences in the expression levels of specific host genes when infected with influenza A viruses of different pathogenicity (Table 2). Microarray analysis can also result in identification of new genes which are not known to be involved in virus infection through 'cluster' analyses. It is based on the principle that genes in a single cluster are involved in similar biological processes. This functional information can be used to design hypothetical/proposed models depicting probable mechanism or effects of virus infection. One such model has been shown in Figure 2 which shows the signaling events which might be occurring in a host cell in response to highly pathogenic avian influenza A (H5N1) virus infection. Interestingly, the genes involved in the same pathway were down-regulated in a low pathogenic reverse genetically modified vaccine strain of H5N1 [34]. Thus, microarray analysis resulted in identification of many host genes getting affected by virus infection which can help in elucidating the mechanism of host responses to virus infection.

By analyzing host transcription profile at different stages of virus replication, valuable information can be obtained about virus life cycle and into how viruses evade host cellular responses to establish an infection or disease. In case of highly pathogenic influenza virus (H5N1) infection when virus entry into the cell is not successfully blocked, it results in infection of epithelial cells resulting in the production of pro-inflammatory cytokines, chemokines and interferons. The expression levels of these cytokines can be measured which can indicate the degree of infection. The excess amount of cytokines produced (cytokine storm) during inflammatory response against the virus is the cause of high amount of tissue damage observed during highly pathogenic avian (H5N1) influenza virus infection [35,36].

Also, microarray based transcription profiling can be used to determine the susceptibility of different cells and tissues towards a virus infection. Reemer et al., 2010 reported differences in microarray based transcriptional profile of immune genes between different lung sections as well as upper and lower respiratory tract in chicken after infection with H9N2 influenza A virus. They concluded that airflow and anatomy of a particular organ can affect virus load which is not uniform across the respiratory tract and thus can affect the degree of

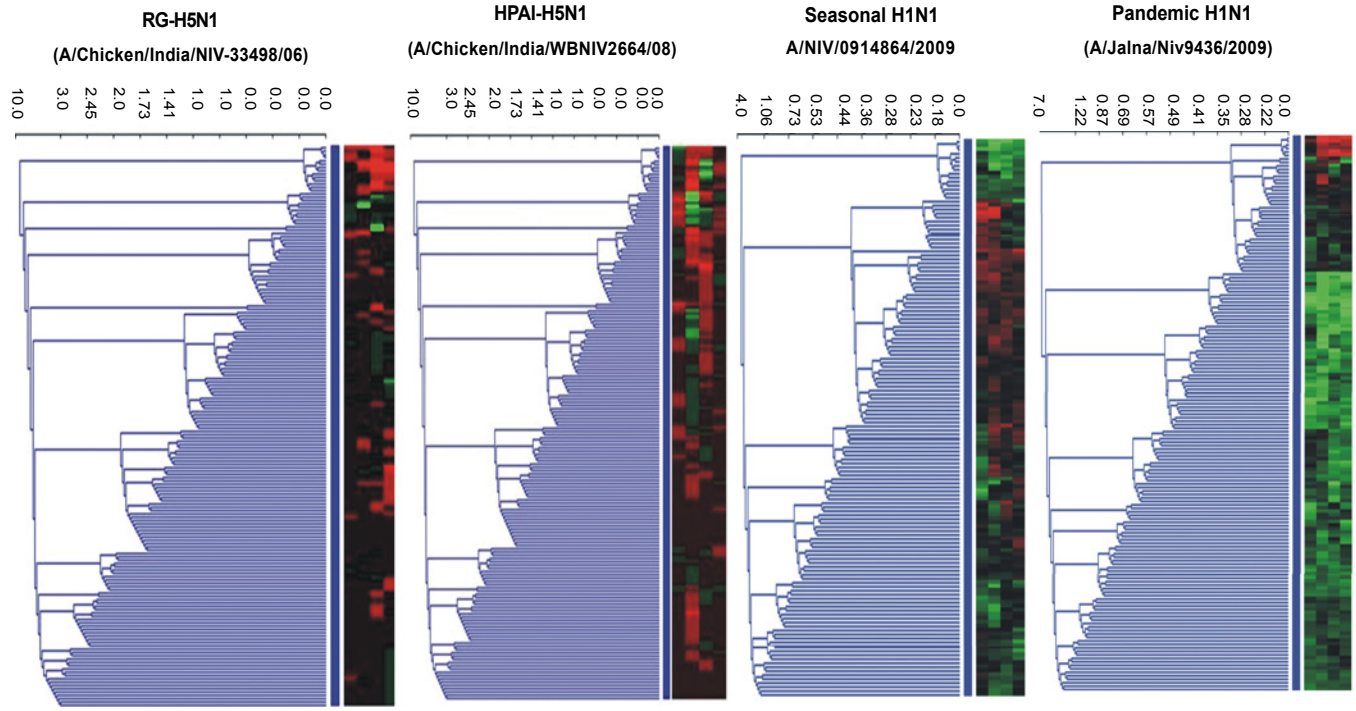

Figure 1: Heat map representation of genes in Human lung epithelial (A549) cells infected with A) Low pathogenic reverse genetics modified vaccine strain of H5N1 B) Highly pathogenic avian influenza(HPAI)-H5N1 C) seasonal influenza H1N1 and D) pandemic influenza H1N1(2009). Cells were analyzed at 4 different time point post-infection (4h, 8h, 16h and 24h). Expression of genes with fold change >+/- 1.5 and $p < 0.05$ were considered as differentially expressed. Red indicates up-regulation whereas green indicates down-regulation. Data presented are averaged gene expression changes for 2 different replicates. There is a clear difference in the expression pattern of host genes in cells infected with influenza A viruses of different pathogenecity.

GENES	HPAI-H5N1 (A/Chicken/India/WB-NIV2664/2008)	Low-pathogenic recombinant vaccine strain of H5N1(A/Chicken/India/NIV-33487/2006)
IL2R-alpha	1.5	-1.5
CXCL10	4.0	-3.0
CCL5(RANTES)	2.0	-2.0
IL1-alpha	2.0	-2.0
IL15R-alpha	2.6	-2.8
JUN	2.0	-2.8
STAT1	3.2	-2.0
FAS	2.0	-2.0
CyclinB1	-2.0	2.0

Table 2: Expression of selective immune genes in human lung epithelial cells infected with highly pathogenic H5N1 and low pathogenic vaccine strain of H5N1 influenza viruses (Chakrabarti et al., 2010).

host cellular responses [37].

DNA microarray has been used to study cellular responses to influenza A virus infections in a variety of hosts including *in vitro* cultured cells, *in vivo* animal models to human patient samples. Along with humans; mouse, ferrets and non-human primates like macaques are also susceptible to influenza A virus infection [38, 39]. High density oligonucleotide microarray was used to study gene expression of peripheral blood of pediatric patients suffering from influenza. The study revealed global host responses to influenza virus infection in young children. The profiles were compared with patients having fabrile convulsions along with flu. The analysis identified many interferon-regulating genes significantly affected by disease condition. The investigators tried to correlate pathogenesis of influenza with its

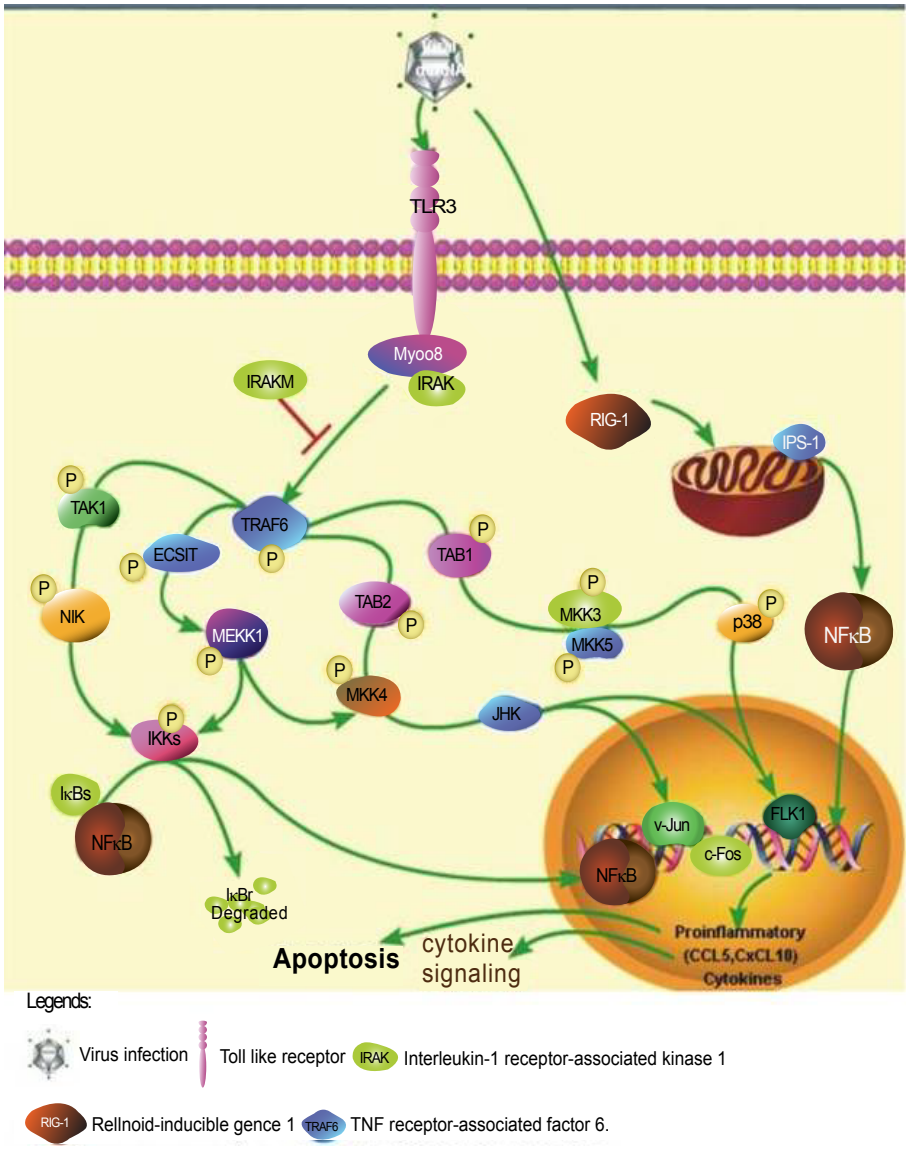

Figure 2: A model depicting a probable cellular response to highly pathogenic avian influenza (HPAI)-H5N1 virus infection in A549 cells which are not activated in response to RG modified H5N1 virus infection. Influenza virus infection results in activation of various signaling events in the host cells. In response to HPAI-H5N1 infection, Toll-like receptor (TLR) mediated signaling events result in activation of inflammatory cytokines like CXCL10, CCL5 through activation of specific transcription factors like NF-_B and v-JUN, as observed in our study. However, this mechanism does not get activated in response to RG modified H5N1 as evident by the down-regulation of cytokine genes. The transcription factors like NF-κB and JUN were also found to be down-regulated during RG modified H5N1 infection [34].

neurological complications [40]. Similar microarray based studies in ferrets showed that H5N1 infection induced severe disease that was associated with strong expression of interferon response genes including the interferon-γ-induced cytokine CXCL10. Treatment of H5N1-infected ferrets with an antagonist of the CXCL10 receptor (CXCR3) reduced the severity of the flu symptoms and the viral titers compared to the controls, clearly demonstrating the potential of biological response modifiers for the clinical management of viral infections [41].

Overall in all these experiments genes involved in immune response were found to be mainly affected by influenza virus infection. The genes specifically getting up-regulated in response to virus infection were interferon inducible genes, pro-inflammatory cytokines, components of JAK-STAT pathway, NF-κB mediated signaling and genes involved in apoptotic mechanism [32-34,41,42]. Interactions of these genes with various other proteins involved in signal transduction mechanism have also been elucidated in these studies.

Microarray technology has also been used to study host responses to influenza viruses having mutations or variation in particular gene sequence. These viruses are either naturally occurring or are produced using reverse genetics technology [43]. These recombinant viruses are used as live attenuated vaccine strains to evoke protective immune responses against pathogenic strains of influenza viruses. In this procedure keeping a similar genome background variations are created in a single gene and viruses are generated which differ only in a single gene sequence. These types of studies help in analyzing the functional mechanism of a particular protein which could be used as therapeutic targets. Using this approach it was demonstrated that NS1 protein derived from the 1918 Spanish H1N1 pandemic influenza virus blocked expression of interferon-regulated genes more efficiently than did the NS1 protein from established seasonal influenza viruses. Genetically engineered influenza A viruses containing NS1 gene of 1918 Spanish H1N1 pandemic influenza A was able to infect mice better than viruses containing other gene segments [44].

Conclusions

Since beginning, new technologies have been continuously added in the field of biomedical sciences which has gradually enriched science and enormously improved the quality and quantity of research output. The microarray technique has over the year contributed significantly towards our understanding of virus biology and interaction with its host. It has not only provided a tool for molecular diagnostics but also has helped in advancing genome wide transcriptome analysis and identifying protein targets for therapeutics. Microarrays have greatly improved our capabilities in biomedical research which is invaluable for patient treatment, efficient use of antibiotic and antiviral agents and prevention of epidemic outbreaks in future.

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